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RISK FACTORS AND AN ASSESSMENT OF CONTROL STRATEGIES FOR IRON DEFICIENCY ANEMIA IN CHILDREN IN NORTHERN ETHIOPIA

by

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Risk factors and control strategies for iron deficiency anemia

ABSTRACT

The aims of the present studies were to determine the magnitude of iron deficiency anemia among preschool children in Northern Ethiopia and to evaluate different control strategies. The cross-sectional study showed that anemia was highly prevalent (42%) and that iron deficiency was the commonest cause of anemia. However, the iron deficiency was not due to lack of iron in the diet but to its poor availability and to other non-dietary risk factors. Unsafe water, mother's illness, older child (24-60 months), family not having food reserves and family income below poverty-line were predictors of anemia. Hookworm and malaria were rare and did not account for the anemia. In the iron pot study three types of Ethiopian foods were cooked in three types of pots (iron, aluminum and clay) and assessed for their total and available iron. After adjusting for cooking time and moisture, there were significantly higher total and available iron in all the three types of foods when cooked in iron pots compared to the aluminum or clay pots. The study also showed that the hemoglobin status and length of children improved significantly when they consumed food cooked in iron pots. In the third study, a randomized, placebo-controlled, and double-blind trial, both iron and vitamin A supplemented children showed increased hemoglobin levels. Combined iron and vitamin A supplementation showed the highest rise. Iron-supplemented children showed increase in length, but no increase in weight. They also showed lower rates of c-reactive protein positivity and decreases in the prevalence and frequency of diarrhea. A single dose of vitamin A did not result in any increase in length or weight but a decrease in the prevalence and frequency of diarrhea was observed. Children supplemented with iron only or vitamin A only showed higher ARI rates, but those children who received combined iron and vitamin A showed significantly lower ARI rates. Iron supplementation did not have any effect on either zinc or copper status of the study children. These studies suggest that significant public health benefits may be realized in the use of iron pots and in the concurrent supplementation of iron with vitamin A in target populations. In addition, they provide sound evidence for a reassessment of existing strategies for the control of iron deficiency anemia.

ABRÉGÉ

Les objectifs de cette recherche sont de déterminer l'importance de l'anémie causée par une carence en fer dans le Nord de l'Éthiopie et d'évaluer différentes stratégies de contrôle. Trois études ont été faites. L'étude transversale a démontré l'ampleur de la prévalence de l'anémie (42 %) et le fait que la carence en fer en est la cause la plus fréquente. Cependant, la carence n'était pas due à la faible teneur en fer dans l'alimentation mais à sa faible disponibilité et à d'autres facteurs de risque reliés indirectement à l'alimentation. L'eau douteuse, la présence de maladie chez la mère, le jeune âge de l'enfant (< 24 mois), l'absence de réserves alimentaires et un revenu familial sous le seuil de pauvreté constituaient des facteurs prédictifs d'anémie. L'ankylostome et la malaria étaient peu fréquents et n'expliquaient pas l'anémie. Dans l'étude des chaudrons de fer, trois types de plats éthiopiens ont été cuits à l'aide de trois différents types de chaudrons (fer, aluminium et argile) et leurs quantités totales et disponibles de fer ont été estimées. Après ajustement pour le temps de cuisson et l'humidité, les quantités totales et disponibles de fer étaient de manière significative plus élevées dans les trois types de plats lorsque cuits dans les chaudrons de fer comparativement aux chaudrons d'aluminium et d'argile. L'étude a également démontré que le taux d'hémoglobine ainsi qu'une augmentation de la taille des enfants anémiques s'étaient amélioré de façon significative lorsqu'ils consommaient de la nourriture cuite dans les chaudrons de fer. Dans la troisième étude, un essai randomisé à double-insu placebo-contrôle, les enfants ayant reçu des suppléments de fer ou de vitamine A ont démontré une augmentation du niveau de l'hémoglobine. Les suppléments combinés de fer et de vitamine A ont donné l'augmentation la plus élevée. Les enfants ayant reçu des suppléments de fer ont présenté une augmentation de la taille mais pas du poids. Ils ont aussi présenté des taux inférieurs de positivité de la Protéine C-réactive et une diminution de la prévalence et de la fréquence de la diarrhée. Une seule dose de vitamine A n'a pas entraîné d'augmentation de la taille ou du poids mais une diminution de la prévalence et de la fréquence de la diarrhée. Les enfants ayant reçu des suppléments de fer ou de vitamine A ont montré des taux plus

élevés d'IRA (infections respiratoires aiguës), mais les enfants qui ont reçu les deux suppléments combinés ont démontré des taux d'IRA nettement plus bas. Les suppléments de fer n'ont pas eu d'effet sur les taux de zinc et de cuivre des enfants participant à l'étude. Ces études suggèrent que, chez les populations cibles, l'utilisation de chaudrons de fer ainsi que la prise de suppléments de fer accompagnée de vitamine A peut entrainer des avantages significatifs en matiére de santé publique. De plus, elles fournissent des preuves solides pour la re-évaluation des stratégies actuelles de controle de la carence en fer.

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PREFACE

The first year of my professional life as a practicing physician was in a city in Northern Ethiopia, one of the worst affected areas by the 1985/86 drought and famine. In this first year, I witnessed unfathomable starvation and human tragedy no amount of drug or clinical care could cure. I also realized micronutrient deficiency was rampant and that children were losing their lives, their sight and their intellect for largely avoidable reasons. Other than making me appreciate the stark reality of life in rural Ethiopia, this experience has also helped me shape my professional career.

When I got the opportunity to do thesis research, I decided to work on micronutrient deficiencies in a community affected by famine and drought. I wrote the proposal and the questionnaire, which were the ground work for the present studies. After securing funding, I went to Ethiopia and directed the field work. I participated in most of the laboratory work and serum analysis for ferritin, zinc and copper. I was also responsible for the data cleaning, analysis and writing of the results.

No amount of lecture and academic training prepared me for the problems I encountered in the field. My first nightmare started when the equipment and drugs shipped from Canada failed to show up in Ethiopia. Ordering the drugs and getting all the equipment together had taken more than eight months and I was devastated when I heard of the loss. Fortunately, help came from several directions and after three months of

tedious search, the shipment was located at the Khartoum airport very far from its destination. The reason for the mishap, I was told, was that the airline responsible for transporting the shipment had found the package to be too big to fit into the door of its airplane. Therefore they left the package in Khartoum and did not bother to notify us or their agents in Addis.

Once every equipment was in place and potency of the drug was verified, organizing and conducting the research was the next challenge. The study has encountered all the harsh realities of life in a developing country. Unexpected power cuts were a challenge to safekeeping of specimens and using the computer for data entry. Communication (fax/telephone) with my supervisors in Canada was one of the biggest challenges I had to live with. Getting access to a fax/ telephone depended on the good will of offices or individuals who had them. Once I got the chance to use the phone, getting connected was the next challenge. I was privileged to have had access to an e-mail service, which in most cases was a convenient and inexpensive way to transfer big files. However, the e-mail also depended on the use of the poor telephone system and inefficient e-mail network (PADIS). The real boost to my morale and what made me love the field work was the smile in the faces of the children and the pride of their mothers. If the children could smile and the mothers could keep their pride and not expect pity for their harsh life and poverty, the least I could do was face the challenges.

In November of 1994, in collaboration with the Relief Society of Tigray (REST) and the Steelworkers Humanity Fund (SHF), I conducted a food security workshop in Mekele, whereby the results of the baseline study and its recommendations were thoroughly discussed. This workshop was attended by not less than 20 international and local NGOs and representatives of the local ministries and health institutions. Based on the baseline results and repeated history of famine and drought in the region, the participants suggested that a nutrition monitoring and disaster early warning unit be organized in the region. It was with this objective that the Nutrition Monitoring Unit was organized under the auspices of REST. The goal of the Nutrition Monitoring Unit is to have an ongoing evaluation of social programs such as health and nutrition, education and economic development. With continuous surveillance one can keep an eye on social programs and detect problems early and avoid disasters. The Unit is now fully functional and it is being funded by the SHF.

Based on the nutritional problems identified by the baseline study, five different types of posters and six types of leaflets were produced and distributed throughout the region. As of December 1995, the program managed to provide three sessions of nutrition education to the public through the local radio. Arrangements are currently underway for a weekly nutritional education program. I also was able to publish the findings of the baseline study into a book in two local languages. This is the first scientific study to be translated into Amharic and Tigrigna. As these languages are very poor in scientific terminology it was a challenge to create and coin scientific terms.

This thesis uses a manuscript-based structure by including three papers to be submitted for publication. In order to inform the external examiner of Faculty regulations, the following five indented paragraphs are reproduced from the Guidelines for Thesis Preparation by the Faculty of Graduate Studies and Research.

"Candidates have the option of including, as part of the thesis, the text of a paper(s) submitted or to be submitted for publication, or the clearly-duplicated text of a published papers(s). These texts must be bound as an integral part of the thesis.

If this option is chosen, connecting texts that provide logical bridges between the different papers are mandatory. The thesis must be written in such a way that it is more than a mere collection of manuscripts; in other words, results of a series of papers must be integrated.

The thesis must still conform to all other requirements of the "Guidelines for Thesis Preparation". The thesis must include: A Table of contents, an abstract in English and French, an introduction which clearly states the literature, a final conclusion and summary, and a thorough bibliography or reference list.

Additional material must be provided where appropriate (e.g. in appendices) and in sufficient detail to allow a clear and precise judgement to be made of the importance and originality of the research reported in the thesis.

In the case of manuscripts co-authored by the candidate and others, the candidate

is required to make an explicit statement in the thesis as to who contributed to such work and to what extent. Supervisors must attest to the accuracy of such statements at the doctoral oral defense. Since the task of the examiners is made more difficult in these cases, it is in the candidate's interest to make perfectly clear the responsibilities of all the authors of the co-authored papers. Under no circumstances can a co-author of any component of such a thesis serve as an examiner for the thesis".

STATEMENT OF ORIGINALITY

With the dire need of reducing the economic and health impact of iron deficiency and improving the existing control strategies, there is a great need for rigorously designed studies on iron deficiency anemia. Most of the existing field studies have raised controversies and there is an obvious lack of knowledge in the field. The three studies conducted for this thesis will address some of the controversies and attempt to fill existing gaps in knowledge. The iron pot study which is the first study of its kind will shed new light on possible alternative strategies for the control of iron deficiency anemia.

There are only few studies on the prevalence of iron deficiency anemia. Most of the existing studies are either hospital-based or small community studies. Moreover, none of these studies had addressed the risk factors that predispose children to iron deficiency anemia. In comparison to these studies, the study presented here had a very large sample size and included both urban and semi-urban communities. The comprehensive number of maternal and child variables collected made adjusting for potential confounders possible and provided sufficient data to determine risk factors for iron deficiency anemia. This is the first study in the country that used an assessment of dietary intake (food frequency and weighed food record) to investigate the association of diet to iron deficiency anemia. The use of up-to-date equipment and highly qualified technicians also made the study unique in the country.

This study is the first study anywhere to assess the role of iron pots in the control of iron deficiency anemia. The role of iron pots was assessed at two levels: 1- Laboratory level (*in-vitro* study) and 2- Community level. The *in-vitro* study compared iron release into foods from iron pots with that from aluminum and clay pots. The community study examined whether the food cooked in the iron pots improved the iron status of preschool children.

Several previous studies that have assessed the effect of iron supplementation on morbidity and growth produced controversial results. Most of these emphasized the need for other placebo-controlled, randomized and double-blind trials. Furthermore, all of these studies were conducted on school children or adults. This study is the first rigorously controlled, randomized and double-blind community trial of preschool children in communities with significant micronutrient deficiency. Therefore, this study has added new information to help resolve the existing controversy. In this study the independent and combined effects of iron and vitamin A on hematological indices and the growth and morbidity of preschool children were examined. This is the first study to report the effect of iron supplementation on zinc and copper status of children. The study is also the first in the country to report on zinc and copper status in children.

As an example of applied research, the studies presented here were exceptional in terms of the level of community involvement and dissemination of the results. The communities organized nutrition committees which participated in the implementation and

monitoring of the field research. For every 20 households one community worker was selected to visit the study children every other day to assess compliance. The results of this research have also been disseminated in the region in general and in the study communities in particular. A seminar was organized to present the preliminary findings to members of the communities and to local and regional government representatives. The results, along with pertinent nutrition education, were broadcast on the local radio, for an audience of over one million people. Finally, pertinent findings have been published into a book in two local languages for free distribution.

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1.0 - INTRODUCTION

Among the essential elements, iron is the most abundant mineral on earth (five percent of the earth's crust), yet iron deficiency affects over half a billion people (Cook *et al* 1994). It affects a significant part of populations in nearly all countries of the world. Preschool children in Africa have some of the highest rates of iron deficiency anemia in the world, nearly 56% (WHO 1959, UN 1990, UN 1991). Although the problem is more prevalent in developing countries (36%), developed countries (18%) also suffer from iron deficiency anemia (FAO 1988). It is generally agreed that an optimal iron intake is one that allows the unrestricted production of hemoglobin (Braunwald *et al* 1987). As some studies have reported, iron intake in Ethiopia and some other developing countries is over and above the recommended requirement (ICNND 1959, Hofvander 1968). Yet, the rates of iron deficiency in these countries are astounding, casting some doubt on the validity and generalizability of their results. In addition, it may be that factors other than dietary intake play important roles in the causation of iron deficiency.

In addition to the effect of anemia on oxygen carrying capacity, the non-hematological consequences of iron deficiency include poor growth, increased susceptibility to infection, deficit in intellectual development and decreased work capacity (WHO 1994). Therefore, meeting at least the minimum iron requirements is an important component of maximizing health and well-being and offers sound investment for development. The World Health Assembly and the World Summit for Children 1990 (UN

1990) adopted a goal of reducing iron deficiency at least among women of child-bearing age by one-third of the 1991 level by the year 2000 (WHO 1994). The staggering economic effect of iron deficiency anemia and its consequences have prompted countries to formulate strategies to control the problem.

Once iron deficiency anemia occurs, even timely and adequate iron therapy seems to be ineffective in reversing the behavioral, cognitive and other consequences which ensue (Walter 1995). A sound approach to this problem therefore is to prevent iron deficiency before it occurs. However, existing control strategies have their shortcomings. Fortification is impractical in most developing countries and not fully effective in persons suffering from severe iron deficiency. The side effects of iron and the logistics of distributing iron tablets deter from successful implementation of iron supplementation programs (Cook *et al* 1994). Therefore, attention must be placed at either improving the effectiveness of existing strategies or developing new approaches to the problem.

Iron is the most studied of the micronutrients. However, several controversies, including the effect of iron on growth and morbidity and the effect of iron supplementation on the absorption of other minerals, exist. The three studies presented here address these issues and provide new evidence to guide the formulation of health policy in this area.

2.0 - LITERATURE REVIEW

2.1 - Iron and iron deficiency anemia

Anemia is defined as a reduction of the red cell volume or hemoglobin concentration below the range of values occurring in healthy persons (Vaughan *et al* 1979). Hemoglobin levels indicative of anemia have been established by a WHO scientific group in 1968, for specific population groups: below 11 g/dl for pregnant women, below 12 g/dl for menstruating women, below 13 g/dl for male adults, below 12 g/dl for children 6-14 years old and 11 g/dl for children under five (WHO 1968). In physiological terms, anemia results when the hemoglobin level is below the body's physiological needs as set by tissue oxygen demand (Andreoli *et al* 1993). Therefore, irrespective of an initial hemoglobin level, an increase of hemoglobin by 1 g/dl after iron supplementation qualifies the individual as anemic.

Anemia resulting from a lack of sufficient iron for the synthesis of hemoglobin is by far the most common hematological disease in infancy and childhood (Andreoli *et al* 1993). Iron deficiency develops when the amount of iron absorbed from the gastrointestinal tract is not sufficient to meet normal body requirements. Iron balance, therefore, is a function of the amount of iron in the diet, its availability and absorption in the intestine, and its increased demand and/or its excessive loss in the body. As shown in Table 1 iron depletion occurs in three progressive stages (Vaughan *et al* 1979).

Table 1: Sequences of Clinical Changes in Iron Deficiency Anemia*

1- Stage I - high risk for iron deficiency

1.1- Decrease in iron stores; decrease in hemosiderin content of liver and bone marrow.

1.2- Decrease in levels of serum ferritin to less than 10 μg/L.

2- Stage II - iron deficiency without anemia

2.1- Decrease in level of serum iron; increase in total iron binding capacity; fall in percent of saturation (< 15%).

2.2- Increase in levels of free erythrocyte protoporphyrin.

3- Stage III - iron deficiency anemia

3.1- Decrease in hemoglobin level

3.2- Progressive hypochromia and microcytosis.

Note: Decrease in the activity of intracellular enzymes may occur in any stage.

* Table modified from Vaughan *et al* (1979)

The first stage is a decrease in iron stores without loss of essential iron compounds. Other than showing vulnerability, this stage has not been associated with adverse physiological consequences. The second stage is characterized by biochemical changes that are due to the inadequacy of iron for normal production of hemoglobin

and other essential iron compounds. This stage is indicated by a decrease in transferrin saturation and an increase in erythrocyte protoporphyrin. This stage is regarded as iron deficiency without anemia, and may be associated with deficits in psychomotor and intellectual performance (Oski *et al* 1983, Lozoff *et al* 1987). The final stage is frank iron deficiency anemia, which occurs when hemoglobin production has been sufficiently depressed, resulting in hemoglobin levels below age and gender-specific norms. At this stage patients usually have obvious clinical signs and symptoms, the most important being a reduced ability of the blood to transport oxygen. This is manifested by pallor, splenomegaly, koilonychia and post cricoid web.

Iron absorption is regulated at the level of the mucosal cells of the small intestine. Its efficiency depends on both the iron status of the individual and the type of iron element. Heme iron (iron from animal sources) is better absorbed than non-heme iron (iron of plant origin). The absorption of iron is enhanced by vitamin C, meat, fish and poultry, and fermentation. It is inhibited by phytates, tannins, coffee, tea and cocoa. Iron is transported by transferrin in the plasma and it is stored as ferritin and hemosiderin in the liver, spleen and bone marrow.

As a constituent of heme, iron is present in hemoglobin, myoglobin and several enzymes which serve a variety of functions. Some of these functions are transport and storage of oxygen, mitochondrial electron transport, catecholamine metabolism and DNA synthesis. The consequences of iron deficiency, therefore, can be explained by

the function of the circulating hemoglobin and the activities of these enzymes in the body (Dallman *et al* 1980).

2.2 - Epidemiology of iron deficiency anemia

The prevalence of iron deficiency anemia is influenced by both host factors (eg. age, gender and physiological state) and environmental factors (eg. diet, socio-economic factors and altitude) (WHO 1994). Hemoglobin levels rise during the first ten years of childhood, with a further increase at puberty. Gender differences start at puberty and diminish gradually with increase in age (WHO 1994). In the United States individuals of African extraction have 0.5 to 1 g/dl lesser hemoglobin level than individuals of European extraction. In developing countries, factors such as prolonged loss of iron from bleeding caused by intestinal parasites, diarrhea due to lack of personal and environmental sanitation, or inadequate iron intake may predispose children to iron deficiency anemia (Committee on Nutrition 1978). These factors are the results of other more deep-rooted factors related to underdevelopment in general.

A study of 242 Bangladeshi children showed mean hemoglobin levels to be significantly higher in children of smaller family size than children of larger family size (Ahmed *et al* 1992). Children from low income families showed significantly lower hemoglobin levels than children from high income families. However, there was no

significant difference in mean hemoglobin between children belonging to low versus middle income groups and between middle versus high income groups. A cohort study by Michaelsen *et al* (1995) reported that children with a high growth velocity and a high intake of bread but a low intake of meat and fish had an increased risk of depleting their iron stores. Another study in non-pregnant women in Western Ethiopia reported that hookworm infection was the major cause of iron deficiency anemia and that multi-parity and low income were risk factors for anemia (Adish *et al* 1996).

2.3 - Assessment of iron status

In iron deficiency, a fairly definite sequence of biochemical and hematological events occurs (Table 1). In the first stage tissue iron stores are assessed by its disappearance from liver and bone marrow (WHO 1994). Serum ferritin which is an iron-binding protein provides a relatively accurate estimate of iron stores. Serum ferritin values fall in iron deficiency anemia before changes in serum iron, total iron binding capacity or hemoglobin levels are manifested. In most individuals the concentration of serum ferritin parallels the total amount of iron stored. Serum ferritin is the only iron status index that can accurately reflect a deficiency, excess and normal iron status (Cook *et al* 1974). In conditions of frank iron deficiency anemia, when classical microcytic hypochromic anemia occurs, serum ferritin levels are very low or zero, reflecting the exhausted iron stores. A low concentration of serum ferritin

(< 10 μg/L) is characteristic only of iron deficiency (Dallman *et al* 1980). According to Guyatt *et al* (1992), even though serum ferritin is an extremely powerful test for the diagnosis of iron deficiency anemia, infection and inflammation are known to cause increases in the rate of ferritin synthesis in the reticulo-endothelial system, leading to increase in serum ferritin levels. Therefore, serum ferritin should be used with caution as an index of iron status in countries where iron deficiency coexists with infection (INACG 1985, Gibson 1990). There are significant variations of serum ferritin levels across age and sex related to vulnerability to iron deficiency (Gibson, 1990). Serum ferritin is relatively high at birth and rise during the first two months. It starts to fall in late infancy and throughout childhood. Adult men and the elderly have higher levels than women of reproductive age.

With increasing severity the red cells become deformed and misshaped. These changes result in characteristic morphologic findings of microcytosis, hypochromia, and poikilocytosis, without which a diagnosis of significant iron deficiency anemia is untenable. In other words, the red blood cells become smaller than normal and their hemoglobin content decreases. The morphological characteristics of red blood cells are best quantified by means of determining red blood cell indices: mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH).

Hemoglobin is first affected at the second stage of iron deficiency. Hemoglobin is an iron-bearing protein which confers blood its red coloring.

Therefore, lower hemoglobin or hematocrit levels reflect the decreased capacity of the blood to carry oxygen to the tissues. There is no one single perfect test to diagnose iron deficiency, nor is the use of multiple tests a practical way of overcoming the limitation of a single test (ESWG 1985). When choosing a test, especially for a community study, ease of operation, accuracy, and cost have to be considered (WHO 1994). The normal ranges for hemoglobin, hematocrit or other hematological tests represent the values obtained for 95% of a normal, healthy population, assuming a normal distribution of individuals by age, gender, and a stable ambient altitude. In poor communities when resources are not available for routine laboratory testing, clinical signs, such as pallor of the palms and the eyelid, can be used to screen very high risk women and children for an intervention.

2.3.1 - Individual-based assessments

Individual-based assessments are used to detect high risk cases for iron supplementation or case management (WHO 1994). The commonly used screening tests are hemoglobin and hematocrit. Hemoglobin and hematocrit only identify anemia and are not specific for iron deficiency. Therefore, more specific tests, such as ferritin (storage protein), or transferrin saturation (transport protein) have to be performed to confirm the diagnosis (Table 2).

2.3.2 - Population-based assessments

Population-based assessments might be used for diagnosis (i.e. assessing prevalence of anemia in a community, screening high risk cases for intervention or monitoring of iron intervention programs). The commonly used measures for these purposes are hemoglobin or hematocrit (Beaton 1986). The prevalence of anemia assessed by hemoglobin or hematocrit measures can also be used to compare a population in one community to a similar population in another (WHO 1994).

Table 2 - Assessing iron status based on the availability of local resources

	Resource conditions		
	Poor	Intermediate	Adequate
Individual-based applications			
Screening	Clinical exam for severe anemia	Hb/Hct for screening	Hb/Hct for screening SF/SAT as additional tests (a)
Confirmation or diagnosis	Hb/Hct clinical response	Hb/Hct response to treatment	Hb/Hct response to treatment SF/EP/SAT
Population based applications			
Special assessment or survey	Hb/Hct(b,c)	Hb optional MCV/SF/SAT/EP	Hb/MCV/SF/SAT/EP/ TR
"Diagnosis" of causes of anemia	Response to iron supplements (d)	Response to iron supplements	--
Long term surveillance (e)	Hb/Hct at selected sites	Hb/Hct from PHC or MCH	Hb/Hct from clinics

(a) Use of serum ferritin or transferrin saturation in addition to Hb or Hct in areas interested in detecting milder forms of iron deficiency or of iron overload.

(b) Specific iron biochemistry tests may not be helpful in areas also having high rates of infections. In addition, it is more difficult to implement serum processing, transport and storage.

(c) In areas with known malaria and/or hookworm transmission, testing and treatment responses for each condition can be considered.

(d) If other relative or absolute nutritional deficiencies, such as folic acid, vitamin C or vitamin A, are suspected of contributors to anemia, trials of iron supplements with and without these other supplements can be considered.

(e) Less than adequate testing procedures for individual based screening are still useful for the purpose of surveillance or to define trends, if the same methods are used throughout.

EP = Erythrocyte protoporphyrin
Hb = Hemoglobin
Hct = Hematocrit
MCH = Maternal and child health
MCV = Mean corpuscular volume
PHC = Primary health care
SAT = Transferrin saturation
SF = Serum ferritin
TR = Transferrin receptors

Table modified from WHO, 1994

2.4 - Iron deficiency anemia in Ethiopia

Ethiopia is among the poorest countries of the world and is greatly affected by both macro and micronutrient deficiencies. The staple foods of its people are of cereal origin; vegetables and meat are rarely consumed. Stunting, diarrhea, and acute respiratory infections (ARI) are the most common health problems in children with prevalence rates of 30%, 17% and 14%, respectively (MOH/ WHO 1987).

In Ethiopia, the magnitude and importance of iron deficiency anemia as a public health problem is still disputed. Some studies have reported iron deficiency anemia rates of less than 18% (Hofander 1968, Gebremedhen *et al* 1976, Wolde-Gebriel 1992) while others have shown prevalences of 25% and above (Zain 1987, Gebresselassie 1997). According to Wolde-Gebriel (1992), the relatively low rate of anemia in most parts of the country, despite the high prevalence of infectious diseases, is attributed to the consumption of the staple cereal, teff, *Eragrostis teff*, and most likely to its contamination with iron rich clay soil (90 mg of iron per 100 gm of teff). Hofander (1968) estimated the iron intake for an Ethiopian adult to be 400 mg/day in a diet of 2500 calories. The Interdepartmental Committee on Nutrition for National Defense (ICNND 1959) study, which covered most parts of the country, reported that the intake of iron was more than adequate, that of protein and niacin were acceptable, and that of riboflavin, vitamin A, and vitamin C were low. Similarly, a survey by the Ethiopian Nutrition Institute (1980) reported that iron, thiamine and riboflavin intakes were adequate, but vitamin A and vitamin C intakes were low in both pre-harvest

(May) and harvest (December) seasons. Studies by Selinus *et al* (1971) in Arsi reported similar results. All these studies seem to agree on the fact that iron intakes of most communities in the country were far above the normal requirements. Yet none of the studies explained why there was still a high prevalence of anemia in the country, even in teff-consuming communities. The fact that iron deficiency anemia remains one of the important factors associated with hospital admissions and death in all regions of the country (MOH 1987, MOH 1995) also requires an explanation. In summary, iron deficiency anemia is by no means rare in Ethiopia, and there are risk factors, other than iron intake, which need to be considered as the determinants of iron deficiency anemia. The fact that the rates of anemia are different in various communities in the country suggests that these risk factors are community and situation specific.

2.5 - Consequences of iron deficiency anemia

There are numerous symptoms of iron deficiency, some are due to the anemia itself, some are due to the effect of iron deficiency on iron dependent enzymes, and some are due to both (Dallman *et al* 1980). The degree of functional impairment generally increases with the severity of the deficiency. Some of the important functional consequences of iron deficiency in children are reduced resistance to infection, growth faltering, and impaired intellectual development (FAO 1988, UN 1990).

2.5.1 - Effect of iron on morbidity

The effect of iron on resistance to infection is controversial (Committee on Nutrition 1978, FAO 1988, UN 1990). Iron deficiency and infection often coexist in disadvantaged communities. Some infections, such as hookworm (Foy 1960, Layrisse 1964, Stephenson 1987), schistosomiasis (Stephenson 1987) and malaria (Stephenson 1987, FAO 1988,) are known to lead to iron deficiency as a result of bleeding or destruction of red blood cells caused by the parasites. Iron deficiency may also lead to infection due to reduced host defense, reduced cell mediated immunity (Joyson *et al* 1972, Chandra and Saraya 1975, Bhaskaram and Reddy 1975, Srikantia *et al* 1976, Badoual and Hercberg 1993), reduced neutrophil function (Chandra and Saraya 1975, MacDugall and Anderson 1975, Joyson *et al* 1976) and depressed skin test to common antigens (Bhaskaram and Reddy 1975, Srikantia *et al* 1976). The humoral component of the immune system seems to be intact (Joyson *et al* 1972, Chandra and Saraya 1975). However, studies in human communities are less conclusive and contradictory (MacDugall and Anderson 1975, FAO 1988). Some community intervention studies in both developed (MacKay 1928, Andelman and Sered 1981) and developing countries (MacDugall and Anderson 1975, Hussein *et al* 1988, Berger *et al* 1992) have provided evidence that iron supplementation reduces the occurrence of diarrhea and acute respiratory infections. Other investigators have reported that supplemental iron reactivates pre-existing infections such as malaria, brucellosis and tuberculosis (Masawe and Muindi 1974, Murray *et al* 1975,

Oppenheimer *et al* 1986, Chwang *et al* 1988). A randomized study by Heresi *et al* (1995) failed to show any difference in the rates of diarrhea and ARI in 872 and 783 urban infants who received iron-fortified powdered milk and non-fortified powdered milk, respectively. However, several researchers have speculated that the increase in the rate of infection is not due to iron therapy *per se*, but to the route of administration of the mineral (Committee on Nutrition 1978, DeMaeyer 1989). Parenteral iron causes transferrin to become saturated making unbound iron available for growth and reproduction of microorganisms in the blood.

2.5.2 - Effect of iron on growth

The biological requirement for iron increases with growth, and iron deficiency is most common during infancy and puberty when velocity of growth is rapid (Chwang *et al* 1988, Brabin and Bernard 1992). A study by Beard *et al* (1995) showed that iron-deficient rats have metabolic inefficiency and less body fat. Another study in Kenya showed that iron deficiency reduces appetite (Lawless *et al* 1994) and affects intestinal nutrient absorption (Herberg and Galons 1989), thereby impairing growth. A follow-up of growth charts of 156 children under 3 years of age with nutritional anemia showed a preponderance of lower weight which shifted towards normal weight following iron therapy (Judisch *et al* 1966). In a cross-sectional study, Bangladeshi children living in a community who were found to have consumed more than 1 mg of iron/day from pipe water, were taller and heavier compared to children in

a control community with less iron in their water (Briend *et al* 1990). In Birmingham, a double-blind, randomized clinical trial in 97 children, 17 to 19 months of age demonstrated that iron-supplemented children gained weight faster than placebo-treated controls (Aukett *et al* 1986). A study in rural Indonesian (Chwang *et al* 1988) and Kenyan children (Latham *et al* 1990, Lawless *et al* 1994) supported this result, but a study in New Zealand showed a gain in weight only, and not in height or head circumference (Tonkin 1970). A cross sectional study in Togo failed to show any difference either in weight or in height, between iron-deficient (n=241) and iron-sufficient (n=56) children (Berger *et al* 1992). A controversial finding by Idjiradinata *et al* (1994) reported retarded growth in 47 iron-replete Indonesian children after daily iron supplementation. Even though the sample size of this sudy was small, its finding is a challenge to long term indiscriminate iron supplementation in communities with disadvantaged nutritional status.

2.5.3 - Effect of iron on cognitive development

Iron deficiency anemia has often been presumed to have few deleterious effects unless severe enough to compromise cardiovascular function (Lozoff *et al* 1982). A study in 11-year-old Thai children showed significantly better performance in language and mathematical tests in iron-supplemented children compared to the placebo group and this difference was observed even in the subgroup of children who had iron deficiency without anemia (Pollitt 1991, Walter 1995). Several other studies,

both in developing and developed countries, showed similar results (Seshadri and Gopaldas 1989, Somantri 1989, Pollitt 1991). Furthermore, iron deficiency is of special concern because its occurrence is more prevalent during periods of rapid brain growth and the changes may be irreversible (Lozoff *et al* 1987). A study in Egypt showed that children who had moderate anemia as infants had lower scores on tests of intelligence and other functions at school entry compared to children who were non-anemic at infancy (Lozoff *et al* 1991). In summary, while iron deficiency can impair cognitive performance at all ages, the effects of iron deficiency in infancy and early childhood are not likely to be reversed by subsequent iron therapy.

In adults, work capacity and productivity were found to be markedly decreased due to iron deficiency (Viteri and Torun 1974, Basta *et al* 1979). Other studies have demonstrated that after iron supplementation the productivity of anemic workers rapidly returned to normal (Woldgemuth *et al* 1982, Li 1993). Following iron supplementation, gains in productivity and take-home pay ranged from 10-30% (Levin 1986). Other important functional consequences of iron deficiency are increased maternal mortality, prenatal and perinatal infant loss in anemic pregnant mothers, hypothermia and depressed thyroid function in cold exposure and increased risk of heavy metal poisoning (WHO, 1994)

2.6 - Control strategies for iron deficiency

In recent years iron deficiency anemia is receiving increased attention because of its staggering social and economic effects on development, availability of reliable indicators of iron status and feasibility of control (WHO 1994). The important control strategies can be classified into drug-based strategies, such as daily or weekly oral iron supplementation or diet-based strategies, such as food fortification and dietary diversification (WHO 1994). A sound control strategy should include public health measures directed towards the cause of iron deficiency (e.g. treatment for hookworm and malaria in endemic regions) as one of its components (United Nations 1991). In iron supplementation, which is the most common practice of treating iron deficiency at the individual and community levels in developing countries, iron has to be taken daily for at least three months. In spite of the quick response iron supplementation provides, the long duration of treatment and the occurrence of side effects are deterrents to treatment compliance (DeMaeyer 1989, Cook *et al* 1994). Recently, studies have shown that weekly supplementation of iron is as effective as daily supplementation (WHO 1993). Other longer-term strategies, such as dietary modification and food fortification (food-based strategy), need to address issues of food production, processing, marketing and preparation which require intensive community education and logistic support (WHO 1994). Therefore, a different strategy of delivering iron, without the aforementioned constraints, would be a step forward in worldwide efforts to control iron deficiency anemia. One such strategy is the use of iron pots as a means of supplying the required iron for iron-deficient persons

in high risk communities. Iron pots are very inexpensive and can last for years.

2.6.1 - Role of iron pots in the control of iron deficiency

It is common knowledge that in the past iron pots were commonly used in most communities in Africa and other developing countries. This cultural practice has been gradually lost with technological development and the availability of cheaper aluminum pots. Mertz (1980) hypothesized that the diminished use of iron cook-ware in the United States over the past 100 years is one of the factors contributing to the higher rate of iron deficiency in American women. A study by Burroughs and Chan (1972) showed that Mexican foods cooked in an iron pot had a higher iron content compared to foods cooked in glass-ware. However, a study in Papua New Guinea showed an increase in iron content only in cooked rice and green vegetables and not in staple roots (Drover and Maddocks 1975). Mistry *et al* (1988) showed that foods cooked in iron pots had more iron compared to food cooked in non-iron pots (1.67 versus 0.54 mg/100 gm of food) and that the iron was as available as native or food iron (1.5%). Another study by Brittin and Cheryl (1986) noted the same result and showed that higher moisture, higher acidity and longer cooking time had a positive effect on the amount of iron leached into the food. A study by Cheng and Brittin (1991) showed that the release of iron into the food by iron pots was not a one-time phenomenon but continued with consecutive cookings. Two other studies (Kuligowski and Halperin 1992, Zhou and Brittin 1994) showed that steel woks were

as effective a source of iron as iron skillets. An intervention study on experimental animals showed that rats depleted of iron through diet recovered after being fed on foods cooked in iron pots (Martinez and Vannucchi 1986). To our knowledge no study has yet investigated the effect of cooking with iron pots on the iron status of human subjects nor investigated its role as a control strategy for iron deficiency anemia.

2.6.2 - Role of vitamin A in the control of iron deficiency anemia

Roodenberg *et al* (1996) speculated that supplementing with vitamin A during iron repletion contributes to optimize erythropoiesis and iron mobilization when baseline vitamin A status is impaired. Studies in laboratory animals reported that rats fed diets deficient in vitamin A showed a reduction in hematopoietic cells in their bone marrow (Findly and Mackenzie 1922, Wolbach and Howe 1925). However, other studies observed that anemia in early vitamin A deficiency improved in both hemoglobin and hematocrit in spite of the increases in the severity of the vitamin A deficiency (Sure *et al* 1929, O'Toole *et al* 1974). Two other studies in children also showed that vitamin A deficiency resulted in iron deficiency anemia (Blackfan and Woldbach 1933, Sweet and K'ang 1935). The beneficial effect of vitamin A on iron deficiency anemia was also reported by Mejie and Chew in 1988. Bloem *et al* (1989) reported that vitamin A deficiency increases the host's susceptibility to infection, which in turn results in impaired hematopoiesis. According to Semba *et al* (1992)

supplementing with vitamin A alone increased the hematological indices in children and the combined effect of iron and vitamin A showed even better results. Suharno *et al* (1993) reported similar results in pregnant women. However, a community trial on 162 anemic Ethiopian children failed to show any effect (Wolde-Gebriel *et al* 1992). The study showed an increase in the red blood cell count, but a decrease in the MCV and no difference in hemoglobin, hematocrit, serum iron, transferrin saturation and serum ferritin between combined iron and vitamin A supplemented and iron only supplemented children. Therefore, the study did not ascertain the effect of vitamin A alone on the hematological indices. In conclusion, animal studies tend to show that vitamin A plays a positive role in iron deficiency anemia, but human studies have not been conclusive.

2.6.3 - Effect of iron supplementation on the zinc and copper status of children

In developing countries iron deficiency anemia continues to be a public health problem of considerable importance. One of the control strategies, oral iron supplementation, is reported to interfere with the absorption of other micronutrients, especially zinc and copper (Solomons and Jacobs 1981, Breskin *et al* 1983, Hambidge *et al* 1987). Hambidge *et al* (1987) reported a decline in serum zinc during iron therapy in pregnant mothers, while studies by Yip *et al* (1985) and Arnaud *et al* (1993) failed to show any effect. No study of this kind has yet been undertaken in preschool children. It seems that the few studies that do exist show inconclusive results on the

effect of iron supplementation on zinc and copper status.

Zinc is a constituent of over 200 metallo-enzymes. It is essential for many functions, including growth and development, normal reproduction, immune and sensory functions. To assess zinc status, serum zinc is most frequently used, although its sensitivity and specificity are poor. Concentrations are usually low only in severe deficiencies but not in marginal deficiencies (Gibson 1994).

Copper is associated with the activities of cuproenzymes. Even though copper deficiency is rare in humans, if it occurs the deficiency signs are neutropenia and hypochromic anemia (Gibson 1994). Serum copper is the most widely used index of copper status in severe copper deficiency. Its sensitivity and specificity in apparently healthy individuals, however, remain in doubt by many researchers (Gibson 1994).

2.7 - The study country

Ethiopia is located in Eastern Africa, in an area commonly referred to as the "Horn of Africa", immediately north of the equator. It is a multi-ethnic and multi-cultural country with 40 tribes, more than 80 languages, and 200 dialects. The total population, projected from 1984 for 1994, is 55 millions (Ministry of Health of Ethiopia 1995). Its altitude ranges from 115 meters below sea level to 4,600 meters above sea level. Two-thirds of the country is 1,800 meters above sea level. Over 80% of the population lives in rural areas, primarily on a subsistence type of agriculture.

The health status of people in Ethiopia is among the poorest in the world. The life expectancy of its people at birth is 52 and 54 years for males and females, respectively. The infant mortality rate is 105 deaths per 1,000 live births (Ministry of Health of Ethiopia 1995). The health service coverage is estimated to be 45% of the total population. At the present time, Ethiopia has 85 hospitals, 1,051 health centers and 2,470 health stations. The physician to population ratio is 1:40,000.

The subsistence level of production coupled with cyclical droughts have worsened the country's marginal food situation. Undernutrition of both macro- and micro-nutrients in children is widespread. The rates of moderate to severe underweight, wasting and stunting are 48%, 8% and 64%, respectively (Esrey *et al* 1995). The daily per capita caloric supply is only 73% of recommended requirements. The average expenditure of households on food is 49% of the household income. The Gross National Product of the country in 1993 was 100 $USD per annum (UNICEF 1996).

3.0 - HYPOTHESES AND OBJECTIVES OF THE STUDIES

The major hypotheses central to the research were as follows:

- Iron pots release iron into cooking food.
- Children of families that cook food in iron pots will have a better iron status than children of families that cook food in conventional pots.
- Iron-supplemented children will show improved growth and less morbidity than placebo-treated children.
- Vitamin A-supplemented children will have more growth and less morbidity than placebo-treated children.
- Combined iron and vitamin A-supplemented children will have more growth and less morbidity than iron only, vitamin A only or placebo-treated children.
- Iron supplementation will reduce the absorption of zinc and copper.

3.1 - General Objective

The overall objective of the three studies conducted to address these hypotheses was to determine the magnitude and risk factors for iron deficiency anemia and to evaluate different control strategies, especially the role of iron pots and combined iron and vitamin A supplementation in the control of iron deficiency anemia.

3.2 - Specific objectives

The specific objectives were addressed in a study population of preschool children between the ages of 6 to 60 months living in urban and semi-urban communities in Northern Ethiopia.

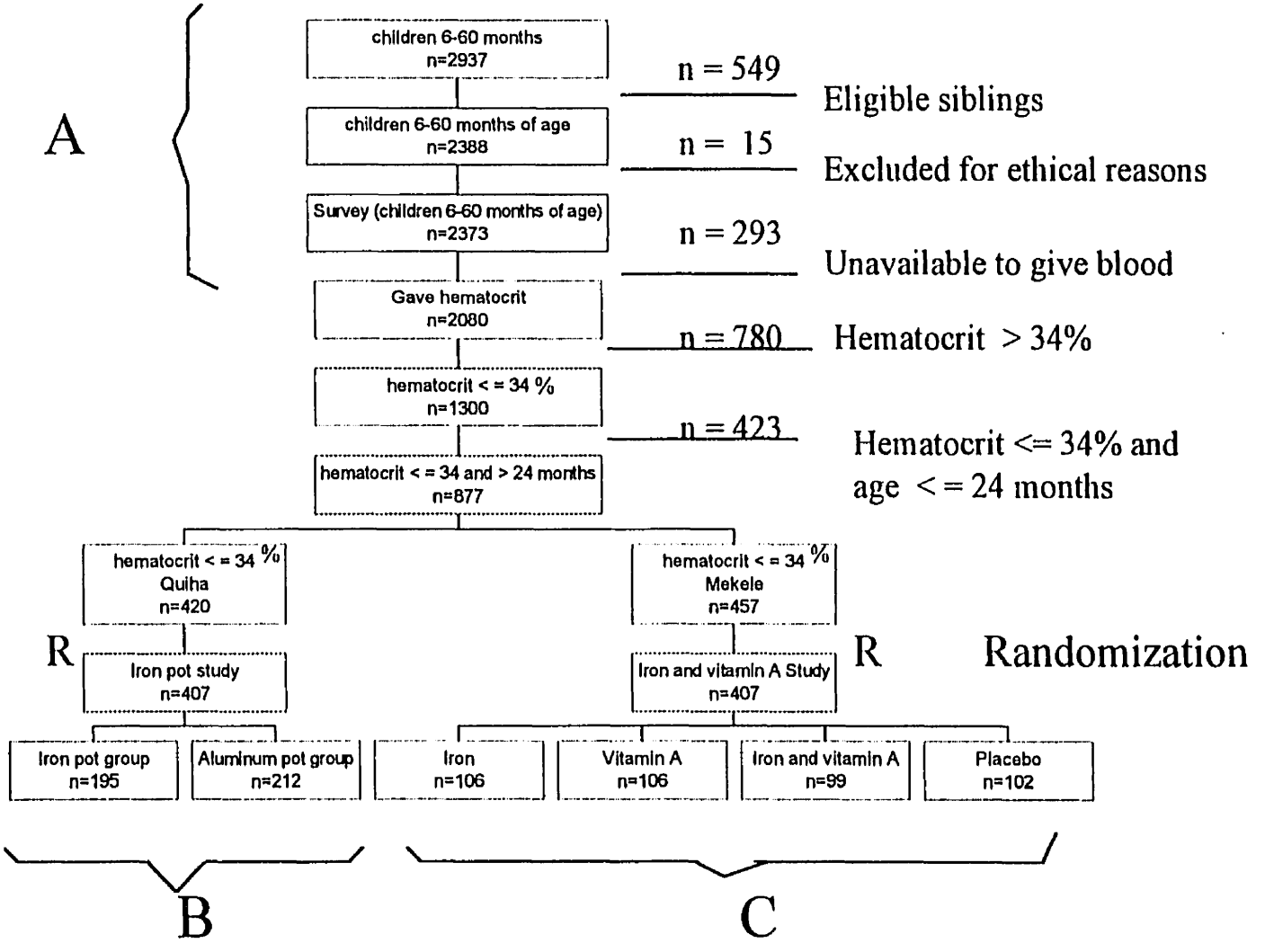
The specific objectives were Ai) To determine the prevalence and type of anemia, Aii) To assess the risk factors for iron deficiency anemia, Bi) To determine the iron content and its availability in traditional Ethiopian foods cooked in iron pots, Bii) To assess the effect of using iron pots for cooking on hemoglobin levels of anemic preschool children, Ci) To assess the individual effects of iron and vitamin A and their combined effect on hemoglobin status, morbidity and growth of anemic children, and Cii) To evaluate the effect of oral iron supplementation on the zinc and copper status of children.

4.0 - GENERAL METHODS

This thesis comprises three studies on iron deficiency anemia in children aged between six and sixty months, conducted in Northern Ethiopia from December 1993 to May 1995 (Figure 1).

Baseline study: The first, a cross-sectional study (Manuscript A), served as the baseline for the latter two studies (Manuscript B and Manuscript C). It was aimed at determining risk factors for anemia in a study population of 2937 children. Fifteen (8 severely anemic, 4 critically ill, 3 with physical disability) were excluded from the study for ethical reasons and because only one child per family was included in the study, 549 children were excluded even though they were eligible aged siblings. Of the 2373 children who participated in the study, 293 children did not give blood for hematocrit. The remaining 2080 children provided blood for screening of which 1300 (62.0%) had a hematocrit of ≤ 34%. Of the 1300, 877 children with a hematocrit of ≤ 34% and aged 24 to 60 months were eligible to participate in the other two intervention studies. Those who were residents of Quiha (n=420) were eligible to participate in the iron pot study while 457 children who lived in the northern section of Mekele were eligible to participate in the combined iron and vitamin A study.

Figure 1. Summary of study populations and study designs of research conducted in Northern Ethiopia



Iron pot study: A randomized community trial was designed to assess the effect of cooking with iron or aluminum cooking pots on hemoglobin status of preschool children. Of the 420 children with hematocrit ≤ 34% and living in Quiha, 407 were randomly selected and assigned randomly into iron (n=195) and aluminum (n=212) pot groups. Because the iron and the aluminum pots were so different, participating families could not be blinded to type of pot. However, the laboratory technicians who collected and analyzed the blood samples and the technicians who measured the children's weight and length were blind to the intervention groups. This study took place from March 1994 to May 1995.

Combined Iron and vitamin A study: A randomized, double-blind, placebo-controlled community trial was conducted to evaluate the individual and combined effects of iron and vitamin A on growth and morbidity of preschool children. From the 457 children with hematocrit ≤ 34% and living in Mekele, based on sample size calculations, 407 were randomly selected and assigned into four intervention groups: Iron (n=100), vitamin A (n=106), iron plus vitamin A (n=99), placebo (n=102). Both mothers and researchers were kept blind to the type of intervention children were receiving. The study was conducted from March 1994 to May 1995.

4.1 - Study Subjects and Place of Study

The city of Mekele and all of the surrounding semi-urban communities (within

a 20 km radius), were included in the study. In these selected communities, a house-to-house census was performed to identify households with children between six to sixty months of age. Those children who were permanent residents of the study area (whose family had lived in the town for at least 12 months), were included in the study. In situations where families had two or more children in the target age group, only one child was selected using a random sampling strategy. Children who were critically ill were excluded from the study for ethical reasons.

4.2 - Sample size determination

In both the iron pot and combined iron and vitamin A studies, growth which was primary outcome of the study, was used to determine sample size. Thus, sample sizes were calculated to detect significant differences in growth using the following formula:

$n= 2[(Z_\alpha+Z_\beta)^2 \sigma^2/\delta^2$; α = Level of significance = 0.05 (Z_α = 1.96); β = Power of test = 0.90 (Z_β = 1.28); σ = Standard deviation (weight = 0.8 kg; length = 1.5 cm (Esrey *et al* 1988)); δ = Expected difference in growth (weight = 0.4 kg; length = 1.0 cm). Substituting the numbers in the above equation, the number of anemic children per group would be 84 children if weight was considered or 47 children if length was considered. The largest sample size calculated (n=84) was taken to increase the power of detecting differences in the other outcomes. Since a twenty percent drop-out rate was anticipated, based on experience in the region, 100 children

per comparison group were finally considered appropriate.

Sub-sample selection: For procedures regarded to be traumatic or expensive such as blood indices, serum ferritin, serum zinc, serum copper and weighed food record, a sub-sample (n=230) was randomly selected from among the children in the intervention studies (n=814). For all the procedures at baseline, the same sub-sample of children was used. For the post-intervention serum analysis a different sub-sample (n=230) was drawn randomly, to avoid traumatizing the same children for the second time.

4.3 - Data collection

The following information was obtained from 2373 mothers during a face-to-face interview and recorded onto a questionnaire form (Appendix 1): health and nutritional status of the child, such as age, gender, illness in the two weeks prior to the interview; presence, frequency and type of diarrhea in the last one week (at least three loose stools in one day); and whether the child was being breast-fed at the time of interview. Maternal caring capacity factors, such as, age, literacy (ability to read and write), religion, ethnicity, marital status, and health status during the interview were recorded. Environmental factors, such as human excreta management (open field versus latrine disposal), and source of drinking water (piped water versus water from unprotected spring, river or well), presence of soap in the house during the interview,

and number of rooms in the family dwelling were included. Food security variables measured were the possession of food reserves and if the family had received any food assistance within six months prior to the interview. Since it was very difficult to acquire information on household income, an indirect method was used. Mothers were asked about income categories they considered to be "very good", "good", "sufficient", "bad" and "very bad". Then they were asked in which category their family belonged. With this indirect method, we hoped to come as close to the true income as possible. According to Kello AB (personal communication), from information acquired using such a technique, the level of income most families considered "sufficient" can be taken as the poverty line. Additional indirect information such as ownership of the residence and of a radio was also used as a proxy measure of economic status. For all the children, food type and frequency of consumption in the one week prior to the interview were reported using a Food Frequency Questionnaire (Gibson 1990).

The questionnaire was developed, translated into the local language (Tigrigna) and pretested in non-study households in a similar community before application. Interviewers were periodically retrained on interviewing techniques and supervised by trained field supervisors to maintain consistency in information gathering. Finally, mothers were given appointments to bring their children into the local clinic for a general physical check-up, anthropometry and laboratory check up.

4.3.1 - Anthropometry:

Both weight and length were measured on all children at baseline, and at 3, 6, and 12 months of the study. Weight was measured using a Seca 770 standing scale. Those children who could not stand were carried by their mother and weighed. The weight of the mother was then deducted from the combined weight of child and mother. All clothing was removed and the child's weight was measured to the nearest 0.01 kg (UN 1986). For all children, recumbent length (crown-heel length) was measured using a wooden length-board (Shorr Corp.). The board was positioned on a flat surface and measurements were taken to the nearest 0.1 cm. All footwear was removed and hair was made loose and flat. The child's age was obtained from the mother and recorded to the nearest month. In all cases the age of the child provided by the mother was confirmed by a local events calendar.

For the baseline study, weight, length, age and gender were then used to create nutritional indices (Z-scores) using the US National Center for Health Statistics as a reference, which is recommended by WHO (Dibley *et al* 1987a; Dibley *et al* 1987b). In children over two years of age, correction factor for length was introduced before calculating the height-for-age Z-score. Each Z-score was then assessed to see if it was above or below -2.00. A child below -2.00 was either underweight (weight-for-age), wasted (weight-for-height) or stunted (height-for-age).

Standardization: The interviewers were trained in interviewing technique, proper use of scales and recording. Their inter- and intra-observer errors were checked before data collection. They were trained to check and adjust the scales before each measuring session (UN 1986).

4.3.2 - Dietary intake

In all children, food type and frequency of consumption in the week prior to interview were reported using a Food Frequency Questionnaire. Since the Food Frequency Questionnaire reports only type of food and its frequency of consumption, it was supplemented with the Weighed Food Record (WFR). WFR, which is known to estimate exact nutrient intakes of the individual child, was collected in the sub-sample of 230 children. In WFR all foods and beverages consumed by the child on two different days were recorded. Then the mean nutrient contents of the foods they consumed over the two days were calculated using a table of Food Composition for use in Ethiopia (Agren *et al* 1964). For children who were being breast-fed 24 hour test-weighing was performed (WHO 1985). In test-weighing to determine the net weight of the milk, the child's weight before breast-feeding was deducted from the child's weight after breast-feeding. Then the amount of milk consumed was converted into its appropriate nutrients using the table of Food Composition for use in Ethiopia (Agren *et al* 1964).

4.3.3 - Laboratory assessment

Laboratory technicians participated in the collecting, transporting and analyzing of the laboratory specimens. Inter- and intra-observer variations were checked to standardize their technique. A senior laboratory technician supervised the specimen collection and randomly re-checked a number of specimens analyzed by the technicians for validation. Hemoglobin and hematocrit were assessed using capillary blood obtained from a finger prick. To obtain blood from the finger, the finger was warmed to promote blood flow, then sterilized and punctured using a sterile disposable lancet. As the first drop was most likely contaminated with a tissue fluid it was wiped away with dry gauze. Then the next drop formed over the puncture was collected with a micro-capillary tube, centrifuged for five minutes and read with a hematocrit reader. For purposes of screening, hematocrit was selected for its ease of operation, accuracy, and cost (WHO, 1994). For hemoglobin determination, two hundredths of a milliliter of blood was collected using a micropipet and read using the HaemoCue system (Lee Diagnostics AB, Medical Equipment Design, Inc.). The HemoCue system is a photometric method that uses undiluted blood which reacts with sodium azide to form methemoglobin azide (Yip *et al* 1990).

From the venous blood collected, 197 specimens were available for blood indices and peripheral blood film. To determine blood indices such as mean corpuscular volume (MCV), hemoglobin, hematocrit, and red blood cell (RBC) count,

a Coulter counter (CBC 5, Coulter Electronics of Canada) was used. Mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated from the above indices. To determine blood morphology, blood films were prepared and stained with Wright's stain (Lawrence *et al* 1989). The same slides were used for the diagnosis of malaria.

For serum analysis, whenever available, as much as 10 milliliters of blood was collected from the antecubital vein using anticoagulant and trace mineral-free vacutainer tubes. The blood was then centrifuged and the serum separated and stored in a serum vial in a deep freezer (-20°C). Finally the serum was transported to Canada in liquid hydrogen. Duplicate samples of serum were analyzed for serum ferritin using a radio immunoassay method (Fisher Scientific Limited). In cases where the amount of serum collected was found to be inadequate for all the tests, the following priority order was followed: serum ferritin (n=230), blood indices (n=200), and blood morphology (n=197).

Fresh stool was collected from 309 of the 814 children, who could provide stool at the time of anthropometric and clinical checkup, for direct microscopic examination. A drop of saline was added to the stool sample and analyzed under the microscope the same day.

During the follow-up period, for both iron pot and combined iron and vitamin A studies, weight, length, and hemoglobin were measured at baseline, at three, six and 12 months. Morbidity in the combined iron and vitamin A study was assessed every week for the duration of intervention (3 months) and for the first three months of the follow-up period.

4.3.4 - Safety precautions and quality control

At baseline and during follow up, all specimens except serum were analyzed in a laboratory in Mekele on the same day. To avoid transmission of hepatitis B and Human Immuno-deficiency Virus (HIV) infections, only disposable lancets and vacutainers were used to draw blood. The laboratory technicians were given appropriate training on sterilization techniques. For all except the stool test duplicate specimens were collected.

4.4 Data entry and analysis

All data were first recorded onto pre-coded questionnaires and laboratory forms and then entered into a computer using Epi Info, version 5, 1994 (Center for Disease Control, Atlanta). Prior to data analysis, all Epi Info files were converted into Stata (Stata Corp. 1993), using DBMS-COPY (SPSS Inc. 1991). All statistical analysis tests were two-tailed, and a type I error rate of 5% was used for p-value and

confidence intervals.

Data were initially described and summarized using univariate statistics to document their distribution in the community. Continuous variables such as age and income were categorized into dichotomous variables (age less than 24 months, and 24 months and above; income below the poverty line of 200 Birr, income at or above the poverty line). Those variables with insufficient variance in the population, less than 2% or above 98%, such as ethnicity, religion and car and motorbike ownership, were excluded from statistical analysis. Multivariate analysis were performed as appropriate (e.g. ANOVA, regression) to assess effects taking into account two or more independent variables simultaneously.

4.5 Ethical consideration

All the three studies reported in this thesis received approval from the ethics review committees of McGill University in Canada and the Jimma Institute of Health Sciences in Ethiopia (Appendix 2). Mothers were informed about the objectives, advantages and potential side effects of treatments prior to obtaining their informed consent. Severely ill and severely anemic children (hemoglobin $\leq$ 7 g/dl) were excluded from the study and treated immediately. At the end of the study, children in the placebo groups who were found anemic were given treatment.

4.6 Outline of research findings

The research findings of this thesis are presented in three manuscripts. Each manuscript is presented in a format as required by the journals to which they will be submitted. An epilogue is provided after each manuscript to summarize the results and link the manuscript to the rest of the thesis.

Manuscript A - Investigates prevalence and importance of iron deficiency anemia as a public health problem. In Ethiopia the importance of iron deficiency anemia is disputed because of the high iron content of the staple *injera*. The study also investigates the role of non-dietary risk factors as a cause of anemia. Anemia was characterized by hematocrit level of less than 34%. This cut-off takes altitude and race into consideration. This manuscript is submitted to the Bulletin of WHO.

Manuscript B - Explores an alternative method of control strategy for iron deficiency anemia. It investigates whether iron pots release iron into the cooking food, and if such iron pots have any role in the control of iron deficiency anemia. The study investigates if food cooked in iron pots raises the iron status of children with hematocrit level of less than or equal to 34%. It also explores the effect of food cooked in iron pots on the weight and length of children. The manuscript is submitted to the Lancet for publication.

Manuscript C - Explores the role of vitamin A in the control of iron deficiency anemia. It investigates the effect of a single dose of vitamin A supplement, with or without iron supplements, on hemoglobin, serum ferritin, weight, length and morbidity of children. In a community where multiple micronutrient deficiencies are common, including a single dose of vitamin A would have the added benefit of tackling vitamin A deficiency at the same time. Manuscript C is submitted to the American Journal of Clinical Nutrition.

5.0 MANUSCRIPT A

RISK FACTORS FOR IRON DEFICIENCY ANEMIA IN PRESCHOOL CHILDREN IN NORTHERN ETHIOPIA

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A cross-sectional study was conducted to determine risk factors for anemia in 2373 children aged six to sixty months. Anemia was highly prevalent (42%) and constituted an important nutritional problem in the region. The fact that 56% of the anemic children had a low RBC count, and 43% had a serum ferritin of less than 12 µg/L confirms that the anemia was largely due to iron deficiency. Unlike other regions in developing countries, hookworm (0.4%) and malaria (0.0%) were rare and contributed little to the anemia. The amount of iron consumed through cereal-based staple foods, was adequate; however, the iron in these foods was not readily available and diets were likely high in iron absorption inhibitors. Primary factors associated with anemia were frequent consumption of inhibitors, such as fenugreek and coffee, and disease in the child such as diarrhea and stunting. Underlying causes were environmental and health service factors, such as lack of safe water and human waste management; maternal caring capacity factors, such as maternal illliteracy and mother being ill; and food security variables, such as having no food reserves. The root cause of these factors is poverty. Low income showed strong associations with anemia. Because the diet in the study area already contains high levels of iron and a combination of risk factors is associated with anemia, the conventional control strategy of iron supplementation may be ineffective. A sound control strategy should ensure the efficiency of iron absorption. The optimal control strategy should also have a holistic approach which includes the alleviation of poverty and the empowerment of women.

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Introduction

Iron deficiency anemia is a common nutritional problem throughout the world and of enormous public health concern in developing countries. An estimated 36% of the developing world's population suffer from anemia. Preschool children in Africa have some of the highest rates of anemia in the world, nearly 56% (*1*). In Ethiopia, the magnitude and importance of iron deficiency anemia as a public health problem is still disputed. Some studies reported iron deficiency anemia rates of less than 18% (*2,3*) while others have reported rates 25% and above (*4,5,6*). Iron deficiency is a function of the imbalance of iron intake, iron absorption and iron loss (*7,8,9*). In several developing countries the intake of iron from diet is more than adequate. For example, in parts of Ethiopia, the daily intake of iron is estimated to be between 180 and 500 mg/day which is 10 to 20 times the suggested daily requirement (*2, 10*). This presumed high intake is attributed to consumption of a staple cereal, teff, *Eragrostis teff* (90 mg of iron per 100 gm of teff), and partly due to its contamination with iron rich clay soil (*2*). In spite of this high intake of iron, some studies have reported a high prevalence of anemia, even in teff-consuming communities, and in 1995, iron deficiency anemia was the most important cause of hospital admissions and deaths all over the country (*4,11,12*). Therefore, the cause of iron deficiency in Ethiopia may not be the inadequate dietary intake of iron. Other factors, ultimately related to poverty and underdevelopment, might also play a role in iron deficiency anemia (*13,14,15*). In such communities with an already high intake of iron, the conventional

supplementation of iron might not be effective or might even be harmful. Therefore, all important risk factors have to be identified and their role in causing anemia evaluated. The objective of this study was to identify these risk factors and assess their role in anemia.

Study Design:

A cross-sectional survey (n=2373) was undertaken between September and December 1993 in Tigray province to determine household food security status of families with preschool children and identify risk factors for iron deficiency anemia in children between the ages of six and 60 months. Hematocrit was used to assess anemia which was defined as a hematocrit of less than 34%, adjusted for altitude (altitude ~ 2,000 meters above see level) and race.

Study Subjects and Place of Study:

This study included the city of Mekele in Northern Ethiopia and the two surrounding semi-urban communities (Quiha and Aynalem). In these communities a house-to-house census was conducted to identify households with children between six months and five years of age. After the census, only those children who were permanent residents of the study area (whose families had lived in the study area for at least 12 months prior to the study), were considered eligible to participate. In

situations where families had two or more children in the target age group, the names of the children were written on a piece of paper and the one child whose name was picked randomly was selected for the study. Children severely febrile, in respiratory distress, or who had a disability were excluded from the study for ethical reasons. Before the interview parental consent was obtained for each child.

Sub-sample selection: Invasive and/or expensive procedures, such as blood indices, serum ferritin, and weighed food record, necessary to determine the type of anemia, were performed in a sub-sample of 230 children selected at random, using a random number generator. The 230 children were selected from the 877 children found to have a hematocrit less or equal to 34% and between 24 and 60 months of age. Out of these 877 children stool specimens were collected from 309 children who could produce stools at the time of the physical checkup and anthropometric measurement.

Data collection

The following information was obtained from 2373 mothers during a face-to-face interview and recorded onto a questionnaire: health and nutritional status of the child, such as age, gender, illness in the two weeks prior to the interview, presence and type of diarrhea in the last one week (at least three loose stools in one day), and whether the child was being breast-fed at the time of interview; maternal caring capacity, such as age of the mother, literacy (mother being able to read and

write), religion, ethnicity, marital status, health status during the interview; environmental services, such as human excreta management (open field versus latrine), and source of drinking water (pipe water versus water from unprotected spring, river or well), presence of soap in the house during the interview, number of rooms in the family dwelling; and food security variables, such as the family having food reserves and if the family had received any food assistance in the six months prior to the interview and family's monthly income. Additional indirect information such as residence ownership, ownership of a radio was also used as a proxy measure of economic status. For all the children, food type and frequency of consumption in the one week prior to the interview were reported using a Food Frequency Questionnaire (*16*).

The questionnaire was developed in English, translated into the local language (Tigrigna), and pretested in non-study households in a similar community before application. Interviewers were periodically retrained on interviewing techniques and supervised by trained field supervisors to maintain consistency in information gathering. Finally, mothers were given appointments to bring their children to the local clinic for a physical check-up by a medical practitioner.

Weight was measured on all children using a standing scale (Seca 770, Hamburg, Germany). All clothing was removed and the child's weight was measured to the nearest 0.01 kg (*17*). For all children recumbent crown-heel length was

measured using a wooden length-board (Shorr Corp.). All footwear was removed and hair was made loose and flat (*17*). The board was positioned on a flat surface and measurements were taken to the nearest 0.1 cm. The child's age was obtained from the mother and recorded to the nearest month. In all cases the age of the child provided by the mother was cross-checked by a local events calendar (*17*). Hematocrit was measured from the volume of erythrocytes as a percentage of the total sample volume after centrifugation of heparinized capillary blood from a finger prick (*18*).

Three months after the interview, in-depth laboratory analyses were undertaken in the sub-sample of the 230 anemic children. Since the food frequency questionnaire records only type of food with frequency of consumption, it was supplemented by the Weighed Food Record (WFR) in the sub-sample of 230 children. WFR was used to identify exact nutrient intakes of individual children (*16*). Interviewers were assigned to follow each child for 24 hours and to record all food and beverages he/she consumed. Duplicate 24 hour measures were taken for each child (*16*). The average nutrient contents of the foods consumed were then calculated using the Table of Food Composition for use in Ethiopia (*19*). Test weighing was performed for children being breast-fed at the time of the interview. In test-weighing the net weight of the milk was determined by deducting the child's weight before breast-feeding from his weight after breast-feeding (*20*). Then the net weight of milk consumed was converted into its appropriate nutrients using the table.

For serum analysis, whenever possible, as much as 10 milliliters of blood was collected from the antecubital vein using anticoagulated and trace mineral-free vacutainer tubes. The blood was then centrifuged and the serum separated and stored in a deep freezer (-20° C). Finally, the serum was transported to Canada in liquid hydrogen. Duplicate samples of serum were analyzed for serum ferritin using a radio immunoassay method (Fisher Scientific Limited) (*21*). In cases where the amount of serum collected was found inadequate for all the tests, the following priority order was followed: serum ferritin (n=230), blood indices (n=200), and blood morphology (n=197).

From the venous blood collected, 197 specimens were available for blood indices and peripheral blood film. To determine blood indices such as mean corpuscular volume (MCV), hemoglobin, hematocrit, and red blood cell (RBC) count, a Coulter counter (CBC 5, Coulter Electronics of Canada) was used. Mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated from the above indices. To determine blood morphology, blood films were prepared and stained with Wright's stain (*22*). The same slides were used for the diagnosis of malaria (thick and thin smear) and sickle cell blood picture. Stool specimens were examined by direct microscopic examination. A drop of saline was added to the stool before being analyzed under the microscope.

All specimens, except serum, were analyzed in a laboratory in Mekele on the same day. To avoid any possible transmission of hepatitis B or the Human Immuno-deficiency Virus (HIV) infections, all lancets and vacutainers used to draw blood were disposable. The laboratory technicians were given appropriate training on sterilization techniques. For all tests except stool, duplicate specimens were collected. The study was approved by ethics review committees of the School of Dietetics and Human Nutrition at McGill University in Canada and the Jimma Institute of Health Sciences in Ethiopia.

Data analysis

All data were first recorded onto pre-coded questionnaires and laboratory forms and then entered into a computer using Epi Info, version 5, 1994 (Center for Disease Control, Atlanta) and analyzed using Stata version 3.1, 1991 (Stata Corp. Texas). All statistical analysis tests were two-tailed, and a type I error rate of 5% was used for p-values and confidence intervals.

Data were initially described and summarized using univariate statistics to document their distribution in the community. The variables were then assessed for their association with anemia using the chi-square test. Continuous variables such as age and income were categorized into dichotomous variables (age < 24 months and $\geq$ 24 months); income below or above the poverty line (200 Birr or 32.2 US$) (*23*).

Those variables with insufficient variance in the population, less than 2% or above 98%, such as ethnicity, religion and car and motorbike ownership, were excluded from the analysis. Those variables found to have a significant crude association with anemia ($p<0.05$) were then entered into a multiple logistic regression analysis, to see if the crude association persisted after simultaneously adjusting for potential confounders. Even though it did not show a significant crude association, gender was included into the model because it was suspected to be a potential confounder *a priori*. To rule out misclassification of anemia due to the cut-off used, a separate analysis was carried out by comparing children at both ends of the anemia spectrum (i.e. children with a hematocrit of less than 30% versus children with a hematocrit of higher than 40%).

Results

Prevalence and type of anemia

Of the initial 2381 children studied, hematocrit results were available for 2080 (88%) children (Figure 1). The mean hematocrit was 35.4%±4.8 , and 42% of children were anemic, as expressed by a hematocrit level less than 34%. Of those 293 children for whom hematocrit was not available, two-thirds did not give blood, while in the remaining one-third, the blood specimens were damaged either during transportation or during laboratory analysis. However, these children were not statistically different for any of the following characteristics: age, gender, the occurrence of illness in the two weeks prior to the interview, presence and type of

diarrhea, whether the child was breast-fed, mother's literacy status, family's human waste management, source of drinking water, number of rooms in the family residence, and family income, compared to children for whom a hematocrit level could be determined.

In the random sub-sample of 230 children with hematocrit less or equal to 34% mean serum ferritin level was 22.5 μg/L. Forty- three percent of these children had serum levels less than 12 μg/L. Results on blood morphology were available from 197 children. Twenty percent of the slides showed either a microcytic hypochromic, anisocytosis or target cell blood picture of iron deficiency anemia while only two slides (1.0%) indicated a macrocytic hyperchromic picture (Table 1). Most of the slides (78.7%) were normocytic and normochromic. None of the slides were positive for malaria or sickle cell blood picture. The mean RBC count was $4.0x10^6$ /mm^3 and 56.2% of children had values below $3.8x10^6$ /mm^3 (Table 2). Almost all the children (92%) had MCV less than the normal cut-off point.

Only 4% of the study children had iron intake of less than their daily Recommended Nutrient Intake (Table 3). Markedly lower intakes compared to RNIs were observed for niacin (61%), riboflavin (59%) and ascorbic acid (41%). The majority (78%) of the children had caloric intakes less than their recommended daily requirements and about a quarter of the children had less than the recommended daily protein requirement.

Among the 309 children for whom stool test for parasites was performed, more than half of the children were found to have at least one intestinal parasite (Table 4). The most common single and mixed parasites observed include *H. nana* (21.6%), *E. histolytica* (21.0%), *A. lumbricoides* (16.1%) and *G. lamblia* (10.0%). Hookworm infection (0.4%) and infections of *Schistosoma mansoni* (0.8%) and *T. trichiura* (0.7%) which are often associated with anemia, were rarely detected.

Univariate analysis of associations of risk factors with anemia

For analysis, similar variables were re-grouped under key risk categories: food intake, disease-related variables, variables related to health and environmental services, maternal caring capacity variables, food security variables and poverty-related variables (Table 5). Variables which do not belong to any of these groups, such as age and gender, were included under the food security variables category for convenience of analysis.

Food intake variables: - *Injera*, a local bread made of teff, was consumed by 83% of all children at least once a week. Meat and vegetables were consumed infrequently by only 5% of the children. Children who were reported to have consumed fenugreek and coffee were 1.4 times more at risk of anemia than children who did not consume these beverages. Breast-fed children were also at increased risk of anemia compared to children who were not breast-fed. Injera and kale, foods high in iron, did not show a significant protective effect against anemia. Oranges (high in ascorbic acid) also did not show a significant protective effect.

not show a significant protective effect.

Disease-related variables: - According to the mothers' perception of the health status of their children, 27% indicated that they considered their children had poor health while the rest were considered to have average or good health. Nearly half of the children had fever in the week prior to the interview and between one-third to one-half of the children had diarrhea or cough. According to their mothers, 68% of children had good or average growth and this corresponded well with the percent of stunted and underweight children in the study. Three of these variables were significantly associated with anemia. Children with diarrhea, fever and stunted children were at significantly greater risk for anemia than their referent counterparts.

Environmental and health services variables: - Half (48%) of the families used open fields for human waste management. When the availability of clean water was assessed, 82% of households were getting piped water. However, the majority of these households bought water from public water stands. A majority of mothers (75%) visited health institutions and 70% had attended health and nutrition sessions in the two weeks prior to the interview.

Children had increased risk of anemia (OR=1.56) if their families used unsafe water sources for drinking or reported inadequate waste management (OR=1.27). Both these crude associations were statistically significant. The presence of soap in

the vicinity at the time of the interview was not indicative of anemia. Children born at home did not have any increased risk of anemia compared with children born in health institutions, nor was attendance to health and nutrition sessions associated with increased risk.

Maternal caring capacity variables: - The mean age of mothers in the study was 29±7.6 years. Thirty eight percent of mothers were 25 years old or younger and only eight percent were 45 years or older. Most mothers were living with their partners (85%) while the remaining 15% were either divorced, separated or widowed. The maternal literacy rate was 48% .

Several maternal caring capacity factors were associated with increased risk of anemia. Children of illiterate mothers and children whose mothers were sick during the interview had 1.27 times higher risks, compared to their referent counterparts. Neither maternal age nor being pregnant at the time of interview was significantly associated with anemia in the child.

Food security variables: - The study children had a mean age of 29 months, and boys (51.7%) and girls (48.3%) were equally represented. Most children lived in crowded and sub-standard houses. Even though half the families owned the house they lived in, the majority (75%) were living in a single room. Almost 26% of families had no separate kitchen, hence the preparation and cooking of food took place in the

same room as other family activities.

Children of families who lived in one room were at 1.28 times increased risk of anemia than children of families with more rooms and children older than 24 months of age were more likely (1.5 times) to be anemic than their referent counterparts. Children of families with no food reserves were 1.21 times more at risk for anemia than children of families with food reserves. Gender differences did not show any association with anemia.

Poverty related variables: - The majority of the study families were classified as living below the poverty line with 81% having a reported monthly income of less than 200 Birr. Less than half of the study families had access to a radio.

Income below the poverty line was a predictor of anemia (OR=1.33). Proxy measure for wealth, such as ownership of a radio, also showed strong protective effects. Ownership of the family residence had no association with anemia in the child.

Multivariate analysis of selected risk factors

The multivariate analysis included only the 1691 children for whom complete information on all variables included in the model was available (Table 6). When variables that showed a significant crude association with anemia were included in a multiple logistic regression analysis, the association of the following variables with anemia persisted after simultaneously controlling for the other variables: older children (OR=1.71), unsafe drinking water (OR=1.36), family not having food reserves (OR=1.31), mother being ill (OR=1.25) and income below poverty line (OR=1.49).

The cut-off of anemia at a hematocrit of less than 34% may result in misclassification bias. To rule out potential misclassification, a subsequent multivariate analysis was undertaken comparing children at the extremes (hematocrit < 30% and hematocrit > 40%) (Table 6). The odds ratios and confidence intervals for nearly all the variables in the model were strengthened: for children more than 24 months of age (OR=2.49), for children of families with unsafe drinking water (OR=1.81), for children of families that did not have food reserves (OR=1.74), and for children of families living below the poverty line (OR=1.71). In addition, children who consumed fenugreek were also at increased risk (OR=1.98). Stunting (OR=1.42), inadequate waste management (OR=1.21), illiterate mother (OR=1.43) and being male (OR=1.39) showed trends of increased risk.

DISCUSSION

Nearly half the study children, residing in urban and semi-urban communities, had anemia. Investigators in other parts of the country have also reported similar results (*4,5,6*). The commonest abnormal blood morphology observed was microcytic hypochromic, a blood picture common in iron deficiency anemia. Only 1.0% of blood slides showed a macrocytic hyperchromic picture of megaloblastic anemia common in vitamin B_{12} and/or folic acid deficiencies. The normal blood morphology observed in most anemic children is due to the fact that these parameters change in the advanced stages of iron deficiency anemia, and are characteristic of severe cases of iron deficiency anemia (*24*). Furthermore, the low mean serum ferritin level (22 μg/L), the high proportion of children with serum ferritin less than 12 μg/L (43.2%) and the high rates of low MCV and MCH in the study children confirm that the anemia was due to iron deficiency.

Malaria, which is one of the commonest causes of anemia in other parts of the country (*25*), was absent in the study communities. Low rates of parasites such as hookworm, schistosomes and whipworm probably reflect the nature of the soil and dry climate of the study area (*26*). The commonest parasites in the study area were *E. histolytica* and *H. nana*. While causing mucoid or bloody diarrhea, it would be difficult to attribute the occurrence of anemia in the study area to these parasites. Although direct microscopy of unconcentrated stool is less precise than the other

microscopic techniques in diagnosing intestinal parasites, it is widely recognized as a suitable method to estimate overall levels of community infection.

This study showed that iron deficiency anemia was quite common and that the anemia was not caused by hookworm or malaria as is common in other parts of the country or other developing countries. No single risk factor was identified as a predominant predictor of iron deficiency anemia, confirming that the iron deficiency in the study area was the combined effect of several risk factors (*27*). It is believed that inadequate intake of iron is one of the most important factors in iron deficiency anemia. This study shows a high rate of iron deficiency anemia in spite of a very high iron intake. In fact iron intake was the least important risk factor for iron deficiency anemia, suggesting that the conventional approach of controlling iron deficiency by iron supplementation alone may have little value. For example, in the study area children get their iron from plant (non-heme) sources, mainly teff. Teff, which is very rich in iron, was consumed by almost all children at least once a week. However, meat and other foods rich in ascorbic acid, which improve the absorption of non-heme iron, were rarely consumed (*28*). Roodenburg (1995) is of the opinion that pregnant women could meet their iron requirement only by increasing iron absorption efficiency (*29*). A study by Sharma and Mathur (1995) showed that 500 mg of ascorbic acid twice a day significantly improved hemoglobin, serum iron, serum ferritin and transferrin saturation levels of 28 strict vegetarians (*30*). Similar results in Chinese children with mild anemia, were reported by Mao (1992) (*31*). It is also known that

diet in developing countries, including the study area, contains substances that inhibit the absorption of iron, such as tannins, phenols and fiber (*32*). Fenugreek, which is rich in mucilaginous fiber (*33*), and coffee and tea, known to inhibit iron absorption (*34*), were also consumed by some children. Therefore, in this study the immediate cause of iron deficiency anemia may be due not so much to inadequate intake as to the low efficiency of iron absorption. In the study community, immediate control strategies should include improving food processing and timing the intake of beverages. Investigators have reported that fermenting cereals and legumes and taking coffee or tea at least three hours after meals will reduce their inhibitory effect (*18*).

No single risk factor was identified as a predominant predictor of iron deficiency anemia. Odds ratios of 2.0 or less suggest the multifactorial nature of iron deficiency anemia. The major risk factors identified were related to inadequate environmental conditions, constrained maternal caring capacity, food insecurity and poverty. Disease related variables, such as diarrhea and fever, were moderately associated with anemia. Sick children are known to have poor appetite, and hence, a low dietary intake (*35*). Low dietary intake in turn leads to low iron intake. Common infections are also known to impair hematopoiesis and consequently cause anemia. Therefore, ill and/or malnourished children often have associated micronutrient deficiencies (*36*). Children in this study had high levels of macronutrient deficiencies as reflected by their low caloric intake and high rate of stunting and wasting. The fact that most micronutrient deficiencies occur along with macronutrient deficiencies is also reported by researchers in other parts of the world (*37*).

The health benefits of clean water and adequate sanitation extend beyond those resulting from reduction of pathogens alone (*38*). Access to clean water will benefit a women's limited time, energy and income. If time and energy saved is used by the mother to care for herself and her child, it will have a direct impact on the health and nutritional status of her children (*38*). In this study the majority of households were getting clean water for drinking. Almost half of the households disposed of their waste in the open field, which is used as a playground by the children. Children in this study lived in crowded and sub-standard houses. Most households had mud floors and only one in four families had a separate kitchen facility. This poor personal and environmental sanitation of the study children is strongly associated with anemia.

Women need to protect their own health and nutritional status to be able to fulfill their role as care-givers. Their level of education and employment status will determine in part whether they will have the understanding and the time to care for their children. Because children of illiterate mothers had a higher risk of anemia, education may enhance mother's ability to protect her child from contaminated environments and her ability to provide an appropriate diet.

Most families depended on relief assistance for most of the year. One can appreciate the poor food security status by the fact that the mean caloric intake of the study children was 78% of their requirement. In contrast, per capita caloric intake for children in developed countries, for example Canada, is 122% (*39*). Having food

reserves and more than one room in the family residence had strong negative associations with anemia. Poverty constrains a family's ability to gain access to and control human, economic and organizational resources (*5*). Human resources include labor, knowledge, skills and practices. Economic resources include assets such as land, tools and credit opportunities. Organizational resources include extended families, credit institutions, and child care organizations. Access to and control of these resources would allow people to gain greater access to food, maternal and child care, and health and environmental services. The study community has been plagued by instability, drought, and civil war, and the people have been deprived of human, organizational and economic resources (*5*). Iron deficiency is undoubtedly more common among groups of low socio-economic status (*40*).

This study found that the cause of iron deficiency anemia was multifactorial. The solution to address the problem must therefore be multidisciplinary. Such a sound iron deficiency control program should place more emphasis on improving the efficiency of iron absorption and tackling the underlying causes of iron deficiency anemia. The control program should not neglect the importance of alleviating general poverty, empowering and educating women, improving personal and environmental hygiene, reducing disease burden, and controlling macronutrient deficiencies.

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Table 1. Blood morphology in anemic (hematocrit ≤ 34%) children, 6-60 months of age, in Tigray region, Northern Ethiopia, December, 1993

Blood Morphology	Frequency	Percent
Normocytic normochromic	155	78.7
Microcytic hypochromic	34	17.2
Macrocytic hyperchromic	2	1.0
Anisocytosis	3	1.5
Target cells	3	1.5
Total*	197	100.0

* Blood morphology was determined in 197 of the 230 children in the sub-sample

Table 2. Red blood cell indices and proportion of anemic (hematocrit ≤ 34%) children*, 6-60 months of age, who fall below the cut-off points in Tigray region, Northern Ethiopia, December, 1993

Indices	Mean ±SD	Cut-off**	%< Cut-off
RBC (mm^3)	$4.0x10^6 \pm 0.87x10^6$	$3.8x10^6$	56.2
MCV (femtoliter)	73±6	80	92.0
Hematocrit (%)	29.3±4.2	34	89.0
Hemoglobin (g/dl)	11.5±1.4	11	30.0
MCHC (%)	40±4.9	32	4.1
MCH (pg 10^{12} g)	30.4±5.5	27	29.5

* Data were available from 200 of the 230 children

** Normal cut-off values for each index were taken from Vaughan *et al* 1979.

Table 3. Mean daily nutrient intakes (weighed food record) as compared to their recommended nutrient intakes (RNIs) in 230 anemic (hematocrit ≤ 34%) children, 6-60 months of age, in Tigray, Northern Ethiopia, December 1993.

Nutrient	RNI[1]	Mean	% of RNI	% < RNI[#]
Calories (Kcal)	1560	1218±460	78	78
Protein (gm)	26	44.6±40	223	27
Iron (mg)	20[*]	110±120	550	4
Calcium (mg)	600	293±168	61	89
Niacin (mg)	9	8.7±6.7	104	61
Thiamin (mg)	0.6	1.7±1.7	283	9
Riboflavin (mg)	0.8	0.8±0.5	102	59
Vitamin A (RE)	300	325	108	60
Ascorbic acid (mg)	20	63±73	315	41

1- RNI- Recommended Nutrient Intake from Health and Welfare Canada (1983)
*- RNI- for children with cereal based diet.
#- After corrections for difference in age were made.

Table 4. Prevalence and type of intestinal parasites in anemic (hematocrit ≤34%) children, 6-60 months of age, in Tigray, Northern Ethiopia, December, 1993.

Parasite	Frequency	Percent
Negative	133	43.0
SINGLE PARASITE		
E. histolytica	42	13.6
H. nana	38	12.3
A. lumbricoides	28	9.1
G. lamblia	26	8.4
S. mansoni	1	0.4
Taenia spp.	1	0.4
Hookworm	1	0.4
E. vermicularis	1	0.4
TWO PARASITES		
E. histolytica and *H. nana*	10	3.4
H. nana and *A. lumbricoides*	8	2.6
E. histolytica and *G. lamblia*	3	1.0
A. lumbricoides and *T. trichiura*	2	0.7
THREE PARASITES		
E. histolytica, H. nana and *A. lumbricoides*	6	2.0
E. histolytica, A. lumbricoides and *Taenia* spp.	3	0.6
H. nana, G. lamblia and *Taenia* spp.	3	0.6
A. lumbricoides, H. nana and *E. vermicularis*	2	0.7
E. histolytica, A. lumbricoides and *S. mansoni*	1	0.4
Total*	309	100.0

* Stool was obtained from 309 of the 877 anemic children

Table 5. Univariate analysis of association of risk factors with anemia in children, 6-60 months of age, in Tigray region, Northern Ethiopia, December, 1993

Risk factors	% anemic n=877	% Non-anemic n=1203	OR(95%C.I.)	Risk factors	% anemic n=877	% Non-anemic n=1203	OR(95%C.I)
Food intake variables				**Maternal caring capacity variables**			
Child ate fenugreek in the past 7 days	8.8	6.4	1.41(1.02-1.95)				
Child drank coffee in the past 7 days	9.4	6.9	1.39(1.01-1.91)	Mother illiterate	50.3	44.2	1.27(1.07-1.52)
Child was breast-fed	71.7	64.9	1.37(1.13-1.65)	Mother ill in the past 7 days	28.6	23.9	1.27(1.04-1.55)
Child ate beef in the past 7 days	4.9	5.4	0.91(0.60-1.37)[1]	Mother older than 40 years	11.5	12.0	1.00(0.76-1.31)
Child ate injera in the past 7 days	83.0	84.6	0.89(0.70-1.12)	Mother pregnant during interview	9.7	8.7	1.12(0.83-1.51)
Child ate kale in the past 7 days	4.6	6.0	0.75(0.51-1.11)	Mother living with a partner	86.0	84.0	1.17(0.91-1.49)
Child ate orange in the past 7 days	18.5	20.5	0.88(0.71-1.10)	Mother did not attend health and nutrition education	69.6	68.9	1.03(0.85-1.24)
Disease-related variables				**Food security variables**			
Child had diarrhea in the past 7 days	34.4	29.0	1.29(1.07-1.55)				
Child had fever in the last 7 days	50.9	46.2	1.20(1.01-1.43)	Child 24-60 months	74.5	65.8	1.52(1.25-1.83)
Child was stunted < -2SD	36.5	32.1	1.22(1.00-1.47)[2]	Family lived in one room	77.5	72.9	1.28(1.04-1.56)
Child had cough in the past 7 days	42.5	43.0	0.99(0.82-1.17)	Family had no food reserves	30.7	26.7	1.21(1.00-1.48)
Child was wasted < -2SD	10.0	9.2	1.09(0.80-1.49)[3]	Male child	54.1	50.4	1.17(0.98-1.39)
Child underweight < -2SD	37.9	33.8	1.20(0.99-1.45)[4]				
Environmental and health services variables				**Poverty related-variables**			
Family used safe water	85.4	78.9	1.56(1.24-1.96)	Family lived below poverty line	83.5	79.9	1.33(1.05-1.68)[6]
Family used open field waste disposal	45.9	51.8	1.27(1.03-1.51)[5]	Family did not own a radio	70.9	64.0	1.32(1.09-1.58)
Presence of soap at their vicinity	61.0	63.6	0.89(0.75-1.07)	Family did not own their residence	45.6	45.6	1.00(0.84-1.19)
Child born at home	72.0	70.0	1.12(0.93-1.37)				

Due to missing information the sample size for the following variables are: 1, n=1920; 2, n=1866; 3, n=1883; 4, n=1897; 5, n=2077; and 6, n=1892.

Table 6. Crude and adjusted associations of selected risk factors with anemia at different cut-off points in children, 6-60 months of age, in Tigray, Northern Ethiopia

	Hematocrit ≥ 34% versus Hematocrit < 34		Hematocrit <30 (n=370) versus Hematocrit >40% (n=296)	
Risk Factor (reference category)	**Crude OR(95% C.I.)**	**Adjusted* OR(95% C.I.)**	**Crude OR(95% C.I.)**	**Adjusted** OR(95% C.I.)**
Food intake variables				
Child consumed fenugreek in past 7 days (did not consume)	1.41(1.02-1.95)	1.28(0.89-1.85)	2.65(1.50-4.68)	1.98(1.04-3.74)
Child drank coffee in past 7 days (did not drink)	1.39(1.01-1.91)	1.24(0.87-1.79)	1.43(0.84-2.46)	1.49(0.78-2.81)
Child not breast-fed (breast-fed)	1.37(1.13-1.65)	1.20(0.90-1.58)	1.86(1.32-2.61)	1.51(0.89-2.55)
Disease related variables				
Child had diarrhea in past 7 days (no diarrhea)	1.29(1.07-1.55)	1.13(0.90-1.41)	1.33(0.96-1.85)	1.22(0.80-1.86)
Child stunted < -2SD (not stunted ≥ -2 SD))	1.22(1.00-1.47)	1.10(0.89-1.37)	1.65(1.18-2.28)	1.42(0.96-2.09)
Child had fever in past 7 days (did not have fever)	1.02(1.01-1.43)	1.14(0.92-1.41)	1.28(0.95-1.74)	1.16(0.77-1.75)
Environment and health services variables				
Family used unsafe water (safe water supply)	1.56(1.24-1.96)	1.36(1.04-1.80)	2.24(1.49-3.36)	1.81(1.09-3.00)
Open field waste disposal (pit latrine)	1.27(1.03-1.51)	1.21(0.98-1.51)	1.27(0.94-1.73)	1.21(0.83-1.81)
Maternal caring capacity variables				
Mother illiterate (literate)	1.27(1.07-1.52)	1.21(0.98-1.50)	1.51(1.11-2.05)	1.43(0.96-2.11)
Mother ill in the past 7 days (mother was not ill)	1.27(1.04-1.55)	1.25(1.00-1.56)	1.00(0.71-1.42)	1.15(0.76-1.74)
Food security variables				
Child 24 to 60 months old (less than 24 months of age)	1.52(1.25-1.83)	1.71(1.26-2.32)	2.07(1.47-2.92)	2.49(1.41-4.39)
Male child (Female)	1.17(0.98-1.39)	1.18(0.96-1.44)	1.29(0.95-1.75)	1.39(0.96-2.02)
Family lived in one room (more than one)	1.28(1.04-1.56)	1.22(0.95-1.57)	1.01(0.72-1.44)	1.07(0.68-1.70)
Family did not have food reserves (had food reserves)	1.21(1.00-1.48)	1.31(1.04-1.64)	1.68(1.21-2.33)	1.74(1.15-2.64)
Poverty related variables				
Family lived below poverty line (lived above poverty line)	1.33(1.05-1.68)	1.49(1.13-1.95)	1.55(1.03-2.35)	1.71(1.04-2.81)
Family did not own a radio (owned a radio)	1.32(1.09-1.58)	1.16(0.92-1.47)	1.50(1.61-2.08)	1.19(0.77-1.85)

The multivariate analysis included children for whom information on all variables included in the logistic regression model were complete were 1691* and 566 **.

Figure 1. Study population for the study on risk factors for iron deficiency anemia in children, 6-60 months of age, in Tigray region, Northern Ethiopia, September-December, 1993

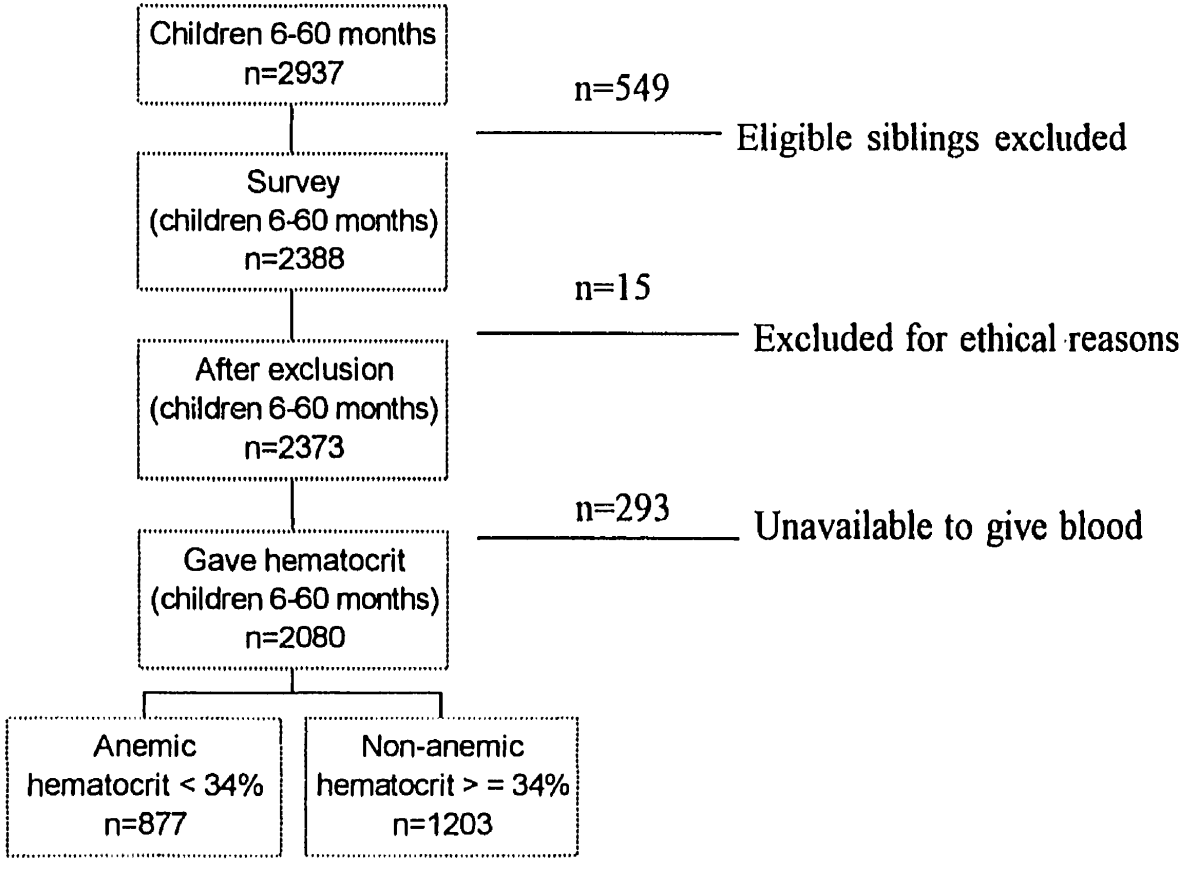
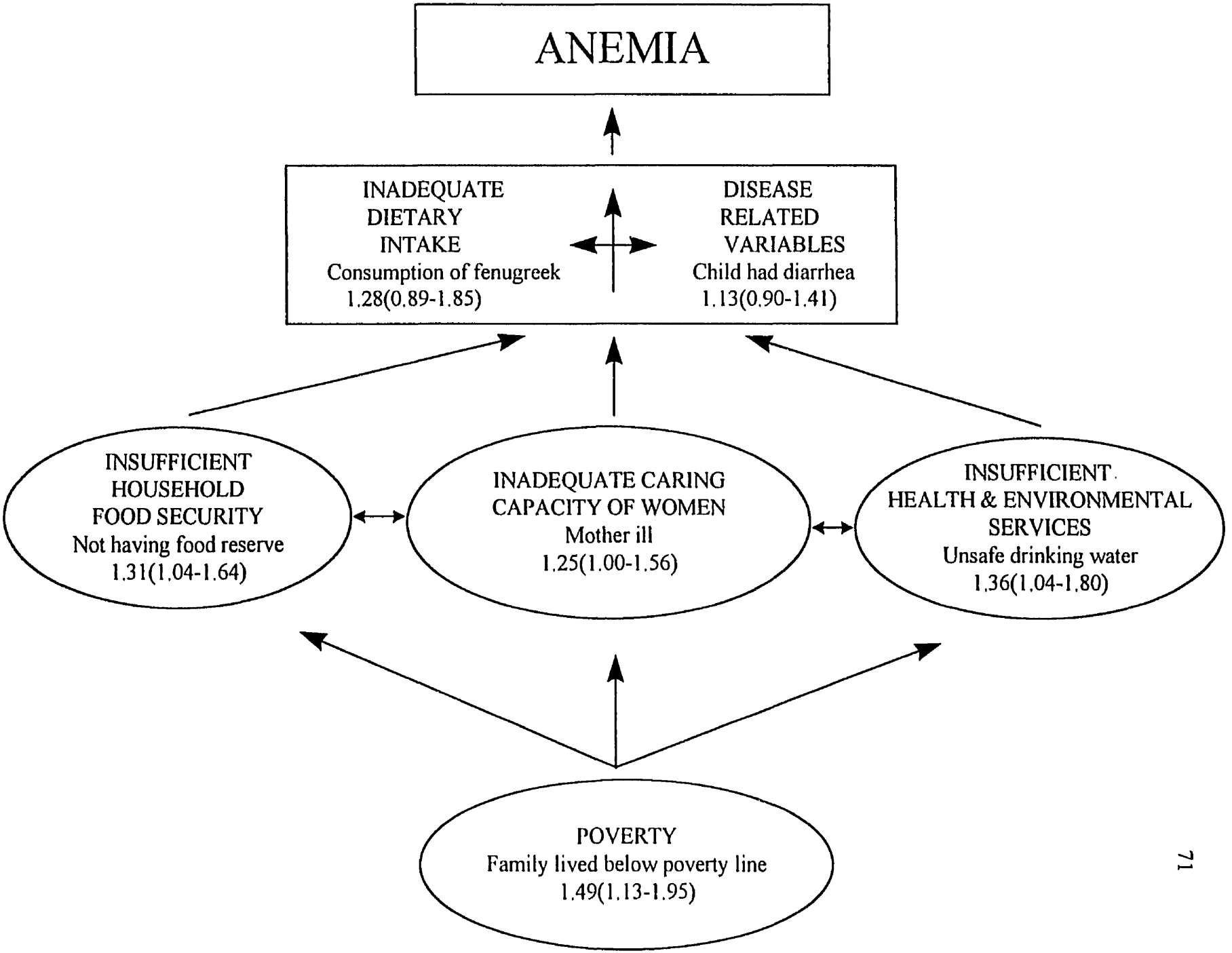


Figure 2 - Conceptual model of risk factors for iron deficiency anemia



Epilogue to Manuscript A

Previous studies in Ethiopia had not reached a consensus on the importance of iron deficiency as a public health problem. There are many known potential causes of iron deficiency, but no study has looked at all of these risk factors simultaneously. In the past, emphasis was placed on the high intake of iron in regions where the staple food was *teff* and where other well known risk factors such as hookworm and malaria occurred. Therefore, the aims of the first manuscript were to identify risk factors for iron deficiency anemia and to determine the role of iron deficiency as a public health problem. The results indicate that there are several dietary and non-dietary factors that play a role in the causation of iron deficiency anemia.

The immediate factors associated with anemia were food intake variables such as consumption of fenugreek and coffee, and disease in the child, such as diarrhea and stunting. The underlying causes were environmental and health service factors such as quality of water and human waste management, maternal caring capacity factors, such as maternal literacy and mother being ill, and food security as measured by possession of food reserves. The root cause of all these factors is poverty. Because the diet in the study area already contains high levels of iron and because a combination of risk factors are associated with anemia, the conventional control strategy of iron supplementation may be ineffective. A sound control strategy should emphasize efficiency of iron absorption. Such a control strategy should also have a holistic

approach and include poverty alleviation, women's empowerment, and disease reduction.

The following manuscript reports on a different control strategy to reduce iron deficiency anemia and its consequences and the report was organized in two studies. The first study *(in-vitro)* was a laboratory analysis to assess whether iron pots release iron into the cooking food and to determine iron availability. Subsequently, the role of iron pots in the control of iron deficiency at a community level was evaluated.

6.0 MANUSCRIPT B

ROLE OF IRON POTS IN THE CONTROL OF IRON DEFICIENCY ANEMIA

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This manuscript reports two investigations, the first being an *in-vitro* study to determine the effect of cooking three types of Ethiopian foods: *Shiro,* legume-based; *Yesiga wet',* meat-based; and *Ye-atkilt Allych'a,* vegetable-based, in three types of pots (iron, aluminum and clay), on total iron (iron released by the food) and available iron (iron available for absorption). There were significant differences of 1.3, 1.6 and 2.6 mg/100gm in total iron in legumes, vegetables and meat, respectively, when cooked in iron pots as compared to when cooked in aluminum pots. The differences persisted after controlling for cooking time and moisture content of food. Available iron was highest in vegetables and meat. The availability of the iron was doubled when vegetables and meat were cooked in the iron pots compared to the aluminum pots. There were no differences in either total or available iron between the foods cooked in aluminum and clay pots, for any of the three food types. The second study was a randomized, controlled community trial to compare hemoglobin levels of anemic preschool children who consumed food cooked in iron pots versus children who ate food cooked in aluminum pots. After a period of three months children in the iron pot group had significantly higher mean hemoglobin levels compared to children in the aluminum pot group and this difference was sustained for the remaining 9 months of the study period. In children in the iron pot group the lower the baseline hemoglobin the greater was the increase over the study period. Three months after children started to consume food cooked in iron pots, there were higher increases in weight and length compared to children who consumed food cooked in aluminum pots, even after adjusting for initial body size. At the end of the study there were still significantly higher gains in length, but not in weight. Therefore, iron pots may provide new strategy to control iron deficiency anemia at the community level. Issues of cultural acceptance and iron overload should be addressed before widespread use.

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Introduction

Iron deficiency anemia is a highly prevalent nutritional disorder. Half of the children and women and a quarter of men in developing countries, and 7-12% of children and women in developed countries, are iron deficient.[1] The magnitude of the problem and its staggering health and economic impact on the population have prompted countries to seek a cost-effective and convenient control strategy.[1,2] The most common practice of controlling iron deficiency at both individual and community levels is oral iron supplementation, with iron taken daily for at least three months. The long duration of treatment and the occurrence of side effects are often deterrents to treatment compliance, in spite of the quick response in iron status.[3,4] Other long-term strategies, such as dietary modification and food fortification (food-based strategy), need to address issues of food production, processing, marketing and preparation which require intensive community education and logistic support.[1] A different strategy of delivering iron, without the aforementioned constraints, would be a step forward in efforts to control iron deficiency anemia and its consequences. One such strategy investigated was cooking with iron pots as a means of supplying the required iron for iron-deficient children.

It is common knowledge that in the past, iron pots were routinely used in many developing communities around the world.[5] This cultural practice has been gradually lost with technical development and availability of cheaper and lighter aluminum pots.

The diminished use of iron cook-ware in developing countries over time may have contributed to the increasing rates of iron deficiency anemia.[6] Several studies suggest that iron from iron pots is leached into the food.[7,8,9,10,11,12] However, its role in the control of iron deficiency anemia in disadvantaged communities has not been assessed.

One reported consequence of iron deficiency is impairment of normal growth.[3,13] Yet evidence accumulated to date is equivocal. Some studies in both developing [14,15,16,17] and developed countries[18] have shown positive effects of iron on growth, while for example, a study in New Zealand showed only gain in weight, not height or head circumference.[19] A cross-sectional study in 220 Togolese children aged 6-36 months failed to show any difference in weight or height.[20] A study by Idjiradinata *et al* [21] reported retarded growth in 47 iron-replete Indonesian children after daily iron supplementation.

We undertook two investigations to examine the potential use of iron pots in the control of iron deficiency anemia. The first study assessed total and available iron in traditional Ethiopian foods cooked in iron pots compared with clay and aluminum pots. The second study assessed the effect of introducing iron pots for cooking on hemoglobin, serum ferritin levels, growth and morbidity of anemic children in a randomized community trial.

Methods

Study design

In-vitro study:

Each of the three typical Ethiopian meals (*Shiro Wet'*, *Yessega Wet'* and *Ye-atiklt Alich'a*) were cooked in aluminum, clay and iron pots and analyzed for total iron and amount of available iron. The clay and iron pots were brought to Canada from Ethiopia and the aluminum pot was purchased in Canada. *Shiro Wet'* is a mixture of chickpea flour (*shiro*) and Ethiopian spiced pepper (*Berberea*). *Yessega Wet'* is beef cut in small pieces cooked in a blend of Ethiopian spices. *Ye-atkilt Alich'a* is a lightly spiced vegetable casserole made from cabbage, carrots and potatoes. The *berberea*, spices and *shiro* were bought in Ethiopia, from the locality in which the community trial took place. The meat for *Yessega Wet'* and the vegetables for the *Ye-atkilt Alich'a* were bought in Canada. All the dishes were prepared in a laboratory at McGill University, according to the recipes specified in a cookbook from the Ethiopian Nutrition Institute.[22] Each type of food was cooked on a hot plate set at 120°C, in each of the three pots on four consecutive days. From each pot, duplicate specimens were taken and digested for 12 hours. Duplicate samples were taken from the digested specimens, duplicate samples were taken making a total of 16 (4x2x2) specimens for each food type in each pot type.

The total iron in the food was measured by an acid digestion procedure.[23]

First, samples of food were dried to a constant weight at 70-80°C and the moisture content was determined. Then the samples were digested for 12 hours in 2 ml of nitric acid at 65°C. A few drops of hydrochloric acid was added, followed by an additional 2 ml of nitric acid. The digestion was continued for 12 hours at 100°C. The total iron in the digested food samples was determined by Flame Atomic Absorption Spectrophotometry (AAS). The available iron (iron available for absorption by the body) from the foods was determined by using the *in-vitro* dialysis assay method of Miller *et al* [24] with Hurrell's[25] modification.

Community trial:

Over 2000 children were screened as part of a baseline study of risk factors for iron deficiency anemia. Of the 877 children with hematocrit of less than or equal 34%, 420 were eligible for this study, and a sample size of 407 children living in Quiha (semi-urban community 8 km from Mekele, Tigray, Northern Ethiopia) were selected for a randomized community trial [26]. The 407 children between the ages of two to five years, with hematocrit of ≤ 34%, were randomly assigned into iron (n=195) and aluminum (n=212) pot groups. Prior to the study children with severe anemia (hematocrit < 20%) were excluded from the study for ethical reasons. Because the iron and the aluminum pots were so different, participating families could not be blinded to the type of pot. However, the laboratory technicians who collected and analyzed the blood samples and the technicians who measured the children's weight

and length were blinded to the intervention groups. Because mothers were likely to also benefit from the iron pots which in turn might benefit their children (e.g. more energy for child care), mothers of aluminum pot group children were supplemented with iron (60 mg/day of elemental iron) for three months. The intent was to minimize differences in the two groups with the exception of iron provided to children through foods cooked in iron pots.

Socio-demographic variables such as age, gender, diarrhea in the child one week prior to interview, illness in the mother a week prior to the interview, mother's literacy status, availability of clean water to the family and adequacy of their waste management were recorded by trained interviewers using a structured and pretested questionnaire. For all the study children, blood hemoglobin using the HemoCue system (Lee Diagnostics AB, Medical Equipment Design, Inc.), weight using standing balance (Seca 770, Hamburg, Germany) and length using crown heel board (Shorr Corp.) were measured at baseline and again at 3, 6 and 12 months by trained technicians.[27] Morbidity information, such as presence, frequency and type of diarrhea (three or more loose stools in 24 hours), presence of ARI (cough and fever or breathing problems) and presence of fever, since the last visit were recorded weekly for the first seven months of the study. Venous blood was collected by a senior laboratory technician for determination of serum ferritin using commercial kits (Fisher Scientific Limited). At baseline serum was collected for ferritin in a sub-sample of 170 randomly selected children. For the post-intervention serum analysis a different sub-

sample (n=84) was drawn randomly, to minimize traumatizing the same children for a second time.

Baseline and follow-up questionnaires were administered to mothers of study children following oral informed consent. During follow-up, all families were briefed on the importance of compliance (to use of the pots) and children in both groups were visited once a week for 12 months. In the event of an illness complaint, interviewers referred the child to a physician for a general check-up and specific examination for side effects of iron overload. This community trial took place from March 1994 to May 1995. The study was reviewed by ethics review committees in the school of Dietetics and Human Nutrition at McGill University in Canada and in the Jimma Institute of Health Sciences in Ethiopia.

Data analysis

All data were recorded directly onto the questionnaires and laboratory forms, subsequently entered into a personal computer using Epi Info, Version 5 1994 (Center for Disease Control, Atlanta). Data were analyzed using Stata version 3.1 1994 (Stata Corp. Texas). All tests were two-tailed, and a type I error rate of 5% was used for p-values and confidence limits.

In-vitro study

Means and standard deviations were used to show the distribution of total and available iron of each food cooked in each type of pot. The availability of iron (percent of the total iron absorbed) was calculated by dividing the available iron by the total iron and multiplying the product by 100. Then, the crude total iron was adjusted for moisture and cooking time of food, which were considered potential confounders.

The individual effects of each pot type and food type and their interaction effects were assessed using multiple linear regression analysis. The categories of legumes and aluminum pot were designated as the reference categories for food type and pot type, respectively. Variables included in the model were: food type variables (meat and vegetables), pot type variables (clay and iron pot) and confounding variables (moisture, cooking time and pH). In the final model the pH was excluded as it was found to be less variable and collinear with moisture.

Community trial

The success of randomization was assessed by comparing characteristics and possible confounders between the intervention groups using the chi-square test and Student's t-test. The variables assessed (age and gender of child, diarrhea in the child one week prior to interview, child consuming fenugreek at least once in the week prior

to interview, maternal illness in the week preceding the interview, mother's literacy status, availability of clean water to the family and adequacy of their waste management and family living below poverty line) were found to be risk factors for iron deficiency anemia in an earlier survey which had included the same children.[28]

Change in hemoglobin at 3, 6 and 12 months of the study were compared between the two groups using a linear regression model where hemoglobin at the specified time was the outcome variable and type of intervention was the independent variable. Then, hemoglobin at baseline was added to the model to adjust for the effect of initial hemoglobin on hemoglobin at 3, 6 or 12 months. Differences in the mean serum ferritin levels were also compared between the groups and within the same group over time using Student's t-test.

The effect of the intervention on growth was also assessed using a linear regression model. In the model weight or length at 3, 6 or 12 months of study was the outcome variable and type of intervention was the independent variable. Then, to control for the effect of weight or length prior to the intervention, weight or length at baseline was included in the model.

During analysis children were stratified by their change in hemoglobin at the end of study and baseline hemoglobin, change in weight and change in height at the end of the study were compared among these strata using ANOVA.

Morbidity information collected weekly for the first seven months of the study period were converted into monthly prevalence rates and compared between the iron pot and aluminum pot groups using ORs and 95% CIs.

Results

In-vitro study

Total iron was highest in all foods cooked in iron pots compared to the other two pots (Table 1). The largest difference was for meat (2.6 mg/100 gm) followed by vegetables (1.6 mg/100 gm) and legumes (1.4 mg/100 gm). This represents a doubling in total iron from meat, 2.5-3.5 times more iron from vegetables and 1.5 times more from legumes. The magnitude of the adjusted results are similar, but not identical to the crude values because of the difference in cooking time. Foods cooked fastest in aluminum pots and slowest in clay pots. The available iron cooked in an iron pot was five times higher for meat and vegetables cooked in an iron pot (0.24 mg/100 gm) compared to meat (0.05 mg/100 gm) and vegetables (0.04 mg/100 gm) cooked in non-iron pots. No difference was observed for legumes. The availability of total iron was more than doubled for both meat (5.13% versus 2.36%) and vegetables (8.24% versus 3.34%) when cooked in iron pots compared to the other two pots. The availability of iron from legumes was not different among the three pot types. There was no significant difference in the mean total and mean available iron between aluminum and clay pots for any of the three foods. Cooking time was lowest for

aluminum pots but similar for iron and clay pots. Legumes cooked in iron pots had higher (3.72 mg/100 gm) total iron than meat cooked in aluminum (2.54 mg/100 gm) or clay (2.64 mg/100 gm) pots. Vegetables (2.32 mg/100 gm) cooked in iron pots had nearly as much total iron as meats (2.6 mg/100g) or legumes (2.4 mg/100g) cooked in clay or aluminum pots.

Community trial

Study children were similar across groups (Table 2). One-third of children were ill in the previous seven days and diarrhea was reported in one out of every five children. Most mothers were illiterate, living in a single room without proper waste management, with family incomes below the poverty line, defined as 200 Birr/month (1 $US=6.2 Birr). Only seven children were lost to follow-up. The main reason for loss to follow-up was emigration from the study area.

Over the 12 months of the study, 28 children presented with complaints at different times and were referred to a physician for check-up and treatment. These children were then treated for illnesses ranging from intestinal parasites to skin disease. None of these children had complaints related to iron overload. Daily use of the iron pots declined from 80-85% during the first 10 weeks to 68-70% in the next 10 weeks. However, almost all families (98%) used the iron pot at least 3 days a week all through the study period.

The initial mean hemoglobin level of children in the iron pot (10.5 g/dl) and aluminum pot (10.8 g/dl) groups were compared ($p = 0.067$) (Table 3). Mean hemoglobin increased by 1.5 g/dl in the first three months in the iron pot group and then plateaued for the remainder of the study period. At the end of the study, hemoglobin among children in the iron pot group was 1.8 g/dl ($p=0.008$) higher than when the study began. No significant change in hemoglobin occurred in the aluminum pot group during the year. At the end of the study a difference of 1.1 g/dl was observed between the two intervention groups and the difference was significant ($p < 0.001$). After adjusting for initial hemoglobin 1.2 g/dl (95%CI: 0.93,1.54) difference was observed at 3 months, which was sustained for the remainder of the year. In the iron pot group the proportion of anemic children (defined by hemoglobin of less than 11 g/dl) decreased from a high of 56.9% to only 13%. However, anemia rates for the aluminum pot group remained unchanged around 50% over the course of the study.

At the beginning of the study, serum ferritin levels were similar in both groups, about 22 to 23 µg/L (Table 4). Children who consumed food cooked in iron pots were found to have an increase of 11.2 µg/L, while children in the aluminum pot group showed no change in their serum ferritin levels. Moreover, at the end of the study a difference of 12.7 µg/L was observed between children in the iron and aluminum pot groups. Both the differences were statistically significant ($p < 0.001$).

At baseline the mean weight of children in the iron pot group was 250 gm less

than that of children in the aluminum pot group, but this difference was not significant (Table 5). At three months children in the iron pot group grew 270 gm (95%CI: 0.13, 0.42) more than children in the aluminum pot group after controlling for initial weight. From three months onwards no difference in weight was observed between the groups, through to the end of the study. The mean length of children at baseline in the iron pot group was 0.8 cm less than the mean length in the aluminum pot group, but the difference was not significant (95%CI: -2.4, 0.9) (Table 6). However, at three months, at six months and at 12 months, after adjusting for initial length, children in the iron pot group grew more than 0.6 cm in length compared to children in aluminum pot group, and the differences were statistically significant. This represents a gain of 4.2 cm at 3 months, 6.9 cm at six months and 8.7 cm at 12 months among children in the iron pot group. The corresponding gain in length in children from the aluminum pot group was 3.3 cm, 5.7 cm and 8.0 cm at 3, 6 and 12 months, respectively.

Children in the iron pot group were subsequently categorized into four groups, according to their level of change in hemoglobin during the study (Table 7). Children who had the lowest hemoglobin at baseline had the highest increase in hemoglobin status at the end of the study. The increase was almost double when the baseline hemoglobin was less than 10 g/dl (1.74-3.31 g/dl) as compared to when it was above 10 g/dl (0.67-1.48 g/dl). There was also a tendency of a higher increase in weight in those children with initial lower hemoglobin compared to those with higher hemoglobin at the baseline. The difference in weight gain between children having the

lowest and highest hemoglobin change categories was 400 gm, a clinically important, but not statistically significant increase. This trend was not observed for changes in length (results not shown).

There was some trend of decrease in prevalence of diarrhea, ARI and fever in the iron pot group compared to the aluminum pot group. However, none of these differences were statistically significant.

Discussion

The *in-vitro* study showed that iron pots release significantly more iron into food compared to the other pots. The iron released into the food was found to be available for absorption by the body. The availability was enhanced more for meat and vegetables than for legumes. Similar results have been reported by other investigators.[8,10,11] There was no difference in either the total or the available iron between the aluminum and clay pots for any of the food types. Both the amount of iron released and its availability were markedly increased when meat and vegetables were cooked in iron pots. Meat factor in meat and vitamin C in vegetables are known to increase the absorbability of non-heme iron.[3] The total iron from legumes cooked in iron pots was higher than the total iron in meat and vegetables cooked in either clay or aluminum pots. This was true even after adjusting for cooking time and moisture of the food. The significance of this finding is that families that cannot afford meat or

vegetables can get a comparable amount of iron from legumes if they use iron pots for cooking. Since the recommended daily intake of iron for preschool children is 6 mg/day, not considering factors that influence availability, a child can acquire his or her requirement by consuming only 300 grams of *shiro* cooked in iron pots. This study did not determine the proportion of heme and non-heme iron in the foods. Therefore, identifying the type of iron is highly recommended for future studies.

There was a significant increase in the mean hemoglobin level of children at 3, 6, and 12 months of the study in children who ate food cooked in iron pots. This change persisted even after controlling for baseline hemoglobin. The increase was significantly higher in children with a lower hemoglobin status at the beginning of the study. Over the study period, little change was observed in the hemoglobin levels of the aluminum pot group. In the iron pot group the increase in hemoglobin was also confirmed by an increase in the serum ferritin levels. Therefore, food cooked in iron pots significantly raised the iron status of children as reflected by their hemoglobin and serum ferritin levels. Prevalence of anemia as assessed by hemoglobin of less than 11 g/dl decreased from a rate of 57% to 13% in the iron pot group. Similar results were observed when a hematocrit less than 33% was used as a cut-off point. No change in prevalence was observed in the aluminum pot group. Even though iron status remained stable, without signs of iron overload after three months, the effect of long term use of iron pots, especially in communities with suspected high dietary intakes of iron, requires further investigation. None of the study children had serum ferritin

levels higher than 175 µg/L. In iron overload serum ferritin levels are often higher than 200 µg/L.[29] In this study the only complaint of families was rusting of the iron pots, which was subsequently resolved by recommending drying of the pots immediately after use.

After adjusting for initial weight and length, at three months, there was an additional 270 gm gain in weight and an additional 0.7 cm gain in length in children consuming food cooked in iron pots compared to children who consumed food cooked in aluminum pots. After six months there was still a significantly higher gain in length, but not in weight. After adjusting for initial hight, at the end of the study, there was a 0.6 cm significantly higher gain in length in the iron pot group compared to the aluminum pot group. In both the study groups increase in length during the first 6 months was 3 times the increase in the second 6 months. A similar pattern of growth was also observed by a study in preschool children in nearby communities receiving iron and/or vitamin A tablets.[26] This might be explained by a decline in rate of length increase with age or seasonal effects of growth.[30] Similar gains in length have been reported from iron supplementation studies conducted in Indonesia, Kenya and Bangladesh. [13,15,16,17]

Public Health Significance of Using Iron Pots

Iron deficiency as a public health problem has received increased attention in recent years along with other micronutrients. The staggering economic effect of iron deficiency anemia and its consequences has prompted countries to seek a cost effective and sustainable strategy to control the problem.[1] The World Health Assembly and the World Summit for Children adopted a goal of reducing the rate of iron deficiency by the year 2000, at least among women of child-bearing age, by one-third of 1991 levels.[31]

Common strategies to improve iron status involve manipulation of one or more of the factors that determine iron absorption or iron loss. The ideal strategy to overcome iron deficiency is to improve the diet by including iron-rich foods, increasing intake of vitamin C and reducing intake of iron absorption inhibitors, such as tea and coffee.[32,33] When food diversification is not possible, food fortification (adding iron to a centrally produced and commonly eaten food) is the next best strategy. The vehicle food selected for fortification should be consumed by the majority of the target group, processed centrally and be inexpensive. Iron supplementation is another widely used preventive as well as therapeutic strategy. Whatever the choice of strategy, it has to be clear that the public health control of iron deficiency anemia is not a simple matter of supplying the missing nutrient. Cultural and religious beliefs, attitudes towards the control strategy, logistic factors, costs and marketing are instrumental in influencing people's decision to adhere to treatment or prevention schedules.[34] According to Levin [34] the cost of iron supplementation for pregnant women alone in a population of

10,000 for one year is $8,000.00 USD. Recently, studies have reported a single weekly dose of iron to be as effective as a daily dose, which would significantly reduce this cost estimate.[35] The cost of iron fortification for the whole population of 10,000 for one year is $20,000 USD.[34] Even though a cost analysis was not part of our study, we estimated the cost of iron pots for a population of 10,000 (with an average family size of 6 people, 1 pot/family) to be $5,000.00 USD. Furthermore, the fact that iron pots could serve the whole family for years might further reduce this estimate. Since iron pots are not foreign to rural communities their introduction might face less cultural resistance than the other control strategies.

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Table 1. Total, adjusted total and available* iron from three types of traditional Ethiopian foods cooked in different types of pots

Foods by pot type	Crude total iron *(mg/100g of food)*	Adjusted total iron (mg/100g)**	Available iron *(mg/100g of food)*	Availability of iron (%)***	Cooking time *(min)*	pH	Moisture (%)
Aluminum Pot							
Legumes (n=16)	2.33±0.11	2.30	0.04±0.01	1.72	35±0	5.88±0.06	83.7±0.6
Meat (n=16)	2.06±0.25	2.54	0.05±0.09	2.43	74±12	5.87±0.01	77.7±5.0
Vegetables(n=16)	1.23±0.23	0.69	0.04±0.02	3.25	50±5	5.84±0.02	90.7±0.01
Clay Pot							
Legumes (n=16)	2.47±0.07	2.51	0.04±0.01	1.62	56±0	5.78±0.05	83.2±0.4
Meat (n=16)	2.18±0.62	2.64	0.05±0.01	2.29	86±2	5.76±0.04	78.2±6.1
Vegetables(n=16)	1.46±0.46	0.98	0.05±0.03	3.42	66±13	5.85±0.02	90.2±0.2
Iron pot							
Legumes (n=16)	3.67±0.17	3.72	0.06±0.02	1.63	51±1	5.87±0.0	82.9±0.3
Meat (n=16)	4.68±0.63	5.17	0.24±0.09	5.13	76±18	5.68±0.0	77.8±7.2
Vegetables(n=16)	2.79±0.24	2.32	0.23±0.08	8.24	66±14	5.97±0.4	90.1±0.2

* Mean±standard deviation
** Adjusted for cooking time and moisture.
*** Availability = (available iron/total iron) times 100

Table 2 Comparison of potential confounders* between the intervention groups in anemic children aged 2-5 years in Tigray, Northern Ethiopia

Variable	Iron pot (n=195)	Aluminum pot (n=212)	p-value
Age of child (months)	31.7±13.6	31.0±14.6	0.61
Gender (% males)	49.7	51.4	0.75
Weight of child (kg)	11.6±2.3	11.9±2.3	0.27
Length of child (cm)	87.2±8.5	88.0±8.7	0.37
Child ill in last 7 days (%)	28.6	33.3	0.33
Child drank coffee at least once in the last 7 days (%)	29.7	30.6	0.83
Child consumed fenugreek at least once in the last 7 days (%)	20.3	19.9	0.92
Child had diarrhea in last 7 days (%)	18.1	19.2	0.79
Mother literate (%)	36.8	31.8	0.31
Mother ill in last 7 days (%)	26.4	22.8	0.43
Family with monthly income below the poverty line (%)	26.7	31.7	0.24
Families that have access to clean water (%)	76.4	73.2	0.48
Families that have adequate waste management (%)	30.2	35.9	0.24

* Continuous variables are expressed as a mean ± SD

Table 3 Change in hemoglobin (g/dl) at different times of the study by type of cooking pot in children 2-5 years of age in Tigray region, Northern Ethiopia

	Iron-pot		Aluminum-pot		Hemoglobin (g/dl) difference between intervention groups (95%CI)	
Time	**Hemoglobin g/dl±SD**	**% < 11g/dl**	**Hemoglobin g/dl±SD**	**%<11g/dl**	**Unadjusted**	**Adjusted***
Baseline	10.5 ±1.2 (n=195)	56.9	10.7 ±1.3 (n=211)	54.5	-0.24 (-0.49, 0.01)	
3-months	12.0 ±1.9 (n=192)	19.8	10.9 ±1.6 (n=206)	50.0	1.08 (0.73, 1.42)	1.24 (0.93, 1.54)
6-months	11.9 ±1.5 (n=177)	20.3	10.8 ±1.3 (n=204)	54.9	1.07 (0.78, 1.35)	1.19 (0.94, 1.44)
12-months	12.2 ±1.5 (n=192)	13.0	11.1 ±1.5 (n=207)	49.8	1.06 (0.80, 1.35)	1.19 (0.98, 1.48)
Difference **	1.7±1.5 (n=192)		0.4±1.0 (n=207)		1.3 (1.07, 1.56)	1.2 (1.00, 1.42)

* Difference of hemoglobin at 3, 6 and 12 months adjusted for hemoglobin level at baseline.
** Mean of the difference between hemoglobin at 12 months and at baseline.

Table 4. Mean difference in serum ferritin (µg/L)* between the iron pot and aluminum pot groups in children aged 2-5 years in Tigray, Northern Ethiopia

	Iron pot	Aluminum pot	Difference	p-value
Baseline	23.3±15.1* (n=95)	22.0±14.1 (n=75)	1.3	0.57
12 months	34.5±12.9 (n=47)	21.8±13.5 (n=37)	12.7	< 0.001
Difference	11.2	0.3		
p-value	< 0.001	0.94		

* serum ferritin mean ±SD

Table 5. Comparison of change in weight(kg)* between intervention groups at different times in children aged 2-5 years in Tigray region, Northern Ethiopia

Time	Iron pot	Aluminum pot	Difference between Intervention groups (95%CI)	
			Unadjusted	Adjusted**
Baseline	11.6±2.3 (n=195)	11.9±2.3 (n=212)	-0.25 (-0.69, 0.19)	
3-months	12.5±2.2 (n=193)	12.4±2.1 (n=208)	0.07 (-0.35, 0.49)	0.27 (0.13, 0.42)
6-months	12.8±2.2 (n=176)	12.9±2.2 (n=204)	-0.16 (-0.60, 0.28)	0.07 (-0.22, 1.13)
12-months	13.7±2.1 (n=192)	13.8±2.2 (n=207)	-0.10 (-0.52, 0.32)	0.10 (-0.07, 0.27)
Difference***	2.0±1.0 (n=192)	1.9±1.0 (n=207)	0.13 (-0.05, 0.31)	0.1 (-0.07, 0.28)

* Mean ±SD
** Comparison of weight at 3, 6 and 12 months adjusted for weight at baseline
*** Mean of difference in length at baseline and at 12 months

Table 6. Comparison of change in length(cm)* between intervention groups at different times in children aged 2-5 years in Tigray region, Northern Ethiopia

Time	Iron pot	Aluminum pot	Difference between intervention groups (95%CI)	
			Unadjusted	Adjusted**
Baseline	87.2±8.5 (n=195)	88.0±8.7 (n=212)	-0.76 (-2.44, 0.91)	
3-months	91.4±8.4 (n=192)	91.3±8.6 (n=208)	0.05 (-1.66, 1.73)	0.7 (0.30, 1.10)
6-months	93.6±8.3 (n=176)	93.7±8.6 (n=204)	-0.09 (-1.80, 1.60)	0.7 (0.22, 1.13)
12-months	95.9±8.3 (n=190)	96.0±8.1 (n=206)	-0.1 (-1.71, 1.52)	0.6 (0.13, 1.00)
Difference***	8.5±2.0 (n=190)	7.9±2.6 (n=206)	0.6 (0.17, 1.08)	0.6 (0.13, 1.00)

* Mean±SD

** Comparison of length at 3, 6 and 12 months adjusted for length at baseline

*** Mean of the difference in length at baseline and at 12 months

Table 7. Change in weight and length at the end of the study by different categories of change in hemoglobin in children, 2-5 years of age, who consumed food cooked in iron pots in Tigray, Northern Ethiopia

Change in hemoglobin (g/dl)*	Number of children	Hemoglobin at baseline (g/dl)	Change in weight (kg)	Change in height (cm)
< 1.0	53	11.0±1.3	1.86±0.9	8.3±2.0
1 - 1.9	68***	10.9±0.9	1.90±0.9	8.7±1.6
2 - 2.9	36	10.5±0.9	2.10±1.1	8.4±1.6
≥ 3.0	33***	08.9±1.3	2.25±1.0	8.7±1.9
P - value**		0.000	0.223	0.724

* Hemoglobin at the end of study minus initial hemoglobin of each child
** ANOVA test
*** 1 child had had missing baseline length and consequently were excluded from calculating changes

Epilogue to Manuscript B

The baseline study determined that iron deficiency was rampant and constituted a public health problem in Ethiopia. Iron in the diet was found to be adequate, but the problem was the efficiency of iron absorption, not the intake. Therefore, the following studies evaluated alternative strategies of controlling iron deficiency. The strategies reported in this thesis were the role of iron pots in the control of iron deficiency, and effect of combined iron and vitamin A supplementation on iron status of children.

The iron pot study had two components, the first being an *in-vitro* study to determine the effect of cooking Ethiopian foods *Shiro*, legume-based; *Yesiga Wet'*, meat-based; and *Ye-atkilt Allych'a*, vegetable-based in three types of pots (iron, aluminum and clay) on total iron and available iron. Iron pots provided more total and available iron than did aluminum or clay pots. The second component was a randomized, controlled community trial to compare hemoglobin levels of anemic preschool children and their growth. Both hemoglobin and growth (length) improved with the use of iron pots compared to aluminum pots. Due to suspected high levels of iron in the diet, signs of iron overload were monitored continuously during the study, both clinically and by assessing serum ferritin. No signs of iron-overload were observed. Before widespread use is promoted, issues of iron overload and cultural acceptance should be addressed by observing use over longer time period and evaluating all family members, including men.

7.0 MANUSCRIPT C

INDIVIDUAL AND COMBINED EFFECTS OF IRON AND VITAMIN A ON GROWTH AND MORBIDITY OF CHILDREN IN NORTHERN ETHIOPIA

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A randomized, placebo-controlled, double-blind community trial of iron and vitamin A supplementation included 407 children in Northern Ethiopia with hematocrit ≤ 34% and aged 24 to 60 months. The study children were randomly selected and assigned into four intervention groups (iron only, vitamin A only, iron plus vitamin A and placebo). Both iron and vitamin A treatments significantly increased hemoglobin levels in children. The combined iron and vitamin A supplementation showed the highest increase in hemoglobin. However, the increase resulting from the supplementation of vitamin A was not as high as the raise due to the iron supplement. After adjusting for initial length there was a 0.6 cm (95%CI:-0.1,1.2) (statistically not significant but clinically important) increase in length in iron supplemented children compared to children in the placebo group, but no significant change in weight. Iron supplementation reduced the frequency of diarrhea and general morbidity as assessed by c-reactive protein. Vitamin A supplementation also resulted in a marked reduction in the prevalence and frequency of diarrhea. Both iron and vitamin A-treated children showed higher rates of ARI (Acute Respiratory Infection), but children who received the combined iron and vitamin A supplementation had significantly lower rates compared to the placebo group. Iron supplementation had no effect on zinc or copper status. These results indicate that a combined iron and vitamin A supplementation strategy provides the most benefit in the treatment of iron deficiency and should be considered as the standard control strategy in preschool children in high risk communities.

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Introduction

The functional impairment of iron deficiency anemia is due either to the reduced capacity of hemoglobin to transport oxygen and nutrients to cells and/or to its effect on redox enzymes, such as cytochrome and catalase (1). Some of the most commonly reported consequences of iron deficiency in children are growth faltering, decreased resistance to infection and impaired cognitive development (2-4).

Existing studies have not reached consensus on the importance of iron deficiency on faltering of growth of children. Investigators in both developing (5-8) and developed countries (9) have shown positive effects of iron on growth, while other studies reported increases in weight (but not in length or head circumference) (10), no increase in weight or height (11) or retarded growth (12).

The findings of the effect of iron on resistance to infection are also controversial (13,14). Iron deficiency and infection often coexist in disadvantaged communities (14). Some community intervention studies in both developed (15,16) and developing countries (11,17,18) have provided evidence that iron supplementation reduces the occurrence of diarrhea and acute respiratory infection (ARI). Other investigators have reported that supplemental iron reactivates pre-existing infections such as malaria, brucellosis and tuberculosis (5,19-21). A randomized study by Heresi and collaborators failed to show any difference in the rates of diarrhea and ARI (22). However, other researchers have reported that an increase in the rate of infection is

not due to iron therapy *per se*, but to the route of administration of the iron (14). Parenteral iron causes transferrin to become saturated making unbound iron available for growth and reproduction of microorganisms in the blood.

Vitamin A is essential for a variety of biological processes, many of which are related to growth, cellular differentiation and interaction of cells with each other or with the extracellular matrix (23). There is general agreement that vitamin A plays an important role in reducing morbidity, particularly from respiratory and diarrheal diseases, and also mortality (24,25). Vitamin A supplementation also seems to improve hematological status. Rats fed diets deficient in vitamin A had reduced hematopoietic cells in their bone marrow (26-28). Some studies in humans have also indicated that vitamin A deficiency contributes to anemia (29). Other studies reported that supplementing with vitamin A alone increases hematological indices and that the combined effect of iron and vitamin A was even greater (30-33). However, a community trial in 162 anemic Ethiopian children failed to show any difference in hemoglobin, hematocrit, serum iron, transferrin saturation or serum ferritin between children receiving combined iron and vitamin A supplementation and children supplemented with iron only (34). Thus, while animal studies tend to show that vitamin A improves iron deficiency anemia, human studies are inconclusive.

Oral iron supplementation is reported to interfere with the absorption of other micronutrients, especially zinc and copper (35-39). Hambidge *et al* reported a decline in serum zinc during iron therapy in pregnant mothers, while Yip *et al* and

Arnaud *et al* failed to show any effect (37-39). None of these studies were conducted in preschool children.

The aim of the present study was to determine whether children with hematocrit ≤ 34% who are supplemented with iron only or vitamin A only experience less morbidity and better growth than control children and whether children supplemented with combined iron and vitamin A have an even greater effect. The study also evaluated the effect of iron supplementation on the absorption of other minerals such as zinc and copper.

Study design

A randomized, placebo-controlled, double-blind community trial was conducted to evaluate the main effects of iron and vitamin A and their combined effect on hemoglobin values, morbidity and growth of preschool children. Children with hematocrit ≤ 34%, were randomly assigned into iron (30 mg of ferrous sulphate per day for 3 months) , vitamin A (single dose of 200,000 IU of vitamin A), iron plus vitamin A (30 mg of ferrous sulphate per day for 3 months and single dose of 200,000 IU of vitamin A) , and placebo (same dose of look alike and taste alike syrup and capsule) groups who received their respective supplements for three months and were followed for nine more months. Both mothers and researchers were kept blind to the type of supplement children were receiving. The study was approved by the ethics committees at the School of Dietetics and Human Nutrition at McGill University in

Canada and the Jimma Institute of Health Sciences in Ethiopia.

Study population

The study sample was drawn from nearly 3000 children who participated in a food security survey. From over 2000 children screened for hematocrit status at baseline, 457 children having a hematocrit of ≤ 34%, aged between 24 and 60 months, and permanent residents of Mekele were eligible for the study. From the 457 children, 407 were randomly selected and randomly assigned into one of the four intervention groups. Children with a hematocrit of less than 21%, and children who were severely ill and in need of immediate medical care, were excluded from the study for ethical reasons. During the survey if two or more children aged two to five years resided in a household, one child was randomly selected. The study area of Mekele is located in Northern Ethiopia in East Africa. The study was conducted from March, 1994 to May, 1995.

Data collection

Socio-demographic and laboratory variables were collected for the purpose of assessing comparability of the different intervention groups following randomization. A pre-testing of the questionnaire took place in non-study households, but in a similar community. Hemoglobin, weight and length were measured on all children at baseline and again at three, six and 12 months of the study. Children's ages were obtained from

their mothers to the nearest month, but also confirmed by a local events calendar (40). For morbidity, a diagnostic format was prepared and administered by trained interviewers once a week all through the study period. The morbidity information collected during treatment and the first three months of the follow up period included: presence, frequency and type of diarrhea (three or more loose stools in 24 hours) and presence of ARI (cough and fever or breathing problems since the last visit). Whenever a child was found sick, he or she was referred to a physician for treatment. After making sure that the physician's treatment had no effect on iron status, the child rejoined the study for the rest of the follow-up period. In a sub-sample of 123 study children, the morbidity information was supplemented with serum C-Reactive Protein test (CRP, Stanbio), which is a more objective measure of infectious status. CRP, a latex agglutination test, is positive in all acute inflammatory processes, both infectious and non-infectious, and in certain malignant conditions as a nonspecific phenomenon.

Laboratory assessment of intermediate variables

In all study subjects, hemoglobin was measured by the HemoCue system (Lee Diagnostics) from capillary blood obtained from a finger prick. In a sub-sample of 135 children at baseline and 123 children post-intervention (12 months after baseline), venous blood was collected in a controlled and standardized manner (same time of the day following the same procedures) for serum ferritin, serum zinc and serum copper determination (41). Since the amount of serum was not always adequate for completion of all the tests, the following priority order was followed: serum ferritin,

zinc and copper. Both sera for zinc and copper were analyzed using Flame Atomic Absorption Spectrophotometer (AAS) at McGill University, Canada. Sera for ferritin assessment were also analyzed at McGill, Canada using a radio-immunoassay technique.

Intervention groups and treatment delivery

Children in the iron group received 30 mg of elemental iron syrup daily (ferrous sulphate, Novopharm Canada) and a single squeezable capsule that resembled and tasted like vitamin A capsule. Children in the vitamin A group received a single dose of 200,000 IU of vitamin A in squeezable capsule and a placebo syrup daily that resembled and tasted like iron syrup (placebo iron). Children in the combined iron and vitamin A group were given 30 mg of elemental iron syrup daily and a single dose of 200,000 IU of vitamin A in a squeezable capsule. Children in the placebo group received placebo iron daily for 30 days and a single dose of placebo vitamin A. Children in all the study groups took iron or its placebo once a day for three months and vitamin A or its placebo capsule once in a single dose at baseline. Every other day community workers checked treatment compliance and encouraged mothers to follow the treatment schedule. To prevent accidental ingestion of excess iron by the child, mothers were given only two week's supply of the syrup at a time.

Data Analysis

All data were recorded onto precoded questionnaires and laboratory forms and were subsequently entered into a personal computer using Epi Info, Version 5 (Center for Disease Control 1994). Data were then analyzed using Stata (Stata Corp. 1993).

Analysis of variance (ANOVA) was used to determine comparability of potential variables between intervention groups (42). Hemoglobin, weight and length were assessed within an intervention group using the paired t-test, among the four intervention groups using ANOVA and between two groups using Student's t-test. Changes in hemoglobin (hemoglobin at 12 months minus hemoglobin at baseline for each child) were calculated and compared among intervention groups using ANOVA. Comparison between each intervention group and the placebo group were made using Student's t-test. The study children (n=407) were re-categorized into iron intake and no iron intake and vitamin A intake and no vitamin A intake and then a multivariate model, where iron, vitamin A and the interaction term (iron*vitamin A) were independent variables and change in hemoglobin (hemoglobin at 12 months minus hemoglobin at baseline) was the dependent variable, was also run to observe main effects of iron and vitamin A and their interaction effects. Similar procedures were followed to compare changes in weight and length.

From the morbidity information, the number of children who had diarrhea or ARI in the previous one month were recorded for convenience of reporting and

compared among the intervention groups using an ANOVA. Odds ratios and 95% confidence intervals were also calculated to compare the monthly prevalence in one intervention group with the prevalence rate in the placebo group for the same month. Frequency of diarrhea was determined to be the number of bowel movements per episode of diarrhea (where a diarrhea-free period of 48 hours distinguished a new episode).

To assess individual effects of iron and vitamin A on the infectious status of the children using CRP, study subjects were re-categorized into iron supplementation (yes or no) and vitamin A intake (yes or no) groups. The CRP status was then compared between these categories using the chi-square test. All tests were two-tailed, and a type I error of 5% was used for p-values.

Effect of iron supplementation on ferritin, zinc and copper statuses

Baseline and final sera were collected from 135 and 123 children, respectively, but results were available for 135 and 123 children for ferritin, 121 and 101 children for zinc and 120 and 119 children for copper, respectively. To verify the hypothesis that iron supplementation reduces the zinc and/or copper status of children, the four intervention groups were re-categorized into iron and no iron supplemented (placebo) groups. Means and differences in serum zinc and serum copper were compared between and within intervention groups using Student's t-test.

Results

Effect of iron supplementation on hemoglobin, growth and morbidity of children

Of the 407 children enrolled, an average of 47% were females with a mean age of 35 months (Table 1). Only 41% of the study children had literate mothers. Most of the study families disposed of their waste in the open and about 17% of families did not have clean drinking water. There was no difference among the four intervention groups in terms of age, gender, quality of drinking water, type of human waste management, illness, diarrhea, and fever in the child in the last 7 days, literacy of the mother, and illness in the mother in the last 7 days at the time of randomization. Only 12 children dropped out of the study, all due to migration out of the area of study.

Mean hemoglobin levels at baseline were similar in children in all four groups (Table 2). At the end of the study hemoglobin levels had increased in all three active intervention groups, while the hemoglobin level of children in the placebo group remained unchanged. The largest increase occurred in the combined iron plus vitamin A group (2.23 g/dl). The changes in the iron only and vitamin A only groups were 1.93 g/dl and 1.26 g/dl, respectively. Compared to the placebo group, these changes were found to be significant. In a model where iron, vitamin A and the interaction term (iron*vitamin A) were independent variables and change in hemoglobin (hemoglobin at 12 months minus hemoglobin at baseline) was the

dependent variable, there was a significant main effect of iron (p=0.001) and vitamin A (p=0.037) but there was no iron and vitamin A interaction effect.

At baseline, no difference in the weight of children was observed among the intervention groups (p=0.111) (Table 3). The mean of the changes in final and initial weights for the iron only (2.25 kg), vitamin A only (2.16 kg), combined iron and vitamin A (2.38 kg) and placebo (2.47 kg) groups were similar. No difference was observed in baseline length (p=0.960) of children in the four groups (Table 4). At the end of the study, there were mean increases in length of 8.7, 8.2, 8.9 and 8.2 cm in the iron, vitamin A, iron plus vitamin A, and placebo groups, respectively, but the changes were not significant (p=0.327). There was a crude difference of 0.5 cm (p=0.271) in length between the iron and the placebo groups. After adjusting for baseline length, this difference increased slightly to 0.6 cm (95% CI: -0.1,1.2). Similarly, the vitamin A group did not show any difference in length when compared with the placebo group (-0.1 cm) (95% CI: -0.3,0.1).

In the first three months of the intervention period there was a progressive reduction in the prevalence of diarrhea in the iron only and iron plus vitamin A groups, but these reductions were not significant (Table 5). Overall, lower rates were observed in five of the six months in the vitamin A group, but only the rates in the third and fifth month were significantly lower (2.5 times lower) compared to the rates in the placebo group for the same months.

A higher frequency of diarrhea was observed in the iron only and iron plus vitamin A groups as compared to the placebo group (Table 6). This difference in frequency was more marked during the iron supplementation period. When the study duration was categorized into supplementation and follow-up periods, there was a marked reduction in frequency of diarrhea, both in the iron only (2.1 versus 1.7 times/person/month) and in the iron plus vitamin A (2.1 versus 1.9 times/person/month) groups after the iron supplementation was stopped. The mean net reduction between supplementation and follow-up period was 0.4 and 0.2 times/person/month in the iron only and iron plus vitamin A groups, respectively. The vitamin A group had a persistently lower frequency of diarrhea than the other three intervention groups .

After a variable period of decline initially, the prevalence of ARI started to increase in all the intervention groups (Table 7). Prevalence was significantly higher in the iron only supplemented children for the 2nd, 3rd, 4th and 5th months compared to the placebo group. Higher rates were also observed in the vitamin A group with significant differences from the placebo group in the 2nd, 3rd and 4th months. The iron plus vitamin A group had lower or similar prevalence rates to the placebo group in all months of the study period, but none of these differences were significant. There was a significant decrease in rate of CRP positivity ($p=0.030$) in iron treated children compared to those who were not supplemented with iron, but no difference was observed between those who received vitamin A and those who did not ($p=0.810$) (table not shown).

Effect of iron supplementation on serum ferritin, zinc and copper statuses

The rates of children with values below the normal cut-off point for serum ferritin, zinc and copper were 25%, 15% and 0%, respectively (table not shown). At baseline there was no significant difference (p=0.432) in the serum ferritin levels between iron supplemented and placebo groups (Table 8). At the end of the study the iron-supplemented children had a mean increase in serum ferritin of 12.1 μg/L (p=0.001), whereas no significant change was observed in the placebo group (1.11 μg/L). The difference in mean serum ferritin levels between the iron and the placebo-treated children at 12 months was 9.38 μg/L (p=0.016) which was highly significant (p=0.016). Serum copper remained unchanged throughout the study. However, after iron supplementation there was an increase in serum zinc in both the iron and the control groups.

6.4.1 Discussion

Both iron and vitamin A supplementation significantly raised hemoglobin levels in high risk children. The increase that resulted from vitamin A supplementation was not as high as the rise due to iron supplementation. Combined iron and vitamin A supplementation resulted in the largest rise but no interaction effect was observed. The results of this study support the view that iron-deficient children benefit markedly by taking a single dose of vitamin A with their iron supplement. Our results support previous findings by Semba *et al*, which reported that supplementing with vitamin A alone increased the hematological indices in children and the combined effect of iron and vitamin A showed even better results (34). A study in Ethiopia (35), however, did not show any difference between anemic children treated with iron alone and anemic children treated with combined iron and vitamin A, perhaps due to a reasonable vitamin A status and absence of nutritional anemia in the study subjects. However, the study did not have a placebo group or a group supplemented with vitamin A only with which to compare the results. Our study was conducted in a different part of the country where both vitamin A and iron deficiency are public health concerns.

Iron supplementation failed to show any effect on weight. In children supplemented with iron gain in length was 0.6 cm (95%CI: -0.1,1.2) more than children not receiving iron. Even though the increase was not statistically significant,

a difference of 0.6 cm is judged to be clinically important. These findings concur with several other community intervention studies (5-9) that reported significant increases in length after iron supplementation. In both the intervention and the control groups the increases in length were most apparent during the first 6 months compared to the latter 6 months. A similar pattern of gain in length was observed in another community study in children of same age group in the same region (43). This might partly be due to the fact that in the age group 24 to 60 months there is a decline in the natural rate of increase in length with increase in age. Season might also have played a role in the difference in the rate of change in stature over time (44).

Single doses of 200,000 IU vitamin A have failed to show any increase in length or weight. Ramakrishnan *et al*, in their study in Tamil Nadu in India also reported a lack of effect of vitamin A supplementation on the growth of preschool children (45).

CRP positivity, an objective measure of infection, was lower in iron supplemented children compared to placebo-treated children. The results from maternal recalls of diarrhea and ARI, however, remain equivocal. There seemed to be a slight reduction in the prevalence of diarrhea in all the three active intervention groups. Reduction in rate of diarrhea in iron supplemented children has been reported by others (15-17). The frequency of diarrhea was also markedly lower in the vitamin A-supplemented children. Iron supplementation showed a reduction in the frequency of diarrhea during the follow-up period but not during the

supplementation period. The increase in diarrheal frequency during supplementation was probably due to the iron. It is well known that diarrhea is one of the commonest side effects of iron treatment (46). There was an increase in ARI in both the iron and vitamin A groups as compared to the placebo group. However, there was a lower rate of ARI in the combined iron and vitamin A supplementation group. Both iron and vitamin A-supplemented children had higher rates of ARI, but significantly lower rates were observed in those children who received the combination of iron and vitamin A.

The significant increase in the serum ferritin levels after iron supplementation confirms that the iron was correctly administered and that the supplementation was effective. Iron supplementation did not show any effect on the zinc and copper status of the study children, supporting previous findings by Yip *et al* (39). The increase in serum zinc in both iron and placebo groups might be due to the change in the mean age of the study children between the baseline and final serum collection at 12 months (41).

In summary, supplementing children with iron and vitamin A significantly improved hemoglobin status of children and the effects were enhanced when both were supplemented together. High rates of malnutrition (47) and zinc deficiency (48,49) may have limited the effect of iron and vitamin A on morbidity and growth. Future studies in iron supplementation with or without vitamin A, should reduce the

limiting effects of other deficiencies when examining the functional consequences of iron.

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Table 1. Distribution of potential confounders among intervention groups in 407 children, 2-5 years of age, in Tigray, Northern Ethiopia

Characteristics	Iron (n=100)	Vit. A (n=106)	Iron + Vit. A (n=99)	Placebo (n=102)	P-value
Risk factors					
Gender (%males)	56.0	60.6	49.0	50.0	0.42
Age (months) mean±SD	35.2±10.4	34.2±10.6	35.7±10.8	34.1±10.6	0.80
Families monthly income less than the poverty line	29.9	36.0	32.8	30.0	0.41
Families with adequate water (%)	84.1	91.6	88.9	80.7	0.08
Families with adequate waste disposal (%)	53.7	48.4	40.0	38.6	0.31
Illness in the last one week (%)	42.7	35.8	43.3	37.5	0.66
Reported diarrhea in the last one week (%)	23.0	18.7	22.0	26.1	0.46
Reported fever in the last one week	35.8	43.1	38.9	41.4	0.76
Child drank coffee at least once in the last 7 days (%)	33.0	33.1	31.2	34.6	0.83
Child consumed fenugreek at least once in the last 7 days(%)	24.6	16.6	28.9	22.8	0.28
Mother able to read (%)	48.8	40.0	35.0	40.9	0.33
Mother ill (%)	29.8	27.3	34.0	26.5	0.60

Table 2. Mean (± SD) of hemoglobin (g/dl) of children, 2-5 years of age, by intervention group at baseline and during follow-up* in Tigray, Northern Ethiopia

Period	Iron	Vit. A	Iron+Vit. A	Placebo	p-value**
	n=100	n=106	n=99	n=102	
Baseline	10.6±1.54 (n=99)	10.2±1.12 (n=106)	10.5±1.21 (n=99)	10.4±1.10 (n=101)	0.105
3-months	11.5±1.54 (n=99)	10.6±1.28 (n=103)	11.5±1.47 (n=97)	10.3±1.06 (n=100)	< 0.001
6-months	11.9±1.46 (n=85)	10.8±1.07 (n=92)	12.3±0.92 (n=86)	10.4±0.87 (n=93)	< 0.001
12-months	12.3±1.18 (n=87)	11.4±1.12 (n=98)	12.7±1.17 (n=94)	10.5±1.04 (n=93)	< 0.001
Change* **	1.93±1.66 (p< 0.001)	1.26±1.02 (p< 0.001)	2.23±1.22 (p< 0.001)	0.09±0.66	<0.001

* Number of study subjects differ due to missing specimens for analysis

** P-value from the ANOVA test

*** Means and SD of the changes in hemoglobin of each child between baseline and 12 months. P-values refer to comparisons made between changes in the specific intervention groups and the change in the placebo group.

Table 3. Mean (±SD) weight (kg) of children, 2-5 years of age, by intervention group at baseline and during follow-up* Tigray region, Northern Ethiopia

Period	Iron n=100	Vit. A n=106	Iron+Vit.A n=99	Placebo n=102	p-value**
Baseline	12.0±2.3 (n=100)	12.7±2.4 (n=106)	12.5±2.6 (n=99)	12.1±2.2 (n=102)	0.111
3-months	12.7±2.3 (n=99)	13.2±2.3 (n=104)	13.2±2.1 (n=98)	12.8±2.2 (n=101)	0.263
6-months	13.4±2.2 (n=85)	13.7±2.3 (n=94)	13.8±2.0 (n=86)	13.5±2.3 (n=92)	0.640
12-months	14.1±2.1 (n=87)	14.5±2.4 (n=94)	14.6±2.2 (n=92)	14.2±2.2 (n=93)	0.978
Change* **	2.25±0.7 (p=0.206)	2.16±1.0 (p=0.103)	2.38±0.9 (p=0.621)	2.47±1.4	0.205

* Number of study subjects differ due to missing children for analysis.

** P-value from the ANOVA test.

*** Means and SDs of changes in weight of each child between baseline and 12 months. P-values refer to comparisons made between changes in the specific intervention groups and the change in the placebo group.

Table 4. Mean (±SD) length (cm) of children, 2-5 years of age, by intervention group at baseline and during follow-up* in Tigray region, Northern Ethiopia

Period	Iron	Vit. A	Iron+ Vit.A	Placebo	p-value**
Baseline	88.9±8.8 (n=100)	90.9±9.0 (n=106)	89.5±8.5 (n=99)	89.4±8.8 (n=102)	0.960
3-months	92.4±8.7 (n=99)	94.4±9.0 (n=104)	93.7±8.0 (n=98)	92.8±8.5 (n=101)	0.670
6-months	95.7±8.7 (n=85)	97.4±8.7 (n=93)	95.8±7.4 (n=86)	95.3±8.3 (n=92)	0.349
12-months	97.3±8.3 (n=87)	98.0±13.0 (n=94)	98.0±7.7 (n=91)	97.3±8.2 (n=93)	0.923
Change***	8.68±2.39 (p=0.271)	8.21±2.57 (p=0.961)	8.90±3.94 (p=0.197)	8.23±3.02	0.327

* Number of study subjects differ due to missing children for analysis

** P-value from the ANOVA test

*** Means and SDs of the changes in length of each child between baseline and 12 months. P-values refer to comparisons made between changes in the specific intervention groups and the change in the placebo group.

Table 5. Number and percent of children, 2-5 years of age, with diarrhea by intervention group during supplementation and follow-up* in Tigray region, Northern Ethiopia

Period	Iron	Vit. A	Iron+Vit.A	Placebo
S - month-1	25 (24.8%)	17 (17.9%)	23 (22.5%)	24 (24.0%)
	1.1 (0.6-2.3)*	0.6 (0.2-1.2)*	1.0 (0.5-1.9)*	
S - month-2	12 (11.8%)	20 (20.8%)	12 (11.8%)	15 (15.3%)
	0.8 (0.4-1.8)	1.4 (0.7-2.9)	0.8 (0.4-1.8)	
S - month-3	11 (11.4%)	8 (8.4%)	10 (10.1%)	18 (18.2%)
	0.5 (0.2-1.2)	0.4 (0.2-0.9)#	0.5 (0.2-1.2)	
F - month-4	10 (9.4%)	12 (12.6%)	12 (11.5%)	16 (16.2%)
	0.6 (0.3-1.5)	0.7 (0.3-1.5)	0.7 (0.3-1.7)	
F - month-5	14 (13.2%)	8 (8.2%)	20 (19.2%)	18 (18.2%)
	0.8 (0.4-1.7)	0.4 (0.2-0.9)#	1.2 (0.6-2.5)	
F - month-6	20 (18.8%)	15 (15.8%)	17 (16.5%)	19 (18.8%)
	1.1 (0.6-2.3)	0.7 (0.3-1.5)	0.9 (0.4-1.8)	

S = supplementation period F = follow-up period
Number of study subjects differ due to missing children for analysis
* OR (95% Confidence Interval) comparing rate in each cell with the respective rate in the placebo group.
# Significantly lower in the intervention group compared to the placebo group.

Table 6. Diarrheal frequency (times/person/month) in children, 2-5 years of age, by intervention group in Tigray region, Northern Ethiopia

	Iron	Vit. A	Iron+vit A	Placebo
S -Total number	623	418	620	449
S - Times/person	2.1	1.3	2.1	1.5
F -Total number	518	472	570	654
F - Times/person	1.7	1.5	1.9	2.1
T -Total number	1141	890	1190	1103
T -Times/person	1.9	1.4	2.0	1.8

S = Iron supplementation period F = Follow-up period T = Total study period

Table 7. Number and percent of children, 2-5 years of age, with ARI by intervention group during supplementation and follow-up in Tigray region, Northern Ethiopia

Period	Iron	Vit. A	Iron+Vit.A	Placebo
S - month-1	29 (28.7%)	27 (28.6%)	22 (21.6%)	35 (35.0%)
	0.8 (0.4-1.4)*	0.6 (0.4-1.2)*	0.5 (0.3-1.0)*	
S - month-2	21 (20.6%)	21 (21.8%)	12 (11.8%)	12 (12.2%)
	2.1 (1.0-4.1)#	2.0 (1.0-4.1)#	1.1 (0.5-2.41)	
S - month-3	28 (26.9%)	24 (25.2%)	12 (11.9%)	12 (12.1%)
	3.1 (1.5-6.4)#	2.2 (1.1-4.6)#	1.0 (0.4-2.4)	
F - month-4	33 (31.0%)	37 (38.9%)	10 (9.6%)	16 (16.2%)
	2.9 (1.5-5.6)#	2.9 (1.5-5.6)#	0.6 (0.3-1.4)	
F - month-5	29 (27.3%)	25 (25.8%)	20 (19.2%)	19 (19.2%)
	1.9 (1.0-3.7)#	1.4 (0.7-2.7)	1.4 (0.6-2.3)	
F - month-6	40 (37.6%)	43 (45.1%)	32 (31.0%)	42 (41.6%)
	1.0 (0.6-1.78)	0.9 (0.5-1.6)	0.7 (0.4-1.2)	

S = supplementation period F = follow-up period
Number of study subjects differ due to missing children for analysis
** OR (95% Confidence Interval) comparing rates in each cell with the respective rate in the placebo group.
# Significantly higher in the intervention group compared to the placebo group.

Table 8. Baseline and 12 month serum ferritin (μg/L), zinc (mg/L) and copper (mg/L) by type of intervention groups in children, 2-5 years of age, in Tigray region, Northern Ethiopia

	Iron (mean±SD)	Placebo(mean±SD)	Difference (p-value)*
Baseline ferritin	n=60	n=75	
	18.27±11	19.87±12	-1.60 (0.432)
Ferritin at	n=60	n=63	
12 months	30.36±25	20.98±17	9.38 (0.016)
Diff. (p-value)**	12.1 (0.001)	1.11 (0.655)	
Baseline zinc	n=57	n=64	
	0.84±0.13	0.86±0.02	-0.02 (0.521)
Zinc at	n=41	n=60	
12 months	0.95±0.17	0.94±0.17	0.01 (0.772)
Diff. (p-value)**	0.12 (0.001)	0.14 (0.018)	
Baseline copper	n=58	n=64	
	0.94±0.18	0.95±0.17	-0.01 (0.755)
Copper at	n=60	n=59	
12 months	0.97±0.10	0.94±0.17	0.03 (0.242)
Diff. (p-value)**	0.03 (0.265)	-0.01 (0.745)	

* Difference and p-value between groups

** Difference and p-value between baseline and at 12 months.

Epilogue to manuscript C

Manuscript C showed that both iron supplementation and vitamin A supplementation significantly raised hemoglobin levels in preschool children, but the increase due to vitamin A was not as high as the rise that resulted from iron supplementation. A combined supplementation of both iron and vitamin A showed the highest increase in hemoglobin. Iron-treated children showed an average gain of 0.6 cm in length, but no gain in weight. Vitamin A supplementation did not result in increased weight or length. Based on the lower c-reactive protein positivity, a slight decrease in prevalence of diarrhea and a marked decrease in its frequency, it is evident that iron supplementation has a beneficial effect on diarrhea and general infection status. Vitamin A supplementation alone also showed a marked reduction in the frequency of diarrhea. Both iron and vitamin A-treated children showed higher rates of ARI, but those children who received the combined iron and vitamin A supplementation showed significantly lower rates. Iron supplementation did not have any effect on the zinc and copper status of the study children. From this study it is apparent that supplementing children with 30 mg of elemental iron resolves hemoglobin deficits, improves length and reduces general infection status. A single dose of 200,000 IU vitamin A did not result in any increase in length or in weight but improved frequency of diarrhea. A combined vitamin A and iron supplementation control strategy may provide the greatest benefit.

8.0 GENERAL DISCUSSION AND CONCLUSION

Several studies in Ethiopia have reported that iron deficiency was not a public health problem (Gebre-medhin *et al* 1976, Wolde-gebriel *et al* 1992). Based on the findings from these studies, the Ministry of Health gave less attention to iron deficiency compared to the other micronutrient deficiencies. Ethiopia is one of the few countries that does not have food fortification policy and does not have a strong iron deficiency control program in place (MOH/WHO 1987). This research was motivated by the need to determine the scope of iron deficiency anemia, and to understand its risk factors and to investigate effective control strategies. It is hoped that the findings will raise awareness among policy makers and bring about an effective control strategy in the country.

The first manuscript reported that the rate of anemia in children aged between 6 to 60 months in Northern Ethiopia was 42%. The high rate of the microcytic hypochromic blood picture (16.6%), the low rate of the macrocytic picture (1.6%), low MCV and MCH and a low mean serum ferritin level in the study children confirm that iron deficiency was the commonest type of anemia in the study area. This study also indicated that there was sufficient iron in the diet of the study children. However, the diet was known to be rich in iron absorption inhibitors such as fiber and tannins and very poor in nutrients that improve absorption of iron, such as ascorbic acid and meat. The problem, therefore, was not the amount of iron in the diet, but poor absorption

and utilization of the iron. As seen from the stool analysis, hookworm and *Trichuris* were not important causes of iron deficiency anemia in the study area. Risk factors that had a strong negative association with anemia were consumption of fenugreek and coffee (inhibitors of iron absorption), diarrhea and stunting in the child, poor quality of drinking water and inadequate human waste management, maternal illiteracy and mother being ill, family having food reserves and family earnings below the poverty line. Therefore, a sound iron deficiency control program in these and similarly affected communities should place less emphasis on iron supplementation and more emphasis on improving the efficiency of iron absorption and tackling the underlying causes of iron deficiency anemia. Such a control program should not neglect the importance of alleviating general poverty, promoting women's empowerment, improving personal and environmental hygiene and controlling macronutrient deficiencies.

The *in-vitro* study found that all the three types of foods cooked in the iron pots had more total and available iron compared to when cooked in non-iron pots. Meat and vegetables cooked in iron pots markedly improved the availability of iron released by the pots. The community intervention study found that the iron released into the food during cooking with iron pots was effective in increasing iron status of children and improving growth (length). Increasing the amount of iron in the food may have a place in the treatment of iron deficiency, but the importance of availability of the iron should not be overlooked. This was further supported by the fact that cooking vegetables and meat in iron pots improved the efficiency of absorption of the iron.

Over the active study period, for the duration of treatment and follow up, no sign of iron overload was observed. However, we recommend that the effect of longer term use of iron pots in communities be further investigated.

The third study evaluated the effect of including a single dose of vitamin A with iron supplementation on the iron status of preschool children. The study reported that both iron and vitamin A supplementation significantly raised hemoglobin levels in children. However, the increase that resulted from vitamin A supplementation was not as high as the increase due to iron supplementation. The combined supplemention of iron and vitamin A showed an even greater increase in hemoglobin level. Other researchers have also found similar results (Mejia and Chew 1977, Suharno *et al* 1993). From this study it is apparent that supplementing children with 30 mg of elemental iron resulted in a slight increase in length but not in weight. A single dose of 200,000 IU vitamin A did not show any increase in weight and length.

There seemed to be a slight reduction in the prevalence of diarrhea in all the three active intervention groups. However, the decrease was higher in the vitamin A group. Iron supplementation showed a reduction in the frequency of diarrhea during follow-up, but was higher during the treatment period. The increase in frequency during treatment was probably a result of the side effects of iron therapy. C-reactive protein also found that infection status was less in iron treated children. The frequency of diarrhea was also markedly lower in the vitamin A-treated children. There was a

marked increase in prevalence of ARI in both the iron and vitamin A groups as compared to the placebo group for the two months during the treatment period and for the first month of the follow-up period. However, the combined iron and vitamin A supplementation showed a marked reduction in ARI prevalence. The results of the study support the view that iron-deficient children markedly benefit by taking a single dose of vitamin A with their iron supplement.

This is the first study to report zinc status in Ethiopia. The high rate of zinc deficiency reported in this study calls for similar studies in other parts of the country. It is also recommended that the role of zinc as an important public health problem be addressed. In this study iron supplementation did not show any effect on the zinc and copper status of the study children. Therefore, lack of effect of iron on growth (weight) was not accounted for by the difference in zinc status which is known to have an effect on growth (Gibson 1994).

Both the iron pot and the combined iron and vitamin A supplementation studies reported increases in length, but not in weight. Controlled studies in Kenya and Indonesia (Chwang *et al* 1988, Latham *et al* 1990, Lawless *et al* 1994) reported increase in weight, but another study in Indonesia (Idjiradinata *et al* 1994) reported retarded growth in iron supplemented children. Compared to children in these studies, children in our study community might have a very low caloric intake (78% of their requirement) and high rate of other micronutrient deficiencies such as zinc (15%). High rates of malnutrition (Black *et al* 1984) and zinc deficiency (Lutter *et al* 1989,

Sazawal *et al* 1995) may account for lack of increase in weight observed in our study. In both the intervention and the control groups changes in length over the first six months of the study across the groups were almost three times the changes reported in the following six months. This might partly be due to the fact that in the age group of 24 to 60 months there is a decline in the rate of increase in length with an increase in age. Season might also play a role in the difference in the rate of change in stature (Marshall & Swan 1971).

The results in manuscript A might have been strengthened with more qualitative and quantitative information on inhibitors of iron absorption. In manuscript B assessing the type of iron (heme or non-heme) in the different types of foods cooked in the iron pots might have shed more information on the type of iron in the foods. In manuscripts B and C information on the caloric intake of all children in the iron pot and combined iron and vitamin A studies might have made stratifying the changes in weight and length by caloric intake possible, to rule out the notion that iron might affect growth only in a situation of an adequate caloric intake.

In summary, iron deficiency is common in Ethiopia and constitutes an important threat to the public's health. Therefore, the following recommendations should be considered in a holistic control strategy:

1. Emphasis should be placed on the efficiency of iron absorption.
2. Identify and minimize the influence of non-dietary risk factors.

3. Iron pots might play a role in the control of iron deficiency anemia.
4. Include vitamin A supplementation in the control of iron deficiency anemia.

Further studies to answer the following questions are highly recommended: A- What are the types of iron absorption inhibitors in different local foods and their role in iron deficiency anemia in high risk communities? B- To what degree and how would improving the non-dietary risk factors such as quality of drinking water, waste management and maternal literacy affect iron status especially of high risk children? C- Does long term use of iron pots lead to iron toxicity? D- What are the factors that affect compliance in the use of iron pots?

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APPENDIX 1

QUESTIONNAIRE AND FORMS

NUTRITION/HEALTH & HOUSEHOLD FOOD SECURITY SURVEY
SECTION 1. HOUSEHOLD LEVEL INFORMATION

FAMILY ID NO. _____ KEBELE_____
ADMINISTRATIVE REGION_____ HOUSE NO._____
DISTRICT_____ WEREDA _____
KFETEGNA_____ TYPE OF AGROECOLOGICAL ZONE___

101 RESIDENTS	102 RELATION TO HEAD	103 SEX 1=Mal 2=Fem	104 AGE (YRS)	105 ETHNIC 1=Tigrai 2=Other	106 RELIGION 1=Christian 2=Moslem 3=Other
1	HEAD				
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
	< 5 YRS		MONTHS		
1					
2					
3					

102: 1=FAMILY HEAD 2=HUSBAND\WIFE 3=CHILDREN 4=BROTHER\SISTER
5=STEP CHILDREN 6=FATHER\MOTHER 7=SERVANT 8=OTHERS

Are there other persons still alive not listed?(ENTER IN TABLE)

Are there any infants that showed any sign of life
or who cried but only survived a few hours or days?

Total neonatal deaths (< 24 hours of birth) 107._____

Are there any other children who are no longer
alive that died before their fifth birthday?
Number of total deaths (24 hrs to 5 years) 108._____

Head of household (1 = Male 2 = Female) 102:_____

Age of mother (years) 103:_____

Ethnicity of mother (1=Tigrai 2 = Other) 105:_____

Religion of mother (see code above) 106:_____

Number of household members 110:_____

Number of live children 5 years or younger 111:_____

Urban/Rural (Urban=1 Semi-urban=2 Rural=3) 112:_____
1=Mekele (urban) 2=Mekele (semiurban) 3=Rural

SECTION 2. ACCESS TO HEALTH SERVICES/WATER & SANITATION

201. What is the major source of drinking water for members of your household? 201:_____

SPRING PROTECTED	1	ROOF CATCHMENT	5
SPRING UNPROTECTED	2	RIVER	6
WELL PROTECTED	3	PIPE	7
WELL UNPROTECTED	4	OTHER _____	8

202. Do you use this source for all your needs? 202:_____
NO=0 YES=1

203. Who in the household in most of the time fetches water? 203:_____

MALE HEAD OF HOUSEHOLD	1
FEMALE HEAD OF HOUSEHOLD	2
ELDEST CHILD (MALE)	3
ELDEST CHILD (FEMALE)	4
OTHER	5

204. What are the names of the containers you usually use to collect water for the and how many times each was filled. 204:_____

Type of container	Number of times filled
_____	_____ x ___litres
_____	_____ x ___litres
_____	_____ x ___litres
_____	_____ x ___litres
_____	_____ x ___litres

205. How long does it take from the water source to your house in dry season?
_____ minutes 205:_____

206. How long does it take from your house to the water source in the rainy season?
_____ minutes 206:_____

207. Where do you dispose of
A. the younger children's feces? 207A:_____

B. others 207B:_____

FIELD OUTSIDE	1
BUCKET/CONTAINER	2
PIT LATRINE	3
IMPROVED FACILITY (FLUSH)	4

208. Do you have, right now, a cake of soap on the premises? 208:_____
NO=0 YES=1

209. What is the nearest health service infrastructure? 209:_____

HOSPITAL	1
HEALTH CENTER	2
MCH. CLINIC	3

210. How long (one way) is it to the nearest health service infrastructure? 210:_____

Less than 10 minutes	1
10 to 30 minutes	2
30 to 60 minutes	3
More than 60 minutes	4

211. How often did you visit the health infrasructure in the last one month? 211:_____
NUMBER OF VISITS_____

SECTION #3: MORBIDITY (CHILDREN UNDER FIVE YEARS ONLY)
CLINICAL SIGNS OF MALNUTRITION/MORBIDITY

Once again can you give me the name, sex and age (in months) of your children under 5 years of age

CHILD 1

Name of Youngest Living Child (YLC)_____

301. SEX (1=MALE 2=FEMALE) 301A:_____

302. AGE IN MONTHS 302A:_____

303 HEIGHT(CMS) #1:___/___/___.___ 303A:___/___/___.___
#2:___/___/___.___

304. WEIGHT (KGS) #1:___/___.___ 304A:___/___.___
#2:___/___.___

CHILD 2

Name of Second Youngest Living Child (SYLC)_____

301. SEX (1=MALE 2=FEMALE) 301B:_____

302. AGE IN MONTHS 302B:_____

303 HEIGHT(CMS) #1:___/___/___.___ 303B:___/___/___.___
#2:___/___/___.___

304. WEIGHT (KGS) #1:___/___.___ 304B:___/___.___
#2:___/___.___

CHILD 3

Name of Third Youngest Living Child (TYLC)_____

301. SEX (1=MALE 2=FEMALE) 301C:_____

302. AGE IN MONTHS 302C:_____

303 HEIGHT(CMS) #1:___/___/___.___ 303C:___/___/___.___
#2:___/___/___.___

304. WEIGHT (KGS) #1:___/___.___ 304C:___/___.___
#2:___/___.___

CHILD 1 CHILD 2 CHILD 3

305. In general, would you say 305A____ 305B____ 305C____
your child's health is?
EXCELLENT=1 FAIR=3 DON'T KNOW=4
GOOD=2 POOR=4

306: How do you think your child 306A____ 306B____ 306C____
is growing?
GOOD=1; AVERAGE =2; BAD =3

307. How is the appetite of 307A____ 307B____ 308C____
your child?
EATS ALL OFFERED =1 WHEN FORCED =3
DO NOT EAT MUCH =2 DON'T KNOW =4

308. Is the child presently 308A____ 308B____ 308C____
breastfeeding
NO=0 YES=1 DO NOT KNOW=2

309. If NO, at what age did 309A____ 309B____ 309C____
the child stop breastfeeding
AGE IN MONTHS _____

310. When were the following foods
introduced? (ANSWER IN MONTH OF LIFE)

(A) WATER	310AA____310BA____310CA____
(B) MILK	310AB____310BB____310CB____
(C) FENEGREEK	310AC____310BC____310CC____
(D) SOFT FOOD	310AD____310BD____310CD____
(E) ADULT FOOD	310AE____310BE____310CE____
(F) OTHER	310AF____310BF____310CF____
(G) NOTHING INTRODUCED	310AG____310BG____310CG____

311. Did the child have any 311A____ 311B____ 311C____
illness in the last 7 days?
NO=0 YES=1 DO NOT KNOW=2

If the answer is YES ask the following:
Has the child had _____ in the last 7 days?

312. Fever?	312A____	312B____	312C____
313. Shivering?	313A____	313B____	313C____
314. Night sweating?	314A____	314B____	314C____
315. Cough	315A____	315B____	315C____
316. Bloody spuptum?	316A____	316B____	316C____
317. Diarrhea?	317A____	317B____	317C____
318. Bloody diarrhea?	318A____	318B____	318C____
319. Watery diarrhea?	319A____	319B____	319C____
320. Number of liquid stools?	320A____	320B____	320C____
321. Observed any worms?	321A____	321B____	321C____
A. Ascaris	321AA____	321BA____	321CA____
B. Tapeworm	321AB____	321BB____	321CB____
C. Pinworm?	321AC____	321BC____	321CC____
D. Other?	321AD____	21BD____	321CD____
322. General weakness	322A____	322B____	322C____

SECTION 4: CARING CAPACITY OF FEMALE CARE GIVER

401. What is your marital status? 401:_____

NEVER MARRIED	0	DIVORCED	3
MARRIED	1	NOT NOW LIVING TOGETHER	4
WIDOWED	2	PARTNER	5

402. Have you had an illness in the past 2 weeks? 402:_____
NO=0 YES=1

403. Are you presently pregnant? 403:_____
NO=0 YES=1

404. How many other times have you been pregnant? # of other pregnancies _____ 404:_____

405. Can you read this? 405:_____
(IMMUNIZATION IS THE RIGHT OF THE CHILD
Did not read = 0 Read = 1

406. Are you presently breast-feeding? 406:_____
NO = 0 YES = 1

407. Where did you deliver the last child? 407:_____

HOME	1	HEALTH CENTER	3
HOSPITAL	2	OTHER	4

408. Who attended the delivery? 408:_____

RELATIVE (UNTRAINED)	1
TRADITIONAL BIRTH ATTENDANT(UT)	2
TRADITIONAL BIRTH ATTENDANT(T)	3
HEALTH PROFESSIONAL	4
OTHER _____	5

409. Most of the time who prepares food for your child 409:_____

FEMALE HEAD OF HOUSEHOLD	1	OTHER =3	
OLDER CHILD (FEMALE)	2	_____	

410. Most of the time who feeds the children? 410:_____

FEMALE HEAD OF HOUSEHOLD	1	OTHER =3	
OLDER CHILD (FEMALE)	2	_____	

411. How many training sessions for health and nutrition have you attended? 411:_____

NONE	=0	FOUR-SIX	=2
ONE-THREE	=1	MORE THAN SIX	=3

412. Do you belong to any associations for women (social, health/nutrition, or political) 412:_____

NO=0 YES=1

If YES to 412 list three important activities you engage in.
See attached coding sheet
_____
_____
_____

413. How many household members live or work elsewhere and assume responsibility for you and the children? 413:_____

NONE	= 0	TWO	= 2
0NE	= 1	3 OR MORE	= 3

414. What type of fuel do you use for cooking? 414:_____

WOOD	1	ELECTRICITY	4
ANIMAL DUNG	2	OTHER _____	5
CHARCOAL	3		

415. (IF WOOD) Who is responsible for for collecting the wood? 415:_____

COLLECT SELF	1	BUY IT	=3
OTHERS COLLECT IT	2	DO NOT USE WOOD	=9

416. (IF MOTHER COLLECTS WOOD) How often do you collect wood weekly? 416:_____

ONCE	1	FOUR	4
TWICE	2	FIVE OR MORE	5
THREE	3		

417. Did you go to the market for food last week? NO=0 YES=1 417:_____

418. (IF YES) How many times did you go to the market? 418:_____

DIDN'T GO	= 0	TWICE = 2	FOUR = 4
ONCE	= 1	THREE = 3	>FOUR = 5

419. How long is the nearest market from your house? _____ minutes 419:_____

420. How do you travel to the market? 420:_____

ON FOOT	=1	MOTORIZED VEHICLE	=4
ON MULE	=2	CART/HORSE	=5
ON HORSE	=3	OTHERS	=6

421. Are you involved in the following work for? (NO=0 YES=1)

	FOOD	KIND	MONEY
(A) AGRICULTURE	421AF____	421AK____	421AM____
(B) SMALL ANIMAL HUSB	421BF____	421BK____	421BM____

(C) WAGE LABOR	421CF____	421CK____	421CM____
(D) COMMERCE	421DF____	421DK____	421DM____
(E) HANDICRAFTS/SEWING	421EF____	421EK____	421EM____
(F) BREWING	421FF____	421FK____	421FM____
(G) POTTERY	421GF____	421GK____	421GM____
(H) CHILDCARE	421HF____	421HK____	421HM____
(I) PREPARING FOOD	421IF____	421IK____	421IM____
(J) OTHER_____	421JF____	421JK____	421JM____

422. What is your involvement in agriculture? (NO=0 YES=1)

(A) CULTIVATION	_____	422A:_____
(B) PLANTING	_____	422B:_____
(C) WEEDING	_____	422C:_____
(D) HARVESTING	_____	422D:_____
(E) OTHER	_____ _____	422E:_____

SECTION 5: PHYSICAL ACCESS TO FOOD

501. I would like to ask you about the foods that you normally feed your children whoo are at least 6 months of age. For each food please tell me how often the family eats the food and the proportion size when eaten

FOOD GROUP	FOOD ITEM	FREQUENCY OF CONSUMPTION	PROPORTION SIZE
1=every meal 3=2-6/week 5=on occasion 2=once a day 4=once/week 6=seasonally 7=never			1 = Small 2 = Medium 3 = Large
MEAT STEWS	BEEF	501A:	501B:
	CHICKEN	502A:	502B:
	FISH	503A:	503B:
	EGGS	504A:	504B:
	SHERO/KEK	505A:	505B:
	OTHER_____	506A:	506B:
CEREAL	INJERA	507A:	507B:
	BREAD	508A:	508B:
	RICE	509A:	509B:
	PASTA	510A:	510B:
	ROASTED BARLEY	511A:	511B:
	OTHER_____	512A:	512B:
MILK	BREAST MILK	513A:	513B:
	COW MILK	514A:	514B:
	GOAT MILK	515A:	515B:
	OTHER_____	516A:	516B:
VEGETABLE	POTATO	517A:	517B:
	CARROT	518A:	518B:
	ONION	519A:	519B:
	TOMATO	520A:	520B:
	OTHER	521A:	521B:
	CABBAGE	522A:	522B:
	KALE	523A:	523B:
	CAULIFLOWER	524A:	524B:
	OTHER_____	525A:	525B:
FRUIT	BANANA	526A:	526B:
	ORANGE	527A:	527B:
	LEMON	528A:	528B:
	CACTUS	529A:	529B:
	MANGO	530A:	530B:
	GUAVA	531A:	531B:

	OTHER_____	532A:	532B:
DRINKS	COFFEE	533A:	533B:
	TEA	534A:	534B:
	TELLA	535A:	535B:
	FENEGREEK	536A:	536B:
	OTHER_____	537A:	537B:

502. How do you think consumption of these foods will change in the next year? 502:_____
LESS = 1 MORE = 3
SAME = 2 DON'T KNOW = 4

503. If more food could be available in the market what foods would you like to have more of (IDENTIFY 3)?

CEREALS	= 1	VEGETABLES	= 5	503A:_____
MEAT	= 2	FRUITS	= 6	503B:_____
LEGUMES	= 3	OTHER	= 7	503C:_____
MILK	= 4	_____		

504. Are you receiving food assistance at present? 504:_____
NO=0 YES=1

505. If YES, how often did this household obtain food relief in the last six months? 505:_____
_____TIMES

506. (IF YES) How was food relief obtained? 506:_____
FOOD AID 1 OTHER 3
FOOD-FOR-WORK 2 _____

507.(IF FOOD RELIEF WITHIN THE LAST 6 MONTHS) 507:_____
How much cereal, oil, sugar, powdered milk, lentils did you receive the last time you obtained food relief?

	QUANTITY
(A) CEREAL (KILO)	_____
(B) OIL (LITER)	_____
(C) SUGAR (KILO)	_____
(D) POWDERED MILK (KG)	_____
(E) FAFA (KILO)	_____
(F) OTHERS	_____

508. Which one has been exchanged for another type of food item, non-food item or cash?
NO=0 /YES=1

A. CEREAL	____ ____ ____	508A1___508A2___508A3___
B. OIL	____ ____ ____	508B1___508B2___508B3___
C. FAFA	____ ____ ____	508C1___508C2___508C3___
D. OTHERS	____ ____ ____	508D1___508D2___508D3___

509. What was obtained in exchange of the above mentioned item? NO = 0 YES = 1

A. SPICES	_____	509A:_____
B. BUTTER	_____	509B:_____
B. MEAT	_____	509C:_____
D. VEGETABLE	_____	509D:_____
E. FRUIT	_____	509E:_____
F. WOOD	_____	509F:_____
G. OTHER	_____	509G:_____

510. Do you have some reserve food at present? NO=0 YES=1 — 510:_____

511. If yes, how much do you have? QUANTITY _____Kg — 511._____

512. How often do you rely on the following to obtain food? NEVER=0 ALWAYS=1 WEEKLY=2 MONTHLY=3

A OWN AGRICULTURAL PRODUCTION	_____	512A:_____
B PASTORAL ACTIVITIES	_____	512B:_____
C SMALL ANIMAL HUSBANDRY/CHICKENS	_____	512C:_____
D PURCHASED AT MARKET	_____	512D:_____
E BARTER FOR FOOD	_____	512E:_____
F COMMERCE/PETTY TRADE	_____	512F:_____
G GIFT FROM FRIENDS	_____	512G:_____
H GIFT FROM FAMILY	_____	512H:_____
I FOOD RELIEF	_____	512I:_____
J FOOD FOR WORK	_____	512J:_____
K GATHERED WILD FOOD	_____	512K:_____
L OTHER _____	_____	512L:_____

513. What are the important crops which you cultivate and how much did you produce last year?

	QUANTITY (BAGS)	
(A) SORGHUM	_____	513A:_____
(B) MILLET	_____	513B:_____
(C) BARLEY	_____	513C:_____
(D) TEFF	_____	513D:_____
(E) MAIZE	_____	513E:_____
(F) WHEAT	_____	513F:_____
(G) LENTILS	_____	513G:_____
(H) OIL SEEDS	_____	513H:_____
(I) BEANS	_____	513I:_____
(J) PEAS	_____	513J:_____
(K) CHICKPEAS	_____	513K:_____
(L) VETCH(GUAYYA)	_____	513L:_____
(M) LINSEED	_____	513M:_____

514. Which of the crops did you...? EXCHANGE=1 SELL=3 TRADE=2 CONSUME =4

(A) SORGHUM	_____	514A:_____
(B) MILLET	_____	514B:_____
(C) BARLEY	_____	514C:_____
(D) TEFF	_____	514D:_____

(E) MAIZE	_____	514E:_____
(F) WHEAT	_____	514F:_____
(G) LENTILS	_____	514G:_____
(H) OIL SEEDS	_____	513H:_____
(I) BEANS	_____	514I:_____
(J) PEAS	_____	514J:_____
(K) CHICKPEAS	_____	514K:_____
(L) VETCH (GUAYYA)	_____	514L:_____
(M) LINSEED	_____	514M:_____

515. What vegetables did you grow this past year?

	QUANTITY (BUSHEL)	
(A) NONE	_____	515A:_____
(B) CABBAGE/LETTUCE	_____	515B:_____
(C) SPINACH	_____	515C:_____
(D) CAULIFLOWER	_____	515D:_____
(E) CARROT	_____	515E:_____
(F) BEETROOT	_____	515F:_____
(G) TOMATO	_____	515G:_____
(H) POTATO	_____	515H:_____
(I) ONIONS	_____	515I:_____
(J) ADRI	_____	515J:_____
(K) PUMPKIN	_____	515K:_____
(L) KALE	_____	515L:_____

SECTION 6. ECONOMIC ACCESS TO FOOD

601. (IF WOMAN IS THE HEAD OF THE HOUSEHOLD) Is there a man or any household member who assumes financial responsibility for you and the children? NO=0 YES=1 — 601:_____

602.(IF MAN IS THE HEAD OF THE HOUSEHOLD Does the head of household belong to any associations for men (social, health, farmers, political)? NO = 0 YES = 1 — 602:_____

If yes, what kind of association?

	RECEIVED FIN. ASSt.	OFFERED FIN. ASSt.		
A.SOCIAL	_____	_____	602AR:____	602BO:____
B.FARMERS	_____	_____	602BR:____	602BO:____
C.OTHERS	_____	_____	602CR:____	602BO:____

(SPECIFY: _____)

603. How would you describe your household's ...

(ASK FOR EACH)

CODING
1= MORE ADEQUATE
2= ADEQUATE
3= LESS ADEQUATE

A. HOUSING	____	603A:_____
B. EDUCATION	____	603B:_____
C. HEALTH	____	603C:_____
D. FOOD CONSUMPTION	____	603D:_____
E. EMPLOYMENT	____	603E:_____
F. SOCIAL STATUS	____	603F:_____

604. How would you describe your neighbor's ...
(ASK FOR EACH)

A. HOUSING	____	CODING	604A:_____
B. EDUCATION	____	1= MORE ADEQUATE	604B:_____
C. HEALTH	____	2= ADEQUATE	604C:_____
D. FOOD CONSUMPTION	____	3= LESS ADEQUATE	604D:_____
E. EMPLOYMENT	____		604E:_____
F. SOCIAL STATUS	____		604F:_____

605. In your opinion how much should one earn annually to be classified as ...? 605:_____
(SPECIFY THE APPROXIMATE AMOUNT OF MONEY)

UP TO_____BIRR VERY GOOD
UP TO_____BIRR GOOD
UP TO_____BIRR INSUFFICIENT
UP TO_____BIRR BAD
UP TO_____BIRR VERY BAD

606. In which category do you put yourself? 606:_____

1 VERY GOOD 3 INSUFFICIENT
2 GOOD 4 BAD
5 VERY BAD

607. If your income were to *increase* by a small abount how would you spend the money?
(SPECIFY THE FIRST THREE THINGS)

FOOD	= 1	PAY DEBT	= 6	607A:_____
CLOTHING	= 2	TAXES	= 7	607B:_____
HOUSING	= 3	SAVINGS	= 8	607C:_____
HEALTH	= 4	BUSINESS/FARM	= 9	
EDUCATION	= 5	DON'T KNOW	= 10	

608. If your income were to *decrease* by a small amount how would you reduce your spending?

FOOD	= 1	PAY DEBT	= 6	608A:_____
CLOTHING	= 2	TAXES	= 7	608B:_____
HOUSING	= 3	SAVINGS	= 8	608C:_____
HEALTH	= 4	BUSINESS/FARM	= 9	
EDUCATION	= 5	DON'T KNOW	= 10	

609. If you are faced with a food crisis, how will your family try to maintain normal consumption? (IDENTIFY THE FIRST THREE THINGS THAT WILL BE DONE)

USE FOOD STOCKS	1	609A:_____
PURCHASE LOWER COST FOODS	2	609B:_____
BORROW FOOD/INCOME	3	609C:_____
SEEK ADDITIONAL EMPLOYMENT	4	
SELL HOUSEHOLD ASSETS	5	
OBTAIN GOVERNMENT ASSISTANCE	6	
REDUCE FOOD COONSUMPTION	7	
SELL LAND	8	
SELL HOUSE	9	
MIGRATE OUT OF AREA	10	
OTHER _____	11	

610. Are you confident that these steps will work? NO = 0 YES = 1 — 610:_____

611. By what means did you get food for the household this past month? (THREE MOST IMPORTANT) — 611A:_____ 611B:_____ 611C:_____

OWN AGRICULTURAL PRODUCTION	01
PASTORAL ACTIVITIES	02
SMALL ANIMAL HUSBANDRY/CHICKENS	03
PURCHASED AT MARKET	04
BARTER FOR FOOD	05
COMMERCE/PETTY TRADE	06
GIFT FROM FRIENDS	07
GIFT FROM FAMILY	08
FOOD RELIEF	09
FOOD FOR WORK	10
GATHERED WILD FOOD	11
OTHER _____	99

612. Of the food that you bought, what is your expenditure from your monthly income? — 612:_____

MORE THAN THAT 1 HALF OF IT 3
ALL OF IT 2 QUARTER OF IT 4

613. How do you expect your household income to be this year compared to last year? — 613:_____

LESS = 1 MORE = 3
SAME = 2 DONO'T KNOW = 4

THE FOLLOWING QUESTIONS PERTAIN TO THOSE WHO HAVE LAND AND USE IT FOR AGRICULTURAL PRODUCTION OR HAVE ANIMALS

620. Have you farming land &/or animals? — 620:_____

NO =0 (GO TO QUESTION # xxx)
YES =1 (GO TO NEXT QUESTION)

621. What is the number of fields (plots) for each type of tenure?

	NUMBER OF FIELDS	
A OWN	_____	621A:_____
B RENT	_____	621B:_____
C SHARECROP	_____	621C:_____
D COOPERATIVE	_____	621D:_____

622. How many persons in the household cultivated planted, weeded and harvested the fields this past year? TOTAL # _____ — 622:_____

623. How many persons were hired outside the the household to work in the family fields this past year? TOTAL # _____ — 623:_____

624. How did you plough your fields last year? — 624:_____

ANIMAL LABOR 1 ANIMAL/HUMAN LABOR 3
HUMAN LABOR 2 OTHERS _____ 4

625. What access do you have to farming animals and equipment?

NONE=0 OWN=1 RENT=2 BORROW=3 WORK=4 EXCHANGE=5

(A) CAMELS	_____	625A:_____
(B) OXEN	_____	625B:_____
(C) HORSES	_____	625C:_____
(D) DONKEYS/MULES	_____	625D:_____
(E) TRACTOR	_____	625E:_____
(F) PLOUGH	_____	625F:_____
(G) PLANTER	_____	625G:_____
(H) HOE\SHOVEL	_____	625H:_____
(I) CART	_____	625I:_____

626. What type of animals and how many of each type of animal are owned and maintained by this household?

TYPE OF ANIMAL	QUANTITY	
(A) CATTLE	_____	626A:_____
(B) SHEEP	_____	626B:_____
(C) GOATS	_____	626C:_____
(D) CAMELS	_____	626D:_____
(E) OXEN	_____	626E:_____
(F) HORSES	_____	626F:_____
(G) DONKEYS	_____	626G:_____
(H) MULES	_____	626H:_____
(I) POULTRY	_____	626I:_____
(J) OTHERS	_____	626J:_____

627. Last year did you exchange or sell any of the following to acquire more food for household members? NO =0 YES =1

(A) HOME	_____	627A:_____
(B) LAND	_____	627B:_____
(C) ANIMALS PREMATURELY	_____	627C:_____
(D) FARM EQUIPMENT	_____	627D:_____
(E) SEWING MACHINE	_____	627E:_____
(F) HOUSEHOLD GOODS	_____	627F:_____
(G) PERSONAL ASSETS/JEWELRY	_____	627G:_____
(H) FUEL WOOD	_____	627H:_____
(I) CHARCOAL	_____	627I:_____

THESE QUESTIONS SHOULD BE ASKED OF ALL HOUSEHOLDS

628. Did someone in the household work for wages on someone else's land at any time last year? 628:_____

NEVER	0	PART TIME	2
FULL TIME	1	SEASONALLY	3

629. (IF FARM WAGE WORK) How was the money earned by working on someone else's land used by the household? (THREE MOST IMPORTANT) 629A:_____ 629B:_____ 629C:_____

PURCHASE FOOD	1
PURCHASE ANIMALS	2
PURCHASE FARM EQUIPMENT	3
PURCHASE LAND	4
HOUSE CONSTRUCTION	5
EDUCATION FEES	6
CEREMONIAL FEES	7
PURCHASE HOUSEHOLD GOODS	8
PURCHASE CLOTHING	9

630. Does a family member work in another part of this country or in another country and send money to the household regularly? NO=0 YES=1 — 630:_____

631. (IF YES) How was the money used? (THREE MOST IMPORTANT) — 631A:_____ 631B:_____ 631C:_____

PURCHASE FOOD	1
PURCHASE ANIMALS	2
PURCHASE FARM EQUIPMENT	3
PURCHASE LAND	4
HOUSE CONSTRUCTION	5
EDUCATION FEES	6
CEREMONIAL FEES	7
PURCHASE HOUSEHOLD GOODS	8
PURCHASE CLOTHING	9
HOUSE RENT	10
OTHERS	11

632. Do you own this residence? NO=0 YES=1 — 632:_____

633. Does any member of your household own any of the following? NO=0 YES=1

(A) SEWING MACHINE	_____	633A:_____
(B) BICYCLE	_____	633B:_____
(C) MOTORCYCLE	_____	633C:_____
(D) CAR	_____	633D:_____
(E) REFRIGERATOR	_____	633E:_____
(F) CLOCK	_____	633F:_____

634. Did any member of this family migrate to another region or country to look for work this year? NO=0 YES=1 — 634:_____

635. Does the family plan on moving to another region or country in the next few months? NO=0 YES=1 — 635:_____

636. (IF YES) Why is the household moving? NO=0 YES=1

A.LAND SHORTAGE	____	636A:_____
B.HUMAN LABOR SHORTAGE	____	636B:_____
C.DRAUGHT ANIMAL SHORTAGE	____	636C:_____
D.INSUFFICIENT RAINFALL	____	636D:_____
E.INSUFFICIENT SEED	____	636E:_____
F.INSUFFICIENT FERTILIZER	____	636F:_____
G.INSUFFICIENT EQUIPMENT	____	636G:_____
H.PESTS	____	636H:_____
I.POOR CONDITION OF LAND	____	636I:_____
J.OTHERS	____	636J:_____

SECTION 7: HOUSEHOLD OBSERVATION SHEET

701. Number of rooms in this dwelling 701:_____

ONE 1	FOUR 4
TWO 2	FIVE+ 5
THREE 3	

702. Is there a separate kitchen? 702:_____

NO=0 YES=1

703. Where are the cattle kept? 703:_____

SAME DWELLING = 1	IN THE BARN = 3
OUTSIDE FIELD = 3	IN THE CAMPS = 4
	NOT APPLICABLE = 9

704. Main material of the floor ? 704:_____

FINISHED (WOOD/TILES)	1
CEMENT	2
EARTH/SAND	3

705. Main material used for wall construction 705:_____

MUD/WOOD	1
BRICK/STONE	2

706. Main material used for roof 706:_____

THATCH \GRASSES	1	WOODS	4
TIN	2	OTHERS	5
TILE	3		

707. Commodities in the dwelling 707:_____

RADIO	1	SOFA	4
TABLE/CHAIRS	2	WORKING WATCH	5
BED	3	OTHERS	6

708. Toilet facilities 708:_____

FIELD OUTSIDE	1
BUCKET/CONTAINER	2
PIT LATRINE	3
IMPROVED LATRINE (FLUSH)	4

709. What kind of light do use for your house? 709:_____

ELECTRICITY	1	CANDLE	3
DIESEL	2	OTHER	4

710. What kind of grain store do you have in your house? 710:_____

GRAIN PIT	1
GRAIN POT	2
OTHERS	3

OBSERVATION SHEET #3 (CHILDREN UNDER FIVE YEARS ONLY)
CLINICAL SIGNS OF MALNUTRITION/MORBIDITY

PART 6: Once again can you give me the name, sex and age (in months) of the YOUNGEST LIVING CHILD (YLC)?

Name of Youngest Living Child (YLC) _____
SEX _____
AGE IN MONTHS _____
HEIGHT(CMS) _____
WEIGHT(KGS) _____

---

601. In general, would you say your child's health is 601._____
EXCELLENT=1 GOOD=2 FAIR=3
POOR=4 DON NOT KNOW=4

602.Did your youngest living child (YLC) have any illness in the last 7 days? 602._____
NO=0 YES=1 DO NOT KNOW=2

603.If 'YES' what do you think is the cause of the illness? 603._____
_____
_____

604. If yes, did the youngest living child (YLC) have fever? 604._____
NO=0 YES=1 DO NOT KNOW=2

605. Did the youngest living child (YLC) have shivering? 605._____
NO=0 YES=1 DO NOT KNOW=2

606. Did the youngest living child (YLC) have night sweating? 606._____
NO=0 YES=1 DO NOT KNOW=2

607. Did the youngest living child (YLC) have cough? 607._____
NO=0 YES=1 DO NOT KNOW=2

608. If YES, was the sputum productive? 608._____
NO=0 YES=1 DO NOT KNOW=2

609. Did the sputum have blood? 609._____
NO=0 YES=1 DO NOT KNOW=2

610. Did your youngest living child (YLC) have diarrhea in the last 24 hours? 610._____
NO=0 YES=1 DO NOT KNOW=2

611. If yes, how many times did the child pass stool in the last 24 hours? 611._____
NO=0 YES=1 DO NOT KNOW=2

612.If yes, did the stool have blood? 612._____
NO=0 YES=1 DO NOT KNOW=2

613. If yes, what was the consistency of the stool? 613._____
WATERY=1 WELL FORMED=2
OTHERS=3 SPECIFY_____

614. Did you notice any worms with the stool? 614._____
NO=0 YES=1 DO NOT KNOW=2

615. If yes, what kind of worms did you notice? 615._____
TAPEWORMS =1
ASCARIASIS =2
OTHERS _____ =3

616.Did your child cry when urinating? 616._____
NO=0 YES=1 DO NOT KNOW=2

617.If yes, was the color of the urine red? 617._____
NO=0 YES=1 DO NOT KNOW=2

618. Did your youngest living child (YLC) have rashes? 618._____
NO=0 YES=1 DO NOT KNOW=2

519. Did your youngest living child (YLC) have itchy skin? 619._____
NO=0 YES=1 DO NOT KNOW=2

620. Did your youngest living child (YLC) have convulsions? 620._____
NO=0 YES=1 DO NOT KNOW=2

621. Did your youngest living child (YLC) have shortness of breath? 621._____
NO=0 YES=1 DO NOT KNOW=2

622. Did your youngest living child (YLC) have general weakness? 622._____
NO=0 YES=1 DO NOT KNOW=2

623. Did your youngest living child (YLC) have ear ache? 623._____
NO=0 YES=1 DO NOT KNOW=2

624. Did your youngest living child (YLC) have ear discharge? 624._____
NO=0 YES=1 DO NOT KNOW=2

625. Did your youngest living child (YLC) have any injury? 625._____
NO=0 YES=1 DO NOT KNOW=2

626.Did your youngest living child (YLC) have visible heart beating? 626._____

NO=0 YES=1 DO NOT KNOW=2

627. Did your youngest living child (YLC) have nose bleeding? 627._____
NO=0 YES=1 DO NOT KNOW=2

628. Did your youngest living child (YLC) have any trouble with his eyes? 628._____
NO=0 YES=1 DO NOT KNOW=2

629. Did your youngest living child (YLC) have eye discharge? 629._____
NO=0 YES=1 DO NOT KNOW=2

630. What did you do for the illness? 630._____
GAVE LOCAL DRUG MEDICINE MYSELF =1
CONSULTED LOCAL HEALER =2
CONSULTED MEDICAL PERSONNEL =3
CONSULTED LOCAL PHARMACIST =4
DID NOTHING =5
OTHERS (SPECIFY) =6 _____

631. Did your child undergo uvula cutting? 631._____
NO=0 YES=1 DO NOT KNOW=2

632.Did your child milk teeth were taken out or burned ? 632._____

114. Has immunization been provided? 120A._____
N0=0 YES=1 B._____
A. INFANT (YLC) C._____
B. CHILD (NYLC) D._____
E._____

C. CHILD (SYLC)
D. MALE HEAD OF HOUSEHOLD
E. YOURSELF

-See vaccination card if she has one

TYPE OF VACCINATION	DAY	MONTH	YEAR
_____	____	____	____
_____	____	____	____
_____	____	____	____
_____	____	____	____

	Week 1		Week 2		Weed 3		Week 4		Weed 5		Weed 6		Week 7		Week 8		Weed 9		Week10		Week11		Weeks12	
	Fq	Cs	Fq	Cs	Fq	Cs	Fq	Cs	Fq	Cs	Fq	Cs	Fq	Cs	Fq	Cs	Fq	Cs	Fq	Cs	Fq	Cs	Fq	Cs
Diarrhea																								
Vomiting																								
Cough																								
Fever																								
Breazing pro.																								
Others																								
Compliance																								

Diarrhea Fq= Number of stool the child passed in the last 24 hours.
Cs= 1 if stool is watery, 2 if stool is mucoid, 3 if stool is bloody, 4 if others, Specify others _____

Cough Fq= Number of days in the last one week the child had cough
Cs= 1 if child has a bloody sputum, 2 if child has pus with his sputum, 3 if others, Specify _____

Fever Fq= Number of days in the last week that the child has fever
Cs= 1 if child has intermittent fever, 2 if child has fever with shivering, 3 if child has fever with sweating, 4 if others, Specify

Breathing difficulty 1 - If yes 0 - If no

Others Record any other symptoms mentioned by the mother.

Compliance Reporting by the mother that treatment had been taken 1- If yes 0 - If no

	Baseline	3 months	6 months	12 months
Weight				
Height				
Hemoglobin				
Hematocrit				
Serum ferritin				
Serum Zinc				
Serum copper				
C-reactive pro.				
Stool				

APPENDIX 2

ETHICAL APPROVAL

McGill Memorandum

McGill University, Macdonald Campus
School of Dietetics and Human Nutrition

To: Steven A. Esrey
Abdulaziz A. Adish

From: Katherine Gray-Donald
Chair, Ethics Committee

Date: July 12, 1993

The Ethics Committee of Macdonald Campus of McGill University has reviewed the changes made to the original proposal entitled "Effect of Iron Supplementation on Morbidity and Growth on Preschool Children in Ethiopia" which was submitted to the Thrasher Foundation.

All changes described have been reviewed and found to be acceptable to our committee. We appreciate the opportunity to review projects as they are finally developed so as to assess any potential ethical problems that may arise as a result of protocol changes.

Katherine Gray-Donald

Katherine Gray-Donald, Chairperson
Ethics Committee

McGill

Faculty of Agricultural and Environmental Sciences

McGill University
Macdonald Campus

School of Dietetics and Human Nutrition

Faculté des sciences de l'agriculture et de l'environnement

Université McGill
Campus Macdonald

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CERTIFICATION OF ETHICAL ACCEPTABILITY FOR RESEARCH INVOLVING HUMAN SUBJECTS

A review committee consisting of:

Position	Field of Research
L. Prichard	Lady Member
D. Buszard	Plant Science
C. Chadee	Parasitology
K. Gray-Donald	Human Nutrition
U. Kuhnlein	Animal Science

has examined the application for funds in support of a project titled:

The effect of Iron Supplementation and other control strategies for Iron Deficiency on morbidity and growth of Preschool Children

As proposed by Abdulaziz A. Adish (Applicant) to IDRC (Granting agency, if any)

and consider the experimental procedures, as outlined by the applicant, to be acceptable on ethical grounds for research involving human subjects.

Comments:

No ethical problems. Permission to interview subjects will be sought in each community.

July 12, 1993
Date

K. Gray-Donald
Chairperson of Ethics Committee

M. Uhland
Dean's Representative

To:- Abdulaziz A. Adish
Department of Dietetics and Human Nutrition
McGill University
Montreal. Canada

From:- The Ethics committee
Jimma Institute of Health Sciences
Jimma, Ethiopia

Subject:- Ethical Clearance

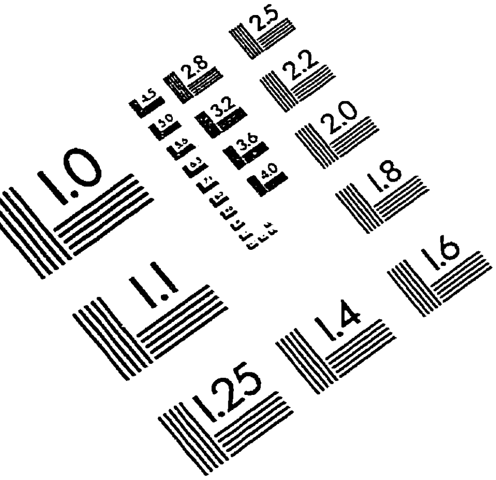
We acknowledge the receipt of your proposal entitled "Effect of Iron Supplementation and Other Control Strategies for Iron deficiency on Growth, Morbidity on Preschool Children in Ethiopia".

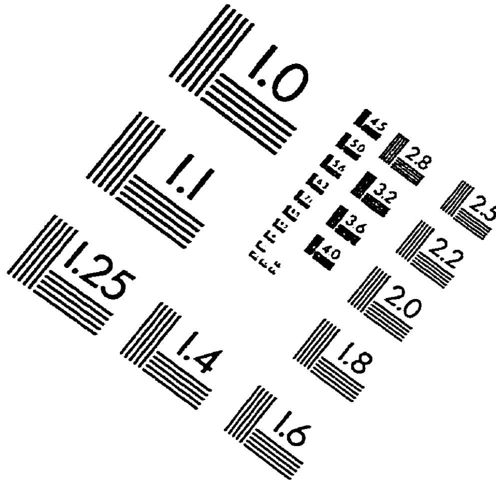
The proposal, upon revision, has been found to be within our procedural and ethical standards. The committee extends its support to your study and wishes you success.

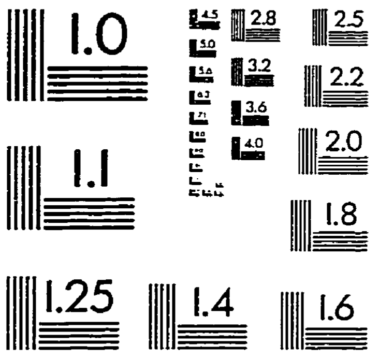
Yours sincerely,

Dr. Tesfaye Siferaw
Research and Publication Officer,
Jimma Institute of Health Sciences
P.O.Box 378
Jimma, Ethiopia

IMAGE EVALUATION TEST TARGET (QA-3)







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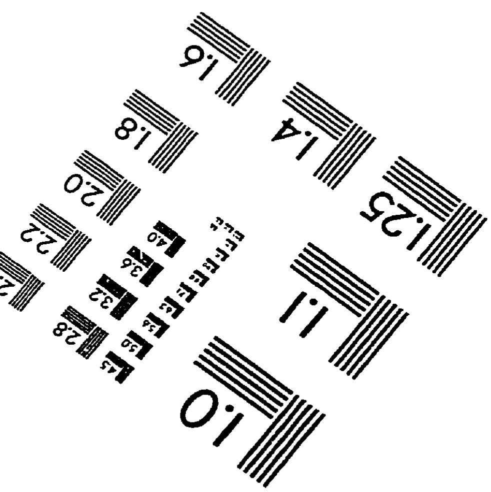

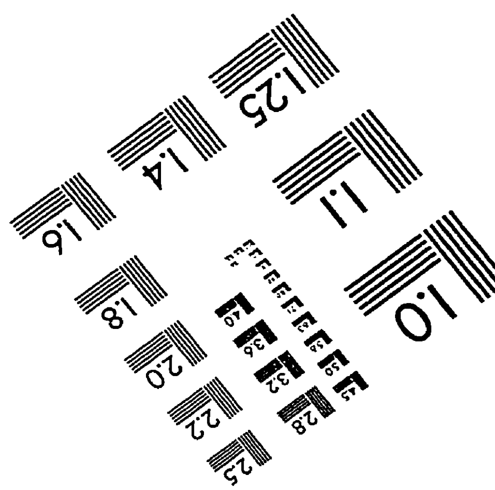

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