Role of Zinc in Stunting of Infants and Children in Rural Ethiopia

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Proefschrift
ter verkrijging van der graad van doctor
op gezag van de Rector Magnificus
van Wageningen Universiteit,
Prof. dr. ir. L. Speelman
in het openbaar te verdedigen
op maandag 17 Maart 2003
des namiddags te vier uur in de Aula

Melaku Umeta (2003)

Role of zinc in stunting of infants and children in rural Ethiopia

Thesis Wageningen University, The Netherlands – with references – with summary in English

ISBN: 90–5808–814–6

To Mirtnesh, Kulani and Jigssa

Abstract

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PhD thesis by Melaku Umeta Deressa, Division of Human Nutrition and Epidemiology, Wageningen University, The Netherlands, 17 March 2003.

Stunting is highly prevalent in children in Ethiopia with 57% of infants aged 6-11 mo being affected. The reasons for stunting are poorly understood but zinc deficiency may play a role in its aetiology. The research described in this thesis was carried out in a rural area of Ethiopia. It comprised a cross-sectional study of 305 breastfed infants aged 5-11 mo and their mothers; a double-blind randomised controlled zinc supplementation trial on growth of 200 breastfed infants aged 6-12 mo for 6 mo, in which children were examined at 6 at the end of the supplementation period, and 42 mo later; and a study of the mineral, phytate and tannin content of traditional diets of the rural population.

The prevalence of stunting, underweight and wasting of infants aged 5-11 mo was 36%, 41% and 13% respectively. The type, quality and quantity of supplementary foods given to infants were the major factors associated with stunting. Malnutrition was also prevalent among the mothers with 27% being chronic energy deficient (body mass index <18.5 kg/m^2) and 20% being night blind indicating that vitamin A deficiency is a serious problem. Supplementation with 10 mg of elemental zinc daily for 6 mo increased both linear and ponderal growth in both stunted and non-stunted infants and the effect was greater in the stunted children. Zinc supplementation resulted in a markedly lower incidence of anorexia and morbidity from cough, diarrhoea, fever and vomiting in stunted children. When the children were followed up 36 mo after supplementation had been discontinued, the gains in height and weight achieved during zinc supplementation had all but disappeared. The diets of the rural Ethiopian population are relatively high in zinc and iron, but because of the high content of phytate and tannins, the bioavailability of zinc and iron is low. An exception to this is the high content of bioavailable iron and zinc in enjera prepared from fermented cereals, especially from tef.

The findings of this study suggest that stunted children in Ethiopia need a continuous supply of zinc to maintain adequate health and growth. Thus policies and strategies should be developed to address the high prevalence of stunting among children, as stunting is a major barrier to people in rural Ethiopia reaching their full potential.

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1

General introduction

Malnutrition remains a common problem among preschool children worldwide with about one quarter of all children under five years of age being underweight (1). The prevalence is very high in less developed countries with children in South-Central Asia (44%) and in Eastern and Western Africa (36%) being the most affected (2). Inadequate diet and infectious diseases are the major causes of malnutrition (3,4), contributing to the high prevalence of child morbidity and mortality in poor countries (5,6). It has been estimated that about 12 million children under five years of age die annually due to infectious disease and malnutrition of which malnutrition alone contributes to more than half of the cases (7).

In the past, malnutrition was thought to be due primarily to insufficient intake of protein and energy but deficiencies of micronutrients also play a role although signs and symptoms are lacking in some cases. During the past two decades however, deficiencies of 3 micronutrients have received the most attention globally, namely of iodine, vitamin A and iron. Zinc deficiency may also be very important because it contributes to low birth weight, growth failure, impaired immunity, and to infant mortality and morbidity (5,6,8). Furthermore, diets in less developed countries are predominantly cereal based and contain high amounts of phytate – a potent inhibitor of zinc absorption – and are low in animal products that are good sources of highly bioavailable zinc (9). Thus zinc deficiency may have far reaching consequences for infant, child and maternal health but has long been overlooked. In recent years, however, zinc deficiency has been acknowledged to be an important public health problem (10).

Stunting is highly prevalent in children in Ethiopia. Its aetiology has been poorly understood but it has been hypothesised that zinc deficiency may play a role. The present thesis investigates the role of zinc deficiency in stunting in infants and children a rural population in Ethiopia and whether stunting can be reversed by supplementation with zinc. Furthermore, the zinc and iron content of traditional foods and the bioavailability of zinc and iron based also on the content of phytate, tannin and calcium in such foods has been investigated.

Growth retardation

Anthropometric indicators of child growth

Child malnutrition leads to growth failure - diminished height and weight - that are manifested as linear growth retardation or short stature, and thinness. These are most commonly known as stunting and wasting respectively. Stunting and wasting can be measured using anthropometric indices of height-for-age or weight-for-height respectively. Children with Z-scores for height-for-age (HAZ or length-for-age, LAZ) or weight-for-height (WHZ) below -2 standard deviations (SD) of the median National Centre for Health Statistics (NCHS) (11) of reference population with similar age and sex, are generally considered to be stunted or wasted respectively. HAZ reflects growth achievement pre- and postnatally while a deficit indicates a slowing in growth resulting from a cumulative long-term effect of inadequate nutrition or repeated infectious disease or both. Wasting is the result of acute severe undernutrition, as found in times of famine or sudden weaning, or to chronic disease.

It indicates an acute situation in which the body is forced to use its own resources, including muscle, to function "normally".

Prevalence of linear growth retardation

It has been estimated that 182 million (about one third) of preschool children in developing countries are stunted and the prevalence is 35% in Africa and 44% in South-Central Asia (1). The highest level of stunting is found in Eastern Africa where 48% of preschool children are currently affected. More alarming is the fact that over the past 20 y the number of stunted preschool children in Eastern Africa has increased substantially from 12.9 million in 1980 to about 22 million in 2000 (1). In rural Ethiopia, the national prevalence of stunting was reported to be 64%, which is one of the highest in the world (12,13). Furthermore, the prevalence of stunting increases from 57% during infancy to 73% in the second year of life. Regional variations within the country have also been observed with prevalences varying from 74% in the Northwest to 49% in the south (Omo). More surprising and difficult to explain, however, is the fact that a very high prevalence of stunting (73%) was observed in a food surplus region of the country (West Gojam).

Causes and mechanisms

Stunting and wasting are used as markers of malnutrition. As mentioned above, they represent different processes. Stunting starts early in infancy and is generally associated with the introduction of complementary feeding (14). It generally begins at around 3 - 4 mo and peaks around 2 - 3 y after which values run parallel to the reference curve. Stunting may also be already present at birth because of malnutrition of mother during pregnancy (15,16). Stunting is associated with a significant functional impairment in adult life such as mental and motor development (17,18), obstetric complications during pregnancy and low birthweight of children of women of short stature (19). Thus once a child is growth retarded, stunting usually remains for life, with limited potential for catch-up growth. This emphasises the importance of addressing linear growth retardation at the earliest possible age when nutritional deficiencies occur. Wasting results from failure to gain weight or from weight loss. It can occur after recent starvation or disease but can be restored relatively easily after feeding. Wasting is the index of choice for monitoring short-term changes in nutritional status. However, it should be noted that stunting and wasting do not have the same aetiology as is evident from the fact that stunting usually starts earlier in life than wasting. The causes and underlying biology of infant stunting are poorly understood (20,21), but poor diet - both in quality and quantity - and infection may play a role (20-22).

Zinc status and deficiency

Zinc is an essential trace element important for good health and for the proper development and functioning of the body. It is the most abundant trace element in all cells of the body, except red blood cells. The biological role of zinc is always as a bivalent cation, and unlike iron it does not undergo reduction or oxidation under physiological conditions, making zinc a

stable component of protein complexes. Zinc is not limited to a few functional roles like some elements such as iron and calcium. It is known to be a constituent of a large number of enzymes. It plays a key role in several metabolic processes such as nucleic acid transcription and translation and protein synthesis, cell division and growth (23), and to date more than 200 enzymes are known to be zinc dependent. The role of zinc in such enzymes can be either structural and/or catalytic. The fact that zinc plays a central role in gene replication and protein synthesis and cellular division makes it extremely important during periods of rapid growth both pre-and postnatally.

Zinc deficiency is thought to be widespread in most developing countries but the magnitude is not known because of lack of specific clinical signs and/or biochemical indicators associated with mild zinc deficiency (24,25). Cases of severe zinc deficiency are rare while mild to moderate zinc deficiency is not uncommon but not easily recognisable (26). Many biochemical methods have been proposed to assess zinc status but many are fraught with problems that affect their use and interpretation. The concentration of zinc in serum or plasma is the most common parameter used as an indicator of zinc status (9,27) although it is influenced by a variety of factors such as infection, stress and physiological and pathological conditions which limit its diagnostic value. Besides, it also represents a small fraction of total body zinc - only 0.1% - and the levels vary by 15-20% within individuals throughout the day (28). The applications of other indices, such as the concentration of zinc in hair, nails and leukocytes, is even less well defined and such indices do not seem to offer any advantages to the concentration of zinc in serum or plasma. At present, the most reliable method for diagnosing zinc status is to monitor the impact of zinc supplementation on zinc dependent variables such as growth and cellular immunity in blind, randomised, placebo controlled intervention studies (29,30). Such an approach is however time consuming, expensive and necessitates good compliance and follow-up.

Zinc supplementation and child growth

Evidence of human zinc deficiency began to emerge in the 1960s when Prasad *et al.* (31) first described that the growth retardation and hypogonadism syndrome in Iranian male adolescents was in part caused by zinc deficiency. Since then, a number of zinc supplementation trials on growth of children have been conducted both in developed and developing countries, but the results are inconsistent. A growth-limiting, mild zinc deficiency syndrome has been identified in children from low-income families in developed countries (32-34). The problem of marginal zinc deficiency may be worse in children in developing countries because their diets are predominantly plant based and rich in phytate (9,35). Despite the suggestion that the growth retardation in children is related to a nutritional deficiency of zinc, the results of studies carried out prior to those reported in this thesis to examine whether zinc supplementation improved linear growth were inconsistent. Only one of the eighteen studies had been carried out in Africa and this study showed no improvement in linear growth with zinc supplementation although changes in body composition were reported (36). Zinc deficiency was also shown to impair immunity and to increase morbidity from common infectious diseases and also mortality (8,37-39)

Prevalence of zinc deficiency

Poor zinc nutriture and possibly zinc deficiency is likely to be widespread in most developing countries (9,26), but the quantitative estimate of the global prevalence is not known because of the difficulties mentioned above in assessing zinc status. However, it may be estimated indirectly using proxy information on zinc intake from global food supply (40) and secondly by reviewing the national prevalence of stunting in children (1).

The national food balance sheets compiled by the Food and Agriculture Organisation (FAO) may provide valuable information on the total amounts of major food commodities and types of foods available for human consumption at national level. By calculating the amount of zinc present in these foods and the amounts that are potentially available for absorption, as estimated from phytate:zinc molar ratios, it is possible to assess whether the food supply is adequate to satisfy the population's theoretical requirement for zinc. Based on these considerations, a model has been developed for categorising diets with potential zinc bioavailability as high (>50%), moderate (30%) and low (<15%). The model has taken into considerations the dietary content of animal protein, daily intake of calcium and the daily phytate:zinc molar ratios. The diets of developing countries are largely based on cereals and legumes staples and based on the phytate:zinc molar ratio exceeded >15, the bioavailability of zinc is estimated as 10 – 20%. Using this approach, the amount of zinc present in the foods and the amounts that are potentially absorbable have been calculated and the adequacy of food supply to satisfy the population's theoretical requirement for zinc has been assessed. Based on these assumptions, current estimate indicated that about 95% of South Asian, 74% North Africa and Mediterranean and 68% of sub-Saharan African population are at risk of low dietary intake of zinc (40). On average, about 48% of the world population are at a risk of zinc deficiency.

The global database also provides information on national prevalence of childhood stunting (1). One of the earliest sign of suboptimal zinc nutriture in infants and children is impaired growth. As discussed earlier, moderate to severe stunting responds to zinc supplementation more than children who are not. Thus those countries with high prevalence of stunting tend to be those with high risks of low zinc intakes. These relationships may suggest that high prevalence of stunting may be used as a proxy indicator for zinc deficiency in the absence of a reliable biomarker.

Causes and mechanisms of zinc deficiency

Zinc deficiency arises when the effective intake of zinc is low and the requirement is increased, for example through infection (Figure 1). Effective zinc intake is determined by food intake, the zinc content of food and its bioavailability. Inadequate food intake arises from limited access and availability to food and parental feeding practices (41,42). Foods with a low zinc intake are those based mainly on plant products and poor in animal products (9,35,43,44). Although cereal and legumes contain relatively large amounts of zinc, they also contain relatively large amounts of substances that inhibit zinc absorption such as phytate and tannins. Inadequate intake of zinc can also result in reduction in appetite and subsequent decrease in food consumption (45). Environmental conditions such as poor hygiene and sanitation as well as poor access to health services may also lead to increased risk of infection

Figure 1. Possible aetiology of zinc deficiency and stunting

and to reduced immunity. Infections also usually suppress appetite causing a substantial decrease in food intake thus exacerbating zinc deficiency. Children with acute lower respiratory infection and diarrhoea have been shown to have lower food and energy intakes than those children without infections (46,47). Diarrhoea and fever, especially in infants and young children, may also increase intestinal losses of zinc and thus may exacerbate zinc deficiency leading to more pronounced linear growth retardation or stunting.

Zinc deficiency contributes to stunting through depression of appetite and growth, increased morbidity and metabolism disorders. Low intake of zinc from diet leads to a suppression of appetite primarily due to an impairment of taste. Taste impairment is mediated through concentrations of zinc in saliva and low levels lead to a reduction of taste hence to a greatly reduced appetite. Zinc deficiency is associated with metabolic disturbances of a wide range of hormones and enzymes involved in growth and bone development. It may impair growth by altering circulating in insulin-like growth factor I (IGF-I) which is a major

postnatal growth factor (48). Plasma IGF-I, was reported to decrease in children with dietary protein-energy malnutrition (49) and zinc deficient children (50). Zinc supplementation however, increases plasma IGF-I and improves growth in stunted children (48). Thus, the growth stimulation might be mediated through changes in circulating IGF-I. Effect of zinc on growth may also be due to a direct role of zinc in protein synthesis and gene expression. Changes in protein synthesis and cell replication contribute to accumulation of lean tissue (30). The impact of zinc on growth may also be through reduction in morbidity that causes an impaired immunity and intestinal mucosal damage, and an increase in appetite.

Ethiopia

Ethiopia is a landlocked country in the horn of Africa with an area of 1,088 million square kilometres. It is a land of great physical diversity where much of the country is highland with some plateau, deep valleys, mountains and plains. The total population is estimated to be 65 million, increasing at an annual rate of 2.6%. There are over 70 different ethnic groups of which Oromo, Amhara, and Tigre are the most prominent ones. About 85% of the population reside in rural area with subsistence farming being the main livelihood. The wide ranges of crops grown in the country include the staple cereal crops maize, tef, sorghum, wheat and barley, while enset (*Enset ventricosum)* also known as "false banana", is a major staple food for the south and southwestern part of the country. Cattle are numerous in many regions of the country but their products make a significant contribution to the diet only of the nomadic population.

Of the population approximately 3.5% are infants aged less than 12 months, 18.3% are children under five years of age and 21% are women of reproductive age. Ethiopia is one of the world's poorest countries with an annual per capita income of about €100, infant mortality rate (IMR) of 99/1000 live births, child birth rate (CBR) of 44/1000, crude death rate (CDR) of 18/1000 and a life expectancy of 43 and 46 y for men and women respectively. While some advances have been made in health care, education, water and sanitation, the country's high population growth and the current high prevalence of HIV/AIDS have eroded some of the gains.

Child malnutrition remains a public health problem, affecting children aged <5 y in rural areas. About 64% children <5 y are stunted while 47% are underweight, and such rates are among the highest in the world. From 1983 to 1992, the nutritional situation deteriorated with the prevalence of stunting, underweight and wasting increasing from 60 to 64%, 37 to 47%, and 8% to 9% respectively (12,51). The surveys, on which these trends were based, were conducted in the postharvest season. Stunting starts earlier in life where 57% of the infants aged 5 – 11 mo are already affected. The prevalence of stunting is also the highest (73%) in the food surplus regions of the country such as West Gojam. In rural Ethiopia, the prevalence of breast-feeding is as high as 93% and breast-feeding continues up to the age of 18-24 mo (52). Micronutrient deficiencies are also widespread with a prevalence of iodine deficiency disorders (IDD) manifested by goitre of 26% (53) and vitamin A deficiency manifested by Bitot's spot in children aged 6-72 mo of 1% (54). There is no figure for the

national prevalence of anaemia including iron deficiency anaemia but a value of 18% has been reported for pregnant and lactating women (55).

Rationale of the study

The high prevalence of stunting in children in Ethiopian is very alarming. The environmental risk factors such as inadequate food intake and frequent infection are common in the country. However, stunting may vary in different settings in which specific micronutrients, in particular zinc, may be limiting because of the crucial role in many enzyme systems and metabolic processes including the synthesis of nucleic acids and proteins.

There are reasons to suspect zinc might be a limiting factor for their poor growth. In rural Ethiopia, infants and children's diets are predominantly based on unrefined cereals and legumes, which are rich in phytate, and often low in animal foods which are good sources of readily available zinc. The predisposing factors for suboptimal zinc status exist in the country but unfortunately to date no study has been carried out on zinc status of any group of the population. Furthermore, information on dietary intake of zinc of the population is lacking due to lack of information on zinc and phytate contents of local foods. Thus, zinc deficiency is likely to exist in this child population.

Objective of the thesis

The objective of this study was to investigate whether or not zinc is a limiting nutrient for the growth of children in rural Ethiopia. The aims of the research describes in this thesis are:

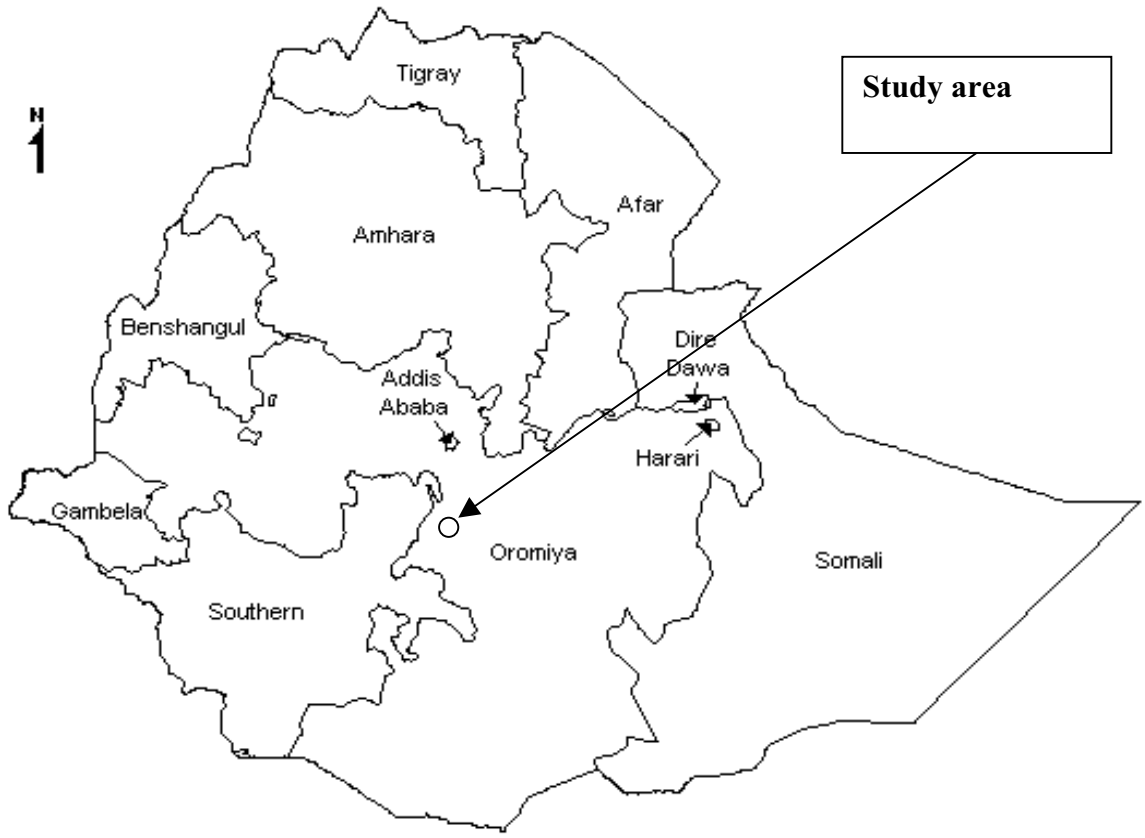
1. To assess the nutritional status of breastfed infants and their mothers and to identify major factors associated with stunting in rural Ethiopia (**Chapter 2).**
2. To investigate the effects of zinc supplementation on growth and morbidity in stunted and non-stunted infants **(Chapter 3**).
3. To investigate whether the effects of zinc supplementation on growth persist after discontinuation of zinc supplementation **(Chapter 4**)
4. To investigate the mineral content of foods commonly consumed in rural areas and to predict the bioavailability of zinc and iron based on the content of phytate, tannin and calcium **(Chapter 5).**

Description of study area

The study described in this thesis was carried out in a rural village of Dodota-Sire District, Arsi Zone (Oromia region), Central Ethiopia, located about 140-170 km east of Addis Ababa (Figure 2). In this district, two villages, Dheera and Hamude, about 40 km apart were selected as working centres. The study area was chosen because of the high prevalence of malnutrition, access to an all weather road, availability of health facilities, and good co-operation of the community and local government officials. The area lies in the Great Rift Valley of Africa and the climate is hot with the main rainy season occurring in July and August. Local staple crops are wheat, maize, sorghum, barley and tef mainly grown for

subsistence. Traditional rearing of cattle and goats is commonly practised, mainly as a means of generating cash income.

Figure 2: Map of Ethiopia showing the study site.



Study design and outline of the thesis

A community-based study was carried out in 18 peasant associations with 22,100 inhabitants living within a 15-km radius of the two working centres, which are 40 km apart. A census of all breastfed infants aged 5-11 mo (n=305) and their mothers was made. With respect to both mothers and infants, anthropometry was carried out, clinical examinations were made, demographic information was collected, and breast milk from the mothers was collected in which concentrations of zinc, copper and calcium were determined. The data were then analysed cross-sectionally to determine the factors associated with stunting in the infants (Chapter 2). After the survey, infants aged 6-12 mo who were apparently healthy and free from intestinal parasites, and whose mothers were willing to allow their infants to participate were recruited for inclusion in a randomised, double-blind, placebo-controlled

supplementation study. Stunted infants (LAZ <-2, n = 100) were selected and pair matched for age and sex with 100 non-stunted infants (≥ -2) for inclusion in the study. Within the stunted and non-stunted infants, matching was carried out by sex, age (within 2 mo) and recumbent length (within 3 cm). Each group was randomly and blindly allocated to be supplemented with either zinc (10 mg) or placebo for 6d/wk for 6 mo. Growth was measured monthly and morbidity data were collected daily for 6 mo. At the end of 6 mo intervention, both blood and hair samples were collected. The data thus obtained were then used to determine the effect of 6 mo of zinc supplementation on growth and morbidity (Chapter 3). After the supplementation was terminated at 6 mo, the infants were followed-up for a further 36 mo and anthropometric measurements were made and blood and hair samples were collected. In addition, anthropometric indices of siblings born subsequent to randomisation were also collected. These data are presented in Chapter 4. In Chapter 5, foods commonly consumed in rural villages in different part of the country collected for analysis of the content of minerals, phytate and tannin. The effect of the phytate, tannin and calcium content on the bioavailability of zinc and iron are discussed. In Chapter 6, the main findings of the study described in this thesis are discussed and a number of conclusions are drawn.

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2

Factors Associated with Stunting in Infants aged 5-11 months in the Dodota-Sire District, Rural Ethiopia

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J Nutr (in press)

ABSTRACT

The contribution of factors to malnutrition, particularly stunting, may differ among areas and communities. This cross-sectional study aimed to estimate the level of malnutrition and identify factors associated with the high level of stunting in breast-fed infants aged 5–11 mo living in Dodota-Sire District, Ethiopia. Infants (n=305) and their mothers were examined physically, and anthropometric and demographic data were collected. The content of zinc, calcium and copper in breast milk was measured, and data collected on the type, frequency of consumption, and time of introduction of supplementary feeding. Overall, 36% were stunted, 41% underweight and 13% wasted. The highest prevalence of malnutrition was seen in infants aged 9-11 mo. Among mothers, 27% had chronic energy deficiency (body mass index, <18.5 kg/m^2) and 20% were night blind indicating that vitamin A deficiency is a serious problem. Infants fed >3 times/d, consuming > 600 mL/d or consuming cows milk in addition to cereals and/or legumes had markedly higher length-for-age Z-scores than their peers fed less frequently, consuming less food or not consuming cows milk (differences: 0.39, 95%CI: 0.04-0.74; 0.17, 95%CI: 0.02-0.32; 0.40, 95%CI: 0.07-0.72, respectively). Infants of mothers with low concentrations of zinc in their breast milk were more stunted. In conclusion, the quality and quantity of foods consumed by infants is insufficient to prevent stunting. Thus it is necessary to increase the nutrient supply to infants by increasing intake and nutrient concentration of breast milk and of supplementary foods they consume, and by providing supplements to infants where appropriate.

INTRODUCTION

Malnutrition remains one of the major public health problems in less developed countries affecting infants and children and women of reproductive age (1). Malnutrition involves deficiencies not only of macronutrients but also of micronutrients. In less developed countries it has been estimated that about 12 million children under five years of age die annually due to infection and malnutrition with malnutrition contributing to half of the mortality. Stunting or linear growth retardation is a common result of malnutrition among young children in poor countries (2). Ethiopia is no exception: nutritional deficiencies and infectious disease are the leading health problems in the country (3-6). At the national level, 64% of the Ethiopian children under five years of age are moderately or severely stunted while 47% are underweight and 8% are wasted (7). These prevalences are among the highest in the world. More surprisingly, a very high prevalence of stunting (75%) has been observed in food surplus regions of the country such as West Gojam. Moreover, approximately 17% of children are estimated to have a low birth weight (< 2500 g) (8).

In developing countries, growth faltering starts within a few months after birth. The causes of early stunting are not yet well understood (9) but may include both a nutritionally inadequate dietary intake and infections (10-12), both of which have their roots in poverty. During the last decade, however, several randomized controlled trials have provided evidence that zinc deficiency contribute to stunting in children both in developing (13-15) and developed (16-18) countries that can be reversed by zinc supplementation.

The risk of stunting is greatest during the period of rapid body growth and development, and slows down at around 3 years of age (19). Early stunting is likely to persist through adolescence if children remain in the same environment. Traditionally, malnutrition has been considered to be solely due to a shortage of food in the community resulting from low economic development, skewed distribution of wealth, poverty, seasonal factors and war. Other factors, such as large family size, poor feeding practices and high prevalence of infectious disease also play a role. Knowledge of the relative contribution of the major risk factors associated with stunting is therefore an important prerequisite for developing nutrition intervention strategies. We have undertaken a cross-sectional study to examine factors possibly associated with stunting in breast-fed infants in Dodota-Sire District, rural Ethiopia. Infants enrolled in this study were subsequently supplemented with zinc, which resulted in a beneficial effect on linear growth and morbidity (20).

SUBJECTS AND METHODS

Study area and population. The study was carried out in July 1996 during the rainy season in a rural area of Dodota-Sire District, Arsi Zone, rural Ethiopia, which is located about 150 km east of the capital, Addis Ababa. In this district, two villages, Dheera and Hamude were arbitrarily selected. They are located about 40 km apart and both have health facilities and access to all weather roads. The area lies in the Great Rift Valley of Africa with hot weather and a short rainy season of less than two months (July and August). During this period, there was an acute shortage of food in the community. The local staple crops are wheat, maize, sorghum, barley and tef, which are grown for subsistence. Diets based on these

cereals contain high levels of phytate that inhibit the absorption of iron and zinc. Traditional rearing of animals, mainly cattle and goats, is commonly practiced as a means of generating cash income. Some milk is consumed, but after the removal of the cream, which is sold. Vegetables are rarely consumed.

A total of 18 farmers associations with a population of 22,100 inhabitants within 15 km of the villages were included in the study. A census of all breast-fed infants aged 5-11 mo was made, and their parents were invited to bring their children for the study. All children invited participated in the study. Demographic information was gathered using a pre-tested questionnaire. Dates of birth of the children were established by a local events calendar with information on anniversaries, festivals, fasting periods and farming seasons. No clinic cards were available from which dates of birth could be established. All infants were born at home with or without assistance of traditional birth attendants, which is the usual practice in rural Ethiopia.

Anthropometry. Length of the infants was measured in a recumbent position to the nearest 0.1 cm using a board with an upright wooden base and a movable headpiece, designed by the Division of Human Nutrition and Epidemiology, Wageningen University. Height of the mothers was measured in middle head, the Frankfurt position without shoes using a wall-mounted stadiometer to the nearest 0.1 cm. Weight of the lightly clothed infants was measured to the nearest 10 g by a metal beam seat balance (Seca, model 725/424, Lameris, Utrecht, Netherlands). Weight of the mothers in light clothes and without shoes was measured to the nearest 100 g by an electronic scale (Tefal, model SC 3218, Rumilly, France). The scales were calibrated each morning and checked at regular intervals throughout the day. Knee-heel length of the infants was measured to the nearest 0.1 mm using an electronic kneemometer (Force Institutterme, model BK5, Brondby, Denmark). Mid upper-arm circumference (MUAC) of both infants and mothers was measured without compression to the nearest 0.1 cm with a flexible non-stretch measuring tape on the left arm with the arm hanging relaxed. Triceps skinfold thickness was measured at the same site to the nearest 0.1 mm by a Harpenden caliper (John Bull British Indicator Ltd., UK). The standardization procedures used to increase accuracy for anthropometric measurements are discussed in detail elsewhere (20). Bioelectrical impedance in children was measured at 100 kHz using a multi-frequency impedance analyzer (Dietosystem, Milan, Italy). The injection electrodes were connected on the foot and on the hand just proximal to the digits, and the sensor electrodes were connected 5 cm proximal to the injection electrodes. The impedance index (length squared /impedance) was calculated as a crude indicator of fat-free body mass.

Clinical examination. Clinical examinations of both infants and their mothers were carried out by a physician. Information was obtained from each mother with respect to the previous 2 wk about her appetite and about the amount and frequency of consumption of supplementary foods by the infant.

Breast milk collection and analysis. Breast milk about 15 mL, obtained from the right breast about 60 min after the last feeding from that breast, was collected in an acid

washed plastic bottle and kept at -20^0C until analysis. Concentrations of zinc, calcium and copper in breast milk were determined by atomic absorption spectrophotometer (Varian Spectra AA 10/20 Plus, Varian Techtrone Pty, Ltd., Mulgrave, Vic, Australia) after centrifugation to remove fat (21).

Stool collection and examination. Each mother was given a cup with a cover in which she collected a sample of her child's stool. The samples were suspended in saline solution immediately upon receipt by the study team in the field and examined by microscopy for the presence of intestinal parasites, particularly *Ascaris*, ameba, hookworm, *Giardia* and strongyloids.

Ethical approval. The study design was explained to officials of the Zonal Health Department of Arsi, administrative officials of Dodota-Sire District, community and religious leaders, and peasant association leaders. The nature of the study was also fully explained to mothers and oral consent was obtained. Permission for the study was obtained from the medical ethical committee of the Ethiopian Health and Nutrition Research Institute.

Statistical analysis. Anthropometric indices were calculated using CASP software (version 3, 1987, Centers for Disease Control and Prevention, Atlanta, GA, USA). Being stunted, underweight and wasted were defined by Z-scores for length-for-age (LAZ), weight-for-age (WAZ) and weight-for-length (WLZ) < – 2 SD below median values of a reference population of US children (22). All other analyses were carried out using SPSS software (SPSS Inc, version 8.0, Chicago, IL). Komolgorov-Smirnov tests were used to check if outcome variables were normally distributed. If data were not normally distributed, the Mann-Whitney tests was used to assess group differences. Group means were compared by independent t-tests. Linear regression analysis was used to examine independent associations between supplementary food feeding variables and stunting as assessed by LAZ.

RESULTS

The study included 305 breast-fed infants aged 5-11 mo of whom 146 (47%) were boys and 159 (52%) were girls. Of the infants, 110 (36%) were stunted. Stunted and non-stunted infants differed with respect to most of the parameters examined with boys more likely being stunted than girls (**Table 1**). Stunted infants were not only shorter but also weighed less, were thinner as measured by triceps skinfold and MUAC and had a lower fat-free body mass as measured by impedance index.

Most mothers (> 80%) had > 4 children, were married (97%), were illiterate (91%), had farming as the major source of income (93%), and were vaccinated against tetanus during pregnancy (72%). Untrained traditional birth attendants attended most deliveries and about 38% of the mothers reported being examined by a nurse after the baby was delivered. Of the mothers, 82, (27%) had chronic energy deficiency (body mass index <18.5 kg/m^2) while mothers of stunted infants were slightly shorter (~ 2 cm) and had a slightly lower triceps skinfold thickness (**Table 2)**. Of the stunted children, 66% were born within 2 y of their older

siblings, compared with 52% for the non-stunted children (P = 0.04). More mothers (12%) of stunted infants reported poor appetite compared with mothers of non-stunted infants (7%) (P = 0.06). Signs and symptoms of vitamin A deficiency such as night blindness, Bitot's spots and corneal xerosis in at least one eye were recorded by 20%, 1% and 1.5% respectively of the mothers. The results were similar for mothers of stunted and non-stunted children.

Table 1: Comparison of anthropometric indices in stunted and non-stunted breast-fed infants[*†]

Characteristics	Stunted	Non-stunted	Difference		P value
			Estimate	95% CI	
n	110	195			
Sex (boys/girls)	58/52	88/107			
Age (mo) [‡]	9.1 ± 0.2	8.6 ± 0.2	0.5	(0.0, 0.9)	0.07
Length (cm)	63.7 ± 0.3	68.2 ± 0.3	-4.6	(-5.5, -3.8)	0.001
Weight (kg)	6.1 ± 0.1	7.3 ± 0.7	-1.2	(-1.4, -0.9)	0.001
Mid-upper arm circumference (cm)	13.2 ± 1.1	14.1 ± 1.0	-0.9	(-1.6, -0.6)	0.001
Triceps skinfold thickness (mm)	5.8 ± 0.1	6.6 ± 0.1	-0.8	(-1.1, -0.5)	0.001
Knee-heel length (mm)	163±11	176 ±13	-12	(-15.5, -9.4)	0.001
Weight-for-age Z-score (WAZ)	-2.79 ± 0.09	-1.33 ± 0.11	-1.47	(-1.72, -1.31)	0.001
Weight-for-length Z-score (WLZ)	-0.84 ± 0.11	-0.87 ± 0.11	0.03	(-0.22, 0.32)	0.84
Length-for-age Z-score (LAZ)	-2.84 ± 0.06	-0.81 ±0.07	-2.02	(-2.21, -1.84)	0.001
Impedance index at 100 kHz (cm^2/Ω)	5.6 ± 0.1	6.5 ± 0.1	-0.9	(-1.1, -0.7)	0.001

*Stunting and non-stunting were defined as length-for-age Z-score (LAZ) < –2 SD and ≥ -2 SD respectinvely of the median of NCHS reference population (Ref 22). [†]Data are age adjusted and expressed as means ±SE. [‡]Marginal difference between groups, P < 0.07, Mann-Whitney U test.

Breast milk was collected from 253 of 305 mothers (83%): 37 refused to give milk due to ritual beliefs and 15 mothers did not produce enough milk. The concentration of zinc in breast milk of mothers of stunted infants was less than that in milk of mothers of non-stunted infants (-1.1 μmol/L, P = 0.02). The same appeared to be true for breast milk calcium concentrations (-1.3 mmol/L; P = 0.08). There were no differences between the groups in copper concentration of breast milk.

The overall prevalence of being stunted, underweight, wasted or MUAC < 12.5 cm were 36%, 41%, 13% and 10% respectively (**Table 3**). Furthermore, the proportion of stunting, underweight and wasting increased with age, the highest being in the age group of 9-11 mo. The prevalence of wasting was 15% (n=16) for stunted infants and 12% (n=23) for non-stunted children.

The most common health problems in infants reported in the 2 wk before the interview were cough (10.8%), fever (7.5%), eye diseases (6.9%), scabies (5.9%) and diarrhea (4.3%). There were no differences between stunted and non-stunted infants.

The results of the stool examinations for helminths were negative for 97% of the infants. Only strongyloids (1.3%) and *Ascaris* (1.0%) were seen.

Table 2: Comparison of indices nutritional status of mothers of stunted and non-stunted infants*

Characteristics	Mothers of stunted infants	Mothers of non-stunted infants	Difference		P value
			Estimate	95% CI	
n	110	195			
Age (y)	26.6 ± 0.6	26.4 ± 0.4	0.3	(-1.1, 1.6)	0.70
Height (cm)	153.3 ± 0.6	155.1 ± 0.4	-1.8	(-3.1, -0.5)	0.008
Weight (kg)	47.3 ± 0.6	47.9 ± 0.4	-0.7	(-2.1, 0.7)	0.34
Mid-upper arm circumference (cm)	22.9 ± 0.2	22.8 ± 0.1	0.1	(-0.3, 0.6)	0.52
Triceps skinfold thickness (mm)	6.9 ± 0.0	7.7 ± 0.2	-0.8	(-1.3, -0.4)	0.001
Body mass index (kg/m^2)	20.1 ± 0.2	19.9 ± 0.1	0.2	(0.3, 0.70)	0.51
Concentration in breast milk†					
Zinc (µmol/L)	9.2 ± 0.4	10.4 ± 0.3	-1.1	(-2.1, -0.1)	0.02
Copper (µmol/L)	2.3 ± 0.1	2.2 ± 0.1	0.1	(-0.3, 0.4)	0.74
Calcium (mmol/L)	0.32±0.02	0.35±0.01	-1.3	(-2.7, -0.2)	0.08
Night blindness‡ (%)	23.6	18.6	5.1		0.30
Bitot's spot‡ (%)	0.9	1.0	-0.1		1.00
Corneal xerosis‡ (%)	1.8	1.0	0.8		0.62

*Data are age adjusted and expressed as mean ± SE. †The number of mothers of stunted and non-stunted infants from whom breast milk samples were obtained and analyzed was 92 and 161 respectively.
‡By Fisher's Exact test.

Table 3: Prevalence of indicators of malnutrition in infants per age class*

Age, mo	Total number of children	Stunted[1]	Underweight[1]	Wasted[1]	MUAC < 12.5 cm
	n	n (%)	n (%)	n (%)	n (%)
5.0 – 7.0	85	27 (32)	23 (27)	1 (1)	5 (6)
7.1 – 9.0	108	37 (34)	27 (25)	8 (7)	7 (7)
9.1 – 11.0	112	46 (41)	76 (68)	30 (27)	17 (15)
Total	305	110 (36)	126 (41)	39 (13)	29 (10)

*Being stunted, underweight or wasted was defined by Z-scores for length-for-age, weight-for-age and weight-for-length respectively, below –2 SD (Ref.22).

Table 4: Independent associations of supplementary food feeding variables with length-for-age of infants as analyzed by multivariate analysis.

Effects adjusted for age	n/n*	Difference in LAZ	95% CI	P value
Frequency of feeding > 3 times/d Compared with <3 times/d	194/111	0.39	(0.04, 0.74)	0.03
Consuming > 600mL/d compared with consuming < 600 mL/d	167/138	0.17	(0.02, 0.32)	0.02
Consumption of cereals and legumes without cows milk†	179/126	0.40	(0.07, 0.72)	0.02
Age of introduction of supplementary foods > 6 mo compared with < 6 mo	213/93	-0.13	(-0.45, 0.19)	0.43

*Number infants in first category/number infants in second category

†All but one infant were fed with cereals, legumes or cows milk.

The frequency, quantity and type of supplementary feeding were strongly associated with stunting while the age of introduction of supplementary foods was not (**Table 4)**. Infants consumed the following types of supplements: cows milk (n = 93, 30.5%), cereals alone (61, 20.0%), legumes only (2, 0.7%), vegetables (1, 0.3%), cereals with legume mix (104, 34.1%) and cereals with cows milk (44, 14.4%). Of the mothers who claimed to give their infants cows milk as supplementary food, further questioning showed that they fed their infants cows milk not more than 2 d /wk because they prefer to sell it as a means of generating cash. Children given cows milk tended to be less stunted. The most common supplementary food used in the household in this village was *attmit* (thin gruel), porridge and partially or fully fermented *enjera* (a pancake-like thin leavened bread) or bread prepared from cereals.

DISCUSSION

Our results demonstrate that the quantity and types of food given to the infants and the frequency of feeding are important factors related to stunting in the infants studied (Table 4). Children who were reported to consume relatively large quantities of food (> 600 mL/d) had higher LAZ than their peers consuming lower quantities (< 600 mL/d). Age of introduction of supplementary foods was less related to stunting. Furthermore poor appetite and nutritional status of mothers, and low zinc and calcium concentration of breast milk also contributed to stunting of infants.

LAZ is considered to be a good indicator of the nutritional status and health of infants and young children (23). Stunting indicates low growth and is the cumulative effect of low or inadequate intake of energy, macronutrients or micronutrients over a long period or results from chronic or frequent infection. The underlying malnutrition may also contribute to

morbidity and mortality from common infectious diseases such as acute respiratory infections, diarrhea, measles and malaria. The high prevalence of stunting found among infants in this study indicates that malnutrition is wide spread among children in the study area. Our prevalence estimate (36%) is relatively low compared with national data for the same age range (57%) (7). Communities may vary regarding the factors studied. Because we only collected data in two villages, our descriptive data may not be representative of the country as a whole. This is less relevant with respect to the relationships observed between variables.

The prevalence of wasting (13%) is alarming and very high compared with estimates for Eastern Africa, 7% (24) and the national figure for Ethiopia 8% (7). Wasting reflects a problem of current or recent starvation, insufficient or inappropriate supplementary foods, or it may be a consequence of acute infectious disease. The survey was undertaken in the wet season before harvesting of crops when most families experienced acute shortages of food. Surveillance of nutritional status and measures to prevent or tackle acute malnutrition should be implemented in this area.

Although stunted infants were thinner (both MUAC and triceps skinfold thickness were lower) than non-stunted infants, such a difference was not reflected in the proportion of wasted children among stunted and non-stunted infants. Of the 110 stunted infants, 16 (15%) were wasted while of the 195 non-stunted infants, 23 (12%) were wasted. Stunted infants also had a lower impedance index than non-stunted infants. Unfortunately no formula is available for predicting fat-free mass from impedance data in infants. Thus, the impedance index can only be used as a crude indicator of fat-free mass. The fact that the index is lower in the stunted children and that it correlates well with LAZ ($y = 0.73x – 6.05$; $R^2 = 0.34$). indicate that the stunted children not only are smaller, but that they also have less adipose tissue. However it should be noted that the etiology of wasting is different to that of stunting, as is evident from the fact that stunting usually starts earlier in life than wasting (25). Helminths are unlikely to contribute to infant malnutrition in this population because very few of the infants had evidence of helminths in their stools.

Breast milk is an important source of both zinc and vitamin A for the infants during infancy. Zinc from breast milk is well absorbed but becomes inadequate for growth after 6 mo of lactation. Both the concentration and bioavailability of zinc in the supplementary foods given to the infants in this study were low and likely to be less absorbed because of the inhibitory effect of phytate. Thus zinc is insufficient in meeting infant requirement for growth. The concentration of zinc in breast milk is not affected by the nutritional status of the mother but by age of lactation thus it is unlikely to increase breast milk zinc concentration. Randomized, controlled trials in lactating mothers did not show any effect of zinc supplementation on breast milk zinc concentration (26,27). In a non-randomized study, it was found that zinc intake in relatively well nourished mothers was associated with breast milk zinc concentrations (28). These studies on zinc were conducted in relatively well nourished women. Thus randomized controlled trials need to be undertaken in developing countries to determine whether increased maternal zinc intake can enhance zinc concentration in breast milk in such countries. The low concentration of zinc both in breast milk and the diet thus may have contributed to the poor infant growth in stunted infants. We and others have shown

that supplementing stunted infants with zinc stimulates growth (20,29). A recent study has shown that deficiencies of vitamin A and zinc occur together in lactating mothers and their infants and that the retinol concentration of breast milk is related to the vitamin A status of infants (30). Randomized controlled trials have shown that supplementation with β-carotene or retinol can lead to increase retinol concentrations in breast milk (31,32). Thus maternal vitamin A status affects the breast milk vitamin A concentration. In this study, 23.6% of mothers of stunted infants had night blindness, a sign of vitamin A deficiency, and their infants are likely at risk of vitamin A deficiency. These findings suggested that poor micronutrient status of mothers is likely to increase the risk in their infants of deficiencies of zinc and of vitamin A, and poor growth. Adequate maternal vitamin A status and dietary intakes are important to improve the vitamin A transfer to their young infants. Therefore soon after the survey, we distributed vitamin A capsule to both the mothers and infants in our study.

Stunting in infants may also be caused by low energy intake. In the present study, the energy and nutrient densities of the supplementary foods were low as the foods were prepared from a limited number of local staple cereals and legumes without the addition of sugar or fat. They were also diluted with water to reduce the viscosity to make them more acceptable to the infants. In addition, only 30% of infants were supplemented with cows milk. Because of the their small stomachs, infants can consume only half or one cup of supplementary food per feed, which makes it difficult for them to satisfy their energy and nutrient requirements. This is in agreement with other studies in developing countries (33,34). Based on data collected from mothers on the food intake of their infants, we also found that infants fed < 3 medium size cups (< ca 200 mL) per day had lower LAZ scores ($p = 0.03$). Therefore, the small amounts consumed and low feeding frequency are probably important determinants of stunting. No data were collected on consumption of foods from various food groups.

We found that infants born < 24 mo after the birth of the previous sibling were more likely to be stunted than those born > 24 mo afterwards (data not shown). The resulting poor nutritional status of their mothers, coupled with the increased burden of childcare, may result in reduced nutritional status of the infants including stunting. Greater spacing of children would enable mothers to provide better nutrition, care and attention to their children. The consequences of poor nutrition of a mother and her fetus extend far into later life of the child and possibly to the next generation. There is mounting evidence for Barker's fetal origins hypothesis (35) in which it is proposed that undernutrition of the fetus during critical periods of development increases the risk in later life of so-called diseases of affluence such as coronary heart disease. Thus it would be interesting to follow up the children in this study into later life.

The prevalence of night blindness (ca 20%, **Table 2**) exceeded 5%, which defines vitamin A deficiency as a public health problem in pregnant women (36). As most of the women were not pregnant, this proportion is extremely high. We did not observe any evidence of xerophthalmia in the infants but this is not surprising because vitamin A deficiency usually becomes manifest in the second year of life (37). However, there is growing awareness that sub-clinical and even clinical vitamin A deficiency is common among infants (38). Vitamin A deficiency has been reported in preschool children living in

the same area (39,40). The main staple foods comprise cereals supplemented with legumes while dark-green leafy vegetables are available only during the rainy season. Such foods are known to be poor sources for vitamin A (41).

In conclusion, this study clearly shows that the quality and quantity of foods consumed by infants is insufficient to prevent stunting. Thus it is necessary to increase the nutrient supply to infants by increasing the intake and nutrient concentration of breast milk and of supplementary foods they consume and by providing supplements to infants where appropriate.

ACKNOWLEDGEMENTS

We would like to thank the children and parents who participated in the study; Dr Kedir Wajisso, Head of Arsi Zone Health Department and Ato Wake, Chief Administrator of Dodota-Sire district for facilitating and allowing us to do the study. The contribution of Wasse Dubale for excellent technical assistance, of Wolde-Mariam Girma for data organization, and of Paul Deurenberg for training in anthropometry is gratefully acknowledged. The study was supported by a grant from the Nestlé Foundation for the Study of the Problems of Nutrition in the World.

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Zinc Supplementation and Stunted Infants in Ethiopia: a randomised controlled trial

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(Lancet, 2000; 355: 2021-26)

Summary

Background Stunting is highly prevalent in Ethiopia and many other developing countries but the reason for it is poorly understood. Zinc is essential for growth but diets in such countries often do not contain zinc in sufficient quantity or of sufficient bioavailability. Thus zinc deficiency may play a major role in stunting. The aim of the study was to investigate whether the low rate of linear growth of apparently healthy breastfed infants in a rural village in Ethiopia could be improved by zinc supplementation.

Methods: A randomised, double-blind, placebo-controlled trial was done on apparently healthy breastfed infants aged 6–12 months. 100 stunted (length-for-age, Z score <-2) were matched for age and sex with 100 randomly selected non-stunted (>–2) infants. Infants, both stunted and non stunted, were matched by sex, age (within 2 months) and recumbent length (within 3 cm) for random assignment, to receive a zinc supplement (10 mg zinc per day, as zinc sulphate) or placebo, 6 days a week for 6 months. Anthropometric measurements were taken monthly, data on illness and appetite were collected daily, and samples of serum and hair were taken at the end of the intervention for the analysis of zinc.

Findings: The length of stunted infants increased significantly more ($p<0·001$) when supplemented with zinc (7·0 cm [SE 1·1]) than with placebo (2·8 cm [0·9]); and the effect was greater ($p<0·01$) than in non-stunted infants (6·6 [0·9] vs 5·0 [0·8] cm for the zinc and placebo groups respectively, $p<0·01$). Zinc supplementation also increased the weight of stunted children (1·73 [0·39] vs 0·95 [0·39] kg for the corresponding placebo group, $p<0·001$) and of non-stunted children (1·19 [0·39] vs 1·02 [0·32] kg for the corresponding placebo group, $p<0·05$). Zinc supplementation resulted in a markedly lower incidence of anorexia and morbidity from cough, diarrhoea, fever, and vomiting in the stunted children. The total number of these conditions per child was 1·56 and 1·11 in the stunted and non-stunted zinc supplemented children versus 3·38 and 1·64 in the stunted and nonstunted placebo-treated children, respectively. At the end of the intervention period, the concentrations of zinc in serum and hair of stunted infants, who had not been supplemented with zinc, were lower than the respective concentrations of zinc in serum and hair of their non-stunted counterparts.

Interpretation: Combating zinc deficiency can increase the growth rate of stunted children to that of non-stunted infants in rural Ethiopia. This would appear to be due, at least in part, to reduction in morbidity from infection and increased appetite.

INTRODUCTION

Zinc has long been recognised as an essential micronutrient for health and normal growth, but only in the past 20 years has the manifestation of mild zinc deficiency been documented in man.[1] Zinc is a constituent of a number of enzymes and as such is involved in a large number of metabolic processes. Mild-to-severe zinc deficiency disturbs several biological functions such as gene expression, protein synthesis, immunity, skeletal growth and maturation, gonad development and pregnancy outcomes, and taste perception and appetite.[2] It has been suggested that zinc deficiency may have a role in stunting, especially in developing countries.[3] The assessment of zinc status is hampered by the lack of a single sensitive and specific biochemical factor. At present, the most reliable method to assess zinc status in children would appear to be to measure increase in growth velocity in response to zinc supplementation in physiological amounts.

The early studies in which adolescents with nutritional dwarfism in Egypt[4] and Iran[5] were supplemented with zinc did not show any consistent effect of zinc on linear growth. However, increased growth with zinc supplementation was found in malnourished infants and children.[6–8] Controlled studies in apparently healthy infants and children from developing and affluent countries have shown a positive effect of zinc supplementation on linear growth,[9,10] and also on lean body mass.[11,12]

57% of infants aged 6–11 months in Ethiopia are stunted.[13] Moreover, the dietary pattern is largely cereal and tubers and is low in animal products. Thus suboptimal zinc status is likely to exist in the population. Three earlier studies in sub-Saharan Africa found no effect of zinc supplementation in linear growth in infants and young children.[12,14,15] Therefore, we decided to test whether zinc deficiency is responsible for the low rate of growth of stunted children in Ethiopia. We did a randomised, double-blind, placebo-controlled study in which stunted and non-stunted infants aged 6–12 months were supplemented with zinc (10 mg/day) or a placebo, 6 days a week) for 6 months. Length and other anthropometric factors were measured monthly and the concentrations of zinc in serum and hair was measured at the end of the intervention. Because their incidence has been reported to be reduced by zinc supplementation, information was collected on anorexia[16] and morbidity six days each week.[17–20]

Before deciding on the design of this study, it was important to address the question of whether or not it would be ethical to include a placebo group. Stunting is a serious problem in Ethiopia but its cause is poorly understood. We regarded zinc deficiency as a possible major contributing factor but there were no data on the extent of severity of the problem in the country when the study was being conceived in 1994 and 1995. As stated above, other studies in Africa, both before and after the present study was done, have not shown any effect on growth[12,14,15] although evidence was building up for a role of zinc in growth[9] that was later confirmed.[10] In Ethiopia, no programmes have ever provided zinc supplements to infants or to any other group of the population. The double-blind placebo-controlled trial is probably the most powerful tool we have to examine whether or not a nutrient deficiency is present that is affecting the health or nutritional status of a group of individuals. It is generally agreed that it is ethical to use a design including a placebo group when there is insufficient evidence

to accept or reject the proposed hypothesis and when the individuals enrolled in the study are not being deprived of prophylaxis or treatment. Thus, we regarded the proposed study as ethical and the ethical committee was of the same opinion.

PATIENTS AND METHODS

Study individuals: The study area was in the Dodota Sire district, Arsi zone, central Ethiopia, which is about 150 km east of the capital, Addis Ababa. In this district two working centres, Dheera and Hamude, about 40 km apart with available health facilities and access to all-weather road, were selected. The area lies in the Great Rift Valley of Africa with hot weather and a short rainy season. The staple crops are wheat, maize, sorghum, barley, and tef, which are grown for subsistence not profit. Traditional rearing of animals, mainly cattle and goats, is commonly practiced mainly for income generation.

To select the study individuals, a census of all breastfed infants aged 5-11 months and their parents living within 15 km of the working centres was made. Demographic information was gathered using a pretested structured questionnaire to produce a list of target households. Date of birth of children was established by a local event calendar with information such as anniversaries, festivals, fasting periods, and farming seasons. No clinic cards were available from which dates of birth could be established. All infants were born at home with the assistance of traditional birth attendants, which is usual practice in rural Ethiopia.

The study design was explained to the Zonal Health Department of Arsi, the Administrative Officials of Dodota Sire district, community and religious leaders, and the peasant association leaders. The nature of the study was also fully explained to mothers and oral consent was obtained. Permission for the study was obtained from the ethics committee of the Ethiopian Health and Nutrition Research Institute. Before the study, a clinical examination was done by a physician and stool samples were checked for intestinal parasites, specifically ascaris, amoeba, and hookworm. None of those enrolled in the study had intestinal parasites nor were they dewormed. Although there is some malaria in the area, the few clinical cases of malaria proved negative by microscopic examination of blood smears.

Methods

Among infants who were apparently healthy, looked well, and were free from intestinal parasites and whose mothers were willing to participate in the study, 100 stunted (length-for-age Z score [LAZ]<-2) infants were randomly selected and matched for age and sex with 100 non-stunted (>-2) infants for inclusion in the randomised, double-blind, placebo-controlled study, with ran from August, 1996, to February, 1997. The infants, both stunted and non-stunted, were matched by sex, age (within 2 months), and recumbent length (within 3 cm) and randomly assigned to receive the zinc supplement or placebo (figure 1). The zinc-supplement groups received 10 mg zinc as zinc sulphate in 3 mL syrup and the placebo groups received 3 mL of a syrup without zinc, both prepared by the Pharmacy Department of the Gelderse Vallei Ziekenhuis (Ede, Netherlands). The supplement and placebo were indistinguishable in colour and the slight metallic taste of the supplement was acceptable to the infants. The syrup comprised zinc sulphate heptahydrate (4400 mg), citric acid

monohydrate (450 mg), saccharin sodium (300 mg), vanilla-coconut essence (15 drops), methylparaben concentrate (2 mL), sorbitol, 70% w/v (180 mL), and water to 300 mL. Trained field assistants gave the supplement and the placebo 6 days a week for 6 months. The supplement was given between 07.00 h and 11.00 h after breastfeeding but before any weaning foods were fed. Mothers complied well with this instruction. The field supervisor made spot checks of the field workers and of the households. In addition, information was gathered via the local peasant associations and community leaders as well as from the mothers during the monthly visit of one of the investigators. Neither the field assistants nor the investigator knew the codes. The codes were revealed only after the study was completed and the data analysis was finalised.

All anthropometric measurements were taken at monthly intervals for 6 months by the same investigator. Recumbent length was measured to the nearest 0·1 cm using a length board with an upright wooden base and a moveable headpiece designed by the Division of Human Nutrition and Epidemiology, Wageningen University. Weight was measured to the nearest 10 g by a metal-beam seat balance (Seca, model 725/424, Lamerus, Utrecht, Netherlands) in light clothes. The knee-heel length was measured to the nearest 0·1 mm by an electronic kneemometer (model BK5, Force Institute DK-2605 Brondby, Denmark). Mid-upper-arm circumference (MUAC) was measured to the nearest 0·1 cm with a flexible non-stretch measuring tape, midway between the acromion and the olecranon of the left arm with the arm hanging relaxed, without compressing the tissue. Triceps skinfold was measured to the nearest 0·1 mm with a Harpenden calliper at the same site. For all anthropometric measurements, three independent measurements were taken except knee-heel length, for which five independent measurements were taken. Three measurements on each of two occasions within 1 h from each of 20 infants were taken to measure the reliability of recumbent length, weight, MUAC, and triceps skinfold. The means of the first series of measurements were higher, but not significantly, than the second series of measurements (technical error=$d^2/2n$ where d=difference between measurements; coefficient of variation of all measurements in parentheses): recumbent length, 0·21 cm (0·51 cm, 0·31%); weight, 0·05 kg (0·08 kg, 0·17%); MUAC, 0·04 cm (0·07 cm, 0·14%); triceps skinfold, 0·13 mm (0·22 mm, 0·18%). For the measurements of knee-heel length, five independent measurements were taken and within each series of measurements, the technical error was as high as 14 mm. During the time of knee-heel measurements, the infants were very uncooperative, crying, moving their legs and hands, and were very unstable. It is difficult to keep them lying or sitting quietly even with the help of their mothers. Thus, the reliability could not be assessed. The SD scores for length for age (LAZ), weight-for- age (WAZ), and weight-for-length (WLZ) were calculated using the CASP program, version 3 (CDC, Atlanta, GA, USA). Stunting, underweight, and wasting were defined as LAZ less than –2, WAZ less than –2, and WLZ less than –2 compared with the standards laid down by the National Center for Health Statistics,[21] respectively.

During each daily visit by the field assistants, information was obtained from the child's mother about the presence or absence of symptoms of illness and on the child's appetite. Any child reported to have any symptom of illness was referred to the health centre for clinical examination and, if necessary, for treatment. All clinical examinations and

treatments were recorded. Data on the consistency and frequency of the passage of stools were first obtained from the mothers and then from observation of the stool samples by the field assistants. The mothers were well informed about keeping stool samples for examination during the field assistants' visits. Diarrhoea was defined as the passage of three or more liquid or semi-liquid stools in a 24 h period. Fever was defined as body temperature over 38·5°C at least once in a 24 h period. Data on appetite were collected by asking the mothers whether the child refused to breastfeed, whether the frequency, duration or intensity of breastfeeding was reduced, or whether the frequency or amount of weaning foods consumed was reduced.

At the end of the study, samples of venous blood (5 mL) were collected from those infants whose mothers gave permission, from the cubital vein between 08.00 h and 11.00 h in the nonfasting state. Samples were taken without anticoagulant into Vacutainer tubes (Venojet, Terumo, Belgium), stored in a cool place for 45 min, and then centrifuged at room temperature for 15 min at 1500 g. The serum was then transferred to vials and stored at –20°C in the health centre before being transferred frozen to the Ethiopian Health and Nutrition Research Institute where they were also stored at –20°C. Scalp-hair samples (50–100 mg) were collected from close to the capital portion of the scalp at the end of the study. Only the proximal 1–2 cm of the hair shaft, which reflects the zinc uptake by the follicles during the intervention, was used for the analysis. Serum zinc was analysed[22] by flame atomic absorption spectrometry (Varian Spectra AA 10/20 Plus, Varian Techtron Pty, Mulgrave, Victoria, Australia). Hair samples were washed with non-ionic detergent, rinsed several times with deionised water, dried, and a sample (40–70 mg) digested with trichloroacetic acid and zinc was analysed by flame atomic absorption spectrophotometry.[23] All glassware used for analysis was washed with acid and rinsed with deionised water. Accuracy and precision of the analyses were monitored by replicate analysis of quality control reference material SRM 1598 bovine serum (National Institute of Standards and Technology, Gaithersburg, MD, USA).

Statistical analysis

Before deciding to use parametric tests, we examined the data to ensure that they were not skewed. Descriptive data are expressed as mean (SD) and the results of the intervention as mean (SE). Significance was set at p less than 0·05. All factors, except incidence of anorexia and morbidity, were compared by ANOVA with "whether or not zinc supplemented" or "whether or not stunted" as dependent variables to test for differences among groups and, if significant, by independent *t* test. The significance of the difference in the effect of zinc between the stunted and non-stunted infants was also tested by independent t test. χ^2 analysis was used to test for differences in anorexia and morbidity among the groups. Anthropometric and biochemical indices were correlated with Pearson product-moment correlation and, if one of the indices was not normally distributed, also by rank-order correlation. All analyses were done with SPSS version 7.5.

RESULTS

The number of children recruited in the census was 305 and, of the 50 individuals in each group, complete results over the 6-month period were obtained from 45–47 individuals per group (figure 1). All children in the study were exclusively breastfed for the first 4 months of life. When the intervention commenced, some mothers had begun to provide traditional cereal-based weaning foods. The practice was observed throughout the study period and, among those children who completed the study, 11 (6%) were weaned because of the mother's lack of breast milk. During the study, information on the health of the infants collected by the field assistants was passed to the health centres where diagnoses were confirmed and recorded and where treatment was done without reference to whether the infant was receiving the zinc supplement or not. No cases of malaria, based on examination of thick blood films, were recorded and no antimalarials were issued. Medicines prescribed included oral antibiotics (ampicillin), antihelminthics (piperazine), aspirin, and chloramphenicol eye drops. There were no differences between the groups in age or between the zinc and placebo groups of the stunted and non-stunted groups with respect to all the factors listed in table 1. Compared with non-stunted infants, the stunted infants were shorter (about 5 cm), lighter (about 1 kg), had smaller mid-upper- arm circumference (about 1 cm) indicating less muscle and fat mass, and had smaller triceps skinfold thickness (about 0·3 mm) indicating less body fat. This was reflected in lower WAZ and higher WLZ. There were no differences between the mothers of stunted and of non-stunted infants (table 1). The proportion of mothers with desirable body weight (body mass index >18·5 kg/m^2) was 66·3% while those with mild (18·4–17·0 kg/m^2), moderate (16·9–16·0 kg/m^2 and severe (<15·9 kg/m^2) chronic energy deficiency were 21·7%, 3·8%, and 8·2%, respectively.

In stunted children, growth (increase in length) over the 6 month period was 2·5-fold higher with zinc supplementation than without (7·0 [1·0] vs 2·8 [0·9] cm) while in non-stunted children length increased only 20% (6·0 [0·9] vs 5·0 [0·8] as shown in table 2 and figure 2. Growth in the zinc-supplemented stunted group was about 6% greater (not significant) than in their non- stunted counterparts. The stimulatory effect of zinc in the stunted children was significantly greater than in the non-stunted children ($p<0·0001$). In fact, growth was sufficient in the zinc-supplemented stunted group to allow the LAZ scores to increase over the intervention period. No other groups could achieve this goal. Growth rates were constant over the 6 months of the study (figure 2). There were no differences in growth between boys and girls in each of the groups (data not shown). In addition, there were no differences between younger (<50th percentile, 9·75 months at the start of the intervention) and older infants except in the non-stunted group where the younger infants grew 10% more than their older counterparts. No differences in the change in knee-heel length over the period of the interventionwere seen among the four groups. Zinc supplementation increased the weight gain over the 6 months by about 20% in the non-stunted group and by about 80% in the stunted group. The increase in weight gain in the zinc supplemented stunted group was sufficient to give a positive change in WAZ scores (table 2). The changes in mid-upper-arm circumference and triceps skinfold thickness tended to follow the weight changes but did not reach significance. Thus with those infants given the placebo, the stunted children

deteriorated markedly in WLZ scores while with infants given zinc supplements, the stunted children deteriorated somewhat less in WLZ score.

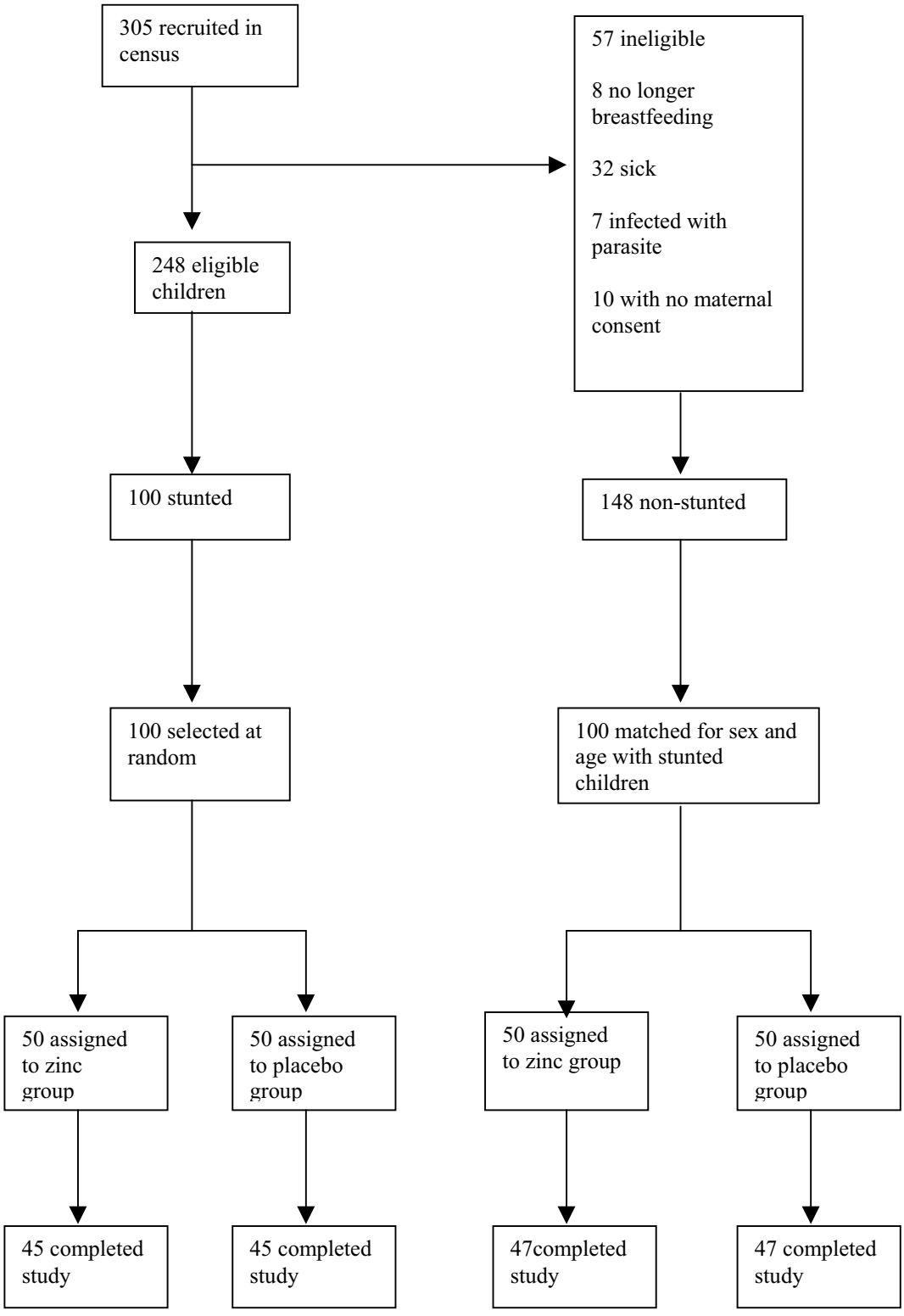

Figure 1: **Trial profile**

	Stunted		Non-stunted	
	Zinc	Placebo	Zinc	Placebo
Infants				
n (M/F)	45 (24/21)	45 (24/21)	47 (22/25)	47 (22/25)
Age (months)	9.5 (2.0)	9.7 (2.0)	9.3 (2.1)	9.2 (2.0)
Length (cm)*	64.6 (3.2)	64.4 (3.2)	69.6 (3.5)	69.9 (3.2)
Knee-heel length (mm)*	168 (12)	165 (11)	179 (11)	180 (10)
Weight (kg)*	6.6 (0.9)	6.4 (0.9)	7.5 (0.9)	7.4 (0.8)
Mid-upper-arm circumference (cm)†	12.3 (1.3)	12.1 (1.5)	13.1 (1.1)	12.9 (1.1)
Triceps skinfold thickness (mm)†	5.3 (0.5)	5.3 (0.7)	5.6 (0.4)	5.6 (0.3)
Z-Scores				
Length for-age *	-2.74 (0.59)	-2.87 (0.60	-0.70 (0.76)	-0.57 (0.74)
Weight-for-age*	-2.46 (0.69)	-2.70 (1.01)	-1.35 (0.75)	-1.45 (0.88)
Weight-for-length ‡	-0.48 (1.05)	-0.69 (1.24)	-1.00 (0.79)	-1.27 (0.98)
Mothers				
Age (years)	25.8 (5.9)	27.1 (6.4)	26.2 (5.3)	25.4 (5.3)
Height (cm)	154.7 (6.4)	153.3 (6.8)	154.3 (5.4)	153.4 (5.4)
Weight (kg)	47.3 (4.8)	46.4 (5.2)	47.7 (5.8)	44.9 (4.8)
Mid-upper-arm-circumference (cm)	23.7 (3.3)	22.8 (1.9)	23.5 (2.3)	22.6 (1.9)
Triceps skinfold thickness (mm)	7.1 (1.8)	6.9 (1.8)	7.0 (1.9)	6.3 (1.8)
Body mass index (kg/m^2)	19.8 (1.9)	19.8 (2.0)	20.0 (2.2)	19.1 (2.0)

Data are means (SD).

Significant difference between stunted and non-stunted groups by independent t test:
*P<0.001; †P<0.05; ‡P<0.01.

Table 1: **Characteristics at baseline of infants and mothers who completed the study**

	Stunted		Non-stunted	
	Zinc	Placebo	Zinc	Placebo
n (M/F)	45 (24/21)	45 (24/21)	47 (22/25)	47 (22/25)
Length (cm)	7.0 (1.0)*	2.9 (0.9)	6.6 (0.9) †	5.0 (0.8)
Knee-heel length (mm)	21.3 (9.6)	21.4 (7.3)	21.9 (6.1)	19.8 (6.3)
Weight (kg)	1.73 (0.39)*	0.95 (0.39)	1.19 (0.39) ‡ §	1.02 (0.32)
Mid-upper-arm circumference (cm)	0.4 (1.3)	0.3 (1.4)	0.1 (1.2)	0.0 (1.1)
Triceps skinfold thickness (mm)	0.1 (1.3)	0.2 (1.1)	-0.2 (1.2)	-0.5 (0.3)
Z-Scores				
Length for-age	0.14 (0.46)*	-1.24 (0.60	-0.18 (0.32)* §	-0.74 (0.42) §
Weight-for-age	0.36 (0.49)*	-0.28 (0.56)	-0.31 (0.49) §	-0.46 (0.55)
Weight-for-length	-0.17 (0.55) †	-0.26 (0.54)	-0.43 (0.50) ‡ ‖	-0.17 (0.47) §

Data are means (SE).

Significant difference by independent t test: with comparable group - * P< 0.001; †P<0.01; ‡P<0.05; with comparable stunted group.- § P <0.001; ‖ P < 0.05.

Table 2: **Changes in anthropometric variables during the intervention**

It was not possible to take blood or hair samples before the intervention period but blood samples were obtained from 25 children from each group at the end of the intervention. Then the mean concentration of zinc in serum ($p<0{\cdot}0001$) and hair ($p=0{\cdot}02$) was lower in the placebo stunted group than in the zinc-supplemented stunted group (table 3). The groups supplemented with zinc had higher concentrations of zinc in serum and hair with the most pronounced differences being seen in serum zinc concentrations and between the stunted groups. The concentrations of zinc in serum and hair were positively associated with increased growth ($r=0{\cdot}59$, $p<0{\cdot}0001$, and $r=0{\cdot}18$, $p=0{\cdot}04$, respectively). Because the values for serum zinc concentration were not normally distributed, the rank-order correlation was also calculated for this relationship ($r=0{\cdot}59$, $p<0{\cdot}0001$). Boys tended to have higher serum zinc concentrations than girls (data not shown). Zinc supplementation reduced the incidence of anorexia, cough, diarrhoea, fever, and vomiting in the stunted children by more than half ($p<0{\cdot}0001$) from 3·38 episodes per child in the placebo group to 1·56 episodes per child in the supplemented group (table 4). The incidence in both non- stunted groups was comparable to that of the zinc-supplemented stunted infants.

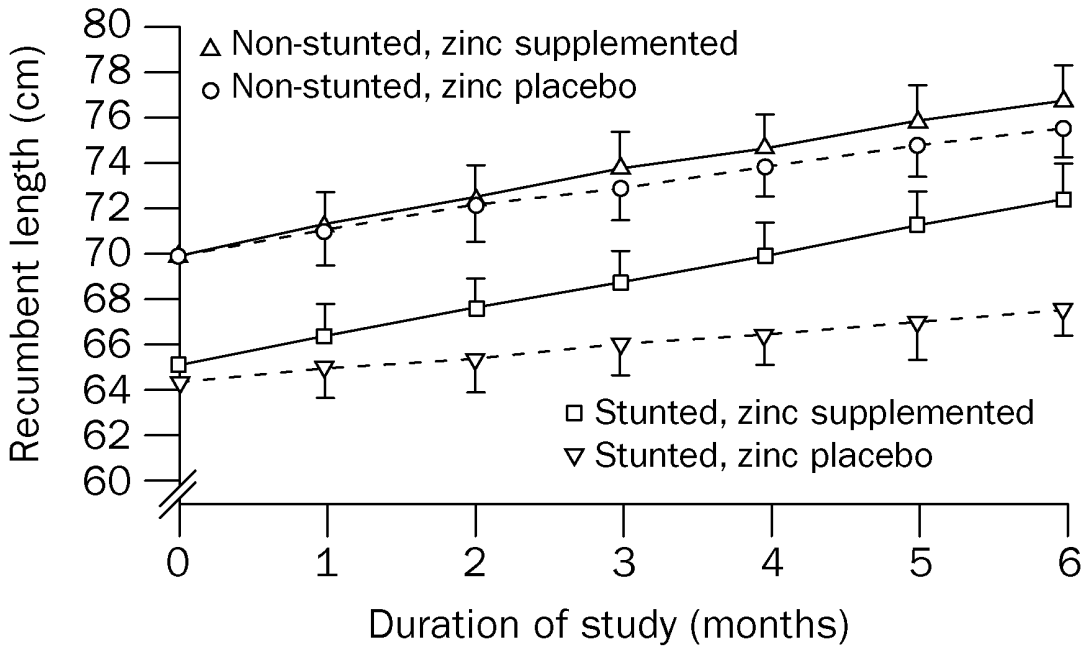

Figure 2: **Recumbent length during the intervention period**
Bars are SE of the mean.

	Stunted		Non-stunted	
	Zinc	Placebo	Zinc	Placebo
Serum				
n (M/F)	25 (13/12)	25 (13/12)	25 (11/14)	25 (11/14)
Serum zinc (μmol/L)	15.8 (3.7)*	11.0 (1.9)	17.9 (5.0) ‡	14.5 (2.1) §
Hair				
n (M/F)	35 (17/18)	35 (17/18)	34 (16/18)	34 (16/18)
Hair zinc (μmol/g)	1.38 (0.42) †	1.16 (0.44)	1.57 (0.51)	1.43 (0.47) ‖

Data are means (SD).

Significant difference using independent t test: with comparable group - * P< 0.001; †P<0.05; ‡P<0.01; with comparable stunted group - § P <0.001; ‖ P < 0.05.

Table 3: **Concentration of zinc in serum and hair at the end of the intervention period**

	Stunted		Non-stunted	
	Zinc	Placebo	Zinc	Placebo
Symptoms				
Anorexia	3*	15	0	4
Cough	15*	32	12	21§
Diarrhoea	13†	40	14	19‖
Fever	27†	41	18	21‖
Vomiting	12‡	24	8	12
Total	70‡	152	52	77‖
Total/child	1.56†	3.38	1.11	1.64‖

Significant difference by χ^2 analysis: with comparable placebo group - * P< 0.05; †P <0.001; ‡P<0.01; with comparable stunted group. - § P <0.05; ‖ P < 0.01.

Table 4: **Incidence of anorexia and selected illnesses during the 6 mo intervention period**

DISCUSSION

This study clearly shows that zinc supplementation can halt the stunting process in stunted infants in rural Ethiopia (table 2, figure 2). This would appear to be due, at least in part, to improved appetite, as judged by recording episodes of anorexia, and reduced morbidity from gastrointestinal and respiratory disease (table 4).

During the 6-month intervention period, the LAZ score increased 0·14 thus suggesting that the catch-up growth during this period was 7% of the 2·04 LAZ deficit at baseline (LAZ of the non-stunted infants, –0·70 minus LAZ of their stunted counterparts, –2·74). Thus, although zinc supplementation can halt the process of stunting, it would take a long time to overcome the length deficit of the stunted infants. Earlier studies of infants and young children in sub-Saharan Africa [12,14,15] showed no effect of zinc supplementation on linear growth. In fact, the increase in LAZ reported here was greater than that observed in most if

not all previous studies.[10] This can be attributed not only to the severity of the zinc deficiency (44% of the non-supplemented stunted group had serum zinc concentrations <10·7 μmol/L) but also to the dose (10 mg/day), frequency of dosing (6 days a week) and age of the infants (6–12 months at onset). In the non-stunted infants, zinc supplementation also resulted in increased linear growth compared with the placebo group, but the effect was less pronounced. This suggests that the beneficial effect of zinc supplementationon growth is related to the degree of stunting, and of zinc deficiency. In those infants not supplemented with zinc, the concentration of zinc in both serum and hair was lower in the stunted children than in their non-stunted counterparts. Zinc supplementation of stunted children resulted in higher concentrations of zinc in both serum and hair bringing the concentrations in line with those in the non-stunted infants (table 3).

In this study, zinc supplementation also increased weight gain in the stunted children and, to a lesser extent, in the non-stunted children. In fact, the increase in WAZ over the 6 month intervention period in the stunted children (0·36) was 32% of the initial WAZ deficit (1·11) compared with 7% of the initial LAZ deficit. Such increases in weight have been reported earlier.[9–12]

Earlier studies have also reported changes in body composition after zinc supplementation.[11,12] Triceps skinfold thickness and mid-upper-arm circumference did not change in the present study. It may be that the supplementation period was too short to find any measurable effects on body composition.

No significant difference was observed in knee-heel length among the groups. This was surprising, as kneeheel length has been reported to be a sensitive and precise measurement of short-term linear growth in infants.[24] However, we found it very difficult to measure knee-heel length because the infants were somewhat restless. Thus the low reproducibility of our measurements is a more likely explanation than that the increase in length was restricted to growth in the femur and trunk.

The marked effect of zinc supplementation on reducing morbidity in stunted infants (table 4) has been observed earlier [16–20] and is analogous to the effects observed with vitamin A supplementation.[25] We are now beginning to understand the role of zinc in immune function.[26] Although some of the effects of zinc are related to its interaction with vitamin A,[27] zinc is unique in its affect on appetite.[16] In part, this could be related to taste perception [28] but is probably an adaptation to preventing the adverse effects of cell proliferation in the absence of sufficient zinc.[29]

The serum concentration of zinc is the most commonly used indicator of zinc status[30] although it is influenced by a variety of factors, such as infection, stress, pregnancy, and growth velocity, which limit its diagnostic value. However, as mentioned earlier, about 44% of the non-supplemented stunted group had serum zinc concentrations below 10·7 μmol/L. This proportion was zero in the zinc-supplemented stunted group as it was in both non-stunted groups. The concentration of zinc in hair is generally regarded as a good indicator of zinc status in children providing that care is taken with sampling, washing, and analysis. The effect of confounding factors such as season and age need to be taken into account when interpreting results. The proportion of infants with hair zinc concentrations below 1·07 μmol/g was higher in the non-supplemented stunted infants (43%) than in their non-stunted

counterparts (21%). Zinc supplementation reduced these proportions to 26% and 11%, respectively.

That zinc concentration in both serum and hair can be good indicators of status is indicated by the positive association of linear growth with the concentration of zinc in serum and in hair in the supplemented stunted infants. Similar results have been reported earlier [7,31] but not in all studies.[11]

Our results provide clear evidence that stunted infants in rural Ethiopia are in need of zinc supplementation to halt the stunting process. It is also evident that prolonged zinc supplementation would be essential for adequate catch-up growth, but other nutrients may become a growth-limiting factor during supplementation. Zinc supplementation in these stunted infants also improved health possibly by stimulating appetite and reducing morbidity of several infant diseases. This is the first study showing the existence of a serious zinc deficiency in rural Ethiopia and the results ask for an immediate national public-health policy on zinc supplementation.

Contributors: The study was designed jointly by M Umeta, C E West, P Deurenberg, and J G A J Hautvast. The fieldwork was executed under the supervision of M Umeta with a major input by J Haidar and supervisory visits by C E West and P Deurenberg. The data was analysed by M Umeta, C E West, and P Deurenberg. All authors contributed to writing the paper.

Acknowledgments

We thank the children and parents who participated in the study and the health workers of Dheera Health Centre and Hamude Clinic who assisted in the study. Thanks are also due to: the Arsi Zone Health Department and the Dodota-Sire district administration for granting permission for the study and for facilitating its execution; Wasse Dubale, Asfaw Hussen, Wolde-Mariam Girma, Abraham H/Mariam, Shibabaw Adgeh, Yimam Negatu, Demelash Haile Meskel of the Ethiopian Health and Nutrition Research Institute for assistance with field work and laboratory analysis; Geert van der Meer of the Pharmacy of Ziekenhuis Gelderse Vallei for supplying the zinc and placebo syrups; Jan Burema and Grietje van der Zee of Wageningen University for statistical advice and logistical support. The study was supported by a grant from The Nestlé Foundation for the Study of the Problems of Nutrition in the World.

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Long-term effects of zinc supplementation on growth of children in rural Ethiopia: a randomised controlled trial.

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(Submitted for publication)

Summary

Background: We have shown earlier that zinc supplementation in stunted Ethiopian infants leads to increased growth. Thus, the aim of this study was to investigate whether the effect persists after supplementation is discontinued.

Methods: Stunted (n = 100; height-for-age z-score, HAZ <-2 SD) and non-stunted (n = 100; HAZ ≥-2 SD) symptom-free breast-fed infants (aged 6-12 mo) were matched by sex and age, and randomly assigned to supplementation with either zinc (10 mg/d as sulphate) or placebo, 6 d/wk for 6 mo. Anthropometric measurements were taken monthly during the intervention, and 42 mo after randomisation. Additional data were collected on the occurrence of illness and appetite, zinc concentrations in serum and hair, and anthropometric indices in siblings born subsequently to randomisation.

Findings: Thirty-six months after discontinuing supplementation, the effect of zinc supplementation of stunted infants on height was –3.4 cm (95%CI: –4.8 to –2.1) and on HAZ was –0.86 (95%CI: –1.26 to –0.52). More than half of stunted children had deficient serum and hair zinc concentrations, irrespective of whether they had been previously supplemented with zinc. Siblings of stunted infants were shorter, and had lower HAZ and weight-for-age z-score than siblings of non-stunted counterparts.

Interpretation: Beneficial effects obtained during zinc supplementation in stunted infants on linear and ponderal growth are reversed once supplementation is discontinued. Poor feeding practices might explain why siblings of stunted infants are more likely to be stunted than those of non-stunted counterparts.

Introduction

Zinc deficiency is widespread in children in developing countries because they consume diets low in animal products and high in phytate. In children, it leads to poor growth, [1,2] impaired immunity, increased morbidity from common infectious diseases[3] and increased mortality.[4]

Assessment of the prevalence of zinc deficiency is difficult because of the lack of adequate indicators of zinc status.[5] Although combinations of biochemical and functional indicators are usually used, these do not indicate zinc demand at the tissue level.[6] Currently, the most reliable method to assess zinc deficiency in children is to show a response to zinc supplementation on health outcomes such as growth in a randomised, placebo-controlled trial.[2] Such trials have shown that zinc supplementation can reverse adverse effects of zinc deficiency on growth (length or height and/or weight),[2] body composition[7-9] and morbidity[4,10] in symptom-free stunted infants and children, malnourished infants and children recovering from protein-energy malnutrition.[11,12] Three earlier studies in sub-African countries found no effect of zinc supplementation in growth in infants and young children.[7-9] Recently, we reported that zinc supplementation resulted in enhanced linear growth and weight gain and reduced frequency of occurrence of anorexia and morbidity in stunted children in rural Ethiopia.[1]

However, the question arises whether effects of zinc supplementation persist after discontinuation of the intervention. We therefore re-examined children taking part in our earlier trial,[1] with the aim to assess whether gains attained in linear growth persisted 3 y after they received their last supplement. We also included new siblings born subsequent to the start of this trial, and analysed the extent to which their anthropometric measurements were associated with those of the children originally taking part.

Subjects and methods

Details on the population and the design of the intervention trial have been reported previously [1] and will be summarised below.

Study area

The study was performed in rural areas in Dodota-Sire District, Arsi Zone, Ethiopia. In the study area, two villages (Dheera and Hamude) 40 km apart were arbitrarily selected. Both villages have health facilities and access to all-weather roads. The climate of the area is hot with the main rainy season occurring in July and August. Local staple crops are wheat, maize, sorghum, barley and tef. Traditional rearing of cattle and goats is commonly practised as a means of generating cash income.

Population and supplements

From a population of 22,100 inhabitants, a census of all infants aged 5-11 mo was made and parents were invited to bring those infants for screening as described earlier[1] (Figure 1). Of the 248 eligible infants, 110 children were stunted infants (height-for-age z-score, HAZ <-2 SD) and 138 were not stunted (HAZ ≥-2 SD) (in our initial paper[1], it was erroneously stated

that 100 were stunted and 148 non-stunted). From the non-stunted infants, 100 were individually matched for age (within 2 months) and sex with an equal number of stunted infants for inclusion in a randomised, double-blind, placebo-controlled zinc supplementation trials for 6 mo. Within the stunted and non-stunted groups thus obtained, infants were randomly and blindly allocated, using a table with random numbers generated by a statistician independent of the research group, to receive supplements with either zinc (10 mg as sulphate in a syrup) or its placebo (the syrup without zinc sulphate). The supplement with the active ingredient and placebo were indistinguishable in colour and flavour. The supplement was given 6 d/wk between 07:00 h and 11: 00 h at least 1 h after breast feeding but before any weaning food was fed. Supplementation was masked to the participants and all field staff and supplements were provided in bottles colour coded at production (two colours for the supplement and two for the placebo). Supplementation continued for 6 mo, whereupon the results were analysed per group, after which the codes were revealed.[1] At 42 mo after the baseline (36 mo after discontinuation of supplementation) the effects were re-assessed. All new siblings born after randomisation from the same families of the study subjects were recorded and also examined at 42 mo after the start of the intervention period.

The study protocol was explained in the local language (Oromiffa) to each mother, and oral consent was obtained. Permission for the study was obtained from the Ethiopian Health and Nutrition Research Institute medical-ethical committee.

Anthropometry

Anthropometric measurements were taken at baseline, at monthly intervals during the supplementation period of 6 mo and at 42 mo. At each of these periods, length or height, weight, mid-upper-arm circumference (MUAC) and triceps skinfold thickness were measured for each of the child. Recumbent length or height was measured to the nearest 0.1 cm using a length board with the child not wearing shoes. Weight was measured to the nearest 10 g by a metal-beam seat balance (Seca, model 725/424, Lamerus, Utrecht, Netherlands) in light clothes and for older children to the nearest 0.1 kg by an electronic scale (Tefal, model SC 3218, Rumilly, France) in light clothes without shoes. MUAC was measured to the nearest 0.1 cm with a flexible non-stretch measuring tape on the left arm and triceps skinfold thickness was measured at the same site to the nearest 0.1 mm by a Harpenden calliper (John Bull British Indicator Ltd., London, UK). For all anthropometric measurements, three replicate measurements were taken by the same investigator (MU) throughout the study to reduce inter-examiner error. The standardisation procedures used to increase accuracy for anthropometric measurements have been described previously.[1]

Blood collection and analysis

Samples of blood (<5 mL) were collected from the cubital vein between 08:00 h and 11:00 h in a non-fasting state at 6 mo after supplementation and at 42 mo after baseline. In some cases, the amount of blood collected was insufficient for analysis. Samples were taken without anticoagulant into tubes (Venoject, Terumo NV, Leuven, Belgium), kept for 45 min at ambient temperature and protected from light, and then centrifuged at room temperature for 15 min at 1500 x g. Serum samples were transferred to cryovials and stored at –20°C until

subsequent analysis. Proximal scalp-hair samples (1-2 cm long) were collected from the occipital portion of the scalp using stainless steel scissors and kept in plastic tubes and stored at -20°C until biochemical analysis.

Serum zinc concentration was determined by flame atomic absorption spectrometry (Varian Spectra AA 10/20 Plus, Varian Techtron, Pty, Ltd, Mulgrave, Vic, Australia). [13] Hair samples were washed with non-ionic detergents, rinsed several times with deionised water, dried, and a sample (40-70 mg) digested with trichloroacetic acid prior to the measurement of the zinc concentration by flame atomic absorption spectrometry.[14] All glassware used for analysis was washed repeatedly with acid and rinsed with deionised water. Serum retinol concentration was measured by high pressure liquid chromatography.[15] Accuracy of the analysis for the concentrations of zinc and retinol in serum were monitored by replicate analysis of quality control material SRM 1598 bovine sera and SRM 968C human sera (National Institute of Standards and Technology, Gaithersburg, MD, USA) respectively.

Statistical analysis

Z-scores of height-for-age, weight-for-age (WAZ) and weight-for-height (WHZ) were calculated from the National Centers for Health Statistics (NCHS) growth chart[16] using CASP software (Centers for Disease Control and Prevention, version 3, Atlanta, GA, USA). Being underweight and wasted were defined as WAZ <-2 SD and WHZ < -2 SD below median NCHS values respectively. All other analyses were carried out using SPSS software (SPSS Inc, version 8.2, Chicago, IL, USA). Komolgorov-Smirnov tests were used to check whether outcome variables were normally distributed. Group differences in anthropometric indices and concentrations of zinc in serum and hair were calculated assuming t-distributions. Multiple linear regression analysis was used to determine treatment effects on height, weight, MUAC, triceps skinfold thickness, HAZ, WAZ and WHZ (all adjusted for baseline measurements) and to determine directly whether these effects differed between stunted and non-stunted children.

Results

Compliance with respect to consumption of the supplements was >98%. Data are presented for children for whom data were available at 42 mo after baseline (figure 1). The stunted and non-stunted infants receiving either zinc or placebo were similar regarding anthropometric indices at baseline (table 1).

After the 6 mo of supplementation, stunted infants who received zinc were 4.1 cm longer and 0.7 kg heavier than their peers who received placebo, while non-stunted infants receiving zinc were 1.6 cm taller than their counterparts receiving placebo (table 2). The effect of zinc supplementation on HAZ and WAZ was also significantly greater in stunted than in non-stunted infants (table 2). After the 6-mo supplementation, blood and hair samples were collected from 78 and 117 infants respectively. Mean serum zinc concentrations in stunted infants receiving zinc were 5.1 μmol/L higher than in their peers receiving placebo, whereas this effect was less (3.5 μmol/L) in non-stunted children. Similarly, zinc

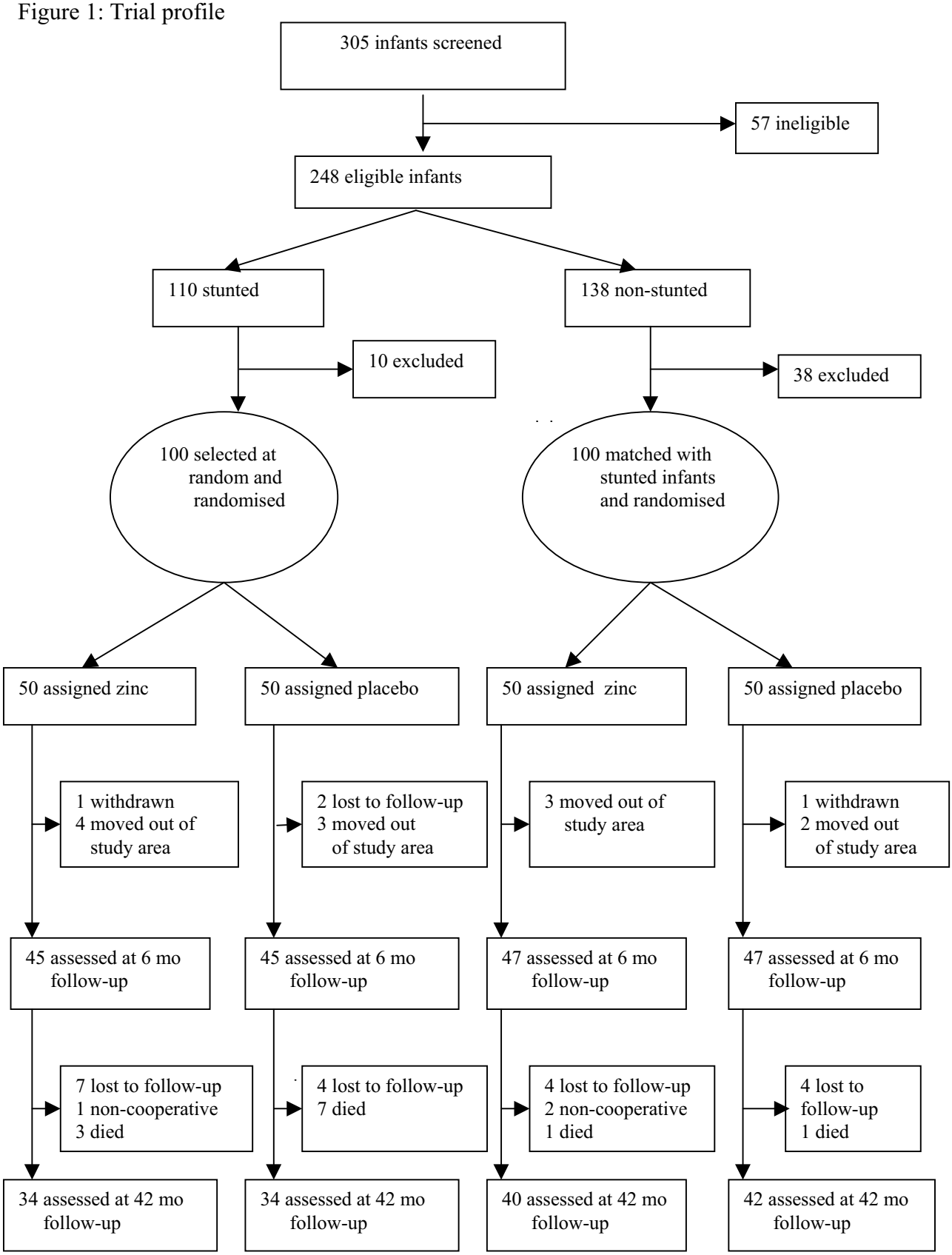
supplementation led to 0.33 μmol/g higher hair zinc concentration in stunted infants, compared with 0.12 μmol/g in non-stunted infants.

At 42 mo after randomisation, there were no marked differences between children who had been supplemented with zinc or placebo in any of the anthropometric parameters of either stunted or non-stunted children (table 1). Thus, the linear growth and weight increase in the 36 mo following supplementation of stunted children who had received zinc was 3.4 cm (95% CI: 2.1 to 4.8) and 0.8 kg (95% CI: 0.0 to 1.6) less respectively than their counterparts who had received placebo (table 2). These differences were reflected in the changes in HAZ and WAZ. In the same time period, there were no such differences between the non-stunted children who had been supplemented or not. In addition, there was no effect of zinc supplementation at 36 mo after supplementation in stunted children on MUAC or triceps skinfold thickness (-0.09 cm and -0.06 mm respectively).

At 42 mo after baseline, there were no differences in zinc concentrations in serum and hair between stunted children who had been supplemented with zinc or with placebo. This was also true for the non-stunted children (table 3). Compared with the values at the end of the supplementation, zinc concentrations in serum and hair decreased by 33% and27% respectively in stunted children who had been supplemented with zinc, while the decreased in the non-stunted children was 23% and 11% respectively, For the stunted children who had been supplemented with zinc, there was also a marked increase in the proportions of children with low concentrations of zinc in serum (<10.7 μmol/L) and hair (<1.07 μmol/g) over the 36-mo period after supplementation had ceased. At 42 mo after baseline, the proportion of children with serum retinol concentrations regarded as marginal (<0.70 μmo/L) was, on average, 24%, indicating a public health problem of vitamin A deficiency.

Mothers of 134 of the 150 children for whom data are reported were seen 42 mo after baseline: 4 mothers had died, 8 were absent for social reasons and 4 were reported to be sick from malaria. The mothers seen had 138 subsequent children who were still alive of whom 112 were first siblings and 26 were second siblings. Siblings from families of stunted infants were shorter, and had lower height-for-age and weight-for-age z-scores than siblings from their non-stunted counterparts (table 4).

Figure 1: Trial profile



	Stunted		Non-stunted	
	Zinc	Placebo	Zinc	Placebo
n	34	34	40	42
Height (cm)				
Baseline	64.9±0.6	64.4±0.6	69.8±0.5	69.9±0.5
6 mo	72.0±0.6	66.9±0.6	76.4±0.5	74.9±0.5
42 mo	91.3±0.3	89.7±0.4	103.9±0.2	102.8±0.2
Weight (kg)				
Baseline	6.6±0.1	6.4±0.2	7.5±0.1	7.4±0.1
6 mo	8.4±0.1	7.3±0.2	8.7±0.1	8.4±0.1
42 mo	14.0±0.1	13.8±0.4	16.9±0.1	16.6±0.1
Mid-upper arm circumference (cm)				
Baseline	12.3±0.2	12.3±0.2	13.1±0.2	12.9±0.2
6 mo	12.8±0.2	12.6±0.2	13.2±0.1	12.9±0.2
42 mo	13.1±0.2	12.9±0.1	14.4±0.1	14.1±0.1
Triceps skinfold thickness (mm)				
Baseline	5.3±0.1	5.4±0.1	5.6±0.1	5.6±0.1
6 mo	5.7±0.1	5.5±0.1	5.8±0.1	5.7±0.1
42 mo	5.8±0.2	5.7±0.2	6.1±0.2	5.9±0.1
Height-for-age z-score				
Baseline	-2.74±0.10	-2.78±0.01	-0.62±0.12	-0.60±0.11
6 mo	-2.55±0.14	-3.56±0.11	-0.79±0.11	-1.05±0.21
42 mo	-3.21±0.06	-3.33±0.05	-1.03±0.11	-1.15±0.10
Weight-for-age z-score				
Baseline	-2.55±0.10	-2.61±0.17	-1.31±0.11	-1.51±0.13
6 mo	-2.12±0.13	-2.94±0.13	-1.61±0.10	-1.66±0.10
42 mo	-2.53±0.06	-2.73±0.06	-1.64±0.06	-1.71±0.11
Weight-for-height z-score				
Baseline	-0.63±0.18	-0.65±0.22	-1.02±0.13	-1.31±0.16
6 mo	-0.76±0.17	-0.33±0.21	-1.43±0.10	-1.47±0.13
42 mo	-1.14±0.08	-1.15±0.10	-1.33±0.08	-1.45±0.08

Mean ± SE

Table 1: Anthropometric indices of stunted and non-stunted children supplemented for 6mo with zinc or placebo

	Stunted children (n=68)		Non-stunted children (n=82)	
	Estimate	(95%CI)	Estimate	(95%CI)
Height (cm)				
At 6 mo	4.1	(3.5 to 4.7)	1.6	(1.3 to 2.0)
At 42 mo	1.6	(0.8 to 2.4)	0.7	(-0.1 to 1.5)
Change (mo 42 – mo 6)*	-3.4	(-4.8 to -2.1)	-0.5	(-1.77 to 0.69)
Weight (kg)				
At 6 mo	0.7	(0.5 to 0.9)	0.2	(0.1 to 0.4)
At 42 mo	0.2	(-0.6 to 0.9)	0.1	(-0.2 to 0.4)
Change (mo 42 – mo 6)†	-0.8	(0.0 to 1.6)	-0.2	(-0.51 to 0.18)
Mid-upper arm circumference (cm)				
At 6 mo	0.1	(-0.4 to 0.6)	0.3	(-0.1 to 0.6)
At 42 mo	0.2	(-0.2 to 0.6)	0.1	(-0.2 to 0.4)
Change (mo 42 – mo 6)‡	-0.09	(-0.74 to 0.55)	-0.2	(-0.64 to 0.29)
Triceps skinfold thickness (mm)				
At 6 mo	0.1	(-0.2 to 0.4)	0.1	(-0.1 to 0.2)
At 42 mo	0.1	(-0.3 to 0.6)	0.1	(-0.3 to 0.5)
Change (mo 42 – mo 6) §	-0.06	(-0.55 to 0.41)	0.04	(-0.37 to 0.45)
Height-for-age z-score				
At 6 mo	0.87	(0.64 to 1.11)	0.59	(0.43 to 0.77)
At 42 mo	0.39	(0.25 to 0.54)	0.28	(-0.03 to 0.59)
Change (mo 42 – mo 6) ¶	-0.88	(-1.26 to -0.52)	-0.13	(-0.61 to 0.33)
Weight-for-age z-score				
At 6 mo	0.71	(0.46 to 0.96)	0.25	(0.07 to 0.43)
At 42 mo	0.17	(0.00 to 0.34)	-0.15	(-0.39 to 0.10)
Change (mo 42 – mo 6) **	-0.63	-0.99 to -0.27)	0.01	(-0.35 to 0.37)
Weight-for-height z-score				
At 6 mo	-0.43	(-0.67 to 0.20)	-0.15	(-0.33 to 0.03)
At 42 mo	- 0.05	(-0.29 to 0.19)	0.11	(-0.09 to 0.32)
Change (mo 42 – mo 6) ††	0.44	(-0.08 to 0.96)	0.08	(-0.27 to 0.43)

Estimates of the effect of zinc supplementation obtained by multivariate regression analyses, adjusted for initial values determined at baseline

When testing for differences between effects determined in stunted and non-stunted children, the p-values were <0.001*, 0.043†, 0.873‡, 0.838§, 0.003¶, 0.002** and 0.151††

Table 2: Effect of zinc supplementation in stunted and non-stunted children at 6mo and 42 mo after the start of the supplementation

	Stunted			Non-stunted		
	Zinc	Placebo	Mean difference [95% CI]	Zinc	Placebo	Mean difference [95%CI]
Serum zinc concentration (μmol/L)						
At 6 mo*	16.5±1.1 (0)	11.4±0.3 (40)	5.1[2.9, 7.2]	17.9±1.0 (0)	14.3±0.5 (0)	3.5 [1.2, 5.8]
42 mo†	11.1 ± 0.5 (56)	10.9 ± 0.4 (52)	0.2 [-1.1, 1.5]	13.7 ± 0.5 (7)	12.9 ± 0.4 (10)	0.8 [-0.5, 2.1]
Hair zinc concentration (μmol/g)						
At 6 mo‡	1.43±0.07 (17)	1.10±0.08 (55)	0.33 [0.10, 0.55]	1.55±0.09 (10)	1.43±0.08 (14)	0.12 [-0.12, 0.37]
At 42 mo§	1.04 ± 0.04 (57)	1.05 ± 0.05 (53)	-0.01 [-0.15, 0.12]	1.74 ± 0.10 (7)	1.81± 0.09 (8)	-0.07 [-0.36, 0.21]
Serum retinol concentration (μmol/L)						
At 42 mo¶	0.79 ± 0.05 (31.6)	0.75 ± 0.04 (47.8)	0.1[-0.9, -0.1]	0.8 ± 0.04 (30)	0.9 ± 0.06 (27.3)	-0.1[-0.2, 0.0]

Mean ± SE. Values in parentheses indicate proportion deficient. Deficiencies are defined as: serum zinc concentration <10.7 μmol/L; hair zinc concentration < 1.07 μmol/g; serum retinol concentration < 0.70 μmol/L.
* n = 15, 20, 23 and 20 respectively. †n = 29, 27, 30 and 31 respectively. ‡n = 18, 23, 30 and 31 respectively. §n = 27, 29, 28 and 35 respectively.
¶ n = 19, 23, 50, and 33 respectively.

Table 3: Effect of zinc supplementation on indicators of zinc and vitamin A status in stunted and non-stunted children at 6 mo and 42 mo after the start of intervention

Anthropometric index measured in the siblings	Effect	(95% CI)	P value
Height (cm)	-2.3	(-3.4 to -1.3)	<0.001
Weight (kg)	-0.1	(-0.4 to 0.6)	0.64
Mid-upper arm circumference (cm)	-0.2	(-0.4 to 0.8)	0.46
Triceps skinfold thickness (mm)	-0.4	(-0.9 to 0.1)	0.07
Height-for-age z-score	-1.28	(-1.51 to -1.04)	<0.001
Weight-for-age z-score	-0.40	(-0.67 to -1.32)	0.004
Weight-for-height z-score	-0.26	(-0.56 to 0.05)	0.10

Mean ± SE.

*First-born siblings of stunted infants (n = 47) and non-stunted infants (n = 65). Age of children, 24.6 ± 0.7 mo. Values adjusted for height of mother, age of both children participating in the trial and of first-born siblings

Table 4: Effects of being stunted or not at baseline in infants participating in the randomised trial on anthropometric indices measured in their first-born siblings

Discussion

This study demonstrates that the effects we reported previously[1] on linear and ponderal growth of stunted infants after 6 mo of zinc supplementation are no longer seen 36 mo after the supplementation is discontinued. In fact the gains in linear and ponderal growth in both the stunted and non-stunted children during the period of zinc supplementation are reversed when supplementation with zinc is stopped. If this trend were to continue, it could be expected that even the small remaining benefit in linear growth of the stunted children would be lost.

The study also demonstrates that subsequent siblings of stunted children are more likely to become stunted than siblings of non-stunted children. Child-rearing practices[17] and, possibly to a lesser extent, other environmental factors are probably responsible for this difference.

Mild zinc deficiency would appear to be one of the dietary risk factors for the poor growth experienced by children in rural Ethiopia. Our results agree with reports from other workers who also found a positive effect of zinc supplementation on growth in young children.[2] We also observed earlier that zinc concentrations in serum are strongly associated with height and HAZ in stunted children suggesting that zinc deficiency impairs growth.[18] The effect on improving growth in these infants may be due to a direct impact of zinc on nucleic acid replication and protein synthesis but could be due in part to increased appetite and an associated decrease in incidence and severity of infectious diseases.

Discontinuation of zinc supplementation, however, disrupted the catch-up growth that occurred in stunted children. In fact, discontinuation of zinc supplementation resulted in a reversal of the gains attained in height and weight. There was a significant decrease (from 6 to 42 mo) both in HAZ and WAZ (table 2). Such an effect has not been reported previously.

As there appear to be no functional stores in the body of zinc[19], such effects could be due to insufficient supply of zinc limiting growth in these children. Zinc requirements are high during periods of rapid growth, which means that the body needs more zinc relative to its size than in periods of slower growth. Cereal-based diets are virtually the sole source of dietary zinc in the population studied, but the bioavailability of zinc in such diets are likely to be low due to the high phytate concentration in cereals.

At the end of the 6 mo supplementation period, 40% of the children who had received the placebo were zinc deficient, based on serum zinc concentrations, compared with none of their non-stunted counterparts (table 3). None of the children who had been supplemented with zinc were deficient. Similar results were also seen for the concentration of zinc in hair. However, the effect of zinc supplementation on the concentration of zinc in serum and hair had disappeared 36 mo after supplementation had been discontinued. More than half of the stunted infants, irrespective of whether they had been previously supplemented with zinc, were zinc deficient based on concentrations of zinc in serum and hair (table 3), indicating that zinc is not stored in the body.[19] Serum zinc concentrations provide an indication of short-term zinc status while hair zinc concentrations provide and indication of zinc status over a longer term. Thus, because the effect of the 6-mo zinc of supplementation on zinc status had disappeared 36 mo after supplementation ceased, so there was no longer any stimulation of growth. An alternative, but less likely explanation, is that factors other than zinc deficiency are responsible for the poor growth of children in the 36 mo subsequent to supplementation.

New siblings born subsequently from the same family with stunted children were also more likely to become stunted. Although genetic factors could play a role, this is most likely due to poor child feeding practices shared within families.[20,21] In addition, use of preventive health services, and the lack of safe water and environmental sanitation may also be important.

Vitamin A deficiency has been reported repeatedly in preschool children living in the same area [22,23]. Although we did not observe evidence of xerophthalmia in these children, 24% of them were marginally vitamin A deficient (serum retinol <0.70 μmol/L). Thus, vitamin A deficiency remains as a public health problem in the area. However, efforts were underway to control the problem through the national programme of vitamin A supplementation.

In conclusion, this study clearly shows that the effects of supplementing stunted infants with zinc on stimulating linear and ponderal growth are reversed once zinc supplementation is discontinued. Thus zinc supplementation must be sustained in order to improve growth in stunted children. In addition, siblings of stunted infants are more likely to become shorter, lighter, thinner, and have lower height-for-age and weight-for-age than siblings of non-stunted counterparts.

Contributors

The study was designed by M. Umeta, C.E. West and J.G.A.J. Hautvast. M. Umeta, with the assistance of W. Dubale, executed the fieldwork and carried out the laboratory analyses. The data were analysed by M. Umeta, C.E. West and H. Verhoef. M Umeta prepared the draft manuscript and all authors contributed to revising the manuscript.

Acknowledgements

We would like to thank the children and parents who participated in the study; the administrations of Arsi Zone Health Department and of Dodota-Sire District; staff of Dheera Health Centre, particularly Tesfaye Deti for field assistance; locally recruited field workers; staff of the Ethiopian Health and Nutrition Research Institute especially Asfaw Hussen (laboratory technician) and Tsegaye Adisu (driver); Paul Deurenberg of Wageningen University for training in anthropometry; and Jan Burema of Wageningen University for statistical advice. The study was supported by a grant from The Nestlé Foundation for the Study of the Problem of Nutrition in the World.

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5

Content of Zinc, Iron, Calcium and Inhibitors of their Absorption in Foods Commonly Consumed in Rural Ethiopia.

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(Submitted for publication)

Abstract

The zinc, iron, calcium, phosphorous, phytate, tannin and moisture content of 36 foods consumed in rural Ethiopia were analysed. The foods analysed included those based on cereals, starchy tubers and roots, and legumes and vegetables as well as some fruits. Although many foods were relatively rich in zinc and iron, many also contained high levels of phytic acid and tannins, which impair bioavailability of zinc and iron. The phytate:zinc molar ratios were >20 for non-fermented cereal foods, >15 for legumes, and <12 for fermented cereals, starchy tubers and roots. Ratios >15 are associated with low bioavailability of zinc. Given the high iron content and the relatively favourable phytate:iron molar ratio, tef enjera was the best source of bioavailable iron of all foods analysed. Foods prepared from tef, enset and kale are rich source of calcium. The consumption of diets based on cereals and legumes, without animal products that are consumed only in limited amounts, can lead to deficiencies, of zinc and iron. However, since fermentation can decrease the phytate content by a factor of 3-4, traditional household practices such as fermentation need to be encouraged to address the problem of zinc deficiency, which is particularly prevalent in Ethiopia.

INTRODUCTION

Zinc and iron are two of the micronutrients that are most often deficient in developing countries with children and women of reproductive age especially at risk of such deficiencies (Gibson, 1994; Sandstead; 1991). In children, zinc deficiency has been shown to lead to poor growth (Umeta *et al*., 2000; Brown *et al*., 2002), impaired immunity, increased morbidity from common infectious diseases (Bhutta *et al*., 1999) and increased mortality (Sazawal, *et al.,* 1998). Zinc deficiency arises to a large extent from impaired bioavailability of dietary zinc, largely attributable to the high phytic acid content of diets (Gibson, 1994). Iron deficiency is the most important cause of nutritional anaemia. This arises from the low bioavailability of non-heme iron (Hallberg and Hulthén, 2000) caused by not only phytate but also tannins in the diet. In general, intakes of calcium in developing countries, although low, are adequate to meet requirements (Latham, 1997). However dietary calcium has been implicated in reducing the bioavailability of iron, not only of non-haem iron but also of haem iron (Hallberg and Hulthén, 2000).

The diets of rural populations in most developing countries are based on cereals and legumes that contain much phytate while the consumption is low of animal based foods that are rich in zinc and iron with high bioavailability (Gibson, 1994). Phytic acid exerts its inhibitory effect on the absorption of zinc and iron by forming insoluble complexes in the gut under physiological condition (Wise, 1995). The formation of such chelates depends on the ratio of the content of zinc, iron or calcium relative to that of phytate in the food. Other minerals of nutritional importance that are chelated by phytate are copper and manganese (Wise, 1983; Hallberg *et al*., 1987).

It is possible to predict the relative bioavailability of zinc from the molar ratio of phytate to zinc in the diet (Fordyce *et al.,* 1987; Morris and Ellis, 1989) and ratios >15 have been negatively associated with growth in children (Ferguson *et al.*, 1989; Allen *et al.*, 1991) and suboptimal zinc status in adults (Gibson *et al.*, 1997). High levels of calcium in the diet have also been shown to exacerbate the inhibitory effect of phytate on zinc absorption in humans by forming insoluble complexes with calcium and zinc in the intestine (Morris and Ellis, 1989; Wise, 1983). Such complexes are even less soluble than complexes of phytate with zinc or with calcium alone. In addition, tannins also interfere with iron absorption thus reducing iron bioavailability. (Hallberg and Hulthén, 2000).

In rural Ethiopia, the diets of the population are predominantly plant-based and low in animal products. Daily consumption of such diets for longer periods may increase the relative risk of deficiencies of zinc and iron. We have demonstrated that zinc deficiency is a problem in infants in an area of rural Ethiopia (Umeta *et al*., 2000) but there is very little data on the extent or severity of deficiencies of iron or calcium. There is also a lack of information on the zinc, iron, calcium, phytate and tannin content of local foods. Thus we have collected such data and assessed the relative bioavailability of zinc and iron of some commonly consumed foods in rural Ethiopia.

MATERIALS AND METHODS

***Sample collection**:* Food samples were collected in rural areas throughout the country. For prepared foods, at least five households at each site were visited to observe the way they were prepared and samples (ca 1 kg) of foods were collected from households that were regarded as preparing foods in a traditional way. No specific guidance was given to those preparing the food. Fruits were purchased from open markets. The samples were packed in plastic bags of which the opening was sealed using a lighted candle, labelled and transferred within 6 h of collection to a deep freeze (-20°C) at a local health centre until sample collection in the area was completed, after which they were transported to the Ethiopian Health and Nutrition Research Institute. All transport of foods was carried out in cool boxes with cooling elements precooled to -20^{0}C. The samples were stored at -20^{0}C until analysis.

***Analysis**:* Food samples were first equilibrated to room temperature and then solid foods were mixed thoroughly while semi-solid foods were homogenised. Food samples were dried at 95-105^{0}C for 24 h. Those with a high water content were first dried at 65^{0}C. Dry weight was determined when the weight was constant after heating for a further 2 h at 95-105^{0}C. The dried samples were then ground to a fine homogeneous powder using a Cyclotec sample mill (Tecator, Hogans, Sweden). A sample (ca 2 g) of this powder was ashed at 550^{0}C for 12 h in a muffle furnace. If ashing was incomplete, concentrated nitric acid (several drops) was added and the samples re-ashed for a further 6 h at 550^{0}C. The ashed samples were dissolved in 6 N HCl (5 mL) and diluted to 50 mL with deionised water. The concentrations of zinc, iron and calcium were determined in an aliquot using an atomic absorption spectrophotometer (Varian Spectra A10/20 Plus, Varian Australia Pty., Ltd., Mulgrave, Vic. 3171, Australia) (Osborne and Voogt, 1978). For the determination of calcium, lanthanum chloride (1% w/v) was added to both standards and samples to suppress interference from phosphorous. The same digest was used to determine total phosphorous (Fiske and Subbarrow, 1925). Phytic acid was extracted from an aliquot (ca 0.5 g) with HCl (0.2M containing Na_2SO_4, 10% w/v; ca 20 mL) for 4 h with a mechanical shaker at room temperature. After centrifugation, the concentration of phytic acid was determined colorimetrically (Hage and Lantzasch, 1983). The concentration of tannins, expressed as catechin equivalents, was measured using the vanillin assay method with catechin as standard (Price *et al.*, 1978).

***Quality control**:* To minimise the risk of contamination, all glassware used was immersed in 10% (v/v) solution of nitric acid for 24 h, washed with distilled water and rinsed with deionised water before use. All chemicals used were obtained from Sigma Chemical Company. For zinc and calcium, recovery of added material was 97.6% and 94.3% respectively. Accuracy of analysis of zinc and iron was determined using durum wheat flour, SRM 8436 (National Institute of Standards and Technology, Gaithersburg, MD, USA). Values measured were 96.4 ± 0.8% and 95.7 ± 1.2% of the certified values for zinc and calcium respectively.

RESULTS

A total of 138 food samples of 36 different foods were collected. The local and scientific names, description and place of collection of the foods analysed are presented in Table 1. The zinc, iron, calcium, phosphorus, phytate and tannin content of the foods analysed are presented in Tables 2-5.

The content of zinc was highest in wheat and lowest in sorghum and maize with the zinc content of tef being between the two. Fermentation of enjera reduced the zinc concentration. The iron content of tef enjera, both fermented and unfermented was very high (>30 mg/100 g) and about 3x that of sorghum enjera and about 8x that of enjera made from wheat or maize (3-4 mg/100g). Tef enjera was also rich in calcium, containing 3x that of maize and wheat enjera and 5x that of sorghum enjera. The differences in phosphorus content among the enjera prepared from the different grains were less pronounced. Fermentation reduced the phytate content of enjera prepared from tef and sorghum by a factor of 3-4 (Table 2) and this effect is also seen in the zinc:phytate molar ration, calcium:phytate molar ratio and the proportion of phosphorous as phytate (Table 6). The tannin content of enjera prepared from tef, sorghum and maize were similar and about 2-3x that of wheat enjera while the tannin content of sorghum porridge was more than 4x that of maize porridge.

The zinc content of starchy tubers and roots (Table 3) was relatively low (0.3-0.7 mg/100 g). Wassa and amicho prepared from enset and anchoottee stew were rich sources of iron (4-5 mg /100 g) but boiled sweet potato, yam and taro were poor sources (0.7-1.3 mg/100g). The enset foods were also rich sources of calcium (ca 80 mg/100g) compared with the other tuber and root foods (20-40 mg/100g) but the enset foods also contained more phytate. In stews based on legumes and vegetables (Table 4), the zinc content was low (<1.0 mg/100g) while the iron content was relatively high (>2.5 mg/100g) with kale foods being particularly rich in iron (4-7 mg/100g). Kale foods were also noteworthy because of their high calcium and tannin content. It is interesting to note that the phytate content of stew prepared from whole kidney beans (325 mg/100g) was twice that of stew prepared from split kidney beans. The zinc content of fruits was low (0.1-0.5 mg/100g) with the iron content being about 3x higher (0.3-1.3 mg/100g) and the calcium content being about 10x higher (10-30 mg/100g) of that of zinc. Four of the 6 fruits contained no tannins but the tannin content was quite high in guava (ca 100 mg/100g) and prickly pear (400 mg/100g).

Table 1 Description of foods analysed

Food type	General description	Food analysed	Site of collection
Enjera	A pancake like bread prepared from cereals such as tef (*Eragrostis tef)*, maize (boqollo, *Zea mays* L.), sorghum (mashilla, *Sorghum* bicolor (L.) Moench), wheat (sinde, *Triticum vulgare*) or a mixture of cereals; served as a main dish.	Tef enjera, unfermented	Gondar (1) Maji(1) North Omo (1) Wollega (1)
		Tef enjera, fermented	Tigray (2) Wollega (3)
		Maize enjera, unfermented	Asossa (2) Maji (1) Tigray (1) Wollega (1)
		Sorghum enjera, unfermented	Gondar (3) Hararge (1) Tigray (2)
		Sorghum enjera, fermented	Asossa (2) Hararge (2) Tigray (1) Wollega (1)
		Wheat enjera, fermented	Hararge (1) Tigray (3) Wollega (1)
Bread (kitta or anbasha)	Unleavened bread prepared from unfermented cereals or a mixture of cereals such as maize, sorghum or wheat.	Maize bread	Gurage (1) Maji (2) Wollega (1)
		Sorghum bread	Maji (1) Metekil (1)

Bread *(continued)*	Unleavened bread prepared from unfermented cereals or a mixture of cereals such as maize, sorghum or wheat	Wheat bread	Bale (1) Hararge (2) Tigray (2)
Porridge (genfo)	Porridge prepared from cereal or mixture of cereals and served as main dish.	Maize porridge	Borena (2) Gambella (2) Maji (1) Sidama (1)
		Sorghum porridge	Afar (1) Asossa (1) Gambella (1) Maji (1)
Boiled cereals or legumes (nifro)	Prepared by boiling cereals such as maize, sorghum, wheat, or legumes such as kidney beans (adenguare, *Phasoelus vulgaris* (L.)), broad beans (*Vicia faba* L.*)*, chick peas (shimbira, *Cicer arietinum* (L.)) or a mixture of the above in water with added salt. The cooking water is drained and the food is served as a snack.	Maize, boiled	Gambella (1) Gamo (1) Kambata (1) Konso (1) Bench (1) Sidama (1)
		Sorghum, boiled	Afar (1) Konso (2)
Enset	Unleavened bread prepared from fermented enset (false banana, *Enset ventricosum)* and served as a main dish.	Wassa	Dorze (1) Gedeo (2) Kambata (1) Keficho (1) Wolayita (1)

Enset (continued)	Root of enset prepared by boiling and draining of the water; served as a snack.	Amicho	Gedeo (1) Guji (1) Sidama (1) Wolayita (1)
Starchy roots and tubers	Stew prepared from anchoottee (*Coccinia abyssinica*), which is a root, seasoned with spices and butter, and served with a main dish.	Anchoottee stew (itto anchoottee)	Wollega (3)
	Sweet potato (metatish, *Ipomoea batatas* L.) prepared by boiling, then drained and served as snack.	Sweet potato, boiled	Gamo (1) Wolayita (1)
	Yam (boyna, *Dioscorea spp.*) prepared by boiling and draining of the water and served as snack.	Yam, boiled	Metekel (1) Sidama (1) Wolayita (1)
	Taro (jong, *Colacasia antiquorum*) prepared by boiling, then drained of water and served as a snack.	Taro, boiled	Gambella (1) Maji (3) North Omo (1)
Pumpkin	Stew prepared from pumpkin (buqqee, *Cucurbita spp.*), cooked with oil and seasoned with spices and served with a main dish.	Pumpkin stew (itto buqqee)	Asossa (1) Sidama (1) Wollega (2)
Kale (gommen)	Kale (gommen, *Brassica carinata* Braun): cooked and seasoned with spices and served as stew with a main dish.	Kale, spiced and cooked (amber)	Gedeo (1) Gurage (1) Maji (1) North Omo (1) Wolyita (1)
	Kale: prepared by boiling, then drained of the water and salted, and served as a stew with a main dish.	Kale, boiled	Gurage (1) Keficho (2)
Stew from ground legumes (shiro wo't)	Stew prepared from legumes such as garden or field peas (atter, *Pisum sativum*), chickpea, grass pea (guaya, *Lathyrus sativus* L*)*, kidney beans or broad beans by roasting, decortication and grinding the grains, seasoning with spices and then cooking. Served with a main dish.	Ground pea stew	Tigray (1) Wollega (1)

Stew from ground legumes (continued)		Ground chick pea stew	Hararge (1) Gambella (1)
		Ground grass pea stew	Tigray (2)
Stew from split legumes (Kik wo't)	Stew prepared from legumes such as kidney beans, broad beans (baqella, *Vicia faba* L.), lentils (misir, *Lens culinarus* L.), chickpea (*Cicer arietinum* L.), by roasting, decortication and splitting the grains, seasoning with spices and then cooking. Served with a main dish.	Split kidney bean stew	Asossa (1) Harar (1) North Omo (1)
		Split broad bean stew	Tigray (1) Wollega (4)
		Split broad bean and lentil stew	Tigray (2)
		Split lentil stew	Wollega (2)
Stew from whole legumes	Stew prepared from whole kidney beans by seasoning with spices and then cooking. Served with a main dish.	Whole kidney bean stew	Gurage (1) Konso (1) Metekel (1)
Fruit	Citron (tringo, *Citrus medica*)	Citron, fresh whole fruit	Metekel (3)
	Guava (zeyitun, *Psidium guajava*)	Guava, fresh whole fruit	Konso (2) Sidama (2)
	Custard apple (gishta, *Annona muricata*)	Custard apple, fresh whole fruit without seeds	Sidama (3)
	Mello (*Ximenia americana* L.*)*	Mello, fresh whole fruit	Tigray (3)
	Passion fruit (hopi, *Passiflora edulis*)	Passion fruit, fresh pulp	Sidama (4)
	Prickly pear (beles, *Opuntia ficus*)	Prickly pear, fresh flesh	Bale (2) Tigray (2)

Table 2 Zinc, iron, calcium, phosphorous, phytate and tannin content of cereal-based foods

Food (n)	Moisture (g/100g)	Zinc (mg/100g)	Iron (mg/100g)	Calcium (mg/100g)	Phosphorus (mg/100g)	Phytate (mg/100g)	Tannin (mg/100g)*
Tef enjera, unfermented (4)	54.5 ± 3.0 [52.2 – 59.1]	1.41 ± 0.30 [1.10 – 1.40]	30.3 ± 3.0 [28.2 – 32.9]	62.7 ± 0.4 [56.7 – 69.2]	179 ± 9 [174 – 183]	389 ± 10 [376 – 394]	60.1 ± 6.2 [54.8 – 64.7]
Tef enjera, fermented (5)	57.8 ± 4.0 [54.3 – 62.7]	1.16 ± 0.20 [1.00 – 1.40]	34.7 ± 4.1 [29.6 – 39.4]	61.4 ± 3.1 [58.3 – 63.5]	164 ± 8 [156 – 168]	126 ± 8 [118 – 134]	49.8 ± 4.2 [45.2 – 51.7]
Maize enjera, unfermented (5)	55.8 ± 3.1 [53.9 – 59.0]	0.88 ± 0.10 [1.10 – 1.80]	4.2 ± 0.7 [3.9 – 5.4]	19.2 ± 2.1 [16.4 – 21.9]	135 ± 7 [128 – 139]	282 ± 6 [274 – 293]	64.6 ± 4.7 [57.1 – 69.1]
Sorghum enjera, unfermented (6)	54.6 ± 4.1 [53.7 – 58.3]	0.91 ± 0.21 [0.75 – 1.24]	9.2 ± 2.1 [7.3 – 10.0]	13.2 ± 1.4 [12.2 - 14.1]	115 ± 8 [110 – 118]	325 ± 12 [317 – 331]	53.2 ± 5.1 [47.3 – 55.8]
Sorghum enjera, fermented (6)	54.9 ± 4.1 [52.6 – 58.2]	0.74 ± 0.21 [0.63 – 0.86]	8.1 ± 1.7 [6.9 10.2]	11.2 ± 1.9 [10.3 – 13.2]	102 ± 9 [96 – 108]	75 ± 2 [70 – 82]	49.8 ± 4.1 [46.2 – 53.6]
Wheat enjera, fermented (5)	47.4 ± 4.7 [45.8 – 52.3]	1.50 ± 0.32 [1.38 – 2.08]	3.5 ± 0.8 [2.3 – 4.3]	23.1 ± 2.1 [18.8 – 26.1]	188 ± 7 [174 – 194]	137 ± 9 [130 – 144]	21.2 ± 2.3 [18.2 – 24.1]
Maize bread (4)	49.8 ± 2.1 [47.6 – 52.2]	1.10 ± 0.30 [0.83 – 1.10]	5.2 ± 1.2 4.4 – 6.8]	8.3 ± 1.4 [6.4 – 9.5]	176 ± 8 [170 – 181]	411 ± 12 [398 – 419]	50.3 ± 6.4 [44.2 – 56.3]
Sorghum bread (2)	53.6 ± 4.0 [50.7 – 56.6]	0.69 ± 0.20 [0.67 – 0.71]	6.8 ± 0.2 [6.6 – 6.9]	13.1 ± 1.9 [11.2 – 14.0]	109 ± 4 [105 – 112]	296 ± 7 [290 –303]	83.0 ± 2.0 [81.4 – 84.6]
Wheat bread (5)	37.3 ± 5.3 34.2 – 40.3]	1.60 ± 0.24 [1.17 – 1.84]	5.4 ± 1.2 [3.8 – 7.4]	23.1 ± 3.1 [20.2 - 26.3]	182 ± 9 [174 – 189]	542 ± 11 [531 – 550]	23.3 ± 3.2 [19.8 – 23.5]
Maize porridge (6)	71.9 ± 4.5 [68.2 – 73.4]	0.60 ± 0.20 [0.49 – 0.84]	3.6 ± 1.2 [2.9 – 5.1]	10.2 ± 1.3 [8.1 – 11.4]	149 ± 5 [146 – 154]	205 ± 9 [197 – 213]	23.9 ± 3.7 [21.4 – 28.6]
Sorghum porridge (4)	65.1 ± 3.1 [62 – 68]	0.69 ± 0.13 [0.62 – 0.89	6.3 ± 1.3 [5.6 – 7.6]	9.2 ± 1.2 [8.1 – 9.4]	101 ± 6 [96 – 104]	237 ± 7 [233 – 246]	111.5 ± 2.5 [108.4 – 114.4]
Maize, boiled (6)	53.8 ± 5.1 [49.3 – 58.3]	1.27 ± 0.23 [1.11 – 1.38]	3.5 ± 0.7 [2.9 – 4.9]	12.1 ± 1.2 [10.4 – 13.1]	184 ± 7 [176 – 189]	344 ± 11 [336 – 358]	16.9 ± 1.4 [15.4 – 18.6]
Sorghum, boiled (3)	65.4 ± 4.1 [62.4 – 69.3]	0.63 ± 0.05 [0.58 – 0.70]	3.6 ± 0.8 [2.9 – 4.4]	11.2 ± 1.0 [10.2 – 12.2]	94 ± 3 [91 – 96]	272 ± 8 [264 – 286]	121.7 ± 2.6 [119.8 – 124.6]

Data are expressed as mean ± SD [range] on a fresh-weight basis; *expressed as D-catechin equivalents

Table 3: Zinc, iron, calcium, phosphorous, phytate and tannin content of foods prepared from starchy tubers and roots

Food (n)	Moisture (g/100g)	Zinc (mg/100g)	Iron (mg/100g)	Calcium (mg/100g)	Phosphorous (mg/100g)	Phytate (mg/100g)	Tannin (mg/100g)*
Wassa (6)	54.6 ± 6.4	0.66 ± 0.10	4.4 ± 0.7	85.5 ± 6.8	47 ± 5	77 ± 6	0.0 ± 0.0
	[48.0 – 61.1]	[0.57 – 0.72]	[3.3 – 5.0]	[80.0 – 89.1]	[46 – 49]	[71 – 82]	[0.0 – 0.0]
Amicho (4)	49.2 ± 5.8	0.64 ± 0.07	4.8 ± 0.4	78.6 ± 6.4	34 ± 4	58 ± 5	341.2 ± 4.6
	[44.3 – 53.2]	[0.56 – 0.72]	[4.2 – 5.0]	[74.2 – 83.4]	[29 – 37]	[53 – 64]	[335.2 – 344.2]
Anchoottee stew (3)	79.4 ± 4.2	0.50 ± 0.08	5.2 ± 0.6	39.3 ± 1.8	80 ± 6	64 ± 5	391.4 ± 7.4
	[77.1 – 83.9]	[0.45 – 0.60]	[4.2 – 6.3]	[37.4 – 41.0]	[76 – 84]	[58 – 67]	[386.3 – 403.2]
Sweet potato, boiled (2)	65.0 ± 0.5	0.30 ± 0.0	0.7 ± 0.0	23.8 ± 0.10	53 ± 1	34 ± 2	75.5 ± 2.2
	[64.6 – 65.4]	[0.30 – 0.30]	[0.7 – 0.7]	[23.6 – 24.8]	[53 – 53]	[31 – 37]	[74.1 – 76.8]
Yam, boiled (3)	71.5 ± 5.1	0.58 ± 0.03	0.8 ± 0.3	18.6 ± 2.8	55 ± 3	30 ± 2	424.1 ± 4.8
	[68.2 – 74.4]	[0.53 – 0.61]	[0.4 – 1.1]	[16.8 – 21.4]	[50 – 58]	[28 – 33]	[420.6 – 428.6]
Taro, boiled (5)	71.5 ± 4.2	0.59 ± 0.09	1.3 ± 0.4	30.8 ± 1.8	65 ± 4	18 ± 1.2	0.0 ± 0.0
	[68.6 – 74.4]	[0.54 – 0.64]	[0.7 – 1.7]	[29.8 – 30.0]	[61 – 72]	[18 –18]	[0.0 – 0.0]

Data are expressed as mean ± SD [range] on a fresh-weight basis; *expressed as D-catechin equivalents

Table 4 Zinc, iron, calcium, phosphorous, phytate and tannin content of stews prepared from legumes and vegetables

Food (n)	Moisture (g/100g)	Zinc (mg/100g)	Iron (mg/100g)	Calcium (mg/100g)	Phosphorous (mg/100g)	Phytate (mg/100g)	Tannin (mg/100g)*
Pumpkin stew (4)	88.5 ± 6.2 [86.0 – 92.0]	0.44 ± 0.08 [0.41 – 0.47]	2.6 ± 0.3 [2.2 – 2.9]	20.8 ± 3.1 [17.8 – 22.7]	34 ± 4 [31 –37]	75 ± 8 [68 – 83]	41.4 ± 3.5 [38.5 – 45.7]
Kale, spiced and cooked (amber) (5)	83.6 ± 4.2 [79.2 – 85.0]	0.59 ± 0.04 [0.54 – 0.68]	6.7 ± 1.7 [4.7 – 7.2]	185.7 ± 7.2 [178.5 – 189.6]	59 ± 4 [55 – 64]	117 ± 11 [109 – 124]	419.3 ± 7.6 [414.6 – 426.4]
Kale, boiled (3)	83.2 ± 4.6 [78.5 – 86.3]	0.71 ± 0.04 [0.66 – 0.74]	4.6 ± 0.7 [3.8 – 5.1]	221.4 ± 4.7 [215.9 – 228.4]	57 ± 4 [54 – 59]	127 ± 8 [123 – 136]	205.3 ± 10.4 [198.5 – 210.2]
Ground pea stew (2)	87.2 ± 1.0 [86.6 – 87.6]	0.43 ± 0.00 [0.42 – 0.43]	3.5 ± 0.1 [3.3 – 3.7]	21.0 ± 1.2 [20.8 – 21.2]	63 ± 0 [63 – 63]	108 ± 3 [106 – 110]	28.2 ± 2.1 [26.8 – 29.5]
Ground chick pea stew (2)	74.4 ± 5.1 [71.2 – 76.9]	0.77 ± 0.06 [0.72 – 0.80]	4.6 ± 0.2 [4.4 – 4.7]	28.1 ± 0.7 [27.6 – 28.5]	118 ± 1 [117 – 119]	292 ± 6 [286 – 299]	0.0 ± 0.0 [0.0 – 0.0]
Ground grass pea stew (2)	82.2 ± 3.3 [79.7 – 84.4]	0.68 ± 0.10 [0.58 – 0.78]	4.2 ± 0.8 [3.3 – 5.1]	25.9 ± 1.0 [25.7 – 26.1]	65 ± 1 [64 – 66]	152 ± 8 [144 – 160]	0.0 ± 0.0 [0.0-0.0]
Split kidney bean stew (3)	80.3 ± 3.4 [78.4 – 83.2]	0.71 ± 0.03 [0.69 – 0.74]	2.9 ± 0.4 [2.4 – 3.3]	34.3 ± 0.6 [33.8 – 35.0]	115 ± 4 [113 – 117]	168 ± 9 [164 – 176]	169.0 ± 6.3 [165 – 173]
Split broad bean stew (5)	77.5 ± 4.1 [73.1 – 81.2]	1.03 ± 0.03 [0.68 – 1.38]	3.9 ± 0.6 [3.4 – 4.7]	16.9 ± 2.8 [15.0 – 20.7]	103 ± 5 [100 – 109]	196 ± 6 [188 – 206]	53.6 ± 2.8 [51.8 – 53.4]
Split broad bean and lentil stew (2)	86.1 ± 0.2 [86.1 – 86.2]	0.56 ± 0.14 [0.51 – 0.61]	3.3 ± 0.0 [3.3 – 3.3]	18.8 ± 1.6 [17.7 – 19.9]	74 ± 5 [70 – 77]	81 ± 1.6 [80 – 83]	51.8 ± 2.4 [49.2 – 54.3]
Split lentil stew (2)	76.6 ± 4.1 [73.4 – 79.8]	0.68 ± 0.10 [0.58 – 0.78]	5.2 ± 0.5 [4.6 – 5.8]	12.8 ± 0.3 [12.7 – 13.0]	75 ± 1 [74 – 76]	158 ± 8 [150 – 166]	29.6 ± 2.2 [27.6 – 31.6]
Whole kidney bean stew (3)	59.8 ± 4.3 [57.1 – 64.3]	0.96 ± 0.12 [0.81 – 1.10]	2.6 ± 0.2 [2.4 – 2.8]	53.2 ± 2.4 [51.4 – 55.0]	125 ± 4 [124 – 126]	325 ± 10 [314 – 336]	168.7 ± 3.7 [167.2 – 172.3]

Data are expressed as mean ± SD [range] on a fresh-weight basis; *expressed as D-catechin equivalents

Table 5 Zinc, iron, calcium, phosphorous, phytate and tannin content of fruits

Food (n)	Moisture (g/100g)	Zinc (mg/100g)	Iron (mg/100g)	Calcium (mg/100g)	Phosphorous (mg/100g)	Phytate (mg/100g)	Tannin (mg/100g)*
Citron, fresh whole fruit (3)	89.9 ± 4.3	0.08 ± 0.01	1.0 ± 0.1	30.9 ± 2.4	13 ± 2	22 ± 3	0.0 ± 0.0
	[87.1 – 90 .5]	[0.06 – 0.09]	[0.8 – 1.01]	[28.1 – 32.3]	[12 – 15]	[20 – 24]	[0.0 – 0.0]
Guava, fresh whole fruit	83.5 ± 2.3	0.24 ± 0.05	0.64 ± 0.04	17.2 ± 2.3	28 ± 3	13 ± 1	113.9 ± 4.6
(4)	[82.2 – 85.4]	[0.22 – 0.26]	[0.59 – 0.67]	[14.3 – 19.5]	[25 – 30]	[11 – 14]	[109.4 - 116.8]
Custard apple, fresh whole	72.3 ± 1.8	0.17 ± 0.04	0.3 ± 0.1	16.1 ± 2.3	55 ± 4	17 ± 2	0.0 ± 0.0
fruit without seeds (3)	[70.4 – 74.2]	[0.15 – 0.19]	[0.2 – 0.5]	[14.2 – 17.5]	[53 – 58]	[15 – 20]	[0.0 – 0.0]
Mello, fresh whole fruit (3)	68.9 ± 2.4	0.30 ± 0.03	1.3 ± 0.2	29.2 ± 3.2	43 ± 4	36 ± 3	0.0 ± 0.0
	[66.2 – 72.1]	[0.2 – 0.4]	[0.9 – 1.6]	[26.5 – 32.1]	[40 – 46]	[34 – 39]	[0.0 – 0.0]
Passion fruit, fresh pulp (4)	77.8 ± 4.6	0.45 ± 0.03	1.2 ± 0.1	11.0 ± 1.0	40 ± 3	83 ± 4	0.0 ± 0.0
	[74.5 – 79.1]	[0.41 – 0.48]	[0.9 – 1.6]	[9.3 – 13.2]	[36 – 43]	[77.2 – 86.8]	[0.0 – 0.0]
Prickly pear, fresh flesh (4)	80.7 ± 3.8	0.15 ± 0.02	1.0 ± 0.1	12.8 ± 2.3	22 ± 3	39 ± 4	395.3 ± 6.4
	[78.6 – 84.3]	[0.12 – 0.17]	[0.7 – 1.3]	[11.4 – 14.6]	[18 – 25]	[35.4 – 43.2]	[387.2 – 401.6]

Data are expressed as mean ± SD [range] on a fresh-weight basis; *expressed as D-catechin equivalents

Table 6: Molar ratios of phytate:zinc, calcium:phytate, [calcium x phytate]:[zinc] and proportion of phosphorous as phytate in cereal-based foods

Food (n)	Phytate:zinc (molar ratio)	Calcium:phytate (molar ratio)	[Calcium x phytate] [zinc] (mol/kg)	Proportion of phosphorous as phytate (%)	Phytate:iron (molar ratio)
Tef enjera, unfermented (4)	28.2 ± 4.1 [26.0 – 32.8]	2.7 ± 0.5 [2.1 – 3.2]	0.5 ± 0.1 [0.3 – 0.6]	61 ± 3 [58 – 63]	1.1 ± 0.2 [0.9 – 1.3]
Tef enjera, fermented (5)	10.8 ± 1.2 [9.3 – 12.7]	8.2 ± 1.1 [6.9 – 9.6]	0.2 ± 0.01 [0.1 – 0.2]	21 ± 1 [18 – 23]	0.3 ± 0.1 0.2 – 0.4]
Maize enjera, unfermented (5)	32.6 ± 4.2 [29.9 – 34.9]	1.1 ± 0.1 [0.9 – 1.4]	0.2 ± 0.05 [0.1 – 0.2]	58 ± 3 [54 – 61]	5.7 ± 0.4 [5.2 – 6.3]
Sorghum enjera, unfermented (6)	38.6 ± 6.1 [35.1 – 42.3]	0.7 ± 0.1 [0.6 – 0.8]	0.1 ± 0.01 [0.10 – 0.04]	79 ± 4 [76 – 82]	3.0 ± 0.3 [2.8 – 3.5]
Sorghum enjera, fermented (6)	11.1 ± 2.1 [9.8 – 13.1]	2.4 ± 0.6 [1.9 – 2.6]	0.1 ±0.01 [0.01 – 0.05]	20 ± 3 [17 – 22]	0.8 ± 0.1 [0.6 – 0.9]
Wheat enjera, fermented (5)	9.4 ± 2.1 [8.2 – 10.8]	2.8 ± 0.9 [2.3 – 3.4]	0.1 ± 0.0 [0.1 – 0.1]	21 ± 2 [17 – 22]	3.3 ± 0.3 [3.1 – 3.5]
Maize bread (4)	37.8 ± 4.3 [34.1 – 43.2]	0.4 ± 0.1 0.4 – 0.5]	0.1 ± 0.01 [0.1 – 0.3]	66 ± 3 [62 – 68]	6.7 ± 0.5 [6.2 – 7.1]
Sorghum bread (2)	38.1 ± 3.9 [37.4 – 39.5]	0.8 ± 0.2 [0.6 – 1.0]	0.1 ± 0.0 [0.1 – 0.1]	76 ± 3 [74 – 79]	3.7 ± 0.3 [3.4 – 4.1]
Wheat bread (5)	34.4 ± 5.3 [29.7 – 36.2]	0.7 ± 0.1 [0.6 – 0.8]	0.2 ± 0.01 [0.1 – 0.3]	84 ± 3 [38 – 43]	8.5 ± 0.4 [8.1 – 8.9]
Maize porridge (6)	32.4 ± 4.1 [27.8 – 35.9]	0.8 ± 0.1 [0.7 – 1.0]	0.1 ± 0.01 [0.1 – 0.2]	37 ± 2 [35 – 41]	4.8 ± 0.3 [4.4 – 5.2]
Sorghum porridge (4)	34.2 ± 4.3 [30.3 – 39.4]	0.6 ± 0.1 [0.6 – 0.7]	0.1 ± 0.0 [0.1 – 0.1]	66 ± 3 [64 – 69]	3.2 ± 0.2 [3.0 – 3.4]
Maize boiled (6)	30.3 ± 3.2 [28.4 – 35.4]	0.5 ± 0.1 [0.4 – 0.7]	0.1 ± 0.04 [0.01 – 0.10]	53 ± 3 [50 – 54]	8.3 ± 0.4 [8.0 – 8.8]
Sorghum boiled (3)	42.7 ± 4.7 [37.4 – 44.4]	0.7 ± 0.1 [0.6 – 0.8]	0.1 ± 0.05 [0.1 – 0.2]	82 ± 4 [78 – 86]	6.4 ± 0.4 [5.9 – 6.7]

Data are expressed as mean ± SD [range] on a fresh-weight basis

Table 7: Molar ratios of phytate:zinc, calcium:phytate, [calcium x phytate]:[zinc] and proportion of phosphorous as phytate of foods prepared from starchy tubers and roots

Food type (n)	Phytate:zinc Molar ratio	Calcium:phytate molar ratio	[Calcium x phytate] [zinc] mol/kg	Proportion of phosphorous as phytate (%)	Phytate:iron molar ratio
Wassa (6)	12.6 ± 3.1	17.7 ± 3.2	0.3 ± 0.01	46 ± 3	1.5 ± 0.2
	[8.2 – 14.3]	[14.3 - 19.3]	[0.2 - 0.4]	[44 – 49]	[1.3 – 1.7]
Amicho (4)	9.1 ± 2.1	20.9 ± 4.1	0.2 ± 0.06	47 ± 2	1.0 ± 0.2
	[8.6 - 10.7]	[17.4 – 23.4]	[0.1 - 0.2]	[44 – 50]	[0.8 – 1.3]
Anchoottee stew (3)	12.6 ± 2.1	20.3 ± 3.2	0.2 ± 0.01	23 ± 2	1.0 ± 0.1
	[11.4 – 14.5]	[18.2 – 24.2]	[0.1 - 0.3]	[20 – 24]	[0.8 – 1.1]
Sweet potato, boiled (2)	13.8 ± 2.2	11.8 ± 1.3	0.1 ± 0.0	29 ± 3	4.1 ± 0.3
	[12.3 – 15.2]	[10.3 – 13.5]	[0.1 – 0.1]	[24 – 32]	[3.8 – 4.4]
Yam, boiled (3)	8.7 ± 1.3	10.3 ± 1.1	0.1 ± 0.01	15 ± 1	5.8 ± 0.4
	[7.9 - 9.2]	[9.5 – 11.4]	[0.08 - 0.10]	[14 – 16]	[5.5 – 6.0]
Taro, boiled (5)	2.9 ± 0.5	28.7 ± 2	2.3 ± 0.3	8 ± 2	1.2 ± 0.1
	[2.6 - 3.2]	[27.4 – 30.5]	[2.0 - 2.4]	[7 – 10]	[1.1 – 1.3]

Data are expressed as mean ± SD [range] on a fresh-weight basis

Table 8: Molar ratios of phytate:zinc, calcium:phytate, [calcium x phytate]:[zinc] and proportion of phosphorous as phytate of stews prepared from legumes and vegetables

Food (n)	Phytate:zinc molar ratio	Calcium:phytate Molar ratio	[calcium x phytate] [zinc] mol/kg	Proportion of phosphorous as Phytate (%)	Phytate:iron molar ratio
Pumpkin stew (4)	17.6 ± 3.2 [15.3 – 23.5]	5.7 ± 2.4 [4.7 - 6.5]	0.1 ± 0.02 [0.10 - 0.08]	77±4 [73 – 79]	2.4 ± 0.2 [2.2 – 2.5]
Kale spiced and cooked (amber) (5)	19.8 ± 2.3 [18.4 – 21.6]	26.9 ± 4.2 [25.3 – 31]	0.9 ± 0.1 [0.7 - 1.2]	57±4 [54 – 61]	1.5 ± 0.1 [1.3 – 1.6]
Kale, boiled (3)	17.9 ± 2.1 [16. 4 – 19.6]	29.3 ± 4.8 [27.3 - 33.4]	1.0 ± 0.1 [0.9 - 1.04]	59±3 [56 – 61]	1.0 ± 0.1 [0.8 – 1.1]
Ground pea stew (2)	23.8 ± 0.9 [23.4 – 24.2]	3.2 ± 0.3 [3.0 – 3.4]	0.1 ± 0.0 [0.1 – 0.1]	48 ± 3 [46 – 50]]	2.6 ± 0.2 [2.5 – 2.9]
Ground chick pea stew (2)	37.3 ± 4.0 [34.1 – 42.4]	1.7 ± 0.5 [1.3 – 1.9]	0.3 ± 0.04 [0.2 – 0.5]	69 ± 4 [65 – 73]	5.4 ± 0.5 [4.8 – 5.7]
Ground grass pea stew (2)	26.2 ± 2.8 [24.4 – 28.0]	2.3 ± 0.1 [2.2 – 2.4]	0.1 ± 0.0 [0.1 – 0.1]	63 ± 2 [61 – 66]	3.1 ± 0.2 [2.8 – 3.3
Split kidney bean stew (3)	23.6 ± 2.3 [21.2 – 25.6]	3.4 ± 0.4 [2.9 – 3.4]	0.3 ± 0.02 [0.2 – 0.5]	41 ± 4 [38 – 43]	4.9 ± 0.5 [4.1 – 5.3]
Split broad bean stew (5)	20.2 ± 4.3 [16.8 – 24.2]	1.5 ± 0.4 [1.1 – 1.7]	0.2 ± 0.03 [0.1 – 0.5]	59 ± 4 [57 – 64]	4.3 ± 0.2 [4.0 – 4.8]
Split bean and lentil stew (2)	14.9 ± 2.0 [13.8 – 16.2]	3.8 ± 0.2 [3.7 – 4.0]	0.1 ± 0.0 [0.1 – 0.1]	31 ± 2 [46 – 50]	2.1 ± 0.3 [1.7 – 2.4]
Split lentil stew (2)	23.2 ±0.4 [22.9 – 23.6]	1.4 ± 0.2 [1.2 – 1.6]	0.1 ± 0.0 [0.1 – 0.1]	60 ± 3 [58 – 64]	3.5 ± 0.2 [3.3 – 3.8]
Whole kidney bean stew (3)	33.6 ± 3.4 [32.2 – 36.4]	2.8 ± 0.4 [2.5 – 3.3]	0.5 ± 0.05 [0.4 – 0.5]	73 ± 4 [70 – 78]	10.6 ± 1.1 [9.2 – 12.1]

Data are expressed as mean ± SD [range] on a fresh-weight basis

Table 9: Molar ratios of phytate:zinc, calcium:phytate, [calcium x phytate]:[zinc], and proportion of phophorous as phytate of fruits

Food type (n)	Phytate:zinc Molar ratio	Calcium:phytate Molar ratio	[Calcium x phytate] [zinc] mol/kg	Proportion of phosphorous as phytate (%)	Phytate:iron molar ratio
Citron, fresh whole fruit (3)	27.3 ± 2.1	23.6 ± 3.1	0.2 ± 0.1	46±3	1.9 ± 0.1
	[25.6 – 28.8]	[22.2 – 26.1]	[0.1 - 0.3]	[43 – 49]	[1.7 – 2.2]
Guava, fresh whole fruit (4)	5.2 ± 0.5	15.5 ± 1.5	0.1 ± 0.0	14±3	1.7 ± 0.3
	[4.2 – 7.4]	[14.2 –17.6]	[0.0 – 0.0]	[12 – 16]	[1.6 – 1.9]
Custard apple, fresh whole fruit without seeds (3)	9.8 ± 1.1	15.6 ± 1.1	0.1 ± 0.0	9±1	4.8 ± 0.2
	[9.3 – 11.1]	[14.4 – 19.1]	[0.1 – 0.1]	[8 – 10]	[4.6 – 4.9]
Mello, fresh whole fruit (3)	11.9 ± 1.2	13.3 ± 1.3	0.1 ± 0.01	22±2	2.4 ± 0.2
	[9.2 – 13.3]	[11.2 - 15.6]	[0.07 - 0.13]	[19 – 24]	[2.3 – 2.5]
Pashion fruit, fresh pulp (4)	18.1 ± 2.1	2.2 ± 0.5	0.1 ± 0.0	57±4	5.9 ± 0.3
	[15.3 – 20.6]	[2.1 –3.0]	[0.1 – 0.1]	[54 – 60	[5.5 – 6.1]
Prickly pear, fresh flesh (4)	25.6 ± 2.2	5.4 ± 0.5	0.1 ± 0.0	50±3	3.3 ± 0.2
	24.3 – 27.8]	[5.2 – 7.0]	[0.1 – 0.1]	[47 – 52]	[3.0 – 3.5]

Data are expressed as mean ± SD [range] on a fresh-weight basis

The phytate:zinc molar ratio in the unfermented cereal foods was >30 but the ratio was <15 (regarded as favourable for zinc bioavailability) in fermented cereal foods (Table 6). High values (>15) were also found in the stews prepared from legumes or vegetables (Table 8) and in citron and passion fruit pulp (Table 9) but were <15 in foods prepared from starchy tubers and roots (Tables 7) and in the other fruits examined (Table 9). The calcium:phytate molar ratio was > 6 (regarded as unfavourable for calcium absorption) in fermented tef enjera, all foods from starchy tubers and roots and from kale, and all fruits except passion fruit. A millimolar ratio of [calcium x phytate]:[zinc] >0.5 (indicative of low zinc bioavailability) was observed only in kale. Phytate:iron molar ratios > 0.15 indicative of low iron bioavailability.were found in all foods. In fact, phytate:iron molar ratios were >1.5 in all foods except foods prepared from tef (unfermented and fermented enjera), enset (wassa and amicho) and kale as well as in anchoottee stew and boiled taro. Fermentation of enjera resulted in a 3 to 4-fold reduction in the molar ratio. For tef, the ratio was 1.3 and 0.3 in unfermented and fermented enjera respectively while for sorghum, the ratio was 3.0 and 1.8 in unfermented and fermented enjera respectively.

DISCUSSION

Diets in Ethiopia are based to a large extent on cereals, starchy roots and tubers, and on legumes. The contribution of these foods varies throughout the country. Tef is the main staple in the northern, western and central parts of the country; in the lowland areas, maize and sorghum are important; while in the south and southwest, starchy tubers and roots including enset are the principle foods (Bezuneh and Feleke, 1966; Pijls *et al.,* 1995). These foods not only provide energy and protein but are also the major sources of zinc, iron, calcium and phosphorus. Vegetables and fruits provide only limited amounts of these nutrients but are important sources of vitamins, particularly vitamin C. The content in foods of the minerals and trace elements measured are comparable to the values reported in the Ethiopian food composition tables (Agren and Gibson, 1968; EHNRI, 1998) although we have concentrated to a greater extent on foods as consumed.

This paper provides the basis for examining the possible contribution of foods commonly eaten in Ethiopia to the supply of zinc, iron and calcium to the population, especially in rural areas. The effective supply of nutrients to individuals depends on the amount of foods eaten, the nutrient content of such foods and nutrient bioavailability. Zinc deficiency appears to be a serious problem in rural Ethiopia as supplementation with zinc increased the linear growth of infants, particularly those who were stunted (Umeta *et al*., 2000). Foods consumed in considerable amounts and rich in zinc were enjera prepared from wheat and tef, bread prepared from wheat (Tabekhla and Donnelly, 1982; Le Francois, 1988) and boiled maize. However the bioavailability of zinc is reduced by phytic acid, which is present in significant amounts in the seed coat of cereals and legumes. This explains the high phytate content of the stew prepared from whole kidney beans compared with that prepared from split kidney beans from which the seed coat is removed. The phytate content of starchy tubers and roots and of fruits was low as has been reported earlier (Maga, 1982). It should be noted that the method used to determine the phytate content of foods did not differentiate

between inositol phosphates containing differing in the number of phosphate groups. The hexaphosphate and pentaphosphate inositol compounds are those primarily responsible for inhibiting mineral absorption (Hallberg and Hulthén, 2000). The proportion of phosphate in phytate was >60% in foods prepared from non-fermented cereals but was reduced to <20% on fermentation. The proportion in roots and tubers was >60% in most of the legume foods but <50% in foods from roots and tubers. Generally diets are regarded as being adequate in bioavailable phosphate. However the high proportion of phosphate as phytate has consequences for bioavailability of minerals and trace elements.

Phytate:zinc molar ratios >15, indicative of poor zinc bioavailability (Morris and Ellis, 1989; Turnlund *et al*., 1984), were found in many of the food consumed, except for fermented enjera (9-11) and foods prepared from starchy foods and roots (3-13). Fermented enjera involves 2-3 d of fermentation during which time phytate is broken down as has been shown for enjera prepared from tef (Umeta, 1986, Urga *et al.*, 1997), maize (Lopez *et al.*, 1983) and pearl millet (Mahajan and Chauhan, 1987). The term 'unfermented' with respect to enjera is misleading as there is limited fermentation during the preparation of unfermented enjera. It is interesting to note that we observed that unfermented enjera is generally preferred to fermented enjera in most rural areas in Ethiopia. Because fermentation reduces the phytate content of foods, it should be encouraged as a means of increasing not only bioavailability of zinc but also of iron. It is known that cooking alone does not reduce the phytate content of foods (Oberleas, 1983). High phytate:zinc molar ratios (24–40) were also reported in diets of populations living in rural area of West Africa (Mbofung *et al.*, 1989) and Iran (Reinhold *et al.,* 1973) who have been shown to be zinc deficient.

The food richest in iron was enjera prepared from tef. The seed of tef is very small and is very difficult to rid completely of soil contamination, which explains the high content of iron in tef enjera. In fact, the iron content of tef is not so different from that of other cereals as the high values reported here and also by other investigators (Besrat *et al.*, 1980; Ramachandran *et al.*, 1984; Umeta, 1986) are due to contamination rather than to intrinsic iron. It may well be that the bioavailability of the iron in tef may be low but this has not been adequately studied. It is known that the prevalence of anaemia is relatively low in populations consuming enjera prepared from tef (Wolde-Gebriel *et al.*; 1993). It may well be that the high iron content of tef enjera may compromise zinc bioavailability and lead to zinc deficiency as we observed earlier (Umeta *et al*., 2000). Although some foods were good source of iron, the bioavailability of iron was low because of the high levels of phytic acid. This resulted in phytate:iron molar ratios >0.15 regarded as indicative of poor iron bioavailability (Siegenberg *et al.,* 1991), in all foods. For fermented tef enjera, the phytate:iron molar ratio (0.3) was less than one third of that in unfermented enjera. Given the high iron content of tef enjera (ca 30 mg/100 g) and the low phytate:iron molar ratio, this was the best source of bioavailable iron of all the foods analysed.

Foods prepared from tef, enset and kale are rich in calcium. The calcium:phytate molar ratio was above the critical molar ratio of 6:1 in fermented tef enjera and foods prepared from enset, starchy roots and tubers and from kale. This may explain why calcium deficiency has not been reported to be a major problem in the country, as in many developing countries (Latham, 1997), but it has probably not received the attention it deserves. The high

calcium content of tef enjera, and of starchy tubers and roots, and vegetables may jeopardise bioavailability of iron (Hallberg and Hulthén, 2000) and zinc (Morris and Ellis, 1989; Wise, 1983). High calcium levels in foods can promote the phytate-induced decrease in zinc availability when the calcium x phytate:zinc millimolar ratio exceeds 0.5 (Cossack *et al.*, 1983; Gibson, 1994). However values >0.5 were observed only in foods prepared from kale, which is rich in calcium. Thus it would appear that the possible contribution of calcium in the diet to exacerbating the low bioavailability of zinc due to phytate is probably minimal.

The bioavailability of zinc and iron is reduced by the tannins present in cereals (Derman *et al.*, 1977; Gillooly *et al.*, 1984) and tannin-rich vegetables (Gillooly *et al.*, 1983) but the tannin content of foods can vary widely. In enjera prepared from tef, sorghum and maize, the tannin content was 2-3x that of wheat enjera. However, the tannin content of sorghum porridge was more than 4x that of maize porridge, possibly because the sorghum porridge was prepared from varieties richer in tannins than the sorghum enjera. The sorghum samples of enjera and sorghum porridge were collected for the most part from different areas of the country. Kale, anchoottee stew, boiled yam and some fruits (guava and prickly pear) were also rich in tannins. Consumption of foods rich in tannins should be taken into account when estimating bioavailability of zinc and iron.

CONCLUSIONS

In conclusion, this paper provides data on the content of zinc, iron, calcium, phytate and tannin and on the relative bioavailability with particular reference to zinc of foods commonly consumed in rural Ethiopia. Although there are foods relatively rich in zinc and iron, high levels of inhibitors of absorption, especially phytic acid but also tannins, can impair bioavailability of these trace elements. Thus, the consumption of diets based on cereals and legumes, without animal products that are consumed only in limited amounts, can lead to deficiencies, particularly of zinc. However, traditional household practices such as fermentation can decrease the phytate content significantly, and thus need to be encouraged to address the problem of zinc deficiency not only in rural areas of Ethiopia but also elsewhere.

ACKNOWLEDGEMENTS

We would like to thank Mengistu Gebre-Tsadik, Benyam Tedla and Yohanes Tesfaye for their technical assistance in the laboratory. The study was supported by a grant from the Nestlé Foundation for the Study of the Problems of Nutrition in the World.

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6

General discussion

In the studies described in this thesis, the possible role of zinc deficiency in the aetiology of the high prevalence of stunting in children in rural Ethiopia was investigated. It was hypothesised that zinc is a limiting nutrient for growth in stunted children in Ethiopia and that zinc supplementation would stimulate growth more in stunted children than in non-stunted children. Furthermore, the question was asked whether or not the stimulation of growth by zinc persists after discontinuation of zinc supplementation. Therefore, a randomised, double-blind, placebo-controlled study was carried out in a rural village of Dodota-Sire District, in Ethiopia. Stunted infants (n=100) and non-stunted infants (n=100) aged 6-12 mo were supplemented 6 d/wk for 6 mo with either 10 mg zinc or placebo. The children were followed up during the first 6 mo, and 36 mo after zinc supplementation had been discontinued. In addition, infants born subsequent to randomisation were also included for examination at this time. These studies were preceded by a cross-sectional study aimed at assessing the nutritional status of breastfed infants aged 5-11 mo and to investigate the major risk factors associated with stunting in the study population. The mineral, phytate and tannin contents of foods commonly consumed in rural parts of the country were examined and estimates of the relative bioavailability of zinc and iron made.

In this chapter, the methodology and design of the studies is discussed. This is followed by a discussion of the main findings. The chapter ends with a discussion of the significance of the studies, policy implications and recommendations for future research.

METHODOLOGY AND DESIGN OF STUDIES

The first study was designed as a cross-sectional study of 305-breastfed infants aged 5-11 mo and their mothers. The study was aimed at assessing the extent to which malnutrition exists in the infants and to identify major factors associated with stunting. Demographic, anthropometric and clinical data were combined with data on the concentrations of zinc, copper and calcium in breast milk to examine possible causes of infant malnutrition in the community. The information obtained from this survey was also used for the selection of subjects and as baseline anthropometric data for the subsequent intervention trial. It was not possible to measure the concentrations of zinc in serum and hair because mothers did not accept that blood be drawn or hair samples taken from infants because of cultural and ritual beliefs.

The supplementation study was carried out in 200 relatively healthy breastfed infants aged 6-12 mo, who were reasonably free from intestinal parasites. Stunted (LAZ<-2) infants (n=100) were matched by age and sex with 100 randomly selected non-stunted (LAZ≥-2) infants. Then infants, both stunted and non-stunted, were matched by sex, age (within 2 mo) and recumbent length (within 3 cm) for random assignment to receive a zinc supplement or placebo, 6 days a week for 6 mo. Zinc supplementation was discontinued after 6 mo of intervention and the children were re-assessed 36 mo after the intervention had been completed. In addition to measuring children enrolled in the intervention study, anthropometric measurements were made on all siblings born subsequent to randomisation.

The ethical issue of including a placebo group was considered during the design phase of this study. In a study in which baseline samples are taken and where a known

micronutrient deficiency such as nutritional anaemia or vitamin A is observed, those responsible for the research are obliged to treat these infants, which usually precludes them for inclusion in the trial. Exclusion of such infants from the trial, on the other hand, creates a bias in the recruited infants, as only infants without prior deficiency would be selected. Stunting is highly prevalent in Ethiopian children and zinc deficiency is purported to be a major contributory factor. However, prior to the study being conceived, and also during the planning phase, no information was available on the zinc status of any group of the population in the country. An earlier trial carried out in Africa (1) did not show any effect on growth. Nonetheless, evidence on the importance of zinc in growth was building up (2,3). Therefore, it was regarded as ethical to include a placebo group to establish whether or not the infants were zinc deficient, and whether zinc supplementation would stimulate linear growth and reduce symptoms of morbidity. The medical-ethical committee of the Ethiopian Health and Nutrition Research Institute accepted these arguments.

In order to increase the possibility of finding an effect of zinc supplementation on growth, a number of decisions were taken because there were several factors in earlier studies that seemed to be associated with a positive effect of zinc supplementation on growth. Thus the study was carried out on an equal number of stunted infants, aged 6-12 mo in an area where the population consumed a diet based largely on cereals which was regarded as being poor in bioavailable zinc. The infants received 10 mg zinc per day, 6 days each week for 6 mo. The importance of these factors is discussed below. In addition, because of the lack on information on which to base a power calculation, the number of subjects included in the study was made greater than any study carried out previously. Randomisation was also carried out at the individual and not the group level to maximize the statistical power.

To examine whether or not foods commonly consumed in rural areas of Ethiopia are good sources of zinc and also of iron, representative samples of food were collected from selected sites in the various agro-ecological zones. These foods were analysed for their content of zinc, iron, calcium, phytate and tannins.

MAIN FINDINGS AND INTERPRETATION

Major factors associated with stunting

In the cross-sectional study **(Chapter 2)**, the first research aim outlined in the introduction was addressed, which was "to assess the nutritional status of breastfed infants and their mother and identify major factors associated with stunting in rural Ethiopia". The prevalence of stunting was 36%, which is relatively low compared with national data for the same age group (57%), but does indicate that malnutrition is widespread among infants in the study area. Malnutrition was also prevalent among mothers, with 27% being chronic energy deficient (body mass index, <18.5 kg/m^2) and 20% night blindness indicating that vitamin A deficiency was a serious problem.

The lower zinc and calcium concentrations of breast milk of mothers of stunted infants, compared with mothers of non-stunted infants would appear to have contributed to stunting of infants. Thus, since we have shown that zinc supplementation improves growth of stunted infants, it must be considered to improve growth by improving the zinc content of

mothers' milk. Unfortunately, supplementation of women with zinc has little or no effect on the zinc concentration of breastmilk (4,5). The mechanisms underlying this lack of response of zinc concentrations in breastmilk to increased maternal zinc intake need to be better understood. Because calcium is an important constituent of bone, it is perhaps not surprising that growth of infants is related to the calcium content of the breast milk they consume. The concentration of other micronutrients such as vitamin A in the breastmilk of stunted infants may also have been low and contributed to infant stunting. However, the analysis of breast milk was restricted to zinc, calcium and copper.

Furthermore, the type, quantity and frequency of supplementary feeding were important factors associated with stunting in the infants studied, while age of introduction of supplementary foods was not. Infants who were reported to be fed >3 times/d or >600 mL/d had higher LAZ than their peers fed ≤ 3 times/d or <600 mL/d respectively. These results are in line with other studies in less developed countries showing that a low frequency of feeding and consumption of small amounts of weaning foods are major factors contributing to growth faltering and stunting in infants (6-9).

Inadequate intake depends not only on quantity and frequency of consumption but also on food quality. One of the main problems with many supplementary foods is their low energy and nutrient density (10). Energy density can be improved by processes such as malting. With respect to micronutrients, a study among five developing countries has shown that most commonly used supplementary foods do not supply sufficient dietary zinc and iron (11). Fermentation of supplementary foods can also improve the bioavailability of nutrients such as zinc and iron by breaking down phytates (12). In the population studied, infants' diets were prepared from local staple cereals, which are limited in variety, monotonous and bulky, and which have low energy and nutrient densities. In addition, the bioavailability of zinc and iron was low because of the high phytate content (see below). Intake of a wider variety of foods including animal based products would increase the likelihood that infants would meet nutrient requirements for growth.

Most studies on the relationships between child feeding practices and nutritional status outcomes have focussed on single factors such as exclusive breast feeding (13), timing of introduction of supplementary feeding (14), or importance of animal products in supplementary feeding (10). These approaches are valuable but they do not allow an examination of the effect of child feeding practices as a whole on children's health and nutrition outcome. The present study, however, emphasized that combined information of some key parameters of child feeding practices such as type, frequency and quality would lead to a better understanding of the determinants of nutritional status.

Effect of zinc supplementation on growth and morbidity

The intervention study **(Chapter 3)**, addressed the second research aim stated in the introduction, namely "to investigate the effect of zinc supplementation on growth and morbidity in stunted and non-stunted infants". The findings showed that supplementation with 10 mg of elemental zinc/d for 6 mo was effective in improving linear and ponderal growth both in stunted and non-stunted children aged 6-12 mo and that the effect was more

pronounced in stunted children. Zinc supplementation also resulted in reduced frequencies of anorexia and morbidity in the stunted children. The observed increase in growth may be due primarily to the direct role of zinc on protein synthesis and gene expression, and in production and action of hormonal mediators, but may also be due to a reduction of the risk of infection and on increasing appetite. The effect on the reduction in morbidity from infection may also be due to the prominent role of zinc in both cellular and hormonal immune functions. In a meta-analysis, zinc supplementation has been shown to reduce the occurrence of diarrhoea (25% for prevalence and 18% for incidence) and pneumonia (41% for incidence) (15). It has also been shown to reduce mortality by 68% among infants (16).

This study is unique in evaluating the effect of zinc supplementation between stunted and non-stunted children. In this intervention study, both linear and ponderal growth were significantly improved in both stunted and non-stunted infants who received zinc compared with their counterparts who received a placebo. The effect of zinc supplementation on height was, however, significantly greater (2.5-fold higher) among stunted infants than among non-stunted infants (20% higher) indicating that it depends on the degree of stunting. No other zinc supplementation study, either before or after this study, has reported such an effect (17).

When linear growth was expressed as LAZ, the stimulatory effect of zinc on growth was greater in stunted children than in non-stunted children who received zinc. In fact the stunted infants who received zinc demonstrated a positive change in LAZ (+0.14) during the 6-mo intervention period indicating that zinc is a limiting in the growth of the stunted children. Non-stunted children who received zinc showed a small but negative change (-0.18) in LAZ indicating that deficiency of other nutrients may have contributed to the poor growth, not only of the non-stunted infants but also the stunted infants. The 6-mo supplementation period was not sufficient for the length of stunted infants to reach that of the non-stunted infants. Such a goal might have been reached with time with continued supplementation but, on the other hand, other nutrients may also be rate limiting, as indicated by the sub-optimal growth of the non-stunted infants supplemented with zinc.

The results observed in our study are consistent with the recently reported findings of meta-analysis of 33 studies that measured growth response to zinc supplementation (17). The overall effect size reported in the meta-analysis was +0.350 (95% CI: 0.189, 0.511). Studies that enrolled children with mean initial HAZ <-2 had a weighted mean effect size of 0.465 (95% CI: 0.179, 0.750) which was almost 2-fold greater than the effect size of 0.254 (95% CI: 0.057, 0.450) in those studies in which the mean initial HAZ was >-2 (Table 1). In the present study, the effect size was 0.432 (95% CI: 0.184, 0.328) for children with an initial LAZ <-2 and 0.220 (95% CI: 0.134, 0.176) for children with an initial LAZ>-2. As would be expected, the effect of zinc supplementation on linear growth was greater in stunted than in non-stunted children.

Table 1: Comparison of effect of zinc supplementation on growth observed with those reported in earlier studies

	Effect size, mean (95% CI)
Present study	
Stunted infants	0.432 (0.184; 0.328)
Non-stunted infants	0.220 (0.134; 0.176)
Meta-analysis (17)	
Stunted children	0.465 (0.179; 0.750)
Non-stunted children	0.254 (0.057; 0.450)
Other studies in stunted children	
Ecuador (18)	0.190 (0.050; 0.330)
Guatemala (19)	0.500 (0.140; 0.810)

As indicated in the introduction, no other studies in Africa, either before or after this study, have shown an improvement in growth (1,20,21). In the Gambian study (1), all children aged 6-36 mo living in the village were included with zinc-supplemented groups receiving 75 mg of zinc twice per week for 15 mo. It may well be that dosing twice per week is not sufficient for there to be an effect. In the study in Zimbabwe, children aged 11-17 y received 15 mg/d zinc for 3 mo (20), while those in Uganda aged 45-47 mo received 10 mg/d zinc for 3 mo respectively (21). The age of the subjects was probably too high for an effect to be observed. In the study described in this thesis, stunted and non-stunted infants aged 6-12 mo were supplemented with 10 mg of zinc/d, 6d/wk for 6 mo and their linear and ponderal growth increased compared with their non-supplemented counterparts. This indicates that zinc deficiency may be responsible for much of the stunting of children in Africa and would benefit from improved zinc nutriture. Recently a study in Bangladesh in which infants were supplemented with zinc for 6 mo failed to show any effect on growth (22), possibly because the children were younger (aged 4 wk) and because the dose was too low (5 mg/d) compared with those in the present study who were aged 6-12 mo and who were provided with 10 mg/d. Before 6 mo of age, infants may have sufficient zinc stores so that zinc supplementation does not stimulate growth while at about 6 mo, infants are becoming progressively more stunted possibly as a result of decreased reserves, increased demands and decreased supply because of the introduction of inadequate supplementary foods. With respect to the optimum dose of zinc to be provided, more work needs to be done as high doses can impair immune function (23) and increase the risk of mortality, especially in children with protein-energy malnutrition (24).

Despite the limitations of serum zinc concentration as an indicator of body zinc status, it has been suggested as a useful indicator of zinc status at the population level (2,25). Unfortunately, serum and hair zinc concentrations could not be measured at baseline and so it is not known whether evidence zinc deficiency existed in stunted and non-stunted children before the intervention. However, the concentrations of zinc in serum at the end of the 6 mo intervention among both stunted and non-stunted children who received zinc supplementation

was appreciably higher than their placebo counterparts. In fact, based on serum zinc concentrations, none of the stunted infants supplemented with zinc were zinc deficient compared with 44% of stunted infants who had received placebo. A similar trend of zinc concentration in hair was also observed. These findings may indicate that stunted infants had lower initial serum and hair zinc concentrations at baseline and had already developed zinc deficiency. The fact that both serum and hair zinc concentrations correlated positively with linear growth in zinc supplemented stunted infants also further emphasized the association between zinc deficiency and stunting. This study failed to demonstrate any positive correlation between the zinc concentration in serum and in hair and this is in agreement with the results of other studies (26,27). Although serum zinc concentrations indicate short-term zinc status while hair zinc concentration reflects long-term zinc status, the lack of correlation does indicate that these parameters can be used for determining the zinc status at a group level but not at an individual level.

Long-term effect of zinc supplementation on growth

Following discussion of what happened during the intervention study, the third research aim **(Chapter 4)** outlined in the introduction - "to investigate whether the effects of zinc supplementation on growth persist after discontinuation of zinc supplementation" – is addressed. The increased rate of linear and ponderal growth that occurred in stunted children after 6 mo of zinc supplementation did not persist 36 mo after zinc supplementation was discontinued and in fact was reversed. The 4.1 cm increase in length and the 0.7 kg in weight in the stunted children achieved during 6 mo of zinc supplementation relative to the placebo group decreased by 3.4 cm and 0.8 kg when measured 36 mo after supplementation had ceased. These changes were also reflected in the HAZ and WAZ. Although growth and /or morbidity were measured during and at the end of zinc supplementation lasting from 2 wk to 14 mo, no studies have examined previously what happens once supplementation has ceased (17-19).

Based on zinc concentrations in serum and hair, 56% of stunted infants, irrespective of whether they had been previously supplemented with zinc became zinc deficient 3 yr after zinc supplementation had been discontinued. This reflects the absence of functional zinc store in the body (28). The positive effect on zinc status had disappeared 36 mo after zinc supplementation was discontinued, thus there was no longer stimulation of growth. It cannot be excluded, however, factors other than zinc deficiency were responsible for the poor growth observed in children 36 mo after discontinuation of zinc supplementation.

It is also interesting to note that new siblings born subsequently into families of the stunted children were also shorter and lighter than their counterparts born into families of the non-stunted children. This is probably due to the common maternal nutritional status during pre-and postnatal period and to the feeding practices (29) shared by siblings. In addition siblings would share the same access to preventive health services, water supply and environmental sanitation.

Minerals, phytate and tannin contents and the bioavailability of zinc in the diets in rural Ethiopia

In this section the fourth research question **(Chapter 5)** outlined in the introduction - "to investigate the mineral contents of foods commonly consumed in rural areas and to predict the bioavailability of zinc based on the content of phytate, tannin and calcium" – is addressed. Diets of the rural population in Ethiopia are based predominantly on cereals, legumes, starchy tubers and roots. These foods contain relatively high amounts of zinc but also high amounts of phytate. Similar to the studies in Ghana and Malawi (30), starchy tubers and roots were found to have a lower zinc content than cereals and were also low in phytate.

The molar ratio of phytate to zinc is important for estimating the relative risk of having an inadequate intake of zinc. Phytate:zinc molar ratios in diets > 15 are associated with suboptimal zinc status (31). Most of the foods analysed in this study had values > 15, suggesting that those consuming diets based on these foods are at risk of having an inadequate intake of zinc and thus an inadequate zinc status. Fermented cereal enjera, however, showed molar ratios < 15 suggesting that zinc bioavailability is high in this food. The low ratio is a result of the activity of phytases during the fermentation process (12,32). The phytate content of foods can also be reduced by malting as phytase activity increases when seeds germinate but not by heat treatment (33).

The calcium content of the foods was relatively low so inhibition of zinc absorption due to the combined chelating action of calcium and phytate was relatively unimportant. Thus the [calcium x phytate]:[zinc] millimolar ratio in almost all the foods analysed, except kale (amber), was < 0.5 mmol/kg. These algorithms may be used in planning menus to select the combination of foods that will supply the most available zinc to the daily diet.

It is also interesting to note that tef enjera contains very high amounts of iron. Earlier studies in Ethiopia indicate that the intake of iron in the daily diet of the population is probably high in regions where cereal tef serve as the main staple (34,35). The high content of iron in tef is of concern because of the potential interaction between iron and zinc that may reduce zinc absorption to such an extent that zinc deficiency occurs.

General conclusions and recommendations

The studies described in this thesis clearly demonstrate that zinc is the primary limiting micronutrient for growth in stunted infants in rural Ethiopia. It also shows that zinc supplementation is effective in halting the stunting process and reducing the incidence of anorexia and morbidity in stunted children. Such beneficial effects depend on the degree of stunting, dose, frequency of dosage, duration of supplementation and age of the infants. Combating zinc deficiency can improve the health and nutritional status of young children in poor communities. Thus, in communities where stunting is highly prevalent and the bioavailability of zinc in the diets is low, zinc deficiency is likely to exist, and infants and young children should be targeted for zinc intervention. The quality, quantity and frequency

of supplementary foods offered to infants are important factors that influence nutritional status of stunted infants whose diet are based on plant foods. Thus there is a need to improve the diets of infants with a variety of foods with a high energy and nutrient density and to increase the intake and frequency of infant feeding. Particular attention should be paid to developing strategies to reduce the phytate content of cereal-based diets to increase the bioavailability of zinc. Such strategies should be in line with the food habits of the community. This would involve food processing methods at the household and community level and perhaps at the industrial level. In addition, since maternal health and nutritional status may also contribute to zinc status of infants, consideration should be given to targeting interventions not only at infants but also at mothers as such interventions would also benefit mothers. However more work needs to be done to find suitable interventions to improve zinc status.

Discontinuation of zinc supplementation reverses the positive effects on linear and ponderal growth built up during zinc supplementation. Thus zinc has no lasting effect on growth emphasizing the need for sustained measures for supplying zinc in order to improve growth and reduce morbidity in stunted children.

Policy implications

Evidence is now accumulating indicating that zinc deficiency is common among stunted children and that zinc supplementation stimulates growth and reduces morbidity. Although this is the first study demonstrating the existence of a serious zinc deficiency in the country, the results are so clear cut that they call for a national public health policy to improve zinc status in areas where the prevalence of stunting is very high. Currently in Ethiopia, there are policy guidelines for the control of three micronutrient deficiencies - vitamin A deficiency, iodine deficiency disorders and iron deficiency anaemia. Because of the potential adverse health effects of zinc deficiency on infant and child health, the control of zinc deficiency should also be given priority. Thus there is a need for national policy guidelines to address this issue.

Unlike other micronutrient deficiencies, no policies or programme have been developed by UN agencies to address the problem of zinc deficiency. In addition, no country has yet developed a national program to control zinc deficiency. It is also unlikely that there is a single solution to controlling zinc deficiency suited to all countries because of variations in ecological backgrounds and dietary patterns. Although zinc supplementation is effective in improving the zinc status of children, daily supplementation is expensive and may not be practical in a country such as Ethiopia. In addition, more work is required to establish effective but safe supplementation regimes. Food-based approaches will need to be developed involving the use of foods now consumed, foods improved by traditional at the household and community level, and foods enriched or fortified at the community and industrial level.

Future research

Although zinc supplementation is the most effective means of improving growth and reducing morbidity in stunted children, it may not be the most appropriate method to combat zinc deficiency in the long term. Thus research is needed to explore ways of increasing the amount of zinc that can be derived from foods, which depends on consumption of foods that are rich in zinc, zinc content of foods and on zinc bioavailability. Appropriate methods of food processing, fortification and plant breading need to be developed. If a decision is made to use zinc supplements, at least in the short term, appropriate doses need to be worked out. In the studies described in this thesis and those carried out by others (16-18) where 10 mg of elemental zinc was provided daily, the dose was effective and no adverse effects related to the dose were observed. However, effects on copper and iron status of children not measured in any of the studies. Thus more work needs to be done to establish dosing regimes that are both effective and safe.

In poor communities where stunting is prevalent in children, deficiencies of micronutrients other than zinc almost certainly co-exist, and may contribute to stunting. Thus more research is needed to investigate the association between several micronutrient deficiencies and stunting.

In Ethiopia information on the adequacy of the zinc intake of the population as a whole and of specific groups is lacking. Apart from infants and young children, attention needs to be directed towards women of child-bearing age, particularly pregnant and lactating women. Diarrhoea and upper respiratory infections are among the top causes of child morbidity and mortality in Ethiopia. In addition, the prevalence of HIV/AIDS infection and tuberculoses is now very high. More research is needed to explore the potential impact of zinc supplementation on reducing the burden of these diseases and on immunity related to the disease process.

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Summary

Malnutrition remains a common problem among preschool children worldwide with about one quarter of all children under five years being underweight. Inadequate diet and infectious diseases are the major causes of malnutrition contributing to the high prevalence of child morbidity and mortality in poor countries. It has been estimated that about 12 million children under five years of age die annually due to infectious disease and malnutrition of which malnutrition alone contributes to more than half of the cases.

Child malnutrition leads to growth failure that is manifested as linear growth retardation or short stature, and thinness. These are most commonly known as stunting and wasting respectively. Stunting and wasting can be measured using anthropometric indices of height-for-age or weight-for-height respectively. Children are generally considered to be stunted or wasted when Z-scores for height-for-age (HAZ) or weight-for-height (WHZ) are below -2 standard deviations (SD) of the median US National Center for Health Statistics reference population with similar age and sex. HAZ reflects growth achievement pre- and postnatally. Wasting is the result of acute severe undernutrition, as found in times of famine or sudden weaning, or to disease. It indicates an acute situation in which the body is forced to use its own resources, including muscle, to function “normally”.

It has been estimated that 182 million (about one third) of preschool children in developing countries are stunted and the prevalence is 35% in Africa and 44% in South-central Asia. The highest level of stunting is found in East Africa where 48% of preschool children are currently affected. More alarming is the fact that over the past 20 y the number of stunted preschool children in East Africa has increased substantially from 13 million in 1980 to about 22 million in 2000. In rural Ethiopia, the national prevalence of stunting was reported to be 64%, which is one of the highest in the world.

In the past, malnutrition was thought to be due primarily to insufficient intake of protein and energy but deficiencies of micronutrients may also play a role. During the past two decades, deficiencies of iodine, vitamin A and iron, received most attention globally. Zinc may also be very important because zinc deficiency contribute to low birth weight, growth failure, impaired immunity, and increased infant mortality and morbidity. Furthermore, diets in less developed countries are predominantly cereal based and contain high amounts of phytate: a potent inhibitor of zinc absorption, and low in animal products that are good sources of highly bioavailable zinc. Thus zinc deficiency may have far reaching consequences on infant, child and maternal health but was long overlooked. In recent years, however, zinc deficiency has been acknowledged to be an important public health problem.

The research described in this thesis was carried out in Dodota-Sire District of Arsi Zone, which is a rural area about 150 km east of Addis Ababa in Ethiopia. It comprised a cross-sectional study of breastfed infants and their mothers; a double-blind randomised controlled trial in which the effect of supplementing breastfed infants with zinc for 6 mo on growth was studied during the supplementation period and 36 mo later; and a study of the mineral, phytate and tannin content of traditional diets of the rural population.

The cross-sectional study aimed to estimate the level of malnutrition and identify factors associated with the high level of stunting in breast-fed infants aged 5–11 mo. Infants

(n=305) and their mothers were examined physically, and anthropometric and demographic data were collected. The content of zinc, calcium and copper in breast milk was measured, and data collected on the type, frequency of consumption, and time of introduction of supplementary feeding. Overall, 36% were stunted, 41% underweight and 13% wasted. The highest prevalence of malnutrition was seen in infants aged 9-11 mo. Among mothers, 27% had chronic energy deficiency (body mass index <18.5 kg/m^2) and 20% were night blind indicating that vitamin A deficiency is a serious problem. Infants fed >3 times/d, consuming >600 mL/d or consuming cows milk in addition to cereals and/or legumes had markedly higher length-for-age Z-scores than their peers fed less frequently, consuming less food or not consuming cows milk (differences: 0.39, 95% CI: 0.04-0.74; 0.17, 95% CI: 0.02-0.32; 0.40, 95% CI: 0.07-0.72, respectively). Infants of mothers with low concentrations of zinc in their breast milk were more stunted. From the cross-sectional study, it could be concluded that the quality and quantity of foods consumed by infants was insufficient to prevent stunting, and that it is necessary to increase the nutrient supply to infants by increasing intake and nutrient concentration of breast milk and of supplementary foods they consume.

The aim of the intervention study was to investigate whether the low rate of linear growth could be improved by zinc supplementation. A randomised, double-blind, placebo-controlled trial was carried out on apparently healthy, breast-fed infants aged 6-12 mo. One hundred non-stunted (length-for-age, LAZ score >-2) were matched for age and sex with 100 randomly selected stunted (LAZ score <-2) infants. Infants, both stunted and non stunted, were matched by sex, age (within 2 mo) and recumbent length (within 3 cm) for random assignment, to receive a zinc supplement (10 mg Zn/d as $ZnSO_4$) or placebo, 6 d/wk for 6 mo. Anthropometric measurements were taken monthly, data on illness and appetite were collected daily, and samples of serum and hair were taken at the end of the intervention for the analysis of zinc. The length of stunted infants increased significantly more ($p<0.001$) when supplemented with zinc (7.0 ± 1.1cm; mean ± SE) than with placebo (2.8±0.9 cm); and the effect was greater ($p<0.01$) than in non-stunted infants (6.6± 0.9 cm vs 5.0± 0.8 cm for the zinc and placebo groups respectively; $p<0.01$). Zinc supplementation also increased the weight of stunted children (1.73±0.39 kg vs 0.95±0.39 kg for the corresponding placebo group; $p<0.001$) and of non-stunted children (1.19±0.39 kg vs 1.02±0.32 kg for the corresponding placebo group; $p<0.05$). Zinc supplementation resulted in a markedly lower incidence of anorexia and morbidity from cough, diarrhoea, fever and vomiting in the stunted children. The total number of these conditions per child was 1.56 and 1.11 in the stunted and non-stunted zinc supplemented children vs 3.38 and 1.64 in the stunted and non-stunted placebo treated children respectively. At the end of the intervention period, the concentrations of zinc in serum and hair of stunted infants, who had not been supplemented with zinc, were both lower than those in serum and hair of their non-stunted counterparts. Thus it was shown that combating zinc deficiency can increase the growth rate of stunted children to that of non-stunted infants in rural Ethiopia. This would appear to be due, at least in part, to reduction in morbidity from infection and increased appetite.

In order to examine whether effects of zinc supplementation persisted after discontinuation, anthropometric measurements were taken monthly 42 mo after randomisation, that is, 36 months after supplementation was discontinued. Additional data

were collected on zinc concentrations in serum and hair, and anthropometric indices in siblings born subsequently to randomisation. The effect of zinc supplementation of stunted infants on height was –3.4 cm (95% CI: –4.8 to –2.1) and on HAZ was –0.86 (95% CI: –1.26 to –0.52). More than half of stunted children had deficient serum and hair zinc concentrations, irrespective of whether they had been previously supplemented with zinc. Siblings of stunted infants were shorter, and had lower HAZ and weight-for-age Z-score than siblings of non-stunted counterparts. Thus, the beneficial effects obtained during zinc supplementation in stunted infants on linear and ponderal growth are reversed once supplementation is discontinued. Poor feeding practices might explain why siblings of stunted infants are more likely to be stunted than those of their non-stunted counterparts.

Diets of the rural population in Ethiopia are based predominantly on cereals, legumes, starchy tubers and roots. These foods contain relatively high amounts of zinc but also high amounts of phytate. Starchy tubers and roots were found to have a lower zinc content than cereals and were also low in phytate. The molar ratio of phytate to zinc is important for estimating the relative risk of having an inadequate intake of zinc. Phytate:zinc molar ratios in diets >15 are associated with suboptimal zinc status. Most of the foods analysed in this study had values >15, suggesting that those consuming diets based on these foods are at risk of having an inadequate intake of zinc and thus an inadequate zinc status. Fermented cereal enjera, however, showed molar ratios <15 suggesting that zinc bioavailability is high in this food. The low ratio is a result of the activity of phytases during the fermentation process. The calcium content of the foods was relatively low so inhibition of zinc absorption due to the combined chelating action of calcium and phytate was relatively unimportant. It is also interesting to note that tef enjera contains very high amounts of iron and explains the high intake of iron in regions where cereal tef serve as the main staple. However, the high content of iron in tef is also of concern because of the potential interaction between iron and zinc that may reduce zinc absorption to such an extent that zinc deficiency occurs.

Thus, the studies described in this thesis clearly demonstrate that zinc may be the primary limiting micronutrient for growth in stunted infants in rural Ethiopia. It also shows that zinc supplementation is effective in halting the stunting process and reducing the incidence of anorexia and morbidity in stunted children. However, discontinuation of zinc supplementation reverses the positive effects on linear and ponderal growth built up during zinc supplementation. Thus, in communities where stunting is highly prevalent and the bioavailability of zinc in the diets is low, zinc deficiency is likely to exist, and infants and young children should be targeted for zinc intervention in such a way that the intervention can be sustained.

Samenvatting

Ondervoeding bij jonge kinderen is een aanhoudend probleem in de wereld: ongeveer een kwart van alle kinderen in de leeftijd onder de 5 jaar zijn te licht voor hun leeftijd. Ontoereikende voeding en infectieziekten vormen de belangrijkste oorzaken en dragen bij aan de hoge prevalentie van kinderziekte en –sterfte in arme landen. Jaarlijks sterven naar schatting 12 miljoen kinderen in de leeftijd onder de 5 jaar als gevolg van infectieziekten en ondervoeding, waarbij ondervoeding verantwoordelijk is voor de helft van deze sterftegevallen.

Ondervoeding bij kinderen leidt tot achterstand in het groeiproces met betrekking tot lengte (‘stunting’) en tot gewicht (‘wasting’). Beide kunnen worden gemeten met behulp als antropometrische indicatoren, namelijk lengte-voor-leeftijd, en gewicht-voor-lengte. Kinderen worden algemeen beschouwd als te kort (‘stunted’) en te mager (‘wasted’) als hun z-scores voor lengte-voor-leeftijd en gewicht-voor-lengte lager zijn dan –2 standaardafwijkingen dan de mediaan-waarden gemeten door de US National Center for Health Statistics in een referentiepopulatie van vergelijkbare leeftijd en vergelijkbaar geslacht. Lengte-voor-leeftijd weerspiegelt de groei die is bereikt voor en na geboorte. Een te lage gewicht-voor-lengte daarentegen wordt daarentegen veroorzaakt door ernstige ondervoeding zoals kan worden aangetroffen tijdens hongersnood of na te snel te zijn overgegaan van borstvoeding op vast voedsel, of door ziekte. Het duidt op een acute gesteldheid waarin het lichaam gedwongen is om haar eigen reserves aan te spreken om ‘normaal’ te kunnen functioneren.

Naar schatting zijn 182 miljoen kinderen onder de 5 jaar (éénderde van het totaal in ontwikkelingslanden) te kort voor hun leeftijd. De prevalentie van te korte kinderen is 35% in Afrika en 44% in zuidoost Azië. De hoogste prevalentie komt voor in oostelijk Afrika, waar 48% van de kinderen onder de 5 jaar te kort zijn. Meer alarmerend is nog dat tijdens de laatste 20 jaar het aantal van deze kinderen behoorlijk gestegen is, van 13 miljoen in 1980 tot ongeveer 22 miljoen in 2000. Op het platteland van Ethiopië is een prevalentie geschat van 64%, hetgeen één van de hoogst gerapporteerde waarden ter wereld is.

In het verleden werd gedacht dat ondervoeding voornamelijk het gevolg is van onvoldoende inname van eiwitten en energie, maar nu is bekend dat gebrek aan micronutrienten ook een rol speelt. Gebrek aan jodium, vitamine A en ijzer hebben in de laatste twee decennia de meeste aandacht gekregen. Zink is echter ook belangrijk omdat gebrek aan dit element bijdraagt aan een laag geboortegewicht, groeiachterstand, verminderde immuuncapaciteit, en een verhoogde incidentie van kindersterfte en –ziekten. Het dagelijks voedsel in ontwikkelingslanden is voornamelijk gebaseerd op granen en bevat daarom hoge gehaltes aan fytaat, een sterke remmer van zinkopname. De voeding is verder arm aan dierlijke producten die een goede bron vormen van opneembaar zink. De verstrekkende gevolgen van zinkgebrek op de gezondheid van kinderen en moeders zijn lang over het hoofd gezien. Sinds recent wordt zinkgebrek echter als een belangrijk probleem gezien voor de volksgezondheid.

Het onderzoek dat in dit proefschrift wordt beschreven, werd uitgevoerd in het District van Dodota-Sire van Arsi Zone, een plattelandsgebied ongeveer 150 km ten oosten van Addis Abeba in Ethiopië. Het behelst allereerst een dwarsdoorsnedeonderzoek onder zuigelingen en hun moeders. Daarnaast werd in een dubbel-blind gerandomiseerd en vergelijkend experiment onder zuigelingen het effect van 6 maanden zinksuppletie gemeten op groei. Deze effecten werden gemeten na afloop van de interventie (na 6 maanden) en nog eens 36 maanden later. Tenslotte werd een studie uitgevoerd naar het gehalte aan mineralen, fytaat en tannine van traditioneel gegeten voedsel van de plattelandsbevolking.

Het doel van het dwarsdoorsnedeonderzoek was om het niveau van ondervoeding te meten, en om risicofactoren te vinden voor achterblijvende lengtegroei bij zuigelingen in de leeftijd tussen 5 en 11 maanden. Deze zuigelingen (n=305) en hun moeders werden lichamelijk onderzocht, en antropometrische en demografische data werden verzameld. Het gehalte aan zink, kalk en koper in de moedermelk werd gemeten, en ook werden gegevens verzameld over het type voedsel, de frequentie van voeding, en de leeftijd waarbij vast voedsel werd gegeven. In deze groep kinderen waren 36% te kort, en 41% te licht voor hun leeftijd, en 13% waren te dun. De hoogste prevalentie van ondervoeding werd waargenomen in zuigelingen in de leeftijd tussen 9 en 11 maanden. Van de moeders had 27% chronisch energietekort (Quetelet-index <18.5 kg/m^2), en 20% waren nachtblind, hetgeen erop duidde dat gebrek aan vitamine A een belangrijk probleem is. Zuigelingen die meer dan 3 keer per dag werden gevoed, die >600 mL melk per dag ontvingen, of die koeienmelk te drinken kregen behalve granen en/of peulvruchten waren beduidend langer voor hun leeftijd dan hun soortgenoten die minder frequent werden gevoed, minder voedsel ontvingen, of die geen koeienmelk te drinken kregen (respectievelijke verschillen in z-scores: 0.39, 95% BI: 0.04-0.74; 0.17, 95% BI: 0.02-0.32; 0.40, 95% BI: 0.07-0.72). Zuigelingen van moeders met lage concentraties van zink in moedermelk waren korter voor hun leeftijd. Uit dit dwarsdoorsnedeonderzoek kon worden geconcludeerd dat de hoeveelheid en de kwaliteit van het voedsel dat door zuigelingen wordt geconsumeerd onvoldoende was om 'stunting' te voorkómen, en dat het nodig is om de aanvoer van voedingsstoffen te verhogen door een verhoogde inname en concentratie te bewerkstelligen van voedingsstoffen in de moedermelk en het vaste voedsel dat deze kinderen consumeren.

Het doel van de interventiestudie was om te onderzoeken of de lengtegroei kon worden versneld door zinksuppletie. Een gerandomiseerd, dubbel-blind placebo-gecontroleerd experiment werd uitgevoerd onder schijnbaar gezonde zuigelingen in de leeftijd tussen 6 en 12 maanden. Honderd zuigelingen die te kort voor hun leeftijd waren, werden gepaard voor leeftijd en geslacht met honderd aselect gekozen zuigelingen die wèl te kort waren. In beide groepen werden kinderen gepaard voor geslacht, leeftijd (binnen twee maanden) en lengte (binnen 3 cm), en aselect toegewezen aan supplementen met daarin zink (10 mg Zn/dag als sulfaat) of placebo, dagelijks voor 6 dagen per week, en gedurende een periode van 6 maanden. Antropometrische bepalingen werden maandelijks uitgevoerd, en gegevens over ziekte en eetlust werden dagelijks verzameld. Monsters van serum en haar werden aan het

eind van de interventieperiode genomen voor de analyse van zinkconcentratie. Onder zuigelingen die bij aanvang te kort waren, en die zinksupplementen kregen, nam de lengte beduidend toe (7.0 ± 1.1 cm; gemiddelde waarde ± standaardfout) ten opzichte van zuigelingen die placebo kregen (2.8 ± 0.9 cm); en dit effect was groter ($P<0.01$) dan in kinderen die bij aanvang niet te kort waren (6.6 ± 0.9 cm tegenover 5.0 ± 0.8 cm in de groepen die respectievelijk zink of placebo ontvingen; $p<0.01$). Suppletie met zink leidde ook tot groter gewicht onder kinderen die bij aanvang te kort waren (1.73 ± 0.39 kg, tegenover 0.95 ± 0.39 kg voor de overeenkomstige placebo-groep), en onder hen die bij aanvang niet te kort waren (1.19 ± 0.39 kg, tegenover 1.02 ±0.32 kg voor de overeenkomstige placebo-groep; $p<0.05$). Onder hen die bij aanvang te kort waren resulteerde zinksuppletie ook in een beduidend lagere incidentie van gebrek aan eetlust en ziekte geassocieerd met hoesten, diarree, koorts en overgeven. Het totale aantal dat deze condities vóórkwamen per kind was 1.56 en 1.11 in kinderen die zink ontvingen en die bij aanvang respectievelijk te kort of niet te kort waren, tegenover overeenkomstige frequenties van 3.38 en 1.64 in kinderen die placebo ontvingen. Aan het eind van de interventieperiode waren de zinkconcentraties in serum en haar van kinderen die placebo hadden ontvangen lager dan de overeenkomstige concentraties in hen die zink hadden gekregen en die niet te kort waren. Hieruit bleek dus dat bestrijding van zinkgebrek bij plattelandskinderen in Ethiopië kan leiden tot een versnelde groei van te korte kinderen ten opzichte van hen die niet te kort zijn. Dit lijkt althans minstens gedeeltelijk te wijten aan de verminderde ziekte als gevolg van infecties and door een verbeterde eetlust.

Om te onderzoeken of de effecten van zink doorwerkten na beëindiging van de suppletie werden antropometrische metingen herhaald na 42 maanden, dus 36 maanden nadat de beëindiging van de suppletie. Zinkconcentraties in serum en haar werden nogmaals gemeten, evenals antropometrische gegevens van broertjes en zusjes die na randomisatie waren geboren. Het effect van zinksuppletie op de lengte in kinderen die bij aanvang te kort waren geweest was –3.4 cm (95% BI: -4.8 tot –2.1 cm), en op z-score voor lengte-voor-leeftijd was –0.86 (95% BI: -1.26 tot –0.52). Meer dan de helft van de kinderen die te kort waren geweest bij randomisatie hadden zinkgebrek zoals aangegeven door zinkconcentraties in serum en haar, ongeacht of ze voorafgaand waren voorzien van zinksupplementen of placebo. Broers en zussen van stunted kinderen waren korter, en hadden lagere z-scores voor lengte-voor-leeftijd dan van broers en zussen van niet-stunted kinderen. Hieruit blijkt dus dat de bevorderlijke effecten van zink die optreden gedurende de suppletie teniet worden gedaan als de suppletie wordt gestopt. Slechte voeding kan verklaren waarom broers en zussen van kinderen die te kort waren een grotere kans hebben om te kort te worden dan broers en zussen van kinderen die niet te kort waren.

Het dagelijks voedsel van deze plattelandsbevolking in Ethiopië is voornamelijk gebaseerd op granen, peulvruchten, en op knollen en wortels rijk aan zetmeel. Dit voedsel bevatten relatief hoge concentraties zink, maar ook hoge gehaltes fytaat. Zetmeel-rijke knollen en wortels bleken lagere zinkconcentraties te hebben dan granen, maar bevatten ook relatief weinig fytaat. De molaire verhouding van fytaat ten opzichte van zink is belangrijk bij het

inschatten van de kans op een onvoldoende inname van zink. Bij verhoudingen in het voedsel >15 zijn geassocieerd met een sub-optimale zinkstatus. De meeste voedingsmiddelen die in deze studie werden geanalyseerd hadden waarden >15, hetgeen erop duidt dat zij die voeding consumeren dat gebaseerd is op deze voedingsmiddelen grote kans hebben op een onvoldoende inname van zink en dus van een onvoldoende zinkstatus. Gefermenteerd graan ('enjera') had echter molaire verhoudingen <15, hetgeen erop duidt dat de opname in de darm van zink uit dit voedsel relatief goed is. De lage molaire verhouding is het resultaat van fytasen gedurende het fermentatieproces. Het kalkgehalte van de onderzochte voedingsmiddelen was relatief laag, zodat de remming van zinkabsorptie in de darm door de gecombineerde chelerende werking van kalk en fytaat niet erg belangrijk was. Het is interessant dat 'tef enjera' erg hoge concentraties van ijzer bevat, hetgeen de hoge inname van ijzer verklaart in gebieden waar graantef het hoofdvoedsel vormt. Deze hoge ijzerconcentratie is echter ook een bron van zorg vanwege de mogelijke interactie tussen ijzer en zink, als gevolg waarvan de zinkabsorptie zodanig laag kan zijn dat zinkgebrek ontstaat.

De in dit proefschrift beschreven studies tonen duidelijk aan dat zink een primair bepalende micronutrient is bij de groeiachterstand die optreedt bij plattelandskinderen uit Ethiopië. Zij laten ook zien dat zinksuppletie effectief deze groeiachterstand een halt toe kan roepen, en om de incidentie van gebrek aan eetlust en ziekte in te korte kinderen te verlagen. Echter, het beëindigen van suppletie leidt tot een ommekeer in de bevorderlijke effecten van zink op lengtegroei en gewichtstoename. In samenlevingen waar achterstand in lengtegroei vaak voorkomt en de biobeschikbaarheid van zink laag is, komt zinkgebrek vaak voor. In zulke omstandigheden moeten zuigelingen en jonge kinderen een doelgroep vormen waarin zinkinterventies op een duurzame wijze worden toegepast.

Acknowledgements

First of all I would like to mention my promoters, Professor Jo Hautvast and Professor Clive West. I gratefully acknowledge Prof. Hautvast for his consistent support, encouragement and guidance since the beginning of my study. Despite your busy schedule you have always found time for fruitful discussions. Clive West, I would like to express my sincere and utmost thanks. It is of a great honor for me to work under your supervision. Thank you for inspiring me to start the first zinc study in Ethiopia. It would have not been possible to start and complete this thesis without your continuous support and guidance in domain of science and scientific writing. I have learned quite a lot from you. I am very much indebted to him for his patience, caring, and sense of humor and all hours of discussions even when his health has not been in good condition. I am also grateful to his wife, Helen for her great patience, tolerance and hospitality; I have taken much of Clive's time at his home in Bennekom while he was ill.

I am grateful to Paul Deurenberg; thanks for your guidance and supervision during the initial period of my study. I also thank Prof. Frans Kok, although you were not directly involved in the thesis; your encouragement during the times I spent in the division is highly appreciated.

I am sincerely indebted to Dr Zewdie Wolde-Gebriel, former Director of Ethiopian Nutrition Institute (ENI), for his support to enable me to join the study programme at Wageningen University and for his continuous moral support. I thank very much Dr Abera Geyid, Director, and Ato Tilahune Woldemichael Deputy Director of EHNRI, and Dr Hailu Yenenh, the former Director, for their understanding and continuous support to finalise my study.

Many thanks to the children involved in the studies and their mothers, without their patience and excellent cooperation this study would not have been possible.

Many thanks are due to the cooperation provided by Dr Kedir Wajisso of Arsi Health Department and Ato Wake, Administrator of Dodota-Sire District for giving me permission to carry out the research in their area. The hard work of my field assistants for their interest, dedication and enthusiasm spent many hours walking under the hot sun in the dust in providing the supplements and data collection deserves my gratitude. Special appreciation also goes to the health workers in Dheera and Hamude health centers for their devotion, keeping records and taking care of the children involved in the study.

My appreciation and special thanks go to Dr Jemal Haidar for his invaluable contributions in taking care of clinical examinations and Wasse Dubale for his excellent technical assistance. A number of the Ethiopian Health and Nutrition Research Institute's Staff are also acknowledged for their contributions - Habtamu Fufa, Asfaw Hussen, Mengistu G/Tsadik, Benyam Tedla, Yonnes Tesfaye and Tsehai Tehome for their assistance in the laboratory work, Wolde-Mariam Girma for data organisation at the baseline measurements of the trial, Shibabaw Adgeh, Yimam Negatu, Demelash H/Meskel and Tsegaye Adisu for their good driving, and the Administration and Finance Department of the Institute for the smooth administrative matters.

Acknowledgements

I am grateful to the staff of Division of Human Nutrition and Epidemiology, for their support, kindness and friendship. My special gratitude go to Hans Verhoef for his assistance in statistical advice and critical comments on some of the articles; Jan Burema for his statistical advice; Grietje van der Zee and Gea Brussen, for logistic support and excellent arrangement of my stay in Wageningen; Lidwien van der Heijden, Eric van Munster and Riekie Jansen for their help in financial matters; Ben Scholte and the late Dirk Joghems for their help in computer- related issues; Lous Duym and Marie Jansen for their helpful hands whenever needed. I also thank very much the PhD students in the division for their moral support.

I thank very much all my friends both at home and abroad and colleaques in the Institute for their encouragement and continuous moral support.

I faithfully thank my father and mother; my brothers and sisters and their families for their continuous moral support and encouragement. My deepest thanks to my wife, for her patience, support and encouragement, during my field work and my visit to Wageningen, when she had to, apart from her busy duty, manage the home and be single parent to our two very busy little kids, Kulani and Jigssa.

Special thanks are due to 'The Nestlé Foundation for the Study of the Problem of Nutrition in the World" both for a scholarship of my study and for research funding.

About the author

Melaku Umeta Deressa was born on 29 April 1956 in Nekemte town in Wollega Zone,Ethiopia where he attended both primary and secondary schools. On matriculation, Melaku enrolled in the Faculty of Science, Addis Ababa University in 1974 but interrupted his studies and served in the National Development Programme for two years. On completion of his studies, he graduated with a BSc in Chemistry in 1980. Then, after working in what was then Ethiopian Nutrition Institute (ENI) for 4 years, Melaku joined the School of Biological Sciences at the University of East Anglia (Norwich), United Kingdom where he studied biochemistry, specialising in nutritional biochemistry, and graduating with an MSc degree in 1986. His thesis was entitled "Studies on the fermentation of *tef (Eragrostis tef)* and its nutritional significance", which he prepared under the supervision of Professor D.A.T. Southgate.

In 1994, Melaku attended the Second International Postgraduate Course on the Production and Use of Food Composition Data in Nutrition, organised by the Graduate School VLAG (Advanced studies in Food Technology, Agrobiotechnology, nutrition and Health Sciences) and Wageningen University. From June 1996 until December 2002, he carried out the work described in this thesis with fieldwork being carried out in Ethiopia, and the preparatory and final phases being undertaken in the Division of Human Nutrition and Epidemiology of Wageningen University.

Melaku has also attended a one-month training course on Methods and Techniques related to Vitamin A Analysis at the National Institute of Nutrition in Hyderabad (India); a three-week training course on Body Composition and Energy Metabolism held in Addis Ababa, organised by International Atomic Agency (IAEA); and several other national and international workshops.

Melaku has worked in what is now the Ethiopian Health and Nutrition Research Institute in various positions: Research Assistant; Expert; Food Chemistry Research Team Leader; Head of the Chemistry Laboratory; and Research Team Leader in Clinical Nutrition. He is also an Honorary Member of the Department of Biochemistry in the Medical Faculty of Addis Ababa University and he has taught biochemistry in School of Pharmacy since 1987. He also served as Guest Lecturer in Biochemistry at Gondar Medical Faculty where he covered a full one-year course in Biochemistry. Furthermore, he is a member of the National Micronutrient Committee. Melaku is married and is the father of two children (a daughter and a son).

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The research described in this thesis was financially supported by a grant from "The Nestlé Foundation for the Study of the Problem of Nutrition in the World".

Financial support for the printing of this thesis was obtained from the Dr Judith Zwartz Foundation, Wageningen, The Netherlands and Wageningen University.

This PhD project was part of the research programme of the Graduate School VLAG (Advanced studies in Food Technology, Agrobiotechnology, Nutrition and Health Sciences).

Cover design: Melaku Umeta

Printing: Grafisch Bedrijf Ponsen & Looijen B.V., Wageningen, The Netherlands.