Human zinc nutrition in arid regions with zinc deficiency in soils and crops – a case study in central Iran

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“Be like the sun for grace and mercy.
Be like the night to cover others' faults.
Be like running water for generosity.
Be like death for rage and anger.
Be like the Earth for modesty.

Appear as you are.
Be as you appear.”

— Rumi
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SUMMARY

Zinc (Zn) deficiency is recognized as a major problem of human nutrition world-wide. It has been estimated to affect up to one-third of the world's population. Inadequate dietary intake of bioavailable forms of Zn is considered the most frequent cause of Zn deficiency. The risk of insufficient dietary Zn intake is particularly high in populations depending on sources with low levels of absorbable Zn such as cereals and with no or only limited access to sources rich in bioavailable Zn such as meat. This situation is wide-spread in arid regions of developing countries. In the developing world; cereal grains provide nearly 50% of the daily calory intake of the population, and up to 70% in rural areas.

While the problem of Zn deficiency in developing countries was recognized already decades ago, it is only rather recently that a relationship between human Zn deficiency and low Zn levels in soils and crops has been found. Building on this link between soil and human nutrition, biofortification of food plants has been proposed as a new strategy to fight Zn malnutrition in developing countries. In addition to breeding for crop varieties with enhanced Zn-efficiency, it also includes the adaptation of farming practices such as fertilization and other soil amendments to improve the Zn concentration of consumed parts of food plants. In contrast to other interventions to abate Zn malnutrition, such as dietary diversification, supplementation and food fortification, biofortification is particularly attractive because it can improve crop production at the same time.

Independent of the choice of strategy, sustainable solutions require an approach that considers the system of land use, agricultural management practices, food production, consumer behaviour, human nutrition as a whole. Such an approach requires the knowledge and understanding of the relevant Zn fluxes through the system. This thesis was part of a larger project with the objective to develop, test and apply – using central Iran as an exemplary case – a system of model-based procedures to assess the fluxes of the essential microelement Zn through the food chain from soil through plants and livestock to the human population, in order (i) to identify dominant pathways of Zn from soils into crops and from there into human diets, (ii) to create a framework for the analysis of the effects of soil, climate, land use and agricultural practices on the nutritional quality of the produced food.
stuffs with respect to Zn availability for humans and (iii) to provide a decision-supporting tool for the evaluation of agricultural options to reduce dietary Zn deficiency. In the framework of this project the objectives of the thesis were: (1) to assess the major Zn sources in the diet of the study population; (2) to analyze phytic acid contents in the diets of the study population as main inhibitory factor of Zn bioavailability in the food; (3) to assess the dietary Zn intake and the nutritional Zn status of the study population, taking account also of iron nutrition status; (4) to develop a Zn intake model for the analysis of intervention strategies; and (5) to assess possible food-based strategies to improve human Zn nutrition.

Central Iran was chosen as case study region for this project because (i) Zn deficiency is considered an important public health concern in Iran (Balali et al. 1998), (ii) policymakers have started to become aware of the concern and are paying increasing attention to improve the nutritional status of the people, (iii) central Iran is representative for many other semi-arid to arid regions in the developing world with diets being based primarily on cereals, and (iv) a well-established basis of scientific collaboration existed between ETH and Isfahan University of Technology (IUT).

In a first step, two surveys were carried out, one in a suburban and the other in a rural community, on dietary habits and food composition in the study region. Major foods and ingredients were collected in the households of the participants of the study and analyzed for Zn, iron and phytic acid (PA). Zn was analysed in rice, wheat flour, bread and legumes (n=111) as well as the main animal source foods (dairy and meat products, n=107) and 9 local cooked dishes (n=38), consumed in a rural and suburban population in central Iran. Phytic acid, which is the main inhibitor of intestinal Zn absorption, was measured in the cereal and legume foods as well as in the local dishes. In addition, iron and calcium were measured in selected rice samples and legumes before and after cooking. The Zn concentration in cooked rice and bread, as major staples, were 0.88 ± 0.34 and 1.32 ± 0.16 mg/100 g DW in the suburb area and 1.29 ± 0.45 and 1.77 ± 0.21 mg/100 g DW in the rural area, respectively. The PA:Zn molar ratio of flat bread was 24 in the suburban area and 22 in the village. Cooked rice and composite dishes had PA:Zn molar ratios between 4 to 13. The results indicate that the local cheese-making processes, rice-polishing and bread-making have a major influence on Zn concentrations in the final products. The PA:Zn molar ratios indicate a low Zn absorption from the common flat breads, but no inhibited absorption from cooked rice and composite dishes.

In the next step we estimated the Zn and iron (Fe) status in the same two sample populations as before, related the Zn status to dietary Zn intake as determined from the data of
the previous surveys, and examined the relationship between Zn and Fe status. Blood samples from 341 subjects (27 preschool children, 157 schoolchildren, 91 women, 66 men) were analyzed for serum Zn, serum ferritin, total iron binding capacity, and hemoglobin concentrations. Daily Zn intake was calculated using the 3-day weighed food records of the previous surveys. The overall prevalences of Zn deficiency were 5.9% in the rural and 7.2% in the suburban community. The prevalence of iron deficiency was 27% in the rural and 31% in the suburban community. There was a positive correlation between Zn and Hb, but no correlation between Zn and Fe status. The prevalence of anemia was higher in the rural than in the suburban community (33.5% vs. 22.7%; p=0.04). Almost half of the anemia in the suburban community and 36% in the rural community were associated with iron deficiency. The hemoglobin levels correlated significantly with the serum Zn concentrations. The low prevalence of Zn deficiency was unexpected. It may be explained by a relatively high Zn intake from animal source foods. Anemia affected some 30% of the subjects, although less than half was due to iron deficiency. Given that Zn plays a role in the production of red blood cells and that there was no correlation between Zn and Fe status, it is possible that Zn deficiency was an independent cause of anemia. The lack of correlation between Fe and Zn status could be due to the frequent consumption of dairy products and tea.

In the final step, we developed a model for the evaluation of different intervention scenarios for abating human Zn deficiency by enhancing dietary Zn intake and demonstrated its applicability for the case of our test population. The model determines dietary Zn intake for different user-defined population groups using the molar PA:Zn ratio as an indicator of Zn bioavailability and taking account of uncertainty in the input data by means of Monte Carlo simulation. Based on the data from the first two steps, Iranian national statistics and other available sources, the model was used to assess the risk of Zn deficiency in the study population and to compare different scenarios of its future development. The scenario analysis revealed that it would take up to 60 years until 97.5% of the population would meet the estimated average Zn requirements if the consumption of major food items would continue to increase at their current exponential rates. With fortification of wheat flour, this goal could hypothetically be reached within 15 years. Biofortification was the next best alternative where after 15 years only 12% of the population would still be at risk of Zn deficiency.

While a more detailed study on trends in the dietary habits of the Iranian population and their variability among groups due to socio-economic and other factors would certainly be a and expedient and warranted sequel to this study, the scenario analysis showed that the Zn intake model developed here is a useful tool for the analysis of possible future trends and to
assist the design and evaluation of appropriate and efficient intervention strategies such as (bio)fortification. Our study in particular also shows that uncertainty analysis is crucial in such studies.
ZINKMANGEL GILT ALS EINES DER GRÖSSTEN GLOBALEN PROBLEME DER HUMANERNÄHRUNG. SCHÄTZUNGEN ZUFOLGE SIND BIS ZU EINEM DREI TEL DER WELTBEVÖLKERUNG BETRAFFEN. UNGENÜGENDE AUFPNMAHNE VON ZINK (ZN) IN BIOVERFÜGBARER FORM WIRD ALS HAUPTRUND FÜR ZN-MANGEL ERACHTET. DAS RISIKO EINER UNZULÄNGLICHEN ZN-AUFPNMAHNE MIT DER NÄHRUNG IST BESONDERE HOCH IN BEVÖLKERUNGSTEILEN, DEREN ZN-VERSORGUNG HAUPTSÄCHLICH VON ZN-QUELLEN MIT NIEDRIGEN GEHALTEN VON ABSORBIERBAREM ZN ABHÄNGIG IST UND DIE KEINEN ODER NUR LIMITIERTEN ZUGANG ZU ANDEREN ZN-QUELLEN HABEN. BESONDERE IN ARIDEN UND SEMIARIDEN GEBIETEN IN ENTWICKLUNGSLÄNDERN IST DIESE SITUATION VERBREITET. FAST 50% DES KALORIENBEDARFS DER STÄDTISCHEN UND BIS ZU 70% DER LÄNDLICHEN BEVÖLKERUNG WIRD HIER ÜBER GETREIDE ABGEGECKT.

DAS PROBLEM DES ZN-MANGELS IN ENTWICKLUNGSLÄNDERN WURDE SCHON VOR JAHRENTEN ERKANNT. ALLERDINGS WURDE ERST IN LETZTER ZEIT EINE BEZIEHUNG ZWISCHEN ZN-MANGEL IN DER BEVÖLKERUNG UND NIEDRIGEN ZN-KONZENTRATIONEN IN BÖDEN UND GETREIDE ENTDECKT. AUFBWAUDEND AUF DER BEZIEHUNG ZWISCHEN BODEN UND HUMANERNÄHRUNG, WURDE DIE BIOFORTIFIKATION VON ZN IN NahrungsPFLANZEN ALS NEUE STRATEGIE IM KAMPF GEGEN ZN-MANGEL IN ENTWICKLUNGSLÄNDERN VORGESCBLAGEN. NEBEN DER ZÜCHTUNG VON GETREIDEVARIETÄTEN MIT ERHÖHTER ZN-EFFIZIENZ BEINHALTET DIESE DAS WISSEN UND VERSTÄNDNIS DER RELEVANTEN ZN-FLÜSSE IM SYSTEM. Diese Doktorarbeit war Teil eines größeren Projekts, welches anhand des Fallbeispiels Iran das Ziel verfolgte, ein System modelbasiert der Massnahmen zu entwickeln, zu testen und anzuwenden um die ZN-FLÜSSE
durch die gesamte Nahrungskette vom Boden über die Pflanzen und Tiere bis zum Mensch zu erfassen, um (i) die dominanten Wege von Zn vom Boden in Lebensmittel und die Ernährung zu identifizieren, (ii) einen Rahmen zu schaffen für die Analyse der Effekte, die Boden, Klima, Landnutzung und Agrarmethoden auf die Qualität der produzierten Nahrungsmittel bezüglich Zn-Verfügbarkeit für den Menschen haben, (iii) ein Werkzeug zur Verfügung zu stellen, das dabei hilft agrartechnische Massnahmen zu evaluieren die als Option zur Verbesserung der Zinkversorgung zur Verfügung stehen. Im Rahmen des Gesamtprojektes hatte diese Doktorarbeit folgende Ziele: (1) die Hauptquellen von Zn in der Ernährung der untersuchten Bevölkerungsteile zu identifizieren, (2) den Phytinsäuregehalt (PA) der Nahrung der untersuchten Bevölkerung als Haupthemmungsfaktor für die Bioverfügbarkeit von Zn zu analysieren, (3) die Nahrungsaufnahme und den Gehalt von Zn in den Nahrungsmitteln der untersuchten Bevölkerung unter Einbezug der Eisen (Fe) Versorgung zu bestimmen, (4) ein Zinkaufnahme-Modell für die Analyse von Interventionsstrategien zu entwickeln, (5) mögliche Ernährungs-basierte Strategien zu beurteilen.

Zentraliran wurde als Fallstudie für dieses Projekt gewählt, weil (i) Zn-Mangel ein wichtiges öffentliches Gesundheitsproblem im Iran ist (Balali et al., 1998), (ii) die Politik begonnen hat sich dieses Problems bewusst zu werden und zunehmend Wert auf die Verbesserung des Zn-Versorgungszustands der Bevölkerung legt, (iii) Zentraliran mit seiner hauptsächlich getreidebasierten Ernährung repräsentativ ist für viele andere semiarid bis aride Regionen in Entwicklungsländern, (iv) bereits eine gut etablierte wissenschaftliche Kollaboration zwischen ETH Zürich und Isfahan University of Technology (IUT) existierte. In einem ersten Schritt wurden zwei Umfragen zu den Ernährungsgewohnheiten und der Nahrungszusammensetzung durchgeführt, eine in einer suburbanen und eine in einer ländlichen Gemeinde im Zentraliran. Die Hauptnahrungsmittel und Zutaten wurden in den Haushalten der Studienteilnehmer gesammelt und auf Zn, Fe und PA analysiert. Zink wurde in Reis, Weizenmehl, Brot und Gemüse (n=111) sowie in den wichtigsten tierischen Nahrungsmitteln (Milch- und Fleischprodukte, n= 107) analysiert. Weiterhin wurden 9 typische lokale Gerichte (n=38) der ländlichen und suburbanen Bevölkerung in Zentraliran untersucht. Phytinsäure, der Haupthemmstoff der intestinalen Zn Absorption, wurde in Getreiden und Gemüsen sowie in typischen lokalen Gerichten gemessen. Ferner wurde die Fe- und Kalziumkonzentrationen ausgesuchter Reisproben und Gemüse vor und nach dem Kochen gemessen. In den Grundnahrungsmitteln lagen die Zn-Konzentrationen bei 0.88 ± 0.34 (gekochter Reis) und 1.32 ± 0.16 mg/ 100 g Trockengewicht (Brot) in der suburbanen Region und 1.29 ± 0.45 und 1.77 ± 0.21 mg/ 100 g Trockengewicht in der ruralen Region.


Im letzten Schritt haben wir ein Modell für die Evaluation von unterschiedlichen Massnahmen zur Verminderung von Zn-Mangel durch eine verbesserte Nahrungsaufnahme von Zn entwickelt, und haben die Anwendbarkeit des Modells auf unsere Testpopulation evaluiert. Das Modell bestimmt die Nahrungsaufnahme von Zn für verschiedene, vom Nutzer-

INTRODUCTION

Micronutrient malnutrition – a global problem
Micronutrient malnutrition – also called “hidden hunger” – is a widespread global problem (Welch 2002). In addition to the direct health effects, the existence of micronutrient malnutrition has profound implications for economic development and productivity, particularly in terms of the potentially huge public health costs and the loss of human capital formation (WHO, 2006). In particular, zinc deficiency is now recognized as one of the most severe problems of human malnutrition world-wide (Prasad, 1984; Gibson, 2006). It is estimated to affect up to one-third of the global human population (Hotz and Brown, 2004) and is particularly frequent in India, Southeast Asia, and equatorial Africa (Figure 1). Furthermore, at least one third of the world’s population is affected with iron, vitamin A and iodine deficiency. Of the three, iron deficiency is the most prevalent. It is estimated that over two billion people are anemic, between one and two billion have inadequate iodine nutrition and a quarter billion preschool-aged children are vitamin A deficient (WHO, 2006). As for zinc, the majority of all these deficiencies are in developing countries.

Zinc nutrition and deficiency
Zinc is an essential component of a large number (>300) of enzymes participating in the synthesis and degradation of carbohydrates, lipids, proteins, and nucleic acids as well as in the metabolism of other micronutrients. It also plays a central role in the immune system, affecting a number of aspects of cellular and humoral immunity (FAO/WHO, 2004). The clinical features of severe zinc deficiency in humans are growth retardation, delayed sexual and bone maturation, skin lesions, diarrhea, alopecia, impaired appetite, increased susceptibility to infections mediated via defects in the immune system, and the appearance of behavioral changes (Hambidge, 1987). The effects of marginal or mild zinc deficiency are less clear. A reduced growth rate and impairments of immune defense are so far the only clearly demonstrated signs of mild zinc deficiency in humans. Other effects, such as impaired taste and wound healing, which have been claimed to result from a low zinc intake, are less consistently observed (Hambidge, 2000).
Low dietary zinc intake is in general the main cause of zinc deficiency. The risk of zinc deficiency is particularly high in populations depending on diets with low levels of absorbable zinc and with no or only limited access to sources rich in bioavailable zinc such as meat. Zinc deficiency is a problem particularly in regions where the population consumes mainly cereals and where soils are low in phytoavailable zinc, as for instance in India (Cakmak, 2008). In the developing world, cereal grains provide nearly 50% of the daily calorie intake of the population. This could be as high as 70% in rural areas. Although cereals provide a major part of the total dietary zinc intake in these areas, they are not ideal as sources of nutritional zinc, because they contain high concentrations of phytate, which complexes zinc and hinders its absorption in the human intestine (Sanstead, 1995; Frossard et al., 2000; Graham et al., 2001; Rimbach et al., 2008). While the problem of zinc deficiency in developing countries was recognized already decades ago (Cakmak et al. 1999), the link between human zinc deficiency and low zinc levels in soils and crops has been recognized only recently. Sunanda et al. (1995) found that soil zinc deficiency translated into low zinc concentrations in rice and these in turn into low serum zinc levels of the local population in Andhra Pradesh, as long as there was no sufficient supply from other sources of protein in the diet. Cakmak et al. (1996 and 1999) showed that there was a clear link between low zinc status in Central Anatolian soils, zinc deficiency in wheat and zinc malnutrition in school children.

**Interventions to fight zinc deficiency**

There are various possibilities to abate mineral malnutrition, including dietary diversification, supplementation, food fortification and biofortification. These strategies do not necessarily exclude each other. Each of them has advantages and problems.

Given that foods differ widely in available zinc, dietary modification or diversification would seem a straightforward and sustainable way to combat zinc deficiency. But changes in dietary habits require individual and societal acceptance, as well as the availability of alternative foods at affordable prices. Supplementation with pharmaceutical zinc preparations can be an effective measure to alleviate zinc deficiency on an individual basis. However, this strategy has often failed on a population level in developing countries, due to lack of adequate infrastructure and education (Graham et al., 2000). Food fortification is a strategy that can be applied rather rapidly at a national level without personal contact to and change of dietary habits by the recipients. Fortification of maize and wheat flour with zinc and other micronutrients has been implemented in Mexico, Indonesia and South Africa (Gibson, 2006). Similar to supplementation, a problem of food fortification in developing countries is that its
successful implementation into society requires safe delivery systems, stable policies, appropriate social infrastructures and continued financial support (White and Broadley, 2005; Gibson, 2006).

Building on the link between soil and human nutrition pointed out before, biofortification of food plants has been proposed more recently as a new strategy to fight zinc malnutrition in developing countries (Welch and Graham, 1999; Frossard et al, 2000). Biofortification is particularly attractive in the case of zinc because it can improve crop production at the same time (Cakmak; 2009). In addition to breeding for crop varieties with enhanced zinc deficiency, also the adaptation of farming practices such as fertilization, co-cropping and organic matter management has potential to increase the bioavailable zinc concentration of consumed parts of food crop plants (Cakmak, 2008; Schulin et al., 2009). For example, Cakmak et al. (1999) found that zinc fertilization not only increased total zinc concentrations in wheat grains on zinc deficient soil, but also its bioavailability for consumers by decreasing the phytate:zinc ratio. The disadvantage of zinc biofortification is that it is still in development and has not yet been demonstrated to work in practice on a large scale.

Independent of the choice of strategy, sustainable solutions require an approach that considers the system of land use, agricultural management practices, food production, consumer behaviour and human nutrition as a whole. Such an approach requires the knowledge and understanding of the relevant zinc fluxes through the entire system.

![Figure 1: Prevalence of countries which are under risk of zinc deficiency](http://www.izincg.org/)
Zinc nutrition and deficiency in Iran

Central Iran was chosen as case study region for this project because (i) zinc deficiency is considered an important public health concern in Iran (Balali et al. 1998), (ii) policymakers have started to become aware of the concern and are paying increasing attention to improve the nutritional status of the people, (iii) central Iran is representative for many other semi-arid to arid regions in the developing world with diets being based primarily on cereals, and (iv) a well-established basis of scientific collaboration existed between ETH and Isfahan University of Technology (IUT).

Although zinc deficiency was recognized as a major human health problem in parts of Iran only in the last two decades, it was first discovered in Iran already in the early 1960’s. Following the first report of human zinc deficiency in adolescent boys from the Fars province in Iran (Prasad et al., 1963), several subsequent studies reported that zinc supplements increased the height and weight of Iranian children (Hakimi et al 2006; Ebrahimi et al 2006; Ranaghy et al 1974). More recently, however prevalence estimates for zinc deficiency in Iran have varied widely depending on the Province and the population studied. Using low serum zinc as the indicator of zinc status, some studies have reported zinc deficiency to be in the region of 30% (Mahmmodi et al 2001; Sharifi et al 1999; Fesharakinia 2009) or higher (Beikheirnia et al 2004), whereas others have reported zinc deficiency to be <10% (Khalili et al 2008, Dehghani et al 2011). The most recent study by Dehghani et al. (2011) reported that zinc deficiency affected only 7.9% of the 3-18 year old children in Shiraz. These latter workers suggested that differences in dietary patterns, the recent wide prescription of zinc supplements by pediatricians, and soil zinc level could all explain the different estimates of zinc deficiency from different Iranian regions.

On average, food supplies of the country provide 16 and 3984 mg/d of zinc and phytate, respectively (Hotz and Brown, 2004). With an average phytate:zinc molar ratio of 24.6 and a stunting prevalence of about 15% Iran has been considered as having medium risk of zinc deficiency (Hotz and Brown, 2004). According to two recent national surveys (National Comprehensive Study on Household Food Consumption Pattern and Nutritional Status in Iran & National Integrated Micronutrient Survey, 2001; cited in the final report by Kalantari et al., 2006), the Iranian diet is plant-based and the staple food is mainly bread. In both urban and rural populations the average intake of vegetables, fruits, meat and dairy products are reported to be lower than recommendations. Although the surveys did not provide data on the zinc intake, the dietary patterns suggest low zinc intake and bioavailability, due to the low
consumption of animal products and high concentration of phytic acid in the cereal-based staple foods.

The phytate:zinc molar ratio in different types of Iranian bread has been studied in some parts of the country (Reinhold, 1971; Faridi et al., 1983; Jahed Khaniki, 2005; Gargari et al., 2007). There are also some sparse studies on population’s zinc intake, using the 24h recall, and Zinc status, measuring the serum zinc concentration (Mahmoodi and Kimiagar, 2001; Kelishadi et al., 2002; Mir et al., 2007; Khalili et al., 2008; Farzin et al., 2009; Dashti-Khavidaki et al., 2010).

The available data on zinc and phytate concentrations of Iranian foods, dietary zinc and phytate intake and nutritional zinc status of the population as well as the understanding and recognition of causes, consequences and intervention options of zinc deficiency are still insufficient in the country to make informed choices on efficient intervention strategies.

Objectives
This thesis was part of a larger project titled “zinc fluxes from soil into the food chain in arid agroecosystems – a case study in Iran”. The overall objective of the project was to develop, test and apply – using central Iran as an exemplary case – a system of procedures which serves to assess the fluxes of the essential microelement zinc through the food chain from soil through plants and livestock to the human population, applicable to countries in arid regions of the developing world at a regional or larger scale, in order (i) to identify the dominant pathways of zinc from soils into crops and from there into human diets in regions where zinc deficiency is a major problem of human nutrition, (ii) to create a framework for the analysis of the effects of soil, climate, land use and agricultural practices on the nutritional quality of the produced food stuffs with respect to zinc availability for humans and (iii) to provide a decision-supporting tool for the evaluation of agricultural options to reduce dietary zinc deficiency. In the framework of this project the objectives of the thesis were to:

1. Assess the major zinc sources in the diet of the study population;
2. Analyze phytic acid contents in the diets of the study population as main inhibitory factor of zinc bioavailability in the food;
3. Assess the dietary zinc intake and the nutritional zinc status of the study population, taking account also of iron nutrition status;
4. Develop a zinc intake model for the analysis of intervention strategies;
5. Use the model to assess possible food-based strategies to improve human zinc nutrition.

To achieve these objectives, we followed the steps shown in the flowchart of Figure 2.

![Figure 2. Steps carried out in this thesis](image)

Outline of thesis

Chapter 1 covers a comprehensive review of zinc and iron metabolism, bioavailability, human requirements, causes and consequences of deficiency, indicators, and intervention strategies.

Chapter 2 presents the results of two surveys carried out, one in a suburban and the other in a rural community, on dietary habits and food composition in the study region. Major foods and ingredients were collected in the households of the participants of the study and analyzed for zinc, iron and phytic acid. Description of the food samples, analytical methods, quality control procedures and statistical treatments of the data are given to permit the end users of the food composition data to evaluate the appropriateness of such data in their projects.

Chapter 3 gives the results of a second survey in which we assessed the zinc and iron status in the same two sample populations of the previous survey. The results were related to the rates of dietary intake in iron and bioavailable zinc determined from the data of the food consumption survey and compared to recommended intake rates. We also analyzed a potential correlation between anemia and serum zinc status.
Chapter 4 describes the zinc intake model developed here and shows how it can be used to analyze the impact of intervention strategies on zinc intake rates and zinc deficiency risks in target population groups.

Finally, general conclusions concerning zinc bioavailability in local foods, diet composition and zinc status of the population, and food management and planning in future are drawn. A number of shortcomings encountered in modeling and data collection are pointed out, and an outlook is provided pointing out the potential applicability of the model developed in this study to include other nutrients and nutritional criteria in Iran and other countries with similar conditions.

REFERENCES


CHAPTER 1 - LITERATURE REVIEW

2.1 Zinc

2.1.1 History
Zinc essentiality was established in 1869 for plants, in 1934 for experimental animals and in 1961 for humans (King and Cousins, 2006). A syndrome of anemia, hypogonadism and dwarfism was reported in a 21-year-old Iranian farmer in 1961 who was subsisting on a diet of unrefined flat bread, potatoes and milk (Prasad, 1963). Shortly after, a similar syndrome was observed in Egyptian adolescents who had similar dietary history to that of the Iranians, mainly subsisting on bread and beans (Sandstead et al., 1967). Administration of supplemental zinc or diets containing adequate animal-protein foods improved growth and corrected the hypogonadism, while anemia responded to oral iron treatment. Subsequent studies showed that the syndrome was primarily the result of low dietary zinc intake in the diet. Since the discovery of zinc deficiency as a human health problem in the 1990s, interest in the biochemical and clinical aspects of zinc nutrition has increased markedly.

2.1.2 Biochemical and Physiologic Functions
Although zinc-dependent biochemical mechanisms in physiologic functions have received extensive study, clear relationships have not been fully defined. Zinc is ubiquitous within cells in contrast to iron, which is contained in defined cellular components and has defined physiological roles. The role of zinc in biology can be grouped into three general functional classes, namely catalytic, structural and regulatory functions (Cousins, 1996).

2.1.3 Metabolism

1) Absorption
Zinc is absorbed in the small intestine by a carrier-mediated mechanism (Cousins, 1985). Under normal physiologic conditions, transport processes of uptake are not saturated. The fraction of zinc absorbed is difficult to determine because zinc is also secreted into the gut. Zinc administered in aqueous solutions to fasting subjects is absorbed efficiently (60–70 percent), whereas absorption from solid diets is less efficient and varies depending on zinc
content and diet composition (FAO/WHO, 2004). Generally, 33 percent is accepted as the average zinc absorption in humans (Cousins, 1985; Turnlund et al., 1984). More recent studies have suggested different absorption rates for different population groups based on their type of diet and phytate:zinc molar ratio (Table 1). Zinc absorption is concentration dependent and increases with increasing dietary zinc up to a maximum rate (Steel et al., 1985; FAO/WHO, 2004). In addition, zinc status may influence zinc absorption. Zinc-deprived humans absorb this element with increased efficiency, whereas humans on a high-zinc diet show a reduced efficiency of absorption (Krebs, 2000).

Table 1. Estimates of dietary zinc absorption, as developed by WHO, FNB/IOM, and IZiNCG, and summaries of the data used to derive them.

<table>
<thead>
<tr>
<th>Diet types represented</th>
<th>WHO</th>
<th>IOM</th>
<th>IZiNCG</th>
</tr>
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<tbody>
<tr>
<td>Diet types represented</td>
<td>Highly refined</td>
<td>Mixed/ refined vegetarian</td>
<td>Unrefined</td>
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<tr>
<td></td>
<td></td>
<td>Mixed, n=5</td>
<td>Mixed, n=1</td>
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<tr>
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<td></td>
<td>Semi-purified, n=4</td>
<td>Refined vegetarian, n=3</td>
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<tr>
<td></td>
<td></td>
<td>EDTA-washed soy protein, n=1</td>
<td>Unrefined, cereal-based, n=1</td>
</tr>
<tr>
<td>Study type</td>
<td>Single meal &amp; total diet</td>
<td>Total diet</td>
<td>Total diet</td>
</tr>
<tr>
<td>Subjects</td>
<td>NA</td>
<td>NA</td>
<td>Men 19-50 yrs</td>
</tr>
<tr>
<td></td>
<td>NA</td>
<td>NA</td>
<td>Men &amp; women 20+ yrs</td>
</tr>
<tr>
<td>Phytate:zinc molar ratio</td>
<td>&lt; 5</td>
<td>5-15</td>
<td>&gt; 15</td>
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<tr>
<td></td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
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<td></td>
<td>4-18</td>
<td>&gt; 18</td>
<td></td>
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<tr>
<td>Zinc absorption</td>
<td>50%</td>
<td>30%</td>
<td>15%</td>
</tr>
<tr>
<td></td>
<td>41%</td>
<td>26% men</td>
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<td></td>
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<td>34% women</td>
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<td>18% men</td>
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<td></td>
<td></td>
<td>25% women</td>
<td></td>
</tr>
</tbody>
</table>

Zinc is released from food as free ions during digestion. These liberated ions may then bind to endogenously secreted ligands before their transport into the enterocytes in the duodenum and jejunum (Tubek, 2007; FAO/WHO, 2004). Specific transport proteins may facilitate the passage of zinc across the cell membrane into the portal circulation. With high intakes, zinc is also absorbed through a passive paracellular route. The portal system carries absorbed zinc directly to the liver, and then released into systemic circulation for delivery to other tissues. About 70% of the zinc in circulation is bound to albumin, and any condition that
alters serum albumin concentration can have a secondary effect on serum zinc levels. Although serum zinc represents only 0.1% of the whole body zinc, the circulating zinc turns over rapidly to meet tissue needs (Tubek, 2007; Hotz and Brown, 2004; FAO/WHO, 2004).

**Zinc Transporters**

There are at least 10 zinc transporters (ZnT) and 15 Zip transporters in human cells (Cousins et al., 2006). They appear to have opposite roles in cellular zinc homeostasis. The expression and cellular distribution of the ZnTs is highly regulated by changes in zinc (Devergnas et al., 2004). ZnTs reduce intracellular zinc availability by promoting zinc efflux from cells or into intracellular vesicles, while Zip transporters increase intracellular zinc availability by promoting extracellular zinc uptake and, perhaps, vesicular zinc release into the cytoplasm (Sekler et al., 2007). Both the ZnT and Zip transporter families exhibit unique tissue-specific expression, differential responsiveness to dietary zinc deficiency and excess, and differential responsiveness to physiologic stimuli via hormones and cytokines (Liuzzi and Cousins, 2004).

The recently characterized ZnTs have significantly increased understanding of the interrelationships of cellular zinc uptake and efflux but do not yet account for observations at the whole body level. ZnT-1 is a ubiquitously expressed protein that has been found to be present in the villi of the proximal small bowel (McMahon and Cousins 1998a). In response to manipulation of dietary zinc, however, expression in rats was increased in response to zinc supplementation but not to zinc restriction (McMahon and Cousins 1998b). These and other observations have led to a current consensus that ZnT-1 functions mainly as a zinc exporter and may play a role in zinc homeostasis as a mechanism for zinc acquisition and elimination under conditions of excess of zinc (McMahon and Cousins 1998b).

The role of metallothionein (MT), an intracellular metal binding protein, in the regulation of zinc absorption, particularly in conjunction with the zinc transporters, also remains unclear. Hepatic and intestinal MT synthesis is stimulated by dietary zinc supplementation, by intraperitoneal zinc injection and by inflammation and the acute phase response. Dietary restriction also results in diminished MT synthesis. In experiments with knockout and transgenic mice, the rise in serum zinc after a single dose of zinc was much greater in the MT knockouts than in the control animals. In contrast, the serum zinc response of the MT transgenic animals was blunted compared with that of the control animals. The expression of ZnT-1 was also measured and found to be directly related to serum zinc levels but unaffected by MT levels (Davis et al. 1998). Thus, MT may function in cellular responses to limit free
zinc concentrations within quite narrow ranges (Cousins 1996) and function as a zinc pool (Davis et al. 1998).

Another transporter potentially involved in zinc and other metal uptake is DCT1, a transmembrane polypeptide that is found in the duodenum in the crypts and lower villi and may be available for the uptake of several metal ions (McMahon and Cousins 1998b).

As these transport proteins are identified and characterized, investigations in the whole animal, under conditions of a range of dietary intake, will be needed. Animal and human studies indicate considerable ability to enhance efficiency of absorption in response to low dietary zinc intake or increased physiologic demand; as yet, the subcellular correlates of these observations are lacking. Observations relating the amount of absorbed zinc to the amount of excreted zinc and to exchangeable pool sizes also await corroboration with the subcellular processes.

Ion gradients are generated by two main mechanisms: 1) A primary pump, utilizing the energy of ATP-hydrolysis; or 2) a secondary active mechanism that uses an ion gradient, such as Na\(^+\), for generating Zn\(^{2+}\) gradients (Sekler et al., 2007). A Zn\(^{2+}\) pump has been demonstrated in bacteria, where several forms of p-type ATPases have been shown to catalyze active Zn\(^{2+}\) transport (Banci et al., 2002). Recently, a similar ATPase, which transports Zn\(^{2+}\) and Cd\(^{2+}\) and to a lesser extent other heavy metals, has been discovered in Arabidopsis (Eren et al., 2006). Surprisingly, there is still no evidence for a Zn\(^{2+}\) pump in either yeast or mammalian cells, though a Cu pump has been identified that is linked to heavy metal ion transport (Petrukhin, 1994).

A Na\(^+\)-dependent secondary active mechanism has, however, been suggested to facilitate formation of the transmembrane Zn\(^{2+}\) gradient in neurons (Sekler et al., 2007). Early studies suggested that the neuronal Na\(^+\)/Ca\(^{2+}\) exchanger mediates Zn\(^{2+}\) extrusion (Sensi, 1997), but more recent findings seem to support the existence of a distinct Na\(^+\)/Zn\(^{2+}\) exchanger. These studies have indicated that a putative Na\(^+\)/Zn\(^{2+}\) exchanger, probably a member of the Na\(^+\)/Ca\(^{2+}\) exchanger superfamily, operates with a stoichiometry of 3Na\(^+\)/1Zn\(^{2+}\), promoting Zn\(^{2+}\) efflux against a 500-fold transmembrane gradient (Ohana et al., 2004). This mechanism is pharmacologically and molecularly distinct from the classical Na\(^+\)/Ca\(^{2+}\) exchangers. Whether this exchanger is the principle plasma membrane extruder of Zn\(^{2+}\) or is accompanied by an as yet unidentified Zn\(^{2+}\) pump, is an open and intriguing question (Sekler et al., 2007).
II) Homeostasis
Maintaining a constant state of cellular zinc, or homeostasis, is essential for survival. In animals and humans, adjustments in total zinc absorption and endogenous intestinal excretion are the primary means of maintaining zinc homeostasis (Hambidge and Krebs, 2001). The adjustments in gastrointestinal zinc absorption and endogenous excretion are synergistic. Shifts in the endogenous excretion appear to occur quickly with changes in intake just above or below optimal intake while the absorption of zinc responds more slowly, but it has the capacity to cope with large fluctuations in intake (King et al., 2000). With extremely low zinc intakes or with prolonged marginal intakes, secondary homeostatic adjustments may augment the gastrointestinal changes. These secondary adjustments include changes in urinary zinc excretion, a shift in plasma zinc turnover rates and, possibly, an avid retention of zinc released from selected tissues, such as bone, in other tissues to maintain function (King, 2000; Hotz and Brown, 2004).

III) Excretion
Loss of zinc through gastrointestinal tract accounts for approximately half of all zinc eliminated from the body. Considerable amount of zinc is secreted through the biliary and intestinal secretions, but most of it is reabsorbed. This is an important process in the regulation of zinc balance. Other routes of zinc excretion include urine and surface losses (desquamated skin, hair, sweat) (Tubek, 2007; Hotz and Brown, 2004; FAO/WHO, 2004). Measurements in humans of endogenous intestinal zinc have primarily been made as fecal excretion; these indicate that amounts excreted are responsive to zinc intake, absorbed zinc and physiologic need (Krebs, 2000).

Typically, human zinc intakes range from 107 to 231 µmol/day (equivalent to 14–30 mg/kg). These intakes support crude zinc balance (i.e., replace fecal and urinary losses) in healthy adults, but balance can be achieved when as little as 22 µmol/day (2.8 mg/kg) or as much as 306 µmol/day (40 mg/kg) is fed (Johnson et al. 1993). With these extreme reductions or increases in zinc intake, zinc losses either fell or increased during the first 6–12 d after the dietary change so that balance was achieved (King et al., 2000). Thus, humans appear to have the capacity to regulate whole body zinc content over a 10-fold change in intake, as has been observed in experimental animals. Body zinc content is regulated, by homeostatic mechanisms, over a wide range of intakes by changes in fractional absorption (normally 20-40%) and urinary (0.5 mg/day) and intestinal (1-3 mg/day) excretion (Fairweather-tait and Hurrell, 1996). A number of fractional zinc absorption studies in humans are summarized in
Table 2 to provide some numbers regarding zinc intake, zinc excretion and retention. All of the studies were performed with healthy adults who consumed diets with adequate amounts of zinc before implementation of the study diet.

Table 2. Zinc absorption and endogenous losses with different levels of zinc intake in adult men (µmol/day).

<table>
<thead>
<tr>
<th>Reference</th>
<th>Day of study</th>
<th>Diet zinc</th>
<th>Total absorbed zinc</th>
<th>% absorbed</th>
<th>Endog fecal loss</th>
<th>Urinary zinc</th>
<th>Balance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wada et al., 1985, n=6</td>
<td>12</td>
<td>252</td>
<td>63</td>
<td>25</td>
<td>29</td>
<td>7</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>54</td>
<td>85</td>
<td>42</td>
<td>49</td>
<td>27</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>Lee et al., 1993, n=8</td>
<td>28</td>
<td>192</td>
<td>85</td>
<td>44</td>
<td>65</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>63</td>
<td>40</td>
<td>63</td>
<td>27</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>Taylor et al., 1991, n=5</td>
<td>6</td>
<td>85</td>
<td>34</td>
<td>40</td>
<td>29</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>12</td>
<td>13</td>
<td>93</td>
<td>13</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Jackson et al., 1984, n=1</td>
<td>8</td>
<td>472</td>
<td>98</td>
<td>21</td>
<td>90</td>
<td>11</td>
<td>-3</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>224</td>
<td>73</td>
<td>32</td>
<td>71</td>
<td>9</td>
<td>-7</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>109</td>
<td>50</td>
<td>47</td>
<td>46</td>
<td>10</td>
<td>-6</td>
</tr>
<tr>
<td>Turnlund et al., 1984, n=4</td>
<td>15</td>
<td>231</td>
<td>79</td>
<td>34</td>
<td>42</td>
<td>8</td>
<td>29</td>
</tr>
<tr>
<td>Turnlund et al., 1986</td>
<td>21</td>
<td>237</td>
<td>78</td>
<td>33</td>
<td>58</td>
<td>14</td>
<td>6</td>
</tr>
</tbody>
</table>

Source: King et al., 2000.

2.1.4 Bioavailability

Bioavailability refers to the fraction of intake that can be absorbed into the blood system and used for physiologic functions of the body. For zinc, in healthy individuals, it is determined by three factors: the individual’s zinc status, the total zinc content of the diet, and the availability of soluble zinc from the diet’s food components (Lonnerdal, 2000). If the individual’s zinc status is discounted, zinc absorption is largely determined by its solubility in the intestinal lumen, which in turn is affected by the chemical form of zinc and the presence of specific inhibitors and enhancers of zinc absorption.
Long-term zinc intake, i.e., zinc status, can also affect absorption of dietary zinc. Although the long-term use of zinc supplements does not appear to cause any down-regulation of zinc absorption compared with normal, healthy subjects not taking any supplements (Sandstrom et al. 1990), low zinc intake and zinc status do affect zinc absorption. Istfan et al. (1983) fed young men a formula diet containing either 1.5 or 15 mg zinc/day and measured zinc absorption using the method of stable tracer isotope neutron activation analysis in a fasted state after 6 days. Zinc absorption was 92% from the low zinc diet and 81% from the high zinc diet. Wada et al. (1985) performed similar stable isotope studies in young men and found that zinc absorption from the diet was 53% when the zinc intake was 5.5 mg/d and that it decreased to 25% when 16.5 mg/d was fed. Similarly, August et al. (1989) found that young adult subjects absorbed 64 ± 5% of zinc from the diet when it contained 2.8 –5 mg/d but only 39 ± 3% when it contained 12.8 –15 mg/d. Differences were also found in elderly subjects (43 ± 7% vs 21 ± 1%), but as can be seen, the extent of zinc absorption was lower in this age group. Thus, it appears that feeding low zinc diets increases zinc absorption in all age groups and that homeostatic mechanisms up-regulate zinc absorption and retention. Previous zinc intake may therefore have an effect on studies on zinc bioavailability (Lonnerdal, 2000).

Inhibitors and Enhancers
Various dietary factors can influence zinc absorption. Phytic acid (inositol hexa- and pentaphosphate) is the principal dietary factor known to limit zinc bioavailability by strongly binding zinc in the gastrointestinal tract (Hambidge et al., 2010). It is the major phosphorus (P) storage compound in plant seeds, especially cereals and legumes, and can account for up to 80% of seed total P (King and Cousins, 2006). Because of its high density of negatively charged phosphate groups, phytate forms mixed salts with mineral cations, which are assumed to play an important role in mineral storage (Lopez et al., 2002). The inhibitory effects of PA on zinc can be predicted by the molar ratios of phytate:zinc in the diet. Molar ratios in excess of 15:1 according to WHO (2006), or 18:1 according to IZiNCG (Hotz and Brown, 2004) progressively inhibit zinc absorption and have been associated with suboptimal zinc status in humans. It appears unlikely that calcium per se has a negative effect on zinc absorption (Lonnerdal, 2000). As calcium has the propensity to form complexes with phytic acid and zinc that are insoluble, it has been proposed that the phytate:zinc molar ratio should be multiplied by the dietary calcium concentration to improve the prediction of zinc bioavailability (Lonnerdal, 2000; Lopez et al., 2002). However, the interactions between zinc and calcium are complex and not all studies have shown that calcium further increases the
impact of phytic acid on zinc absorption (Lonnerdal, 2000; Hunt and Beiseigel, 2009). Techniques such as soaking, germination, and fermentation promote enzymatic hydrolysis of phytic acid in whole grain cereals and legumes by enhancing the activity of endogenous or exogenous phytase enzyme (Hurrell, 2004). Also nonenzymatic methods such as milling have been successful in reducing phytic acid content in plant-based staples (Schlemmer et al., 2009). Thermal processing and extrusion cooking may cause only modest phytate losses (Hurrell, 2004).

The potential interaction between iron and zinc has been a cause of concern. Solomons and Jacob (1981) found that high doses of inorganic iron decreased zinc uptake as measured by changes in plasma zinc over the next 4 h after an oral dose. Human adults were administered 25 mg of zinc (as ZnSO4) in water solution, and iron was added at 25, 50 or 75 mg. Plasma zinc was reduced significantly with increasing dose of iron. Lonnerdal (2000) used a dose of zinc similar to that obtained from most meals and studied zinc absorption by using radiolabeled zinc and whole-body counting. He found a significant reduction in zinc absorption in the fasting state when iron was added to the zinc dose in water solution at a 25:1 molar ratio but not at a 2.5:1 ratio, which is similar to the ratio used in the study by Solomons and Jacob (1981). Thus, the interaction appears much less pronounced when zinc intake is closer to a “physiological” level. He concluded that the effect of iron on zinc is exerted only at a very high ratio of iron to zinc and in water solution. This suggests that iron fortification will not affect zinc absorption. Some inhibitory effects would be seen only if very high iron to zinc ratio is administered apart from a meal. It was demonstrated by Davidsson et al. (1995) that iron fortification of foods is unlikely to affect zinc absorption. They examined the effect of iron fortification of bread (65 mg/kg), weaning cereal (500 mg/kg) and infant formula (12 mg/L) in human adults with the use of stable isotopes. No significant negative effect on zinc absorption was found compared with the same foods without iron fortification. Similar results were obtained by Fairweather-Tait et al. (1995), who studied the effect of iron fortification of a weaning food on zinc absorption in infants with the use of stable isotopes.

Proteins generally have positive influence on zinc absorption, because zinc absorption tends to increase with protein intake (Lonnerdal, 2000; McDowell, 2003). Consumption of animal proteins (e.g. beef, eggs and cheese) improve the bioavailability of zinc from plant food sources possibly because amino acids released from the animal protein keep zinc in solution (Lonnerdal, 2000) or the protein binds the phytate. Generally, binding of zinc to soluble ligands or chelators has a positive effect on zinc absorption as they increase the zinc solubility (Hambidge et al., 1986; Lonnerdal, 2000).
2.1.5 Human Requirements

Since the mid-1990s, the World Health Organization (WHO), the Food and Agriculture Organization (FAO), the International Atomic Energy Association (IAEA) and the Food and Nutrition Board (FNB) of the Institute of Medicine (IOM) have convened expert committees to develop estimates of human zinc requirements and dietary intakes needed to satisfy these requirements (WHO/FAO/IAEA, 2002; FNB/IOM, 2002). For most age groups the committees used a factorial method to estimate the average physiological requirement, which is defined as the amount of zinc that must be absorbed to offset the amount of zinc lost through both intestinal and non-intestinal pathways. For growing children and pregnant women the amount of zinc retained in newly accrued tissues is added to the requirements, and for lactating women the zinc secreted in breast milk is added. More recently, the International Zinc Nutrition Consultative Group (IZiNCG) reported revised estimates of zinc requirement and recommended dietary intake as given in Table 3 (Hotz and Brown, 2004).

Table 3. Estimated physiological requirements for absorbed zinc by age group and sex

<table>
<thead>
<tr>
<th>Age</th>
<th>WHO Reference wt. (kg)</th>
<th>Requirement (mg/day)</th>
<th>FNB Reference wt. (kg)</th>
<th>Requirement (mg/day)</th>
<th>IZiNCG Reference wt. (kg)</th>
<th>Requirement (mg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-12 mo</td>
<td>9</td>
<td>0.84</td>
<td>9</td>
<td>0.84</td>
<td>9</td>
<td>0.84</td>
</tr>
<tr>
<td>1-3 yr</td>
<td>12</td>
<td>0.83</td>
<td>13</td>
<td>0.74</td>
<td>12</td>
<td>0.53</td>
</tr>
<tr>
<td>3-6 yr</td>
<td>17</td>
<td>0.97</td>
<td>22</td>
<td>1.20</td>
<td>21</td>
<td>0.83</td>
</tr>
<tr>
<td>6-10 yr</td>
<td>25</td>
<td>1.12</td>
<td>22</td>
<td>1.20</td>
<td>21</td>
<td>0.83</td>
</tr>
<tr>
<td>10-12 yr</td>
<td>35</td>
<td>1.40</td>
<td>40</td>
<td>2.12</td>
<td>38</td>
<td>1.53</td>
</tr>
<tr>
<td>12-15 yr</td>
<td>48</td>
<td>1.82</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15-18 yr M</td>
<td>64</td>
<td>1.97</td>
<td>64</td>
<td>3.37</td>
<td>64</td>
<td>2.52</td>
</tr>
<tr>
<td>15-18 yr F</td>
<td>55</td>
<td>1.54</td>
<td>57</td>
<td>3.02</td>
<td>56</td>
<td>1.98</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>-</td>
<td>2.27</td>
<td>-</td>
<td>4.1-5.0</td>
<td>-</td>
<td>2.68</td>
</tr>
<tr>
<td>Lactation</td>
<td>-</td>
<td>2.89</td>
<td>-</td>
<td>3.8-4.5</td>
<td>-</td>
<td>2.98</td>
</tr>
</tbody>
</table>

* Different stages of pregnancy/lactation

The estimates of zinc physiological requirements by the IZiNCG in 2004, however, were conspicuously low in comparison with those estimated by the IOM in 2001 Hambidge et al. (2011) explained that this difference was due to an error in zinc menstrual losses, as well as a minor error in the linear regression of endogenous fecal zinc (EFZ) vs. total daily zinc
absorption (TAZ) by IOM. The review also revealed an error by IZiNCG in selecting two data points for the linear regression of EFZ on TAZ. A second major reason for the "gap" was attributable to weighting of the data in the regression analysis by number of subjects per study by IZiNCG. Adjusting for these factors, together with use of the same reference data for body weights, resulted in satisfactory agreement between the two estimates of physiological requirements (Hambidge et al., 2011).

Different types of dietary reference intakes are derived depending on whether they are being used to assess the intakes of individuals or populations. Methods for calculating these reference intake values and their uses have been described by the FNB/IOM Dietary Reference Intake Committees (FNB/IOM, 2002.), and the same terminology and methods were used by IZiNCG. The EAR represents the mean dietary requirement, or the dietary intake level at which 50% of individuals would meet their physiological requirement. The EAR is thus derived by dividing the mean physiological requirement for absorbed zinc by the estimated average absorption of zinc. For example, the EAR for adult women (55 kg) consuming unrefined, cereal-based diets would be calculated as: 1.86 mg absorbed zinc/day ÷ 0.25 = 7.4 mg zinc/day, and rounded to 7 mg/day.

2.1.6 Groups at High Risk

Compared to adults, infants, children, adolescents, pregnant and lactating women have increased requirements for zinc and thus are at increased risk of zinc depletion (King and Cousins, 2006).

Infants and children

Young children are at greater risk of zinc deficiency because of increased zinc requirements during growth. Exclusively breast-fed infants of mothers with adequate zinc nutriture obtain sufficient zinc for the first 5-6 months of their life (Hotz and Brown, 2004). After this age, complementary foods containing absorbable zinc are required to satisfy their requirements. In many low-income countries, complementary feeding is delayed and cereal foods are then used for feeding. These foods have low content of total and absorbable zinc and thus fail to meet the needs for zinc. Conversely, early introduction of such foods may interfere with the absorption of zinc from breast milk due to their high phytate content (FNB/IOM, 2002).

Zinc requirements of malnourished children are estimated to be between 2-4 mg/kg body weight (Muller et al., 2001). These requirements are much higher than those for healthy
children (0.17 mg/kg at 1-3 years), presumably because of prior zinc depletion and reduced zinc absorption due to changes in the intestinal tract.

**Adolescents**
The physiological requirements for zinc peak during adolescence at the time of the pubertal growth spurt, which generally occurs in girls between 10-15 years and in boys between 12-15 years. Even after the growth spurt has ceased, adolescents may require additional zinc to replenish depleted tissue zinc pools (Maret and Sanstead, 2006).

**Pregnant and lactating women**
Increased nutritional demands during pregnancy and lactation predispose women to zinc deficiency (King, 2000). These demands are greater during lactation, although physiological adjustments in zinc absorption help to meet the needs for lactation. A number of studies have demonstrated a negative impact of therapeutic supplemental iron on zinc absorption during pregnancy (O’Brien et al., 2000; Hambidge et al. 1987) and lactation (Fung et al. 1997). In pregnant women, where dietary intakes of zinc were low, supplemental iron, in dosages as low as 60 mg/ day prevented them from meeting their needs for zinc (O’Brien et al., 2000). Situations that seem most likely to encounter problematic interactions are those in which the iron is administered in solution or as a separate supplement rather than incorporated into a meal (Whittaker 1998).

**Elderly**
Diet surveys indicate that zinc intakes by elderly persons are often inadequate, even in rich countries (Andriollo-Sanchz et al., 2005). Several factors may contribute to poor zinc nutrition among the elderly, in particular, reduced consumption of zinc-rich foods such as red meat. In addition, there is some evidence that the efficiency of zinc absorption may decrease with age (Andriollo-Sanchz et al., 2005).

**2.1.7 Consequences and Causes of Zinc Deficiency**

**Consequences of Zinc Deficiency**
Due to the multitude of basic biochemical functions of zinc in the cells of human body, there is a broad range of physiological signs of zinc deficiency. These signs vary depending on the severity of the condition. Organ systems known to be affected clinically by zinc deficiency
states include the epidermal, gastrointestinal, central nervous, immune, skeletal and reproductive systems (Hambidge and Walravens 1982).

Clinical signs of severe zinc deficiency were identified in industrialized countries notably in persons suffering from acrodermatitis enteropathica, a rare genetic disorder that specifically affects zinc absorption (Van Wouwe, 1989). Severe zinc deficiency resulting from other causes such as prolonged parenteral nutrition with inadequate zinc content produced similar clinical signs as in acrodermatitis enteropathica (Hambidge, 2000).

Although less impressive in their clinical presentation, milder zinc deficiency is of numerically much greater importance. Moreover, most of the clinical features of acrodermatitis enteropathica were documented also in milder forms of zinc deficiency. Functional impairments identified in community-based trials may be more representative of mild or moderate deficiency. Some of these functional impairments are as follows:

**Growth and development**

One of the most studied clinical features related to zinc deficiency is the impairment of physical growth and development (Brown et al., 2002; Anderson, 2004). The mechanisms involved, however, are not well understood. This effect is of most significance during the periods of rapid growth such as pregnancy, infancy and puberty during which zinc requirements are highest (Hotz and Brown, 2004).

**Risk of Infections**

*Diarrhea.* Diarrhea is characteristically, although not inevitably, a prominent feature of acrodermatitis enteropathica (Hambidge 1992). Plausible explanations for a link between zinc deficiency and diarrhea include impairment of the immune system and of intestinal mucosal cell transport (Ghishan 1984). A causal relationship between zinc deficiency and diarrhea is indicated by the beneficial effects of zinc supplements and concurrent increase in growth velocity (Bhutta et al. 1999; Brown et al., 1998).

*Pneumonia.* Community zinc supplementation studies in children have demonstrated a substantial and statistically significant reduction in the prevalence of pneumonia in developing countries (Bhutta et al. 1999).
Malaria. Among other infectious diseases, malaria also appears to be reduced by zinc supplementation (Bates et al. 1993, Black 1998, Shankar et al., 2000). However, further studies are required to establish this effect.

Relationship between zinc deficiency and age

Also degenerative changes associated with aging may partly be due to zinc deficiency, including a decline in immunocompetence, delayed wound healing and certain neurological and psychological changes (Andriollo-Sanchz et al., 2005).

In general, clinical manifestations of zinc deficiency vary with age. In early infancy, diarrhea is a prominent symptom. Zinc deficiency also leads to impaired cognitive function, behavioral problems, impaired memory, learning disability and neuronal atrophy (Hambidge et al., 1986; Hotz and Brown, 2004). Skin problems become more frequent as the child grows older. Alopecia, growth retardation and recurrent infections are common in school-age children. Chronic non-healing skin ulcers and also recurrent infections are common among the elderly. These effects have been observed in controlled clinical trials showing positive response to supplemental zinc (Hotz and Brown, 2004).

Infectious diseases and malnutrition are the principal causes of childhood morbidity and mortality globally. Providing adequate zinc nutriture is perhaps the most effective preventive measure at present that can be undertaken to decrease the rates of morbidity and mortality in children of the developing world (Hambidge, 2000; Muller et al., 2001).

Causes of Zinc Deficiency

The general causes of zinc deficiency include inadequate intake, increased requirements, malabsorption, increased losses and impaired utilization (King and Cousins, 2006). Inadequate dietary intake of absorbable zinc is the primary cause of zinc deficiency in most situations (Lonnerdal, 2000; Hotz and Brown, 2004). This may result from low dietary intake or heavy reliance on foods with little or poorly absorbable zinc. Inadequate dietary zinc intake is common in many parts of the world. It is often exacerbated by physiologic conditions associated with elevated zinc requirements. (Andriollo-Sanchz et al., 2005; King and Cousins, 2006)

Malabsorption of zinc may occur in a number of situations for example, acrodermatitis enteropathica (Moynahan, 1974; Van Wouwe, 1989). Malabsorption syndromes and inflammatory diseases of the bowel, resulting in poor absorption and loss of zinc, may lead to secondary zinc deficiency particularly in the presence of marginal dietary intakes (Aggett and
Harries, 1979; King and Cousins, 2006). Utilization of zinc is impaired in the presence of infection as decreased circulation of zinc reduces the availability of zinc to the tissues.

Conditions of impaired intestinal integrity not only reduce absorption, but also result in increased endogenous losses of zinc. Fecal excretion of zinc is increased during acute diarrhea (Brown et al., 1998). It is not clear to what extent this represents unabsorbed zinc or zinc of endogenous origin (Hotz and Brown, 2004). Diarrheal diseases are common in many low-income countries. The fact that zinc deficiency increases the susceptibility to childhood diarrhea while increased losses of endogenous zinc associated with diarrhea further deplete body zinc, results in a vicious cycle that merits further study (Maret, 2001).

2.1.8 Evaluation of zinc status

Assessing the nutritional status of a population is critical in developing intervention programs. Regrettably, there are no simple markers of marginal, mild or moderate zinc deficiency in individuals. Nevertheless, there is sufficient evidence to suggest that zinc deficiency is common in many low-income countries. For example, animal foods that are particularly rich sources of zinc are not easily accessible to many of the world’s poorer population. Diets based on cereals and legumes and poor in animal products make it difficult to meet the zinc requirements because their high phytate content reduces the bioavailability of zinc. Evidence for widespread zinc deficiency in developing countries also results from intervention trials in children, which showed that zinc supplementation improved growth among stunted children. Although other nutritional and environmental factors can also cause stunting, an elevated prevalence of this condition is considered as suggestive evidence of zinc deficiency in a population.

In recent years, efforts have been made to derive more precise and reliable indicators of zinc deficiency using direct measures of zinc status. These include assessment of dietary zinc intakes and biochemical markers.

**Blood plasma or serum zinc concentration**

The concentration of zinc in blood plasma or serum is currently the best available biomarker of the risk of zinc deficiency in a population. Suggested lower cutoffs for serum zinc concentration are based on data collected in the second National Health and Nutrition Examination Survey in US population and are given in Table 4 (Hotz et al., 2003). Although serum zinc concentrations may have limitations in validity and reliability for identification of mild or moderate zinc deficiency in individuals, this index is useful for assessing zinc status at
the population level. The risk of zinc deficiency is considered to be elevated and of public health concern when low serum zinc concentration is prevalent in >20% of the population.

Table 4: Suggested lower cutoffs for assessment of serum zinc concentration (μg/dl) in population studies

<table>
<thead>
<tr>
<th></th>
<th>Morning fasting</th>
<th>Morning non-fasting</th>
<th>Afternoon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children &lt;10 yr</td>
<td>---</td>
<td>65</td>
<td>57</td>
</tr>
<tr>
<td>Males aged ≥ 10 yr</td>
<td>74</td>
<td>70</td>
<td>61</td>
</tr>
<tr>
<td>Non-pregnant females aged ≥ 10 yr</td>
<td>70</td>
<td>66</td>
<td>59</td>
</tr>
<tr>
<td>Pregnant women (1st trimester)</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Pregnant women (2nd/3rd trimester)</td>
<td>56</td>
<td>56</td>
<td>56</td>
</tr>
</tbody>
</table>

Serum zinc concentrations fluctuate by as much as 20% during a 24-hour period (Hambidge et al., 1989), largely due to the effects of food ingestion. Following a meal, there is an immediate initial increase, after which the concentration declines progressively for the next 4 hours and then rises until food is eaten again. During an overnight fast, the concentration of serum zinc increases slightly, so the highest levels of the day are generally seen in the morning (Couturier et al., 1988; Goode et al., 1991). However, diurnal variations in serum zinc concentration among fasted individuals have also been observed, whereby serum zinc decreased from morning to mid-afternoon and then began to rise again to morning levels (Guillard et al., 1979).

Low serum zinc concentrations can occur in the presence of several conditions, representing a normal physiologic response and not necessarily indicative of low zinc status. Serum zinc concentrations are reduced during acute infections and inflammation, which is likely due to the redistribution of zinc from the plasma to the liver (Singh et al., 1991); cytokines released during the acute phase response activate hepatic metallothionein synthesis (Schroeder et al., 1990), a metal-binding protein which appears to alter the hepatic uptake of zinc (Rofè et al., 1996). Elevated concentrations of C-reactive protein or other markers of the acute phase response can be used to indicate the presence of infection and should be considered in the interpretation of results. Stress and myocardial infarction also reduce serum zinc levels (Prasad, 1985). Because zinc is transported in plasma bound to albumin, diseases, such as cirrhosis and protein-energy malnutrition, that produce hypoalbuminemia result in
lower serum zinc concentrations (Solomons, 1979). Hemodilution, as observed during pregnancy, oral contraceptive use, and other hormonal treatments, also results in a lower serum zinc concentration (Halsted et al., 1968; Hobisch-Hagen et al., 1997). On the other hand, conditions resulting in intrinsic or extrinsic hemolysis of blood cells can result in extremely high serum zinc levels because the concentration of intracellular zinc is considerably greater than in serum.

**Dietary zinc intake**

Given that chronic inadequate dietary intake of zinc is the most likely cause of zinc deficiency, quantitative dietary intake surveys are useful to evaluate zinc intake and the risk of zinc deficiency in populations. Based on the type and bioavailability of the diets, the risk of zinc deficiency is estimated by comparing the intakes with the respective EAR values. The risk of zinc deficiency is considered to be elevated and of public health concern when the prevalence of inadequate intake is >25%.

**Methods of assessing dietary intake**

Food consumption data may be collected at the national, household or the individual level. Although data collected at the level of the individual are the most useful for assessing dietary adequacy and adherence to food-based dietary guidelines, food supply and household data provide information that is useful for many other purposes.

1. **National food supply data**

Food supply data at the national level, such as food balance sheets or food disappearance data provide gross estimates of the national availability of food commodities. These data may also be used to calculate the average per capita availability of energy and the macronutrients. A major limitation of national supply data is that they reflect food availability rather than food consumption. Other uses, such as animal feed and industrial applications, as well as losses due to cooking or processing, spoilage and other sources of waste are not easily accounted for. Despite these limitations, national food supply data are useful for tracking trends in the food supply and for determining availability of foods that are potentially good sources of nutrients or of food groups targeted for dietary guidance. Food supply data are not useful for evaluating individual adherence to dietary reference values (DRV) nor for identifying subgroups of the population at risk of inadequate nutrient intakes.
2. Household data

Information regarding food availability at the household level may be collected by a variety of methods (Flores et al., 1988). Such data are useful for comparing food availability among different communities, geographic areas and socioeconomic groups, and for tracking dietary changes in the total population and within population subgroups. However, these data do not provide information on the distribution of foods among individual members of the household.

3. Individual data

The five general methods for assessing dietary intake for individuals are described below, along with the major strengths and limitations of each:

- Food records. Food records, also called food diaries, require that the subject (or observer) report all foods and beverages consumed for a specified period (usually one to seven days). Amounts of each food item may or may not be recorded, depending on the study objectives. If nutrient intakes are to be calculated, the amounts consumed should be estimated as accurately as possible. Amounts may be determined by weighing or by estimating volumes. In some situations, only those foods of particular interest are recorded. For example, to estimate intake of a food component found only in animal products, food records might be limited to foods containing meat, poultry, fish, eggs or dairy products. However, if total energy intake is required, the food record should include all foods consumed.

- 24-hour dietary recalls. The 24-hour dietary recall consists of a listing of foods and beverages consumed the previous day or the 24 hours prior to the recall interview. Foods and amounts are recalled from memory with the aid of an interviewer who has been trained in methods for soliciting dietary information. The interview is usually conducted face to face, but may also be conducted by telephone. In some situations, the recall is self-administered by the subject, but this approach may not yield sufficiently reliable data. A brief activity history may be incorporated into the interview to facilitate probing for foods and beverages consumed.

- Food frequency questionnaires. A food frequency questionnaire (FFQ), sometimes referred to as a "list-based diet history", consists of a structured listing of individual foods or food groupings. For each item on the food list, the respondent is asked to estimate the frequency of consumption based on specified frequency categories which indicate the number of times the food is usually consumed per day, week, month or year. FFQs are generally self-administered but may also be interviewer-administered. The number or types of food items may vary, as well as the number and types of frequency categories. FFQs may be unquantified, semi-
quantified or completely quantified. The unquantified questionnaire does not specify serving sizes, whereas the semi-quantified tool provides a typical serving size as a reference amount for each food item. A quantified FFQ allows the respondent to indicate any amount of food typically consumed. Some FFQs include questions regarding usual food preparation methods, trimming of meats, use of dietary supplements, and identification of the most common brand of certain types of foods such as margarines or ready-to-eat cereals. The answers to these questions are then incorporated into the calculation of nutrient intakes. FFQs are commonly used to rank individuals by intake of selected nutrients. Although FFQs are not designed for estimating absolute nutrient intakes, the method may be more accurate than other methods for estimating average intake of those nutrients having large day-to-day variability and for which there are relatively few significant food sources (e.g. alcohol, vitamin A and vitamin C). Brief FFQs may focus on one or several specific nutrients. Comprehensive FFQs designed to estimate a large number of nutrients generally include between 50 and 150 food items.

• Diet histories. The meal-based diet history is designed to assess usual individual intake. It consists of a detailed listing of the types of foods and beverages commonly consumed at each eating occasion over a defined time period which is often a "typical" week. A trained interviewer probes for the respondent's customary pattern of food intake on each day of the typical week. The reference time frame is often the past month or the past several months, or may reflect seasonal differences if the time frame is the past year.

• Food habit questionnaires. May be designed to collect either general or specific types of information, such as food perceptions and beliefs, food likes and dislikes, methods of preparing foods, use of dietary supplements, social settings surrounding eating occasions. This type of information is frequently included along with the other four methods, but it may also be used as the sole data collection method. These approaches are commonly used in rapid assessment procedures (RAP). The questionnaires may be either open-ended or structured, self- or interviewer-administered, and may include any number of questions depending on the information required. Use of food habit questionnaires is further discussed in the section on rapid assessment methods.

• Combined methods Different types of dietary assessment methods may be combined to improve accuracy and facilitate interpretation of the dietary data. Methods may also be combined for practical reasons. For example, food records have been combined with 24-hour recalls to make the best use of resources in past surveys of the US Department of Agriculture. An FFQ focused on selected nutrients was used in addition to the 24-hour recall in the Third National Health and Nutrition Examination Survey (NHANES III). A 24-hour recall is
frequently used to help establish the typical meal plan for conducting a diet history (Briefel, 1994), and a FFQ may be used as a cross-check for the other three types of methods. (See Table 5, Summary of dietary assessment methods).

Table 5. Summary of dietary assessment methods

<table>
<thead>
<tr>
<th>Type of method</th>
<th>Major strengths</th>
<th>Major limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food record</td>
<td>- does not rely on memory</td>
<td>- high participation burden</td>
</tr>
<tr>
<td></td>
<td>- easy to quantify amounts</td>
<td>- requires literacy</td>
</tr>
<tr>
<td></td>
<td>- open-ended</td>
<td>- may alter intake behavior</td>
</tr>
<tr>
<td>24-h dietary recall</td>
<td>- little respondent burden</td>
<td>- relies on memory</td>
</tr>
<tr>
<td></td>
<td>- no literacy requirement</td>
<td>- requires skilled interviewer</td>
</tr>
<tr>
<td></td>
<td>- does not alter intake behavior</td>
<td>- difficulty to estimate amounts</td>
</tr>
<tr>
<td>Food frequency questionnaire</td>
<td>- relatively inexpensive</td>
<td>- relies on memory</td>
</tr>
<tr>
<td></td>
<td>- preferable method for nutrients with very high day variability</td>
<td>- requires complex calculations</td>
</tr>
<tr>
<td></td>
<td>- to day to estimate frequencies</td>
<td>- requires literacy</td>
</tr>
<tr>
<td></td>
<td>- does not alter intake behavior</td>
<td>- limited flexibility for describing foods</td>
</tr>
<tr>
<td>Diet history (meal-based)</td>
<td>- no literacy requirement</td>
<td>- relies on memory</td>
</tr>
<tr>
<td></td>
<td>- does not alter intake behavior</td>
<td>- requires highly trained interviewer</td>
</tr>
<tr>
<td></td>
<td>- open-ended</td>
<td>- difficulty to estimate amounts</td>
</tr>
<tr>
<td>Food habit</td>
<td>- rapid and low cost</td>
<td>- may rely on memory questionnaires</td>
</tr>
<tr>
<td></td>
<td>- does not alter intake behavior</td>
<td>- may require a trained interviewer</td>
</tr>
<tr>
<td></td>
<td>- open-ended</td>
<td></td>
</tr>
</tbody>
</table>

Source: FAO/WHO, 1996

**Stunting prevalence**

Height-for-age, a measure of nutritional stunting, is the best known and easiest to measure of the adverse outcomes associated with zinc deficiency in populations. Stunting prevalence is
expressed as the percentage of children under 5 years of age with height-for-age below the expected range of a reference population (i.e., less than -2.0 standard deviations with respect to the reference median). WHO considers a prevalence of stunting greater than 20% of the population to indicate a public health concern (Hotz and Brown, 2004). As zinc deficiency is not the only factor affecting children’s growth, assessment of dietary zinc intake and serum zinc levels can be used to confirm the risk of zinc deficiency in these high-risk countries (Hotz and Brown, 2004).

The causes and etiology of stunting include the following: 1) nutrition (energy, macronutrients, micronutrients and toxic factors); 2) infection (injury to gastrointestinal mucosa, systemic effects and immunostimulation); and 3) mother-infant interaction (maternal nutrition and stores at birth, and behavioral interactions) (Frongillo, 1999).

Growth stunting could be the consequence of deficiency of one or several nutrients. In communities in which stunting is prevalent, it is highly likely that several nutrient deficiencies occur simultaneously in the stunted children. In a study in a rural community in Mexico, 82% of children 18 – 36 mo of age were deficient in at least two micronutrients out of five that were determined (iron, zinc, vitamin A, vitamin B-12, and riboflavin) (Rosado, 1999). Results of studies with single nutrient supplementation are conflicting; thus that there is no consistent evidence for any nutrient that its use for supplementation will promote linear growth. In the case of Mexican preschoolers in whom deficiencies of multiple micronutrients were demonstrated, no effect on linear growth was found after 1 y of supplementation with zinc and/or iron. Although supplementation with multiple micronutrients produced a significant increment in linear growth, the actual increment in height was much less than the potential increment expected (Rosado, 1999).

**Other biochemical indicators of zinc status**

*Metallothionein*

Several zinc-dependent enzymes have been shown to be affected by zinc intake or zinc status in experimental animal models and human populations. Metallothionein is a metal storage protein that is present in serum at a low concentration; the circulating concentration of metallothionein appears to correlate with zinc intake. However, similar to several of the enzymes and to serum zinc, metallothionein may be affected by other factors, such as infection and stress, although this has not been confirmed by direct studies. Because of these limitations and the relative difficulty of performing these assays outside the research...
laboratory, it is presently unlikely that they will be useful for assessment of zinc status at the population level (Lowe et al., 2009; Hotz and Brown, 2004).

Exchangeable zinc pools (EZPs)
The zinc that is available for maintaining zinc-dependent functions is thought to be mobilized from small, rapidly exchanging zinc pools found primarily in the plasma and liver (Miller et al., 1994; Wastney et al., 1986). The size of this pool can be estimated from the tracer-tracee disappearance curves using kinetic modeling software. The total exchangeable mass varies with the length of time over which the decay curves are measured. For example, if tracer disappearance is followed for 3 hours, the EZP mass is approximately 18 mg in healthy men; if the tracer disappearance is followed for 192 hours, or eight days, it is approximately 150 mg or about 10% of the whole-body zinc pool. A decline in one or more of the EZPs could be associated with a reduction in the zinc available for zinc-dependent functions, especially among rapidly turning over proteins (Golden, 1989). If so, then EZP mass would provide a good indication of tissue zinc status.

EZP mass has been measured in individuals freely selecting their diets (Miller et al., 1994), in men fed zinc-depleted diets (King et al., 2001; Pinna et al., 2001), and in populations with chronically low zinc intakes. Among individuals freely selecting their zinc intake, EZP mass varied directly with dietary zinc, both in individuals (Miller et al., 1994) and populations (Sian et al., 1996). Also, experimental acute, severe zinc depletion induced in adults by feeding a diet providing 0.23 mg zinc/day for five weeks (King et al., 2001) lowered total EZP by 36%. Plasma zinc concentrations declined 65% in that study suggesting that plasma zinc is more sensitive to severe zinc depletion than is EZP mass. When dietary zinc was reduced to a marginal level (4.6 mg/day) in a group of healthy men, EZP mass did not change (Pinna et al., 2001). Thus total EZP mass does not appear to be a good indicator of modest short-term changes in zinc intake. However, longer-term low intakes or acute zinc depletion causing a reduction in whole-body zinc content appears to cause a concomitant reduction in EZP.

2.1.9 Prevention of zinc deficiency

Intervention Strategies
Numerous zinc supplementation trials have shown that a wide range of health benefits can be realized by increasing the intake of zinc where diets are inadequate in this micronutrient (Maret and Sanstead 2006; Shankar et al. 2000). The results of these trials strongly argue for
the development of programs to improve zinc status in high-risk populations. To give best results such efforts should be integrated into existing health and nutrition programs. The major intervention strategies are supplementation, dietary diversification/modification, fortification and biofortification. These strategies are not mutually exclusive but can be used in a complementary way. Their choice depends upon the available resources and technical feasibility.

*Dietary diversification/modification*

Dietary diversification or modification is a sustainable long-term approach to improving the intake of several nutrients simultaneously. Dietary diversification or modification strategies at the community or household level have the potential to increase the intake of bioavailable zinc. Such strategies include 1) Agricultural interventions 2) Production and promotion of animal-source foods through animal husbandry or aquaculture 3) Processing strategies at the commercial or household level to enhance zinc absorption from plant-based diets (Gibson and Anderson, 2009). Agricultural interventions focused on plant-based foods may have little impact on intake of bioavailable zinc. Some benefit may be realized if accompanied by processing strategies to reduce the levels of substances that inhibit zinc absorption, such as phytate, but this is likely to be insufficient to meet zinc needs of infants and young children. Animal husbandry efforts that increase red meat or liver consumption by infants and young children can have a positive impact. These foods generally have a higher content of readily absorbed zinc than poultry, eggs, dairy products or fish. Nutrition education to promote dietary diversification or modification can lead to greater intakes of animal-source foods and thus bioavailable zinc. Care must be taken to avoid potential adverse effects of the above strategies, such as aflatoxin contamination of germinated cereals, loss of water-soluble nutrients from soaking cereal flours, and displacement of breast milk by increased intakes of other foods (Gibson and Anderson, 2009). However, more information is required on zinc content and zinc absorption modifiers in local foods to identify suitable sources of absorbable zinc. Although dietary modification and diversification is the most sustainable approach, change of the dietary practices and preferences is difficult and foods that provide highly bioavailable zinc (such as red meat) are generally expensive.

*Supplementation*

Supplementation programs are useful for targeting vulnerable population subgroups, which are at a particular high risk of micronutrient deficiencies. The easiest way to supplement zinc
could be to include it in programs already delivering daily or weekly nutrient supplements for the prevention of iron deficiency anemia and other micronutrient deficiencies. The recommended zinc dosages are 5 mg/day for children between 7 months to 3 years and 10 mg/day for older children (Müller et al., 2001; Brown et al., 2002). When formulating multi-nutrient supplements, it is recommended that salts providing readily absorbable zinc, like zinc sulfate, zinc gluconate or zinc acetate are used to avoid antagonistic interactions between zinc and other minerals, in particular iron.

Supplemental zinc is also recommended as an adjunct therapy during the treatment of diarrhea in children (Fontaine, 2001). The recommended daily dosage is twice the age-specific RDA per day for 14 days; that is 10 mg/day for children under 3 years and 20 mg for older children. Several clinical trials have demonstrated that zinc supplements reduce the severity and duration of acute and persistent diarrhea (Müller et al., 2001; Shankar et al., 2000).

Fortification

Food fortification is a more cost-effective and sustainable strategy to overcome micronutrient malnutrition than supplementation. Where micronutrient deficiency is widely distributed in a population and dietary modification or diversification is difficult to achieve, fortification of centrally processed foods is an appropriate alternative. Mexico provides an example of a country with a nationwide zinc fortification program. Apart from zinc, other micronutrients are added to wheat and corn flours that are used in preparing bread and tortilla, the two principal staple foods in the country. For such multiple interventions synergistic and antagonistic interactions between micronutrients have to be taken into account during the development of appropriate formulations (FAO/WHO, 2004).

Fortification programs can also be specifically targeted to increase the intake of zinc in groups of high risk such as infants and young children who consume particular type of food. In many countries, infant formulas and complementary foods are currently fortified with zinc and other micronutrients. Commercially available standard infant formulas contain zinc in concentrations of around 1 mg/L, following current recommendations. In general, the food selected for fortification should be one that is widely consumed in stable and predictable amounts. Among several zinc compounds that are available for fortification, zinc oxide and zinc sulfate are least expensive and most commonly used by the food industry. Suggested levels for fortification of flour are 30-70 mg zinc/kg (WHO, 2009). Zinc sulfate theoretically provides more absorbable zinc because of its greater solubility, but it is more expensive.
Further information is required on the bioavailability of zinc, acceptability and cost of fortifying food products with different chemical forms of zinc.

**Biofortification**

Recently, plant breeding or genetic engineering strategies that either increase the level of zinc, reduce the content of inhibitors (e.g. phytate), or increase the expression of compounds that enhance zinc absorption (e.g. amino acids) have been considered to improve the bioavailability of zinc from plant foods (Lonnerdal, 2003). Biofortification differs from ordinary fortification because it focuses on intrinsic enrichment of micronutrients in plant parts that are used for food while the plants are still growing, rather than having nutrients from external resources added to the foods when they are being processed. (Banuelos and Lin, 2009) This is an improvement on ordinary fortification when it comes to providing nutrients for the rural poor, who rarely have access to commercially fortified foods (Banuelos and Lin, 2009). As such, biofortification is seen as an upcoming strategy for dealing with deficiencies of micronutrients in the developing world. Its additional benefits include higher yield where micronutrients are limiting plant growth and increased vitality of seedlings emerging from zinc-enriched seeds.

**Methods of zinc biofortification**

Biofortification is an agricultural strategy that aims to increase the content of select micronutrients, including zinc, in staple food crops such as rice, wheat, maize, pearl millet, and others (Hotz, 2009). Biofortification strategies include the application of zinc fertilizers and the development of crop genotypes that acquire more zinc from the soil and accumulate it in edible portions (White and Broadley, 2011). The biofortification strategy seeks to take advantage of the consistent daily consumption of large amounts of food staples by all family members, including women and children as they are most at risk for micronutrient malnutrition. As a consequence of the predominance of food staples in the diets of the poor, this strategy implicitly targets low-income households (Bouis 2003).

Dietary zinc intakes can be increased through a variety of interventions (Stein, 2010). These include both agronomic and genetic biofortification of edible crops (Graham et al., 2007; White and Broadley, 2009; Bouis and Welch, 2010). Agronomic biofortification can be achieved by increasing soil zinc phytoavailability or by applying zinc fertilizers. This requires appropriate infrastructures, but can be very successful in regions where mineral fertilizers are used to increase crop yields and zinc is added to these at the point of manufacture or
distribution (Cakmak, 2009). Genetic biofortification is predicated on increasing zinc acquisition from the soil and its accumulation in edible portions. In most agricultural soils there is sufficient zinc to produce biofortified crops for many years, provided it becomes phytoavailable (Graham et al., 1999). Genetic biofortification strategies are, of course, ineffective if there is insufficient zinc present in the soil. Most economic analyses suggest that genetic strategies toward zinc biofortification are more practical, enduring, and cost effective than dietary diversification, supplementation, or food fortification programs for increasing dietary zinc intakes of vulnerable populations (Graham et al., 2007; Stein et al., 2007; Ma et al., 2008; Bouis and Welch, 2010; Stein, 2010).

Application of zinc fertilizers to soil and/or foliar seems to be a practical approach to improving grain zinc concentration (e.g., agronomic biofortification). Very recently, a global zinc fertilizer project has been initiated, so called HarvestZinc project (www.harvestzinc.org) under HarvestPlus program. This project aims at evaluating the potential of zinc containing fertilizers for increasing zinc concentration of cereal grains (e.g., wheat, rice and wheat) and improving crop production in different target countries (e.g., India, China, Pakistan, Thailand, Turkey, Mozambique, Zimbabwe and Brazil). The zinc fertilizer strategy represents an important complementary approach to ongoing breeding programs for developing new genotypes with high zinc density in grain. As described in HarvestZinc project (www.harvestzinc.org), biofortification of cereal grains through use of zinc fertilizers (e.g., agronomic biofortification) is required for i) keeping sufficient amount of available zinc in soil solution, ii) maintaining adequate zinc transport to the seeds during reproductive growth stage and iii) optimizing the success of biofortification of staple food crops with zinc through use of breeding tools.

Increasing evidence is available indicating that soil and/or foliar applications of zinc fertilizers greatly contribute to grain zinc concentrations (Cakmak, 2008). In the past, numerous studies have been published on the role of soil- and foliar-applied zinc fertilizers in order to correct zinc deficiency and increase yield. However, there are only few studies that investigated the effects of zinc fertilizers on grain zinc concentrations (or in other edible parts). Zinc sulfate (ZnSO4) is the widely applied source of zinc because of its high solubility and low cost. In Central Anatolia, application of ZnSO4 fertilizers was very effective in increasing grain zinc concentration of wheat. Applying zinc fertilizer into soil doubled grain zinc concentrations (Figure 1). As presented in Figure 2, foliar-applied zinc resulted in much greater increases in grain zinc concentration than the soil application of zinc. It seems that combined application of soil and foliar zinc fertilizers is the most effective way to maximize
grain zinc accumulation. Besides improving grain zinc concentrations, these soil or foliar zinc applications resulted also in significant increases in plant growth (Figure 2) and grain yield in various locations in Central Anatolia (Cakmak et al., 1996).

![Figure 1: Effect of various zinc application methods on grain zinc concentration of wheat grown in Central Anatolia (Source: Yilmaz et al., 1997).](image1)

![Figure 2: Effect of foliar applied zinc on growth of barley plants in Central Anatolia (Source: Cakmak et al., 1996).](image2)

Due to significant effects of zinc fertilizers on grain yield production in Central Anatolia, farmers showed a growing interest in zinc containing fertilizers in Turkey since the mid of 1990s. In the past 10-15 years increasing amount of zinc supplemented fertilizers has been
produced and applied in Turkey, especially in Central Anatolia. The total amount of zinc containing compound fertilizers applied in Turkey increased from zero in 1994 to a record level of 400,000 tons per annum. Use of such high amounts of zinc containing fertilizers increases in grain zinc concentration, and obviously contributes to human health and nutrition in Turkey, especially in rural areas where wheat provides more than 50% of the daily calorie intake (Cakmak, 2008). Little information is, however, available about the effectiveness of zinc containing compound fertilizers in improving grain zinc concentrations in other countries. In India, zinc enriched urea fertilizers are becoming an important source for zinc application to wheat and rice. Applying zinc coated urea fertilizers (up to 3 % zinc) increased both grain yield and grain zinc concentration in rice (Shivay et al., 2008; Table 6).

Table 6: Effect of zinc enriched urea (ZEU) (up to 3 % zinc in urea) on grain yield and grain zinc concentrations of aromatic rice grown in India. Data show average values of 2-year field trials.  

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Zinc Added (kg ha⁻¹)</th>
<th>Grain Yield (ton ha⁻¹)</th>
<th>Zinc Concentration (mg kg⁻¹ DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prilled Urea</td>
<td>-</td>
<td>3,87</td>
<td>27</td>
</tr>
<tr>
<td>0.5% ZEU</td>
<td>1,3</td>
<td>4,23</td>
<td>29</td>
</tr>
<tr>
<td>1.0%</td>
<td>2,6</td>
<td>4,39</td>
<td>33</td>
</tr>
<tr>
<td>2.0%</td>
<td>5,2</td>
<td>4,60</td>
<td>39</td>
</tr>
<tr>
<td>3.0%</td>
<td>7,8</td>
<td>4,76</td>
<td>42</td>
</tr>
</tbody>
</table>

Source: Shivay et al., 2008

Recent studies also indicate that intercropping systems contribute to grain zinc and iron concentrations. Various field tests in China with peanut/maize and chickpea/wheat intercropping systems showed that gramineaceous species are highly beneficial in biofortifying dicots with micronutrients. In the case of chickpea/wheat intercropping, zinc concentration of the wheat grains was 2.8-fold higher than those of wheat under monocropping (Zuo and Zhang, 2009). In many wheat-cultivated countries, continuous wheat cropping is a widely used cropping system. Inclusion of legumes in the crop rotation system may contribute to grain concentrations of wheat plants. Elevated soil organic matter content of soils up to a certain level improves solubility and root uptake of zinc, especially in alkaline soils. There are several reports on combined applications of zinc fertilizers together with organic materials (like farmyard manure and green manures) being particularly effective in facilitating zinc uptake by roots and correcting zinc deficiency (Cakmak, 2008).
2.2 Iron

2.2.1 History
From ancient times man has recognized the special role of iron in health and disease (Beard and Dawson, 1997). Iron had early medicinal uses by Egyptians, Hindus, Greeks and Romans (Wood and Ronnenberg, 2005; McDowell, 2003). During the 17th century, iron was used to treat chlorosis (green disease), a condition often resulting from iron deficiency (Guggenheim, 1995). However, it was not until 1932 that the importance of iron was finally settled by the convincing proof that inorganic iron could be used for hemoglobin synthesis (Yip and Dallman, 1996). For many years, nutritional interest in iron focused on its role in hemoglobin formation and oxygen transport (Underwood and Suttle, 1999). Nowadays, although low iron bioavailability plays a central role in the etiology of anemia in developing countries and is considered to be responsible for around 50% of anemia (see WHO 2006), infectious and inflammatory diseases (especially malaria), blood loss from parasitic infections, and other nutrient deficiencies (vitamin A, riboflavin, folic acid and vitamin B 12) are also important causes (Brabin et al., 2001).

2.2.2 Biochemistry and Physiology
In contrast to zinc, iron is an abundant element on earth (Wood and Ronnenberg, 2006; Quintero-Gutiérrez et al., 2008) and is a biologically essential component of every living organism. (Aisen 2001; Lieu et al., 2001). However, despite its geologic abundance, iron is often a growth limiting factor in the environment (Quintero-Gutiérrez et al., 2008). This apparent paradox is due to the fact that in contact with oxygen iron forms oxides, which are highly insoluble, and thus is not readily available for uptake by organisms (Wood and Ronnenberg, 2006). In response, various cellular mechanisms have evolved to capture iron from the environment in a biologically useful form. Examples are siderophores secreted by microbes to establish iron in form of highly specific complex (Guerinot, 1994) or mechanisms to reduce iron from the insoluble ferric iron (Fe$^{3+}$) to the soluble ferrous form (Fe$^{2+}$) as in yeasts (Askwith and Kaplan, 1998). Many of the mechanisms found, have analogous counterparts in higher organisms, including humans. In the human body, iron mainly exists in complex forms bound to protein (hemoprotein) as heme compounds (hemoglobin or myoglobin), heme enzymes, or non-heme compounds (flavin-iron enzymes, transferrin and ferritin) (McDowell, 2003). The body requires iron for the synthesis of its oxygen transport proteins, in particular hemoglobin and myoglobin, and for the formation of heme enzymes and other iron-containing enzymes involved in electron transfer and oxidation-reductions.
Almost two-thirds of the body iron is found in the hemoglobin present in circulating erythrocytes, 25% is contained in a readily mobilizable iron store and the remaining 15% is bound to myoglobin in muscle tissue and in a variety of enzymes involved in the oxidative metabolism and many other cell functions (IOM, 2001).

Iron is recycled and thus conserved by the body. Figure 3 show a schematic diagram of iron cycle in the body. Iron is delivered to tissues by circulating transferrin, a transporter that captures iron released into the plasma mainly from intestinal enterocytes or reticuloendothelial macrophages. The binding of iron-laden transferrin to the cell-surface transferrin receptor 1 results in endocytosis and uptake of the metal cargo. Internalized iron is transported to mitochondria for the synthesis of heme or iron–sulfur clusters, which are integral parts of several metalloproteins, and excess iron is stored and detoxified in cytosolic ferritin.

Figure 3. Iron is bound and transported in the body via transferrin and stored in ferritin molecules. Once iron is absorbed, there is no physiologic mechanism for excretion of excess iron from the body other than blood loss i.e. pregnancy, menstruation or other bleeding.

Source: [http://www.cdc.gov/ncbddd/hemochromatosis/training/pathophysiology/iron_cycle_popup.htm](http://www.cdc.gov/ncbddd/hemochromatosis/training/pathophysiology/iron_cycle_popup.htm)
2.2.3 Metabolism

1) Absorption

Since iron is required for a number of diverse cellular functions, a constant balance between iron uptake, transport, storage and utilization is required to maintain iron homeostasis (Lieu et al., 2001). As the body lacks a defined mechanism for the active excretion of iron, it is mainly regulated at the point of absorption (Hurrell and Egli, 2010; Finberg, 2011). The fraction of iron absorbed from the amount ingested is typically low, but may range from 5% to 35% depending on circumstances and type of iron (McDowell, 2003).

Iron absorption occurs by the enterocytes by divalent metal transporter 1 (DMT1), a member of the solute carrier (SLC) group of membrane transport proteins. This takes place predominantly in the duodenum and upper jejunum (Muir and Hopfer, 1985) (Figure 4). It is then transferred across the duodenal mucosa into the blood, where it is transported by transferrin to the cells or the bone marrow for erythropoiesis (producing red blood cells) (Hurrell, 1997; Frazer and Anderson; 2005; Nadadur et al., 2008). A feedback mechanism exists that enhances iron absorption in people who are iron deficient. In contrast, people with iron overload dampen iron absorption via hepcidin. It is now generally accepted that iron absorption is controlled by ferroportin which allows or not iron from the mucosal cell into the plasma. Hepcidin controls ferroportin. Hepcidin is high with good iron status (and infection) and degrades the ferroportin so preventing iron absorption. Hepcidin is low in iron deficiency and ferroportin remains intact so iron can be absorbed.

The physical state of iron entering the duodenum greatly influences its absorption. At physiological pH, ferrous iron (Fe^{2+}) is rapidly oxidized to the insoluble ferric (Fe^{3+}) form. Gastric acid lowers the pH in the proximal duodenum reducing Fe^{3+} in the intestinal lumen by ferric reductases and the subsequent transport of Fe^{2+} across the apical membrane of enterocytes. This enhances the solubility and uptake of ferric iron. When gastric acid production is impaired (for instance by acid pump inhibitors such as the drug, prilosec), iron absorption is reduced substantially.

Dietary heme can also be transported across the apical membrane by a yet unknown mechanism and subsequently metabolized in the enterocytes by heme oxygenase 1 (HO-1) to liberate Fe^{2+} (Wang and Pantopoulos, 2011). This process is more efficient than the absorption of inorganic iron and is independent of duodenal pH. It is thus not influenced by inhibitors such as phytate and polyphenols. Consequently red meats are excellent nutrient sources of iron. Directly internalized Fe^{2+} is processed by the enterocytes and eventually exported across the basolateral membrane into the bloodstream via the solute carrier and Fe^{2+}
Transporter ferroportin. The ferroportin-mediated efflux of Fe$^{+2}$ is coupled by its re-oxidation to Fe$^{+2}$, catalysed by the membrane-bound ferroxidase hephaestin that physically interacts with ferroportin (Yeh et al., 2009), and possibly also by its plasma homologue ceruloplasmin. Exported iron is scavenged by transferrin, which maintains Fe$^{+3}$ in a redox-inert state and delivers it into tissues. The total iron content of transferrin (∼3 mg) corresponds to less than 0.1% of body iron, but it is highly dynamic and undergoes more than ten times daily turnover to sustain erythropoiesis. The transferrin iron pool is replenished mostly by iron recycled from effete red blood cells and, to a lesser extent, by newly absorbed dietary iron. Senescent red blood cells are cleared by reticuloendothelial macrophages, which metabolize hemoglobin and heme, and release iron into the bloodstream. By analogy to intestinal enterocytes, macrophages export Fe$^{+2}$ from their plasma membrane via ferroportin, in a process coupled by re-oxidation of Fe$^{+2}$ to Fe$^{+3}$ by ceruloplasmin and followed by the loading of Fe$^{+3}$ to transferrin (Wang and Pantopoulos, 2011).

Theil et al. (2012) discovered a new mechanism for absorption of iron from vegetables and legumes that could provide a solution to iron deficiency problems. They state that ferritin iron from food is readily bioavailable to humans and has the potential for treating iron deficiency. Whether ferritin iron absorption is mechanistically different from iron absorption from small iron complexes/salts remains controversial. In their recent work they studied iron absorption...
from radiolabeled ferritin iron (0.5 mg) in healthy women with or without non-ferritin iron competitors, ferrous sulfate, or hemoglobin. A 9-fold excess of non-ferritin iron competitor had no significant effect on ferritin iron absorption. Larger amounts of iron (50 mg and a 99-fold excess of either competitor) inhibited iron absorption. Intestinal transport of iron absorbed inside exogenous ferritin was 14.8% of the rate measured for iron absorbed from chelated iron. In the steady state, endogenous enterocyte ferritin contained >90% of the iron absorbed from iron-NTA or ferritin. Theil et al. (2012) found that ferritin is a slow release source of iron, readily available to humans or animals, based on RBC iron incorporation. Hoppler et al. (2008) showed that ferritin iron is readily released from ferritin molecule during cooking and at gastric digestion. They state that the iron is dissolved in the gastric juice and would be expected to be absorbed by DTM1. In their study, they investigated changes of ferritin iron and protein during cooking and in vitro gastric digestion. Water soluble, native ferritin iron, measured in different legumes represented 18% (soybeans) to 42% (peas) of total seed iron. Ferritin iron was no longer detectable after boiling the legumes for 50 min in excess water. When they applied the same cooking treatment to recombinant bean ferritin propagated in Escherichia coli, some ferritin iron remained measurable. They found that during in vitro gastric digestion of recombinant bean ferritin and red kidney bean extract, ferritin iron was fully released from the protein and dissolved at pH 2. Their stability tests at varying pH at 37°C showed that the release of ferritin iron starts at pH 5 and is complete at pH 2. Hoppler et al. (2008) concluded that ferritin iron should be absorbed as efficiently as all other non-heme iron in food.

Regulation of iron homeostasis
Hepcidin is a peptide hormone secreted by the liver that plays a central role in the regulation of iron homeostasis. Hepcidin may be the principal iron-regulatory hormone, the key mediator of anemia of inflammation, and a bridge between innate immunity and iron metabolism (Ganz 2003). Increased hepcidin levels result in anemia while decreased expression is the causative feature in most primary iron overload diseases (De Domenico, et al., 2007). In all species, the concentration of iron in biological fluids is tightly regulated to provide iron as needed and to avoid toxicity, because iron excess can lead to the generation of reactive oxygen species (Braun and Killmann, 1999). Iron homeostasis in mammals is regulated at the level of intestinal absorption, as there is no excretory pathway for iron. Hepcidin, a circulating peptide hormone, is the master regulator of systemic iron homeostasis, coordinating the use and storage of iron with iron acquisition (Nemeth and Ganz, 2006). This hormone is primarily
produced by hepatocytes and is a negative regulator of iron entry into plasma (Figure 5). Hepcidin acts by binding to ferroportin, an iron transporter present on cells of the intestinal duodenum, macrophages, and cells of the placenta. Binding of hepcidin induces ferroportin internalization and degradation (Nemeth et al., 2004). The loss of ferroportin from the cell surface prevents iron entry into plasma (Figure 5A). Decreased iron entry into plasma results in low transferrin saturation, and less iron is delivered to the developing erythroblast. Conversely, decreased expression of hepcidin leads to increased cell surface ferroportin and increased iron absorption (Figure 5C) (De Domenico, et al., 2007).

Plasma hepcidin levels are regulated by different stimuli, including cytokines, plasma iron, anemia, and hypoxia. Dysregulation of hepcidin expression results in iron disorders. Overexpression of hepcidin leads to the anemia of chronic disease, while low hepcidin production results in hereditary hemochromatosis with consequent iron accumulation in vital organs (Figure 5). Most hereditary iron disorders result from inadequate hepcidin production relative to the degree of tissue iron accumulation. Impaired hepcidin expression has been shown to result from mutations in any of 4 different genes: transferrin receptor 2 (TFR2), hemochromatosis (HFE), hemochromatosis type 2 (HFE2), and hepcidin antimicrobial peptide (HAMP). Mutations in HAMP, the gene that encodes hepcidin, result in iron overload disease, as the absence of hepcidin permits constitutively high iron absorption. The role for other genes (TFR2, HFE, and HFE2) in the regulation of hepcidin production has been unclear (De Domenico, et al., 2007).

Storage

Ferritin concentration together with that of hemosiderin, reflect the body iron stores. They store iron in an insoluble form and are present primarily in the liver, spleen and bone marrow (Wood and Ronnenberg, 2006). The majority of iron is bound to the ubiquitous and highly conserved iron-binding protein, ferritin (Nadadur et al., 2008). Hemosiderin is an iron storage complex that less readily supplies iron than ferritin. Under steady state conditions serum ferritin concentrations correlate well with total body iron stores (Hunt, 2001). Thus, serum ferritin is the most convenient laboratory test to estimate iron stores.
Figure 5. Hepcidin-mediated regulation of iron homeostasis. (A) Increased hepcidin expression by the liver results from inflammatory stimuli. High levels of hepcidin in the bloodstream result in the internalization and degradation of the iron exporter ferroportin. Loss of cell surface ferroportin results in macrophage iron loading, low plasma iron levels, and decreased erythropoiesis due to decreased transferrin-bound iron. The decreased erythropoiesis gives rise to the anemia of chronic disease. (B) Normal hepcidin levels, in response to iron demand, regulate the level of iron import into plasma, normal transferrin saturation, and normal levels of erythropoiesis. (C) Hemochromatosis, or iron overload, results from insufficient hepcidin levels, causing increased iron import into plasma, high transferrin saturation, and excess iron deposition in the liver. Source: De Domenico, et al., 2007.

**Excretion**

Apart from iron losses due to menstruation, other bleeding or pregnancy, iron is highly conserved and not readily lost from the body (Hunt et al., 2009). There are some obligatory loss of iron from the body that results from the physiologic exfoliation of cells from epithelial surfaces (Hunt et al., 2009), including the skin, genitourinary tract, and gastrointestinal tract (McDowell, 2003). However, these losses are estimated to be very limited (≈1 mg/day) (Fairbanks, 1999). Iron losses through bleeding can be substantial and excessive menstrual blood loss is the most common cause of iron deficiency in women.

**2.2.4 Bioavailability**

Dietary iron occurs in two forms: heme and non-heme (Hurrell and Egli, 2010). The primary sources of heme iron are hemoglobin and myoglobin from consumption of meat, poultry and fish whereas non-heme iron is obtained from cereals, pulses, legumes, fruits and vegetables (FAO/WHO, 2001). Heme iron is highly bioavailable (15-35%) and dietary factors have little
effect on its absorption whereas non-heme iron absorption is much lower (2-20%) and strongly influenced by the presence of other food components (Hurrell and Egli, 2010). On the other hand, the quantity of non-heme iron in the diet is many fold above that of heme-iron in most meals. Thus despite its lower bioavailability, non-heme iron generally contributes more to iron nutrition than heme-iron (Monsen et al., 1978). Major inhibitors of iron absorption are phytic acid, polyphenols, calcium and peptides from partially digested proteins (Hurrell and Egli, 2010). Enhancers are ascorbic acid and muscle tissue which may reduce ferric iron to ferrous iron and bind it in soluble complexes which are available for absorption (Hurrell and Egli, 2010).

**Factors enhancing iron absorption**

A number of dietary factors influence iron absorption. Ascorbate and citrate increase iron uptake in part by acting as weak chelators to help to solubilize the metal in the duodenum (Table 7) (Conrad and Umbreit, 1993). Iron is readily transferred from these compounds into the mucosal lining cells. The dose-dependent enhancing effect of native or added ascorbic acid on iron absorption has been shown by many researchers (Lynch and Cook, 1980). The enhancing effect is largely due to its ability to reduce ferric to ferrous iron but is also due to its potential to chelate iron (Conrad and Schade, 1968). Ascorbic acid will overcome the negative effect on iron absorption of all inhibitors, which include phytate (Hallberg et al., 1989), polyphenols (Siegenberg et al., 1991), and the calcium and proteins in milk products (Stekel, 1986), and will increase the absorption of both native and fortification iron. In fruit and vegetables the enhancing effect of ascorbic acid is often cancelled out by the inhibiting effect of polyphenols (Ballot et al., 1987). Ascorbic acid is the only main absorption enhancer in vegetarian diets, and iron absorption from vegetarian and vegan meals can be best optimized by the inclusion of ascorbic acid–containing vegetables (Lynch and Cook, 1980). Cooking, industrial processing, and storage degrade ascorbic acid and remove its enhancing effect on iron absorption (Teucher et al., 2004).

The enhancing effect of meat, fish, or poultry on iron absorption from vegetarian meals has been shown by (Lynch et al., 1989), and 30 g muscle tissue is considered equivalent to 25 mg ascorbic acid (Monsen et al., 1978). Bjorn-Rasmussen and Hallberg (1979) reported that the addition of chicken, beef, or fish to a maize meal increased non-heme iron absorption 2–3-fold with no influence of the same quantity of protein added as egg albumin. As with ascorbic acid, it has been somewhat more difficult to demonstrate the enhancing effect of meat in multiple meals and complete diet studies. Reddy et al. (2006) reported only a marginal
improvement in iron absorption (35%) in self-selected diets over 5 days when daily muscle tissue intake was increased to 300 g/day, although, in a similar 5-day study, 60 g pork meat added to a vegetarian diet increased iron absorption by 50% (Bach et al., 2005).

Table 7. Factors that influence iron absorption

<table>
<thead>
<tr>
<th>Physical State (bioavailability)</th>
<th>heme &gt; Fe(^{2+}) &gt; Fe(^{3+})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhibitors</td>
<td>phytates, polyphenols, calcium, proteins, tannins</td>
</tr>
<tr>
<td>Competitors</td>
<td>lead, cobalt, strontium, manganese, zinc</td>
</tr>
<tr>
<td>Facilitators</td>
<td>ascorbate, citrate, amino acids, meat, fish, poultry</td>
</tr>
</tbody>
</table>

**Factors inhibiting iron absorption**

In plant-based diets, phytate (myo-inositol hexakisphosphate) is the main inhibitor of iron absorption (Hurrel and Egli, 2010). The negative effect of phytate on iron absorption has been shown to be dose dependent and starts at very low concentrations of 2–10 mg/meal (Hallberg et al., 1989; Hurrell et al., 1992). The molar ratio of phytate to iron can be used to estimate the effect on absorption. The ratio should be 1:1 or preferably, 0.4:1 to significantly improve iron absorption in plain cereal or legume-based meals that do not contain any enhancers of iron absorption, or, 6:1 in composite meals with certain vegetables that contain ascorbic acid and meat as enhancers (Hurrell, 2004).

Polyphenols occur in various amounts in plant foods and beverages, such as vegetables, fruit, some cereals and legumes, tea, coffee, and wine. The inhibiting effect of polyphenols on iron absorption has been shown with black tea and to a lesser extent with herbal teas (Hurrell et al., 1999; Hallberg and Rossander, 1982). In cereals and legumes, polyphenols add to the inhibitory effect of phytate, as was shown in a study that compared high and low polyphenol sorghum (Hurrell and Egli, 2010).

Calcium has been shown to have negative effects on nonheme and heme iron absorption, which makes it different from other inhibitors that affect nonheme iron absorption only (Hallberg et al., 1993). Dose-dependant inhibitory effects were shown at doses of 75–300 mg when calcium was added to bread rolls and at doses of 165 mg calcium from milk products (Hallberg et al., 1991). It is proposed that single-meal studies show a negative effect of calcium on iron absorption, whereas multiple-meal studies, with a wide variety of foods and
various concentrations of other inhibitors and enhancers, indicate that calcium has only a limited effect on iron absorption (Lynch, 2000).

Animal proteins such as milk proteins, egg proteins, and albumin, have been shown to inhibit iron absorption (Cook and Monsen, 1976). The two major bovine milk protein fractions, casein and whey, and egg white were shown to inhibit iron absorption in humans (Hurrell et al., 1988). Proteins from soybean also decrease iron absorption.

**Competition with iron**

Competition studies suggest that several other heavy metals share the iron intestinal absorption pathway. These include lead, manganese, cobalt and zinc (Table 7). As iron deficiency often coexists with lead intoxication, this interaction can produce particularly serious medical complications in children (Piomelli et al., 1987). Interestingly, copper absorption and metabolism appear to be handled mechanisms different to those of iron.

Lead is a particularly pernicious element to iron metabolism (Goyer, 1993). Lead is taken up by the iron absorption machinery, and secondarily blocks iron through competitive inhibition. Further, lead interferes with a number of important iron-dependent metabolic steps such as heme biosynthesis. This multifaceted attack has particularly dire consequences in children, were lead not only produces anemia, but can impair cognitive development. Lead exists naturally at high levels in ground water and soil in some regions, and can clandestinely attack children's health. For this reason, most pediatricians in the U.S. routinely test for lead at an early age through a simple blood test.

**2.2.5 Human Requirements**

During early infancy iron requirements are met by the little iron contained in the human milk (FAO/WHO, 2004). The need for iron rises markedly 4-6 months after birth and amounts to about 0.7-0.9 mg/day during the remaining part of the first year (FAO/WHO, 2004). Between 1 and 6 years of age, the body iron content is again doubled (FAO/WHO, 2004). Iron requirements are also very high in adolescents, particularly during the period of rapid growth. Girls usually have their growth spurt before menarche, but growth is not finished at that time. In boys there is a marked increase in hemoglobin mass and concentration during puberty. In this stage iron requirements increase to a level above the average iron requirements in menstruating women (FAO/WHO, 2004).

The average adult stores about 1 to 3 grams of iron in his or her body. An exquisite balance between dietary uptake and loss maintains this balance. About 1 mg of iron is lost each day.
through sloughing of cells from skin and mucosal surfaces, including the lining of the
gastrointestinal tract (Cook et al., 1986). Menstruation increases the average daily iron loss to
about 2 mg per day in premenopausal female adults (Bothwell and Charlton, 1982). The
augmentation of body mass during neonatal and childhood growth spurts transiently boosts
iron requirements (Gibson et al., 1988).

A dietary intake of iron is needed to replace iron lost in the stools and urine and through
the skin. These basal losses represent approximately 0.9 mg of iron for an adult male and 0.8
mg for an adult female. The iron lost in menstrual blood must be taken into consideration for
women of reproductive age (see Table 8).

Table 8. Iron requirements of 97.5% of individuals in terms of absorbed iron\(^a\), by age group and sex

<table>
<thead>
<tr>
<th>Age/sex</th>
<th>mg/day(^b)</th>
</tr>
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<tbody>
<tr>
<td>4-12 months</td>
<td>0.96</td>
</tr>
<tr>
<td>13-24 months</td>
<td>0.61</td>
</tr>
<tr>
<td>2-5 years</td>
<td>0.70</td>
</tr>
<tr>
<td>6-11 years</td>
<td>1.17</td>
</tr>
<tr>
<td>12-16 years (girls)</td>
<td>2.02</td>
</tr>
<tr>
<td>12-16 years (boys)</td>
<td>1.82</td>
</tr>
<tr>
<td>Adult males</td>
<td>1.14</td>
</tr>
<tr>
<td>Pregnant women(^c)</td>
<td></td>
</tr>
<tr>
<td>First trimester</td>
<td>0.8</td>
</tr>
<tr>
<td>Second &amp; third trimester</td>
<td>6.3</td>
</tr>
<tr>
<td>Lactating women</td>
<td>1.31</td>
</tr>
<tr>
<td>Menstruating women</td>
<td>2.38</td>
</tr>
<tr>
<td>Post-menopausal women</td>
<td>0.96</td>
</tr>
</tbody>
</table>

\(^a\) Absorbed iron is the fraction that passes from the gastrointestinal tract into the body for further use.
\(^b\) Calculated on the basis of median weight for age.
\(^c\) Requirements during pregnancy depend on the woman's iron status prior to pregnancy.

2.2.6 Groups at High Risk

The highest probability of suffering iron deficiency is found in those parts of a population that
have inadequate access to foods rich in absorbable iron during stages of high iron demand.
These groups correspond to children, adolescents, and women of reproductive age, in

In the case of infants and adolescents, the increased iron demand is the result of rapid
growth. For women of reproductive age the principle reason is the excessive blood loss during
menstruation. During pregnancy, there is a significant increase in iron requirement due to the
rapid growth of the placenta and the fetus and the expansion of the globular mass (Dallman,
1990). In contrast, adult men and postmenopausal women are at low risk of iron deficiency.
and the amount of iron in a normal diet is usually sufficient to cover their physiological requirements (Dallman, 1990).

2.2.7 Consequences and Causes of iron Deficiency

Consequences of iron Deficiency

Iron deficiency can exist with or without anemia. Some functional changes may occur in the absence of anemia, but the most functional deficits appear to occur with the development of anemia (Wood and Ronnenberg, 2006). Even mild and moderate forms of iron deficiency anemia can be associated with functional impairments affecting cognitive development (Beard and Connor, 2003), immunity mechanisms (Failla, 2003) and work capacity (Viteri and Torun, 1974). Iron deficiency during pregnancy is associated with a variety of adverse outcomes for both mother and infant, including increased risk of sepsis, maternal mortality, perinatal mortality, and low birth weight (CDC, 2010). Iron deficiency and anemia also reduce learning ability and are associated with increased rates of morbidity (CDC, 2010).

Causes of iron Deficiency

Iron deficiency results from depletion of iron stores and occurs when iron absorption cannot keep pace over an extended period with the metabolic demands for iron to sustain growth and to replenish iron loss, which is primarily related to blood loss (Wood and Ronnenberg, 2006). The primary causes of iron deficiency include low intake of bioavailable iron, increased iron requirements as a result of rapid growth, pregnancy, menstruation and excess blood loss caused by pathologic infections, such as hook worm and whipworm causing gastrointestinal blood loss (Cooper et al., 1987; WHO, 1996; Crompton and Nesheim, 2002; Larocque et al., 2005) and impaired absorption of iron (Zimmermann and Hurrell, 2007). The frequency of iron deficiency rises in female adolescents because menstrual iron losses are superimposed with needs for rapid growth (Harvey et al., 2005). Other risk factors for iron deficiency in young women are high parity, use of an intrauterine device, and vegetarian diets (Beard, 2000).

Nutritional iron deficiency arises when physiological requirements cannot be met by iron absorption from the diet (Zimmermann and Hurrell, 2007). Dietary iron bioavailability is low in populations consuming monotonous plant-based diets with little meat (Zimmermann and Hurrell, 2007). In many developing countries, plant-based weaning-foods are rarely fortified with iron, and the frequency of anemia exceeds 50% in children younger than 4 years (WHO/UNICEF/UNU, 2001).
When iron stores are depleted and insufficient iron is available for erythropoiesis, hemoglobin synthesis in erythrocyte precursors become impaired and hematologic signs of iron deficiency anemia appear.

2.2.8 Evaluation of iron status

Iron deficiency and eventually anemia develop in stages and can be assessed by measuring various biochemical indices. Although some iron enzymes are sensitive to iron deficiency (Dallman, 1990) their activity has not been used as a successful routine measure of iron status (Wood and Ronnenberg, 2006).

Laboratory measurements are essential for a proper diagnosis of iron deficiency. They are most informative when multiple measures of iron status are examined and evaluated in the context of nutritional and medical history.

Early stages of iron depletion are characterized by reduced serum/plasma iron and elevated plasma transferrin concentration, measured by serum/plasma total iron-binding capacity (TIBC) (Wood and Ronnenberg, 2006). However, marked biologic variation can occur in these values as a result of diurnal variation, the presence of infection or inflammatory conditions and recent dietary iron intake.

Zinc protoporphyrin reflects the shortage of iron supply in the last stages of hemoglobin synthesis so that zinc is inserted into the protoporphyrin molecule in the place of iron. Zinc protoporphyrin can be detected in RBCs by fluorimetry and is a measure of the severity of iron deficiency (WHO/CDC, 2004).

Serum ferritin is a good indicator of body iron stores under most circumstances. However, ferritin is an acute phase reactant protein and its serum concentrations can be elevated, irrespective of a change in iron stores, by infection or inflammation (WHO/CDC, 2004; Wood and Ronnenberg, 2006).

The concentration of transferrin receptor (TfR) in serum is another indicator of iron status. It is mostly derived from developing RBCs and therefore reflects the intensity of erythropoiesis and the demand for iron. The concentration rises in iron deficiency anemia, when iron stores have been exhausted, indicating severe iron insufficiency provided that there are no other causes of abnormal erythropoiesis (WHO/CDC, 2004). Clinical studies indicate that the serum TfR is less affected by inflammation than serum ferritin (Beguin, 2003). The major advantage of TfR as an indicator is the possibility of estimating the magnitude of the functional iron deficit once iron stores are depleted (Baynes, 1996).
The ratio of TfR to ferritin (TfR/ferritin) was designed to evaluate changes in both stored iron and functional iron and was thought to be more useful than either TfR or ferritin alone (Cook et al., 2003). TfR/ferritin has been used to estimate body iron stores in both children and adults (Cook et al., 2005). However, the high cost and the lack of standardization of the TfR assay so far have limited the applicability of the method (Yang et al., 2008).

Low hemoglobin concentration is a measure of anemia, the end stage of iron deficiency (WHO/CDC, 2004; Wood and Ronnenberg, 2006).

2.2.9. Anemia and its Causes

Anemia describes the condition in which the number of red blood cells in the blood is low. A person who has anemia is called anemic. The purpose of the red blood cell is to deliver oxygen from the lungs to other parts of the body. The hemoglobin molecule is the functional unit of the red blood cells and is a complex protein structure that is inside the red blood cells. Even though the red blood cells are made within the bone marrow, many other factors are involved in their production. For example, iron is a very important component of the hemoglobin molecule; erythropoietin, a molecule secreted by the kidneys, promotes the formation of red blood cells in the bone marrow.

Having the correct number of red blood cells and prevention of anemia requires cooperation among the kidneys, the bone marrow, and nutrients within the body. If the kidneys or bone marrow are not functioning, or the body is poorly nourished, then normal red blood cell count and functions may be difficult to maintain.

Anemia is actually a sign of a disease process rather than a disease itself. It is usually classified as either chronic or acute. Chronic anemia occurs over a long period of time. Acute anemia occurs quickly. Determining whether anemia has been present for a long time or whether it is something new, assists doctors in finding the cause. This also helps predict how severe the symptoms of anemia may be. In chronic anemia, symptoms typically begin slowly and progress gradually; whereas in acute anemia symptoms can be abrupt and more distressing.

Red blood cells live about 100 days, so the body is constantly trying to replace them. In adults, red blood cell production occurs in the bone marrow. Doctors try to determine if a low red blood cell count is caused by increased blood loss of red blood cells or from decreased production of them in the bone marrow. Knowing whether the number of white blood cells and/or platelets has changed also helps determine the cause of anemia.
In the United States, 2% to 10% of people have anemia. Other countries have even higher rates of anemia. Young women are twice as likely to have anemia as young men because of regular menstrual bleeding. Anemia occurs in both young people and in old people, but anemia in older people is more likely to cause symptoms because they typically have additional medical problems.

Many medical conditions cause anemia. Common causes of anemia include the following:

**Iron deficiency anemia:** The bone marrow needs iron to make red blood cells. Iron plays an important role in the proper structure of the hemoglobin molecule. If iron intake is limited or inadequate due to poor dietary intake, anemia may occur as a result. This is called iron deficiency anemia. Iron deficiency anemia can also occur when there are stomach ulcers or other sources of slow, chronic bleeding (colon cancer, uterine cancer, intestinal polyps, hemorrhoids, etc).

**Anemia of chronic disease:** Any long-term medical condition can lead to anemia. This type of anemia is the second most prevalent after anemia caused by iron deficiency, and occurs in patients with acute or chronic immune activation. The condition has thus been termed “anemia of inflammation” due to elevated hepcidin (Weiss and Goodnough, 2005).

**Anemia from active bleeding:** Loss of blood through heavy menstrual bleeding or, wounds can cause anemia. Gastrointestinal ulcers or cancers such as cancer of the colon may slowly ooze blood and can also cause anemia.

**Anemia related to kidney disease:** The kidneys release a hormone called the erythropoietin that helps the bone marrow make red blood cells. In people with chronic (long-standing) kidney disease, the production of this hormone is diminished, and this in turn diminishes the production of red blood cells, causing anemia (O’Mara, 2008).

**Anemia related to pregnancy:** Water weight gain during pregnancy dilutes the blood, which may be reflected as anemia.

**Anemia related to poor nutrition:** Vitamins and minerals are required to make red blood cells. In addition to iron, vitamin B12 and folate are required for the proper production of hemoglobin. Deficiency in any of these may cause anemia because of inadequate production of red blood cells. Poor dietary intake is an important cause of low folate and low vitamin B12 levels. Strict vegetarians who do not take sufficient vitamins are at risk to develop vitamin B12 deficiency.

**Pernicious Anemia:** There also may be a problem in the stomach or the intestines leading to poor absorption of vitamin B12. This may lead to anemia because of vitamin B12 deficiency.
Sickle cell anemia: In some individuals, the problem may be related to production of abnormal hemoglobin molecules. In this condition the hemoglobin problem is qualitative, or functional. Abnormal hemoglobin molecules may cause problems in the integrity of the red blood cell structure and they may become crescent-shaped (sickle cells). There are different types of sickle cell anemia with different severity levels. This is typically hereditary and is more common in those of African, Middle Eastern, and Mediterranean ancestry.

Thalassemia: This is another group of hemoglobin-related causes of anemia. There are many types of thalassemia, which vary in severity from mild (thalassemia minor) to severe (thalassemia major). These are also hereditary, but they cause quantitative hemoglobin abnormalities, meaning an insufficient amount of the correct hemoglobin type molecules is made. Thalassemia is more common in people from African, Mediterranean, and Southeast Asian ancestries.

Alcoholism: Poor nutrition and deficiencies of vitamins and minerals are associated with alcoholism. Alcohol itself may also be toxic to the bone marrow and may slow down the red blood cell production. The combination of these factors may lead to anemia in alcoholics.

Bone marrow-related anemia: Anemia may be related to diseases involving the bone marrow. Some blood cancers such as leukemia or lymphomas can alter the production of red blood cells and result in anemia. Other processes may be related to a cancer from another organ spreading to the bone marrow.

Aplastic anemia: Occasionally some viral infections may severely affect the bone marrow and significantly diminish production of all blood cells.

Hemolytic anemia: The normal red blood cell shape is important for its function. Hemolytic anemia is a type of anemia in which the red blood cells rupture (known as hemolysis) and become dysfunctional. This could happen due to a variety of reasons. Some forms of hemolytic anemia can be hereditary with constant destruction and rapid reproduction of red blood cells (for example, as in hereditary spherocytosis, hereditary elliptocytosis, and glucose-6-phosphate dehydrogenase or G6GD deficiency).

Anemia related to medications: Many common medications can occasionally cause anemia as a side effect in some individuals. The mechanisms by which medications can cause anemia are numerous (hemolysis, bone marrow toxicity) and are specific to the medication. Medications that most frequently cause anemia are chemotherapy drugs used to treat cancers. Other common medications that can cause anemia include some seizure medications, transplant medications, HIV medications, some malaria medications, some antibiotics (penicillin, chloramphenicol), antifungal medications, and antihistamines.
Obesity and anemia: Obesity is characterized by chronic, low-grade, systemic inflammation, elevated hepcidin, which, in turn has been associated with anemia of chronic disease. Ausk and Ioannou (2008) hypothesized that obesity may be associated with the features of anemia of chronic disease, including low hemoglobin concentration, low serum iron and transferrin saturation, and elevated serum ferritin. Overweight and obesity were associated with changes in serum iron, transferrin saturation, and ferritin that would be expected to occur in the setting of chronic, systemic inflammation. However, overweight and obese persons were not more likely to be anemic compared with normal-weight persons. Obesity-related inflammation may increase hepcidin concentrations and reduce iron availability. Aeberli et al. (2009) compared iron status, dietary iron intake and bioavailability, as well as circulating levels of hepcidin, leptin and interleukin-6 (IL-6), in overweight vs normal weight children. They indicated that there is reduced iron availability for erythropoiesis in overweight children and that this is likely due to hepcidin-mediated reduced iron absorption and/or increased iron sequestration rather than low dietary iron supply.

Other less common causes of anemia include thyroid problems, cancers, liver disease, autoimmune diseases (lupus), paroxysmal nocturnal hemoglobinuria (PNH), lead poisoning, AIDS, malaria, viral hepatitis, mononucleosis, parasitic infections (hookworm), bleeding disorders, and insecticide exposure. It is noteworthy that there are many other potential causes of anemia that are not included in this list as these are only some of the more common and important ones.

2.2.10 Prevention of iron deficiency

Intervention Strategies

The four principle strategies described for zinc are also applicable for correcting iron deficiency in populations, alone or in combination: education combined with dietary modification or diversification, or both, to improve iron intake and bioavailability; iron supplementation (provision of iron, usually in higher doses, without food), iron fortification of foods and the new approach of biofortification. However, there are some difficulties in the application of some of these strategies when considering iron.

I) Food Diversification

Dietary modifications for reducing IDA involve increased intake of iron rich foods such as flesh foods, consumption of fruits and vegetables rich in ascorbic acid to enhance non-heme
iron absorption and reduce the intake of tea and coffee, which inhibit non-heme iron absorption (Hurrell, 2002; FAO/WHO, 2004).

II) Supplementation

For oral iron supplementation, ferrous iron salts (ferrous sulfate and ferrous gluconate) are preferred because of their low cost and high bioavailability (Zimmerman and Hurrell, 2007). Although iron absorption is enhanced when iron supplements are given on an empty stomach, nausea, and epigastric pain might develop. If these side-effects arise, lower doses between meals should be attempted or iron should be provided with meals, although food reduces absorption of medicinal iron by about two-thirds (Cavalli-Sforza et al., 2005). Iron supplementation during pregnancy is advisable in developing countries, where women often enter pregnancy with low iron stores (CDC, 2002). Although the benefits of iron supplementation have generally been considered to outweigh the putative risks, there is some evidence to suggest that supplementation at levels recommended for otherwise healthy children carries the risk of increased severity of infectious disease in the presence of malaria and/or undernutrition (Oppenheimer, 2001; Sazawal et al., 2006).

III) Fortification

Fortification of foods with iron is more difficult than it is with other nutrients, such as zinc in flour, iodine in salt and vitamin A in cooking oil (Zimmerman and Hurrell, 2007). The most bioavailable iron compounds are soluble in water or diluted acid, but often react with other food components to cause off-flavors, color changes or fat oxidation (Hurrell, 2002). Thus, less soluble forms of iron, although less well absorbed, are often chosen for fortification to avoid unwanted sensory changes (Zimmerman and Hurrell, 2007). Fortification with low iron doses is more similar to the physiological environment than in supplementation and might be the safest intervention (WHO, 2007).

Iron compounds recommended for food fortification by the WHO (2006) include ferrous sulfate, ferrous fumarate, ferric pyrophosphate, and electrolytic iron powder. Hallberg and Rossander-Hulthen (1991) estimated that 25% of the total iron intake in Sweden and the United States comes from fortification iron. When they calculated the bioavailability factors for the complete diet, they assumed the fortification iron was mainly low-bioavailability elemental iron powders and they estimated that it was only 15% as well absorbed as native food iron. Food-fortification practices vary nationally and the need to adjust the dietary iron
bioavailability factor for fortification iron will depend on the proportion of fortification iron in the total iron intake and the iron compounds used.

**IV) Biofortification**

Iron contents vary from 25 to 56 mg/kg in wheat and 7 to 23 mg/kg in rice grains. However, most of this iron is removed during the milling process. Similar to zinc, iron absorption from cereals and legumes, many of which have high native iron content, is generally low because of their high contents of phytate and polyphenols (Hurrell et al., 1999). In a biofortification study Lucca et al. (2002) increased the iron content in rice endosperm to improve its absorption in the human intestine by means of genetic engineering. They introduced a ferritin gene from Phaseolus vulgaris into rice grains, increasing their iron content up to twofold. To increase iron bioavailability, they introduced a thermo-tolerant phytase from Aspergillus fumigatus into the rice endosperm. They indicated that this rice, with higher iron content and rich in phytase has a great potential to substantially improve iron nutrition in those populations where iron deficiency is so widely spread (Lucca et al., 2002).

Another strategy is to reduce anti-nutrient contents in order to make the iron supplied from their food sources more available. Iron availability may also increase by some techniques such as soaking, germination and fermentation, which promote enzymatic hydrolysis of phytic acid in whole grain cereals and legumes by enhancing the activity of endogenous or exogenous phytase enzyme (Schlemmer, 2009). Even use of non-enzymatic methods such as thermal processing, soaking, and milling for reducing phytic acid content in plant-based staples has been successful to improve the bioavailability of zinc and iron (Cook, 2005; Liang, 2008).

### 2.2.11 Iron deficiency in Iran

A national survey in Iran in 1999 showed a large prevalence of anemia as indicated in Table 9. In 2001, National Integrated Micronutrient Survey (NIMS) in Iran divided the country into 11 regions with similar epidemiological indicators (Kalantari et al., 2001). The study showed the prevalence of anemia as measured by hemoglobin levels as well as iron deficiency based on ferritin levels. Non-pregnant women of child-bearing age were not sampled separately in the survey. The results are given in Table 10. Depleted or low iron stores were found in low socioeconomic areas, such as the south and southeast (Sistan and Baluchestan, Hormozgan, Bushehr, Fars, Kerman), and also in the north and northwest (Gilan, Mazandaran, Golestan, East and West Azarbayjan and Ardebil) where the socioeconomic situation is better. Except
in adult men, most of the anemia seemed to be caused by iron deficiency. The percent of adult men with anemia was significantly higher than the percent of adult men with iron deficiency. This in part may be due to existence of thalassemia major in the region (Kalantari et al., 2001).

The national survey did not measure folate status, but smaller studies in Iran did reveal folate deficiency. The results of a study in Golestan province showed a mean daily folate intake from food of 198 micrograms (Abdollahi et al., 2008). The recommended daily allowance of folic acid is 400 micrograms for women of childbearing age. Iran does not have a national birth defect monitoring system, but several studies indicate an incidence rate of about 28-32 per 10,000 births (Golalipour et al., 2009).

Regional workshops identified iron deficiency anemia as a key public health concern throughout the Middle East, and health leaders in Iran included iron deficiency reduction as a key component of the country’s nutrition strategy. “Consultation on Strategies for the Control of Iron Deficiency Anemia” held by WHO and UNICEF in Tehran, Iran, in 1995 agreed upon dietary improvements, iron supplementation for vulnerable groups, and food fortification as strategies to control iron deficiency.

A legislation was passed in 1983, which called for iron supplementation for pregnant women and children up to 2 years of age. This policy has been revised since and there was a national campaign to promote improved iron status via dietary change and iron supplementation for women and children (6-24 months). There is also a national campaign to promote improved iron status via dietary change. Iron supplementation for adolescent schoolgirls has been started in 6 districts, with ferrous sulfate tablets given twice a week in school.

With regard to fortification, after the small pilot project in Isfahan, three larger studies were conducted in Bushehr, Fars, and Golestan provinces. These efforts were instrumental in revealing the feasibility and impact of fortifying flour in Iran. These provincial projects helped food safety authorities and industry leaders develop effective and sustainable quality control processes. These studies, starting in Bushehr in 2001 and ending in Golestan in 2008, showed that iron deficiency significantly decreased after bread fortification. In October 2007, all mills throughout the country started flour fortification with iron and folic acid. Fortification rates were set at 1.5 parts per million (ppm) for folic acid and 30 ppm of ferrous sulfate. Iran decided to fortify flour with extraction rates between 80% and 88% which is about 88% of the flour in the country.
Table 9. Anemia survey of Iranian population in 1999

<table>
<thead>
<tr>
<th>Age group</th>
<th>Sample size</th>
<th>Indicator</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-14 yrs female</td>
<td>7728</td>
<td>Hb &lt; 110 for under 6</td>
<td>14.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hb &lt; 120 g/L for over</td>
<td></td>
</tr>
<tr>
<td>2-14 yrs male</td>
<td>8012</td>
<td>Hb &lt; 110 for under 6</td>
<td>15.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hb &lt; 130 g/L for over</td>
<td></td>
</tr>
<tr>
<td>15-39 yrs female</td>
<td>12061</td>
<td>Hb &lt; 120 g/L</td>
<td>17.4</td>
</tr>
<tr>
<td>15-39 yrs male</td>
<td>8548</td>
<td>Hb &lt; 130 g/L</td>
<td>10.2</td>
</tr>
<tr>
<td>40-69 yrs female</td>
<td>5083</td>
<td>Hb &lt; 120 g/L</td>
<td>17.8</td>
</tr>
<tr>
<td>40-69 yrs male</td>
<td>4150</td>
<td>Hb &lt; 130 g/L</td>
<td>11.4</td>
</tr>
<tr>
<td>Over 70 yrs female</td>
<td>811</td>
<td>Hb &lt; 120 g/L</td>
<td>17.1</td>
</tr>
<tr>
<td>Over 70 yrs male</td>
<td>868</td>
<td>Hb &lt; 130 g/L</td>
<td>23.5</td>
</tr>
</tbody>
</table>

Source: [http://www.tulane.edu/~internut/Countries/Iran/iraniron.html](http://www.tulane.edu/~internut/Countries/Iran/iraniron.html)

Table 10. Prevalence of anemia and low iron stores in Iran

<table>
<thead>
<tr>
<th>Age group</th>
<th>Anemia (%)</th>
<th>Iron deficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children 15-23 months</td>
<td>38</td>
<td>33</td>
</tr>
<tr>
<td>Children 6 years</td>
<td>22</td>
<td>26</td>
</tr>
<tr>
<td>Adolescent 14-20 years</td>
<td>17</td>
<td>23</td>
</tr>
<tr>
<td>Pregnant women ≥ 5 months</td>
<td>21</td>
<td>43</td>
</tr>
<tr>
<td>Adult women 50-60 years</td>
<td>19</td>
<td>14</td>
</tr>
<tr>
<td>Adult men 45-60 years</td>
<td>10</td>
<td>4</td>
</tr>
</tbody>
</table>

Source: Kalantari et al., 2001
REFERENCES


with iron and folic acid on admission to hospital and mortality in preschool children in a high malaria transmission setting: community-based, randomised, placebo-controlled trial. Lancet 367:133–143.


CHAPTER 2

Zinc and Phytic Acid in Major Foods Consumed by a Rural and a Suburban Population in Central Iran

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Abstract

There are no comprehensive food composition data on minerals and phytic acid (PA) contents of Iranian foods. This information is an important prerequisite when assessing nutrient intake and the nutritional status of the population. In this study, zinc was analysed in rice, wheat, bread and legumes (n=111) as well as the main animal source foods (dairy and meat products, n=107) and 9 local cooked dishes (n=38), consumed in a rural and suburban population in central Iran. PA was additionally measured in the cereal and legume foods as well as the local dishes. Iron and calcium were also measured in selected rice samples and legumes before and after cooking. The Zn concentration in cooked rice and bread, as major staples, were 0.88 ± 0.34 and 1.32 ± 0.16 mg/100 g DW in the suburb area and 1.29 ± 0.45 and 1.77 ± 0.21 mg/100 g DW in the rural area, respectively. The PA:Zn molar ratio of flat bread was 24 in Khomeini Shahr and 22 in Rooran. Cooked rice and composite dishes had PA:Zn molar ratios between 4 to 13.

Keywords: food composition, food analysis, bioavailability, nutrient intake, Iranian food

1. Introduction

An adequate intake of Zn is necessary to maintain good health, growth and development (Hambidge, 2000; Hotz and Brown, 2004). Deficiency results from an inadequate dietary intake of bioavailable Zn and is common in many parts of the world (Lönnerdal, 2000). Adequate Zn nutrition is particularly important during childhood (Hotz and Brown, 2004).

The importance of Zn nutrition for human health and development is well documented (Hambidge et al., 2010). Multiple physiological and metabolic functions, such as physical growth, immuno-competence, reproductive function, and neuro-behavioral development are affected by Zn status (Hambidge, 2000; Hotz and Brown, 2004). The first case of human Zn deficiency ever detected was in Iran in 1961, when a dwarf male adolescent with retarded sexual maturation responded positively to Zn supplementation (Prasad et al., 1963). Since then, human Zn deficiency has been found in many other regions of the world, in particular in developing countries (Lönnerdal, 2000).

While the total concentrations of Zn in plant-based foods are comparable to some animal source foods, its bioavailability is usually much less (Sandström, 1997; Gibson, et al., 2010). Poor Zn bioavailability, as well as that of Fe and Ca, is associated with the presence of inositol phosphates, commonly known as phytic acid or phytates (PA), (Gibson, et al., 2010) in staple cereal foods and legumes. PA is the principal storage form of phosphorus in most
plant seeds. It can be bound to Zn, Fe and Ca in the seeds but more importantly it can form complexes with these seed minerals, and the same minerals from other dietary sources, in the digestive tract. (Schlemmer et al., 2009; Reddy, 2002). When complexed with PA, these minerals become insoluble and cannot be absorbed in the intestine (Schlemmer et al., 2009). High PA concentrations in foods become a nutritional concern where populations consume mainly plant-based diets and have a low dietary intake of animal source foods. This situation is widespread in many developing countries but, in relation to an adequate supply of bioavailable Zn could be especially severe in arid rural regions where not only do populations consume mainly locally grown cereal-based diets, but the Zn concentrations in the grain tend to be low due to low concentrations of plant-available Zn in the soils (Cakmak et al., 1999).

In the absence of food, deficiencies of Fe and Zn often occur together in the same populations because they have similar dietary sources and because PA is the major absorption inhibitor of these trace minerals (Gibson, et al., 2010).

In order to predict Fe and Zn absorption from meals containing PA, it has been customary to use their molar ratio in the meal in relation to PA. For Fe, in cereal or legume meals containing no enhancers of Fe absorption, a PA:Fe molar ratio of 1 (or preferably 0.4) has been recommended so as to achieve a meaningful Fe absorption, although this molar ratio was increased to 6 for composite meals containing ascorbic acid or meat as enhancers of Fe absorption (Hurrell and Egli 2010). For Zn, Hotz and Brown (2004) reported that PA:Zn molar ratios above 18 would decrease Zn absorption, however, lower molar ratios would also be expected to have a negative effect. For example a molar ratio of 4-8 in non-zinc-fortified foods should result in Zn absorption in adults of around 20% or about half of Zn absorption in the absence of PA (Hurrell, 2003). It has been estimated that reducing the phytic acid level of whole-grain legumes or cereals from 1 to 0.1% would approximately double Zn absorption whereas complete removal could result in a further twofold increase. Zinc absorption from high phytate foods is around 10% and a doubling absorption by lowering phytate would be of great benefit to Zn nutrition (Hurrell, 2003).

Much less is known about the PA:Ca molar ratios that influence Ca absorption. The influence is presumably much less than for Fe or Zn as Ca concentrations in diets are much higher. Using isotopic studies in human volunteers, Heaney (1991) reported PA to decrease Ca absorption in 16 normal women and based on Ca balance studies Morris and Ellis, (1985) suggested PA:Ca ratios above 0.2 would inhibit absorption.

In addition to depending on soil composition and climatic conditions, Zn concentrations in local plant-based and animal source foods also depend on crop plant cultivars, agricultural
management practices, food processing techniques and food preparation procedures (Sunanda et al., 1995; Alloway, 2004). National or regional food composition data are therefore needed to evaluate the Zn intake. Unfortunately, comprehensive food composition data covering indigenous agricultural products and prepared foods are missing in many developing countries (Abede et al., 2007), including Iran. The food composition data bases used in Iran are mainly derived from the USDA National Nutrient Database with some adjustments for Iranian diets. It is uncertain how well these nutrient data bases represent Iranian foods; however PA contents of cereal and legume foods are not included so the bioavailability of Zn and other minerals cannot be evaluated.

In this study, we have analyzed the Zn concentrations in typical raw and prepared foods consumed in a rural and a suburban community in central Iran. We have measured PA in the cereal and legume foods so as to estimate Zn bioavailability based on PA:Zn molar ratios. The work was performed as a case study in central Iran as an example of an arid region in a developing country with a population depending on cereal staple foods and suspected to be at risk of Zn deficiency. The food composition data generated are part of a larger project aiming to improve Zn bioavailability from cereal-based diets in arid regions of the developing world.

2. Materials and methods
2.1. Study area
The study was carried out in the Province of Isfahan, which is situated in central Iran, covering an area of about 107,000 km². According to the Statistical Centre of Iran (SCI, 2009), the population of the province counted 4,559,256 people in 2006, of which approximately 83.3% were urban residents and 16.7% resided in rural areas. The climate is arid with an average annual rainfall of 116.9 mm. Two populations were selected for this study, one in a rural (Rooran) and the other in a sub-urban (Khomeini Shahr) community. Rooran is a village located 40 km to the south east of Esfahan city. The main economic activity here is farming. The main crops cultivated are rice (Oryza sativa), wheat (Triticum) and maize (Zea mays). In addition, vegetables such as cucumber, tomato and pepper are some produced in green houses, while onion and potato are cultivated in this area as rotation crops. Local livestock provide most of the red meat (beef and lamb) and most of the consumed milk (about 60%). Poultry provide about 30% of the eggs consumed in Rooran, while chicken meat is mainly imported from the city. Khomeini Shahr is a suburb of Isfahan and, although it has not yet fully developed the city infrastructure, people have a more urban life style and consume mostly the same products as are consumed in the city of Esfahan.
2.2. Selection and collection of food samples

We conducted a dietary intake survey in each of the two communities to assess habitual dietary patterns. For these surveys, 28 households in Rooran and 25 households in Khomeini Shahr were randomly selected, and food samples in these households were collected. The criteria for sampling foods were to select the most commonly consumed foodstuffs as well as those considered rich in Zn and PA. Our collection included the most frequently consumed composite meals, types of bread and rice, which are the staple foods of the Iranian population (Pirzadeh et al., 2010), as well as major legumes including beans, lentils, chick peas and split peas. The main varieties of rice consumed in Khomeini Shahr included the local varieties Lenjan and Tarom and unidentified imported varieties. In Rooran, the rice consumed was mainly from local production. In Khomeini Shahr, bread samples were obtained from local bakeries, whereas all bread was homemade in Rooran. Cooked foods were collected from the households and raw foods primarily from the local stores. One exception was the local rice in Rooran, which was collected from the households. Some dairy products, such as unpasteurized cow milk, were collected directly from the families, while well-known brands of dairy products were obtained from the stores (n=3 per brand). Milk consumed in the rural households was usually obtained from own or neighbour’s local cows, while in suburban areas the milk was usually pasteurized and commercially packed. Cheese consumed in both communities was mainly the Iranian white cheese, which is a close textured brined cheese (Madadlou et al., 2006) made from the milk of cows, sheep, or mixtures of both. It resembles Feta cheese in appearance, but differs from Feta in the way it is made. Among dairy products, cream and cream cheeses were consumed only in the suburban area and not in the village. Doogh, a traditional Iranian fermented yogurt drink that is produced from stirred full fat yogurt diluted with water was widely consumed (FAO/WHO, 2009). Raw samples of red meat were collected from the households, and it was not possible to determine the exact tissue which was provided. A brief description on each food sample is given in Table 1.

2.3. Sample preparation

All food samples were packed into airtight, self-closing, acid-washed, pre-weighed polyethylene containers and stored in a freezer at -18 °C until further processing. The cooked samples were freeze-dried, weighed and stored at 4 °C in the laboratory of the Soil Science Department of Isfahan University of Technology (IUT). For chemical analyses, all collected samples were transported to the Human Nutrition Laboratory at ETH Zurich where they were
ground to a fine homogenous powder using a laboratory mill (Retsch ZM1, Retsch GmbH, Haan, Germany).

2.4. Analyses of Zn
For the analysis of Zn, a sub-sample of 500 mg was taken in duplicate from each food sample and placed into a trace-element free Teflon bomb. After adding 7 ml of nitric acid, HNO₃ 65% (suprapur, sub boiled), and 3 ml of hydrogen peroxide, H₂O₂ 30% (pa Merck) to each bomb, the samples were digested in a microwave oven (Microwave MLS-ETHOS plus, MLS GmbH, Germany, software easyWAVE) for an hour. After cooling, the mineralized samples were transferred into previously tarred and acid-washed 50-ml PE bottles. Bread samples and dairy products were analysed for Zn by means of flame atomic absorption spectroscopy (F-AAS), using a Varian AA240FS instrument (Varian Inc., Mulgrave, Australia). Zn concentrations of all other samples, including the cooked dishes were determined by inductively coupled plasma mass spectrometry (Flame-ICP-OES, Varian Vista MPX Pro instrument). External calibration was used for all mineral determinations. Fe and Ca values were also obtained from the ICP OES analysis.

2.5. Phytic acid determination
Phytic acid was determined using a modification of the Makower (1970) method as briefly described in the following. Triplicate sub-samples of 500 mg were taken from each powdered food sample. After adding 3 ml trichloroacetic acid, TCA 12% (TCA, puriss pa, Fluka) to each sub-sample, the mixtures were shaken vigorously, left at room temperature (RT) for 30 min and then centrifuged (Centrifuge Omnifuge 2.0RS, Heraeus Sepatech, Germany) at 3600 rpm for 15 min. The resulting supernatants were transferred into another PE-tube for a second extraction, which was performed in the same way as the first one. The second supernatant was combined with the first one. After adding 2 ml cerium solution (5% Ce(SO₄)₂,4H₂O, pa, Merck in H₂SO₄ 6% [w/v]) and 0.8 ml concentrated sulfuric acid, H₂SO₄ 95-97% (pa, Merck) to each extract, the composite mixtures were again shaken vigorously, left at RT for 30 min, and then centrifuged under the same program as before. The resulting supernatants were discarded, while the precipitates were dissolved in 3 ml H₂SO₄ 95-97% for 3 1/2 hours and heated at 338°C in a heating block (Liebisch, MBV AG). If a solution was not clear and colourless after this time, 10 drops of H₂O₂ 30% were added, and then the sample was heated for another 1 1/2 hours at the same temperature. The clear and colourless solutions obtained by this procedure were transferred into 50-ml volumetric flasks. After rinsing the
mineralization tubes 3 times, the flasks were filled up to 50 ml with nanopur water. According to van Veldhoven (1987), inorganic phosphate binds specifically to ammonium molybdate and produces a complex, which reacts with malachite green to give a coloured compound. Therefore, 150 µl 0.5M H2SO4 were pipetted into each well of a micro-well plate and 150 µl of sample as well as a phosphate standard solution were added to the first row. The standard solution, containing 10 µg/mL phosphorus, was prepared from 1.25 mL phosphate stock solution (175.75 mg KH2PO4 in 500 mL H2O) diluted to 10 mL with nanopur water. After sequential 1:1 dilution from row to row and addition of 30 µL molybdate solution (1.75% (NH4)Mo7O24.4H2O, puriss, Fluka in H2O [w/v]) to each well, the plate was shaken for 10 min at RT. Finally, each well was reacted with 30 µl of malachite green (0.035% malachite green, pa, Fluka in 0.35% polyvinyl alcohol, 15’000, Fluka [w/v]), and after shaking for 45 min at RT, the liberated inorganic phosphate was determined spectrophotometrically (MRX, Dynatech Laboratories) at a wavelength of 639 nm.

2.6. Quality control
All mineral analyses were carried out in duplicate in a clean lab under a laminar flow box, using acid washed consumables and wearing powder-free disposable plastic gloves. With each batch, also a blank without any sample material was analysed.

All PA analyses were performed in triplicate. Wheat bran (50 ml AACC certified hard red wheat bran) with a certified PA concentration of 4.5-5 g/100g was analysed as reference material in duplicate with each batch of analysis. The PA concentrations of this certified standard material averaged 4.84 ± 0.28 g/100g. A reagent blank was also included in each batch of analysis.

3. Results
Table 1 gives a brief description of all non-cooked and cooked food samples collected and analysed in this study. The population in Khomeini Shahr consumed a larger variety of commercial food commodities such as snacks, chocolates, ice cream, etc. and industrial products, including different types and brands of dairy products, rice and bread, than the population in Rooran.

Zn concentrations in animal source foods are reported in Table 2 and Zn and PA in rice, wheat and breads are reported in Table 3.
Table 2 reports and compares the Zn concentrations of milk, white cheese and yoghurt consumed in Rooran and Khomeini Shahr. Among these commonly consumed dairy products for which we had samples from both communities, only milk showed a significant difference in Zn concentration (using the method of one sample t-test and assuming that variability in the Zn concentration of milk was the same in both communities). Variation within the milk samples of Khomeini Shahr was very small. There were no significant differences in the Zn concentrations of yogurt and cheese between the two study areas. Although not significant, the slightly higher Zn concentration of rural yogurt would correspond to the higher Zn concentration of the rural milk, as yogurt is often homemade in the village. On fresh weight basis, white cheese contained the highest concentration of Zn among all sampled dairy products, whereas the Zn concentration of cream was the lowest (P<0.05). The Zn concentration in raw red meat was higher in lamb and mutton than in veal.

Table 3 reports and compares the Zn and PA concentration of flat bread and rice types consumed in Rooran and Khomeini Shahr. Cooked rice and flat breads in Rooran were on average higher in Zn than those collected in Khomeini Shahr (P<0.01), whereas the PA:Zn molar ratios were similar in both communities (P=0.4). While Zn concentration of the rural bread was not significantly different from the rural flour, the PA concentration was lower by 24% resulting in a lower PA:Zn ratio, which still remained above 18.

Five different types of flat bread (Mashini, Taftoon, Tanouri, Ghopeh, Khanegi) consumed in Khomeini Shahr were analysed separately for Zn and PA. They did not differ in their Zn and PA concentrations (data not shown), whereas the baguette had the lowest concentration of Zn.

Table 4 shows the Zn and PA contents of three domestic rice types and in the imported rice consumed in Khomeini Shahr, both before and after cooking. Fe and Ca levels are added for comparison. The imported rice consumed in Khomeini Shahr has a lower Zn concentration than the domestic rice varieties consumed in both areas. The Zn concentration of the raw imported rice sampled in Khomeini Shahr was less than half that of the three domestic varieties, which had similar Zn concentrations (1.9-2.1 mg/100g). Imported rice was also much lower in PA and Ca concentrations. Lenjan rice, which is produced in the Province of Isfahan, contained around twice as much average Fe as the other rice samples. Lenjan rice also contained the highest average concentration of PA and Ca. However, the variability of Fe, Ca and PA in the rice varieties was also very large and the differences were not statistically significant. Although all raw rice had PA:Zn molar ratios above 18, all cooked
samples of rice had PA:Zn molar ratios ranging from 7-13. This compares to molar ratios of Fe:PA of 11 to 32 in the cooked rice and molar ratios of Ca:PA of 0.12 to 0.54.

Cooking slightly decreased the Zn and Fe concentrations (per 100 g DW) in most of the analysed rice varieties (Table 4), whereas it consistently increased their Ca concentrations, although the latter was only significant for the imported rice (P<0.01). The effect of cooking on PA levels (per 100g DW) in rice was greater than that for minerals and the PA:mineral molar ratios were consequently much lower in the cooked than in the raw samples.

Table 5 shows the mineral and PA concentrations and the respective molar ratios in different legumes. Fe and Ca concentrations are added for comparison. It should be emphasized that the raw and cooked beans and lentils were from different origins so it is not possible to evaluate the influence of cooking.

Raw lentils had the highest Zn concentration followed by split peas, chick peas and beans. The difference was only significant between beans and lentils (P=0.009). The PA concentration in chick peas was not significantly different from other legumes. The raw lentils had significantly lower PA than beans (P=0.001) and split peas (P=0.036) causing a wide variation in the PA:Zn molar ratios from 4-30 in the raw legumes. However, the nutritionally important molar rations in the cooked legumes were extremely high from 35-60.

The Fe content of raw legumes (6-9 mg/100g) was higher than that of cereals (Table 4) and the PA:Fe molar ratios were somewhat lower. Although a ratio of 25 was found in the only cooked sample analysed (lentils) the cooked lentils contained a modest amount of Ca with a PA:Ca molar ratio of 0.86.

A list of the prepared dishes, which were analysed, and their main ingredients is given in Table 6. Since the preparation methods for cooked dishes were similar in the two communities and the variation in the Zn and PA concentrations were very small, the results were pooled together for the cooked dishes consumed in Khomeini Shahr and Rooran. The Zn concentration in prepared dishes ranged from 1.6 to 3.8 mg/100 g with the highest levels in the meat dishes; kebab and Shami. The eggs and cooked chicken had intermediate Zn levels with the lowest levels in the cereal and legume dishes devoid of animal source foods. The highest PA concentration was in the split pea dish Gheimeh, although the highest PA:Zn molar ratio was in the Ash dish which, with no meat and a mixture of 3 legumes and noodles, had a low Zn level and a PA:Zn molar ratio of 27. The PA:Zn molar ratios in the other cereal and legume containing dishes ranged from 4-13.
4. Discussion

Many of Iran’s soils are considered Zn deficient (Alloway, 2004), because calcareous soils of arid regions are generally low in Zn that is available for plant uptake (Cakmak et al., 1999). It has been suggested therefore that Iran’s grain products are low in Zn (Malakouti, 2008) that, because the Iranian diet is largely cereal based, Zn deficiency might be highly prevalent (Kalantari et al., 2006). In this study we have measured the Zn and PA content of foods commonly consumed in a rural and a suburban population. While it was not possible to make a comprehensive analysis of all foods, the major foodstuffs, estimated to provide most of the Zn and phytic acid were analysed together with the most common composite dishes. The diets of the 2 locations were surprisingly similar, being largely based on bread, rice, legumes, with a relatively high intake of milk, yoghurt and cheese but also frequently including red meat, chicken and some fish dishes. The data included in this paper will provide a basis to evaluate Zn intake and bioavailability in these 2 communities and generally provides information on Zn and PA composition of common Iranian foods. There were only small differences between the food samples collected in the rural and the suburban communities. These related to the use of locally grown rice, homemade bread and un-processed fresh cow milk in the rural community, while commercial bread and dairy products were consumed in the suburban households. The Zn concentrations of the bread, white rice, and major legumes consumed in Isfahan were in the same range as that reported by other countries (Chan et al., 2007; Ma et al., 2005; Karunaratne et al., 2008). So from our small study there seems no reason to assume Iranians have a lower Zn supply from cereals and legumes.

While the imported rice consumed in Khomeini Shahr did contain less Zn, the differences in the mineral and PA concentrations of different varieties of rice analyzed in our study can be attributed mainly to differences in the degree of milling and polishing. Polishing is the final process in fully whitening the rice. Polished rice contains the lowest amounts of PA and Zn among cereals (Reddy, 2002; Gibson et al. 2010; Prasad, 2010). The Iranian varieties of white rice sampled in this study were abrasively ground but unpolished, whereas the imported variety of the foreign rice was fully polished, explaining its lower concentrations of minerals and PA. The values of 1.9-2.1 mg of Zn in 100 g DW of the domestic rice in our study agree well with those reported by Zazoli et al. (2006), who found a Zn concentration of 1.95 mg in 100 g DW of Iranian raw rice. The concentrations of Zn, Fe and PA were decreased in all rice varieties by cooking. Rice is usually prepared by boiling and steaming discarding the excess water. Because PA and minerals are water soluble, they are leached during cooking. Cooking rice decreased the PA concentration more than that of Zn and Fe presumably because of
greater leaching although PA may also degrade (Hurrell, 2004). The higher Ca concentration in the cooked rice can be explained by the use of hard water for cooking in the communities of our study (Ma et al., 2005; Chan et al., 2007).

The Zn and PA content of bread also vary widely with flour type, flour extraction rate and baking method (Ma et al., 2005). Furthermore, phytic acid content can be strongly affected by fermentation (Schlemmer et al., 2009). However, in our study we did not see wide variations in the Zn and PA concentrations among different breads. The reason probably is that they were all made of wheat flour with similar extraction rate (87-88% extraction) and comparable baking methods. In both study areas, the bread was leavened using sourdough together with commercial yeasts for 90-120 min. Fermentation thus explains the relatively low PA concentration of our bread samples. Nonetheless, the PA:Zn molar ratios were still above the critical value of 18. The significantly lower concentration of the baguette bread compared to the flat types can be related to the relatively low extraction rate of the wheat flour (78-80%) used for its production. The average Zn and PA concentrations found in our flat breads are consistent with studies carried out in other parts of the country (Reinhold, 1971; Faridi et al., 1983; Jahed Khaniki, 2005; Gargari et al., 2007). The PA analyses allow us to make a prediction on the Zn bioavailability by calculating the PA:Zn molar ratios. We would therefore predict relatively low Zn absorption (ca 10%) from flat breads where the molar ratio was 22-24. However, with the molar ratios of PA:Zn in cooked rice and the composite dishes varying from 4-13, we could perhaps predict a more modest Zn absorption of 10-20% (Hurrell, 2003). In the absence of PA, Zn absorption from meat and dairy products would be expected to be 30-40% (Hurrell, 2003). The PA:Fe molar ratios were also high in cooked rice and lentils and would be predictive of low Fe absorption, however we did not evaluate the PA:Fe molar ratios in composite dishes nor the intake of the more bioavailable heme Fe (Hurrell and Egli, 2010). The PA:Ca molar ratios in rice were not predictive of reduced Ca bioavailability and with the high intake of calcium with dairy foods, this would not be expected.

In general, the variety of dairy products consumed in Iran is much smaller than in Western countries. This is particularly striking for cheese, which is mainly produced as white cheese in Iran, while it varies from cottage fresh cheese, to different types of hard and blue cheeses in the USA, Switzerland and Germany. Cheese is a widely consumed dairy product all over Iran the processing of which includes acidifying, heating, salting, draining and ripening (aging) (Madadlou et al., 2007). A difference in any of these processes during cheese making results in considerable changes in the flavor, texture, taste and mineral concentration of the final
product (Fox, 1999; Tarakci and Kucukoner, 2008). Cheese in the US, Germany and Switzerland refers to a diverse group of milk-based food products and is produced in wide-ranging flavors, textures, forms, and mineral concentrations. The high Zn concentration in many of these types, especially that of hard and blue cheeses, results in their considerably higher average Zn content compared to the white cheese that is typically consumed in Iran.

In contrast to the large variation in Zn concentrations in cheese, yoghurt and cream (Table 2), variation in the mineral composition of milk was almost negligible. However, this is perhaps not surprising as our results are based only on a small number of samples that were all taken within a few days. It has been reported that the composition of milk can show seasonal fluctuations with type of feed and other conditions (Tarakci and Kucukoner, 2008). Nevertheless, the average Zn value of milk in this study (0.31 ± 0.04 mg/100 g FW) is consistent with values given in the literature for other countries. Lönnéradal et al. (1981) reported Zn concentrations of 0.3-0.4 mg/100 ml for fresh milk, while Martino et al. (2001) gave an average Zn concentration of 0.38 ± 0.03 mg/100 ml for untreated cow milk and 0.31 ± 0.02 mg/100 ml for UHT (Ultra-high-temperature processed) cow milk.

We analysed only 5 red meat samples of which the exact tissues were unknown. It is doubtful therefore whether the Zn levels we measured for meat could be used in other situations. Apart from species, age, gender and feeding regime of the animals, the variation in nutrient composition of meat depends on tissue type, body part and retail cut (Giuffrida-Mendoza et al., 2007; Mioč et al., 2009; Park, 1998). The Zn concentration of different beef and lamb tissues have been previously reported to vary from 3.2 ± 0.8 mg/100 g in braising steak to 5.1 ± 1.4 mg/100 g in ribs in beef and from 2.3 ± 0.4 to 2.4 ± 0.4 in chop and loin of lamb, respectively (Gerber et al., 2009). Similarly, Zn in chicken has been reported to vary between 0.7 mg/100 g to 1.4 mg/100 g in the breast and legs of chicken, respectively (Gerber et al., 2009).

The Zn levels in basic foodstuffs vary widely in different parts of the world making national or regional food databases necessary to accurately measure Zn intake. Different local processing methods influence the Zn levels in dairy products, rice and breads.

5. Conclusions
This study has generated Zn and PA data on the major basic foodstuffs and composite meals consumed in a rural and semi urban community in Isfahan province. It will allow the estimation of Zn intake and bioavailability in these 2 communities and provides useful information on the Zn and PA content on common Iranian foods. While we could not show
that the reported low soil Zn in Iran resulted in substantially lower Zn levels in rice and bread, our results indicate that the local cheese-making processes, rice-polishing and bread-making have a major influence on Zn concentrations in the final product. Our PA analyses, and the calculated PA:Zn molar ratios, indicate a low Zn absorption from the common flat breads (PA:Zn molar ratio 20-22) but more modest absorption from composite dishes containing cereals and legumes with meat and milk products which have PA:Zn molar ratios ranging from 4-13.
References


Table 1

Types, local names, descriptions and number of the food samples, other than cooked dishes, collected from the rural (R) and the suburban (Kh) communities

<table>
<thead>
<tr>
<th>Foods</th>
<th>State</th>
<th>Collected from</th>
<th>n</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice, Lenjan, milled</td>
<td>Raw</td>
<td>Market</td>
<td>3</td>
<td>0 Produced in Lenjan county of Isfahan</td>
</tr>
<tr>
<td>Rice, Lenjan, milled</td>
<td>Cooked</td>
<td>Households</td>
<td>4</td>
<td>0 Excessive water discarded, steamed</td>
</tr>
<tr>
<td>Rice, Tarom/North, milled</td>
<td>Raw</td>
<td>Market</td>
<td>6</td>
<td>0 Produced in the North of Iran</td>
</tr>
<tr>
<td>Rice, Tarom/North, milled</td>
<td>Cooked</td>
<td>Households</td>
<td>2</td>
<td>0 Excessive water discarded, steamed</td>
</tr>
<tr>
<td>Rice, Foreign, milled</td>
<td>Raw</td>
<td>Market</td>
<td>9</td>
<td>0 Imported from other countries (India, Pakistan, Thailand)</td>
</tr>
<tr>
<td>Rice, Foreign, milled</td>
<td>Cooked</td>
<td>Households</td>
<td>14</td>
<td>0 Excessive water discarded, steamed</td>
</tr>
<tr>
<td>Rice, Local, milled</td>
<td>Raw</td>
<td>Households</td>
<td>0</td>
<td>5 Produced locally in the village</td>
</tr>
<tr>
<td>Rice, Local, milled</td>
<td>Cooked</td>
<td>Households</td>
<td>0</td>
<td>7 Excessive water discarded, steamed</td>
</tr>
<tr>
<td>Wheat flour</td>
<td></td>
<td>Households</td>
<td>0</td>
<td>2 Wheat flour 90-93% extraction</td>
</tr>
<tr>
<td>Flat Bread bakeries/families</td>
<td></td>
<td>Bakery/households</td>
<td>25</td>
<td>8 Wheat flour (87-88%) or (90-93%) extraction</td>
</tr>
<tr>
<td>Baguette</td>
<td></td>
<td>Market</td>
<td>3</td>
<td>0 Cylindrical or round bread baked out of white wheat flour 78-80% extraction, usually used in fast food services in</td>
</tr>
<tr>
<td>Beans, Pinto and red</td>
<td>Raw</td>
<td>Market</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Beans, Pinto and red</td>
<td>Cooked</td>
<td>Households</td>
<td>0</td>
<td>4 Excessive water discarded</td>
</tr>
<tr>
<td>Lentils</td>
<td>Raw</td>
<td>Market</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Lentils</td>
<td>Cooked</td>
<td>Households</td>
<td>0</td>
<td>2 Without discarding the excessive water</td>
</tr>
<tr>
<td>Chickpeas</td>
<td>Raw</td>
<td>Market</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Split peas</td>
<td>Raw</td>
<td>Market</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Milk</td>
<td>Raw</td>
<td>Market/households</td>
<td>4</td>
<td>1 High fat, low fat, local</td>
</tr>
<tr>
<td>White cheese</td>
<td>Raw</td>
<td>Market/households</td>
<td>11</td>
<td>18 A close textured brined cheese Brands: Faleh, mahali, Ruzaneh, Pegah, Basir, Nasim</td>
</tr>
<tr>
<td>Cream cheese</td>
<td>Raw</td>
<td>Market</td>
<td>3</td>
<td>0 Brands: Pegah</td>
</tr>
<tr>
<td>Cream</td>
<td>Raw</td>
<td>Market</td>
<td>4</td>
<td>0 Brands: Pegah, Mihan</td>
</tr>
<tr>
<td>Yogurt</td>
<td>Raw</td>
<td>Market/households</td>
<td>6</td>
<td>15 High fat, low fat, local, homemade</td>
</tr>
<tr>
<td>Doogh</td>
<td>Raw</td>
<td>Market</td>
<td>4</td>
<td>0 A traditional Iranian fermented milk drink</td>
</tr>
<tr>
<td>Red meat</td>
<td>Raw</td>
<td>Households</td>
<td>3</td>
<td>0 Muton, composite of trimmed retail cuts</td>
</tr>
<tr>
<td>Red meat</td>
<td>Raw</td>
<td>Market</td>
<td>1</td>
<td>0 Lamb, shoulder, lean and fat</td>
</tr>
<tr>
<td>Red meat</td>
<td>Raw</td>
<td>Market</td>
<td>1</td>
<td>0 Veal, sirloin, lean only</td>
</tr>
</tbody>
</table>

1 The local names and ingredients of the prepared dishes are given in Table 2.

n, number of samples analysed

Kh, Khomeini Shahr; R, Rooran
<table>
<thead>
<tr>
<th>Food</th>
<th>Community (n)</th>
<th>Zn (mg/100g FW)</th>
<th>Zn (mg/100g DW)</th>
<th>Moisture (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk</td>
<td>R (1)</td>
<td>0.38</td>
<td>3.5</td>
<td>89.1</td>
</tr>
<tr>
<td></td>
<td>Kh (4)</td>
<td>0.29 ± 0.006</td>
<td>2.6 ± 0.02</td>
<td>88.9 ± 0.3</td>
</tr>
<tr>
<td></td>
<td><em>P value</em></td>
<td><em>&lt; 0.001</em></td>
<td><em>&lt; 0.001</em></td>
<td>0.31</td>
</tr>
<tr>
<td>White cheese</td>
<td>R (18)</td>
<td>1.1 ± 0.4</td>
<td>2.75 ± 0.8</td>
<td>59.8 ± 3.8</td>
</tr>
<tr>
<td></td>
<td>Kh (11)</td>
<td>1.2 ± 0.2</td>
<td>3.23 ± 0.84</td>
<td>63.5 ± 3.4</td>
</tr>
<tr>
<td></td>
<td><em>P value</em></td>
<td>0.71</td>
<td>0.14</td>
<td>0.01</td>
</tr>
<tr>
<td>Yogurt</td>
<td>R (15)</td>
<td>0.36 ± 0.10</td>
<td>2.13 ± 0.41</td>
<td>82.5 ± 5.7</td>
</tr>
<tr>
<td></td>
<td>Kh (6)</td>
<td>0.29 ± 0.04</td>
<td>1.85 ± 0.38</td>
<td>83.9 ± 3.1</td>
</tr>
<tr>
<td></td>
<td><em>P value</em></td>
<td>0.11</td>
<td>0.16</td>
<td>0.58</td>
</tr>
<tr>
<td>Cream</td>
<td>Kh (4)</td>
<td>0.21 ± 0.01</td>
<td>0.47 ± 0.1</td>
<td>53.48 ± 10.92</td>
</tr>
<tr>
<td>Cream cheese</td>
<td>Kh (3)</td>
<td>0.42 ± 0.06</td>
<td>1.1 ± 0.16</td>
<td>62.8 ± 1.7</td>
</tr>
<tr>
<td>Doogh</td>
<td>Kh (4)</td>
<td>0.11 ± 0.1</td>
<td>1.9 ± 0.3</td>
<td>94.2 ± 0.6</td>
</tr>
<tr>
<td>Mutton, raw</td>
<td>Kh (3)</td>
<td>4.55 ± 1.52</td>
<td>18.8 ± 6.4</td>
<td>75.7 ± 1.3</td>
</tr>
<tr>
<td>Lamb, raw</td>
<td>Kh (1)</td>
<td>4.62</td>
<td>25.3</td>
<td>81.7</td>
</tr>
<tr>
<td>Veal, raw</td>
<td>Kh (1)</td>
<td>3.50</td>
<td>15.8</td>
<td>78.0</td>
</tr>
</tbody>
</table>

n, number of samples analysed
Values are expressed as mean ± SD in units of mg per 100 g of fresh (FW) and dry weight (DW)
Table 3
Zinc (Zn) and phytic acid (PA) contents and PA:Zn molar ratio of major cereal based foods consumed in the two study communities1

<table>
<thead>
<tr>
<th>Food</th>
<th>Origin (n)</th>
<th>Moisture (%)</th>
<th>Zn (mg/100g)</th>
<th>PA (mg/100g)</th>
<th>PA:Zn (molar ratio)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice (raw)</td>
<td>R (5)</td>
<td>2.7 ± 2.4</td>
<td>2.1 ± 0.3</td>
<td>382 ± 87</td>
<td>18 ± 4</td>
</tr>
<tr>
<td></td>
<td>Kh (18)</td>
<td>0.66 ± 2.1</td>
<td>1.32 ± 0.63</td>
<td>238 ± 134</td>
<td>19 ± 8</td>
</tr>
<tr>
<td>P value</td>
<td></td>
<td>0.070</td>
<td>0.002**</td>
<td>0.036*</td>
<td>0.781</td>
</tr>
<tr>
<td>Rice (cooked)</td>
<td>R (10)</td>
<td>58.4 ± 5.1</td>
<td>1.29 ± 0.45</td>
<td>146.5 ± 87.0</td>
<td>11 ± 5</td>
</tr>
<tr>
<td></td>
<td>Kh (27)</td>
<td>59.7 ± 5.8</td>
<td>0.88 ± 0.34</td>
<td>63 ± 21</td>
<td>8.5 ± 4</td>
</tr>
<tr>
<td>P value</td>
<td></td>
<td>0.53</td>
<td>0.008</td>
<td>&lt; 0.001</td>
<td>0.134</td>
</tr>
<tr>
<td>Flat bread</td>
<td>R (8)</td>
<td>13.2 ± 3.5</td>
<td>1.77 ± 0.21</td>
<td>395 ± 90</td>
<td>22 ± 4</td>
</tr>
<tr>
<td></td>
<td>Kh (23)</td>
<td>20.3 ± 9.0</td>
<td>1.32 ± 0.16</td>
<td>313 ± 61</td>
<td>24 ± 5</td>
</tr>
<tr>
<td>P value</td>
<td></td>
<td>0.003**</td>
<td>&lt; 0.001**</td>
<td>0.006**</td>
<td>0.377</td>
</tr>
<tr>
<td>Wheat flour</td>
<td>R (2)</td>
<td>7.9 ± 0.5</td>
<td>1.47 ± 0.58</td>
<td>519 ± 50</td>
<td>35</td>
</tr>
<tr>
<td>Baguette bread</td>
<td>Kh (3)</td>
<td>29.1 ± 0.9</td>
<td>0.75 ± 0.2</td>
<td>ND</td>
<td>--</td>
</tr>
</tbody>
</table>

1Values are expressed as mean ± SD in units of mg per 100 g of dry weight (DW)
n, number of samples analysed
Kh, Khomeini Shahr; R, Rooran
ND, Not detected
Table 4
Zinc (Zn), iron (Fe), calcium (Ca) and phytic acid (PA) concentrations, as well as PA:mineral molar ratio of different types of raw and cooked rice consumed by the studied populations

<table>
<thead>
<tr>
<th>Food type</th>
<th>Number of samples analysed (Zn, Fe, Ca, PA)</th>
<th>Moisture (%)</th>
<th>Zn (mg/100g)</th>
<th>Fe (mg/100g)</th>
<th>Ca (mg/100g)</th>
<th>PA (mg/100g)</th>
<th>PA:Zn (molar ratio)</th>
<th>PA:Fe (molar ratio)</th>
<th>PA:Ca (molar ratio)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lenjan (raw)</td>
<td>(3, 3, 3, 3)</td>
<td>0.5 ± 2.6</td>
<td>1.92 ± 0.21</td>
<td>1.27 ± 1.16</td>
<td>13.5 ± 8.8</td>
<td>464 ± 126</td>
<td>22.6 (19.7, 29.2)</td>
<td>30.3 (20.7, 169.6)</td>
<td>2.5 (1.6, 3.2)</td>
</tr>
<tr>
<td>Lenjan (cooked)</td>
<td>(4, 3, 4, 4)</td>
<td>61.9 ± 3.2</td>
<td>1.06 ± 0.32</td>
<td>0.46 ± 0.07</td>
<td>23.3 ± 6.5</td>
<td>78 ± 15</td>
<td>6.7 (6.5, 9.4)</td>
<td>14.6 (9.0, 18.1)</td>
<td>0.20 (0.18, 0.24)</td>
</tr>
<tr>
<td>Tarom/North (raw)</td>
<td>(6, 6, 6, 6)</td>
<td>0.6 ± 2.8</td>
<td>1.93 ± 0.19</td>
<td>0.45 ± 0.34</td>
<td>8.0 ± 1.9</td>
<td>245 ± 92</td>
<td>14 (9.8, 15.8)</td>
<td>49.7 (32.9, 279.4)</td>
<td>2.1 (1.4, 2.7)</td>
</tr>
<tr>
<td>Tarom/North (cooked)</td>
<td>(2, 2, 2, 2)</td>
<td>63.7 ± 2.2</td>
<td>0.95 ± 0.53</td>
<td>0.73 ± 0.44</td>
<td>28.6 ± 16.0</td>
<td>73 ± 23</td>
<td>9.9 (4.2, 15.5)</td>
<td>11.3 (4.6, 17.9)</td>
<td>0.17 (0.14, 0.20)</td>
</tr>
<tr>
<td>Foreign (raw)</td>
<td>(9, 9, 9, 9)</td>
<td>0.77 ± 1.62</td>
<td>0.72 ± 0.06</td>
<td>0.65 ± 0.22</td>
<td>2.6 ± 0.8</td>
<td>159 ± 52</td>
<td>25.6 (14.0, 28.0)</td>
<td>20.8 (14.0, 27.0)</td>
<td>3.5 (2.7, 4.8)</td>
</tr>
<tr>
<td>Foreign (cooked)</td>
<td>(14, 11, 13, 14)</td>
<td>60.1 ± 7.1</td>
<td>0.8 ± 0.4</td>
<td>0.41 ± 0.09</td>
<td>37.7 ± 24.5</td>
<td>60 ± 16</td>
<td>10.2 (4.0, 13.0)</td>
<td>12.1 (7.5, 17.3)</td>
<td>0.12 (0.07, 0.17)</td>
</tr>
<tr>
<td>Rural (raw)</td>
<td>(5, 5, 5, 5)</td>
<td>2.7 ± 2.4</td>
<td>2.11 ± 0.30</td>
<td>0.70 ± 0.23</td>
<td>4.9 ± 3.5</td>
<td>382 ± 87</td>
<td>18.3 (14.4, 21.9)</td>
<td>41.3 (37.4, 65.9)</td>
<td>3.9 (2.8, 27.3)</td>
</tr>
<tr>
<td>Rural (cooked)</td>
<td>(7, 3, 3, 7)</td>
<td>57.3 ± 5.2</td>
<td>1.29 ± 0.23</td>
<td>0.45 ± 0.37</td>
<td>24.6 ± 14.7</td>
<td>168 ± 90</td>
<td>12.6 (8.8, 17.0)</td>
<td>32.1 (22.2, 675.9)</td>
<td>0.54 (0.49, 1.14)</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for Zn, Fe, Ca and PA and median (1st, 3rd quartile) for the PA:mineral molar ratios in units of mg per 100 g of dry weight

1The cooked measurements are not made on identical samples to the raw

n, number of samples analysed
Table 5
Zinc (Zn), iron (Fe), calcium (Ca) and phytic acid (PA) concentrations as well as PA:mineral molar ratio of major legumes consumed by the study populations

<table>
<thead>
<tr>
<th>Food type</th>
<th>n</th>
<th>Moisture (%)</th>
<th>Zn (mg/100g)</th>
<th>Fe (mg/100g)</th>
<th>Ca (mg/100g)</th>
<th>PA (mg/100g)</th>
<th>PA:Zn (molar ratio)</th>
<th>PA:Fe (molar ratio)</th>
<th>PA:Ca (molar ratio)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beans (raw)</td>
<td>7</td>
<td>2.3 ± 1.0</td>
<td>2.61 ± 0.54</td>
<td>9.3 ± 4.6</td>
<td>172.8 ± 49.9</td>
<td>716.8 ± 150</td>
<td>30 (22, 32)</td>
<td>9.8 (4.1, 11.5)</td>
<td>0.26 (0.17, 0.34)</td>
</tr>
<tr>
<td>Lentils (raw)</td>
<td>3</td>
<td>1.6 ± 0.7</td>
<td>4.1 ± 0.75</td>
<td>7.2 ± 2.1</td>
<td>54.8 ± 5.0</td>
<td>239.7 ± 94.0</td>
<td>4.1 (3.96, 10.5)</td>
<td>2.1 (1.7, 6)</td>
<td>0.22 (0.21, 0.34)</td>
</tr>
<tr>
<td>Chick peas (raw)</td>
<td>2</td>
<td>1.8 ± 0.8</td>
<td>2.9 ± 0.4</td>
<td>5.6 ± 0.8</td>
<td>130.7 ± 2.5</td>
<td>420 ± 87</td>
<td>14.4 (13.7, 15.2)</td>
<td>6.5 (4.9, 8.1)</td>
<td>0.19 (0.17, 0.22)</td>
</tr>
<tr>
<td>Split peas (raw)</td>
<td>3</td>
<td>0.8 ± 0.5</td>
<td>3.7 ± 0.06</td>
<td>8.2 ± 2.6</td>
<td>70.7 ± 6.8</td>
<td>586.3 ± 78</td>
<td>16.3 (13.6, 17.4)</td>
<td>6.6 (4.8, 7.3)</td>
<td>0.48 (0.47, 0.56)</td>
</tr>
<tr>
<td>Beans (cooked)</td>
<td>4</td>
<td>57.8 ± 2</td>
<td>2.41 ± 0.16</td>
<td>NA</td>
<td>NA</td>
<td>828.6 ± 34.4</td>
<td>35.2 (27.8, 40)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Lentils (cooked)</td>
<td>2</td>
<td>69 ± 4.5</td>
<td>1.97 ± 0.35</td>
<td>4.4 ± 0.25</td>
<td>87.6 ± 4.7</td>
<td>611.3 ± 125.3</td>
<td>60 (30, 90)</td>
<td>25 (9.7, 40.4)</td>
<td>0.86 (0.38, 1.3)</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for Zn, Fe, Ca and PA and median (1st, 3rd quartile) for the PA:mineral molar ratios in units of mg per 100 g of dry weight (DW)

The cooked measurements are not made on identical samples to the raw

n, number of samples analysed

NA, not analysed for lack of sample material
<table>
<thead>
<tr>
<th>Dishes</th>
<th>n</th>
<th>Main ingredients</th>
<th>Moisture (%)</th>
<th>Zn (mg/100g)</th>
<th>PA (mg/100g)</th>
<th>PA:Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macaroni</td>
<td>7</td>
<td>Macaroni, meat</td>
<td>57.1 ± 8.2</td>
<td>0.76 ± 0.29</td>
<td>92 ± 35</td>
<td>13 ± 6</td>
</tr>
<tr>
<td>Istamboli</td>
<td>7</td>
<td>Rice, meat, green beans, potato</td>
<td>61.2 ± 3.6</td>
<td>0.64 ± 0.10</td>
<td>44 ± 26</td>
<td>7 ± 3</td>
</tr>
<tr>
<td>Adas polo</td>
<td>3</td>
<td>Rice, lentil</td>
<td>54.3 ± 10.1</td>
<td>0.72 ± 0.13</td>
<td>92 ± 13</td>
<td>13 ± 0.6</td>
</tr>
<tr>
<td>Kookoo</td>
<td>3</td>
<td>Herbs, egg</td>
<td>51.9 ± 14.9</td>
<td>0.95 ± 0.29</td>
<td>NA</td>
<td>--</td>
</tr>
<tr>
<td>Ghormeh sabzi</td>
<td>3</td>
<td>Herbs, meat</td>
<td>66.5 ± 2.1</td>
<td>0.79 ± 0.45</td>
<td>0.48 ± 0.83</td>
<td>0.04 ± 0.06</td>
</tr>
<tr>
<td>Gheimeh</td>
<td>4</td>
<td>Split peas, meat, potato</td>
<td>66.5 ± 3.7</td>
<td>1.13 ± 0.29</td>
<td>137 ± 42</td>
<td>12.5 ± 4</td>
</tr>
<tr>
<td>Shami</td>
<td>5</td>
<td>Meat, chick pea flour, egg</td>
<td>42.9 ± 8.4</td>
<td>2.2 ± 0.46</td>
<td>82 ± 29</td>
<td>4 ± 2</td>
</tr>
<tr>
<td>Abgousht</td>
<td>2</td>
<td>Meat, potato, red and pinto beans, chick peas</td>
<td>73 ± 3.9</td>
<td>0.65 ± 0.23</td>
<td>65 ± 2</td>
<td>10.5 ± 3</td>
</tr>
<tr>
<td>Ash</td>
<td>4</td>
<td>Noodles, red beans, lentils, chick peas, herbs</td>
<td>80.8 ± 4.9</td>
<td>0.33 ± 0.12</td>
<td>74 ± 46</td>
<td>27 ± 19</td>
</tr>
<tr>
<td>Fish, trout, broiled</td>
<td>3</td>
<td>Fish, condiments</td>
<td>55.4 ± 9.3</td>
<td>0.61 ± 0.26</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Egg, fried/hard boiled</td>
<td>7</td>
<td>Egg</td>
<td>67.0 ± 7.8</td>
<td>1.53 ± 0.40</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Chicken, cooked</td>
<td>19</td>
<td>Chicken, tomato paste, condiments</td>
<td>62.7 ± 13.5</td>
<td>1.42 ± 1.06</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Red meat, cooked</td>
<td>3</td>
<td>Veal, mutton (ground)</td>
<td>66.0 ± 4.4</td>
<td>1.48 ± 0.36</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Kebab, grilled</td>
<td>4</td>
<td>Beef, mutton (ground)</td>
<td>61.2 ± 6.1</td>
<td>3.44 ± 0.94</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

1Values are expressed as mean ± SD in units of mg per 100 g of dry weight
n, number of samples analysed
Kh, Khomeini Shahr; R, Rooran
NA, not analysed for lack of sample material
CHAPTER 3

Assessment of zinc and iron status in rural and suburban populations in Isfahan province, Iran

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Abstract
Zinc (Zn) and iron (Fe) deficiencies are of concern in Iran. The aim of this study was to estimate the Zn and Fe status of different age groups in a rural (Rooran) and a suburban (Khomeini Shahr) population in central Iran, to relate the Zn status to Zn intake from animal and plant foods, and to examine the relationship between Zn and Fe status. Blood samples from 341 subjects (27 preschool children, 157 schoolchildren, 91 women, 66 men) were analyzed for serum zinc (SZn), serum ferritin, total iron binding capacity and hemoglobin (Hb) concentrations. Daily Zn intake was estimated using a 3-day food record. The prevalence of Zn deficiency was 5.9% in Rooran and 7.2% in Khomeini Shahr. The prevalence of iron deficiency (ID) was 27.0% in Rooran and 30.7% in Khomeini Shahr. There was no association between Zn and Fe status. The prevalence of anemia was higher in the village than in the suburban area (33.5% vs. 22.7%; p=0.04). Almost half of the anemia in Khomeini Shahr and 36% in Rooran was associated with ID. The Hb levels correlated significantly with the SZn concentrations. The low prevalence of Zn deficiency can be explained by a relatively high Zn intake from animal source foods. Anemia however is a serious public health problem affecting some 30% of the subjects, although less than half was due to ID. The lack of correlation between Fe and Zn status could be due to the frequent consumption of dairy products and tea.

Introduction
Following the first report of human zinc (Zn) deficiency in adolescent boys from the Fars province in Iran [1], several subsequent studies reported that Zn supplements increased the height and weight of Iranian children [2 - 4]. More recently, however prevalence estimates for Zn deficiency in Iran have varied widely depending on the Province and the population studied. Using low Serum Zn (SZn) as the indicator of Zn status, some studies have reported Zn deficiency to be in the region of 30% [5 - 7] or higher [8], whereas others have reported Zn deficiency to be <10% [9, 10]. The most recent study by Dehghani et al. [10] reported that Zn deficiency affected only 7.9 % of the 3-18 year old children in Shiraz. These latter workers suggested that differences in dietary patterns, the recent wide prescription of Zn supplements by pediatricians, and soil Zn level could all explain the different estimates of Zn deficiency from different Iranian regions.

As in other countries, the prevalence of anemia, iron deficiency (ID) and iron deficiency anemia (IDA) in Iran varies with population group. For example, Keikhaei et al. [11] reported 43.9% anemia and 29.1% IDA in children in southwest Iran, whereas the prevalence of anemia and IDA in adult women and men in northwest Iran were reported to be 9.7% and 7%, and
9.7% and 2% respectively [12]. Additionally, Barooti et al. [13] reported the prevalence of anemia during pregnancy to be between 4.3-21.5%.

Deficiencies in iron (Fe) and Zn would be expected to have negative health consequences. Many enzymes require Zn and Zn deficiency negatively affects physical growth, immune competence, reproductive function and neural development [14]. Fe is required for oxygen transport by heme and for enzymes related to energy metabolism and immune function [15]. IDA has adverse effects on pregnancy outcome, infant growth, cognitive performance, immune status and work capacity; and even mild to moderate ID without anemia may decrease work capacity, resistance to fatigue, and impair cognition [15].

The risk of Zn and Fe deficiencies is particularly high in populations who consume a plant-based diet with few animal source foods [16]. However, although the intake of bioavailable Fe and Zn is strongly dependent on the intake of animal source foods, the correlation between Fe and Zn status is sometimes significant [17], and sometimes not [18]. Zn deficiency is more frequent in developing countries, whereas ID and IDA are common in children and young women of both developing and developed countries [15].

The bread and rice based diets widely consumed in Iran [19, 20] could also lead to Zn and Fe deficiencies when the intake of animal source foods is low. In such a situation, the most at risk groups would be small children, adolescents and pregnant women, since these populations have a particularly high demand for Zn for growth and Fe for increased blood volume. Additionally, women of childbearing age need more Fe to compensate for menstruation losses. Finally, urban populations usually enjoy a higher quality of life, and consume a more diverse diet than rural populations [21, 22] and therefore might be expected to have a lower prevalence of Zn and Fe deficiencies [23, 24]. There is little evidence, however, for these assumptions and a lack of age and gender-specific data on the Zn and Fe status of the Iranian population.

The aim of the present study was, 1) to measure Zn and Fe status in children and adults from a rural (Rooran) and a suburban (Khomeini Shahr) population in Isfahan province, 2) to relate Zn status to Zn intake and bioavailability (based on PA:Zn molar ratio in the diet), 3) and to evaluate the link between Zn status and Fe status in the different population groups.

**Subjects and methods**

**Location**

The survey was conducted from November to December 2009 in Khomeini Shahr, a suburb of Isfahan with a population over 200,000 inhabitants, and in Rooran, a rural community with around 2000 inhabitants. Khomeini Shahr is a small city in the north west of Isfahan and is
now a part of the Isfahan Metropolitan area. Rooran, which is located 40 km to the south east of Isfahan city, still maintains typical characteristics of a village, depending on agriculture as the main occupation and for the self-production of rice and wheat for bread. Bread and rice are the main staple foods of both populations.

**Dietary assessment**

We conducted the three day weighed food record to assess the dietary Zn intake of the two selected communities. The data were collected on three consecutive days, including 2 weekdays and one weekend day, during which the households were asked to maintain their usual food habits. The total food prepared for consumption, the individual portions consumed by each household member, as well as the uneaten remains were weighed. Zn and PA intakes were estimated separately for men, women, pre-school- and school aged-children. They were based on the analyzed Zn and PA contents reported previously for the foods and dishes consumed by these same communities [25] with additional data from food composition tables [26]. The PA:Zn molar ratios were calculated for the whole diet and the degree of Zn bioavailability from the diet (low, moderate or high) was estimated as recommended by the World Health Organization (WHO) [27]. The Estimated Average Requirements (EAR values) for different age and gender groups were based on a moderate Zn bioavailability diet as recommended by WHO [27]. The Zn estimated intakes of the different age and gender groups were then compared with their respective EAR values.

**Subjects and enrollment**

The study subjects were primarily from the same 24 households in Khomeini Shahr and 28 households in Rooran that had previously participated in the dietary survey in 2008 [25]. In each of the two communities, the target enrollment was 40 preschool children aged <5 yrs, 40 school-age children aged 5–14 yrs, 40 females aged ≥15 yrs, and 40 males aged ≥15 yrs. The sample size of 320 participants was selected based on an expected moderately high prevalence of Zn deficiency [14]. The age classes were in accordance with the age classification used by the WHO for hemoglobin (Hb) cut-off values. Due to the small number of children in the subject households, additional children of preschool (<5 yrs) and school age (5-18 yrs) were recruited by informing their parents through schools, local mosques and health centers. However, despite these efforts we did not reach the target number of preschool children in either community with <15 per group compared to approximately 30 for men, 45 for women and around 70 for school aged children. (Table I).
A representative of each household that had participated in the previous study and a parent of the newly participating children were invited to attend a meeting in which the survey was presented orally and questions were answered. Volunteers were enrolled after written consent was obtained from all full-aged participants (over 18) and oral consent from the children. In addition, permission to conduct the survey was obtained from the respective health centers for both communities. The protocol was reviewed and approved by the Ethics Committees of ETH Zurich and the Research Affairs office of Isfahan University of Technology (IUT).

Socio-economic data
Socio-economic data were collected for the study subjects using especially prepared questionnaire designed to rank the subject families as deprived, low middle class, middle class, semi-wealthy or wealthy. The ranking was based on reported monthly expenditure, profession, ownership of house, cars etc., residential area, household size and education. Each family characteristic was given a score and the total score defined the socio-economic class.

Anthropometric measurements and blood sampling
Height and weight of all study subjects were measured and their medical history was recorded using a questionnaire. For the height and weight measurements, the participants were barefoot and dressed in light clothes. All measurements were taken in the morning, according to standardized procedure [28]. Body mass index (BMI) was calculated as weight (Kg) divided by height squared (m$^2$). Immediately after these measurements, approximately 6 mL venous blood was taken from each participant by a nurse. The blood samples were all taken in the morning so as to avoid diurnal and physiological variations in SZn [18]. The subjects were classified as fasting or non-fasting. About 2 mL of each blood sample was transferred into EDTA-treated tubes and the rest (≈ 4 mL) into trace-element free tubes without added anticoagulants. The 4 mL blood samples were allowed to clot for at least 40 minutes in a cooling chest before the serum was separated by centrifugation (High-Speed Refrigerated Centrifuge, SIGMA 3K30, Germany) at room temperature (RT) for 10 min at 3000 × g and divided into aliquots. The serum aliquots and the whole-blood samples in the EDTA-tubes were kept on ice and transported to a laboratory affiliated to the Isfahan University of Medical Sciences (IUMS) for analysis. The whole-blood samples in the EDTA-tubes were used for complete blood count (CBC) analysis within 12 hours after they arrived to the laboratory. The serum samples were frozen and stored at -20°C until further analysis. For some of the pre-school children, less than 5 mL blood was drawn and not all the analyses below could be performed.
Serum Zn (SZn) measurement

Serum Zn was analyzed by flame atomic absorption spectrophotometry (Atomic Absorption Spectrophotometer, Perkin-Elmer 2380, Norwalk, Connecticut, USA) using deproteinized samples at the School of Pharmacy of the IUMS. All analyses were performed in duplicate and a certified reference material, Seronorm™ (Sero AS, Billinstad, Norway) was analyzed in parallel for quality control [14]. Analyses were repeated when the difference between duplicates was >10% and sufficient serum sample was available. For morning non-fasting blood samples of children < 10 yrs, non-pregnant females ≥ 10 yrs and males ≥ 10 yrs, we used SZn cut-offs of < 65 μg/dL, < 66 μg/dL and < 70 μg/dL, respectively. For morning fasting blood samples the cut-offs were < 70 μg/dL and < 74 μg/dL, for non-pregnant females ≥ 10 yrs and males ≥ 10 yrs, respectively (Hotz and Brown, 2004). For morning fasting blood samples of children < 10 yrs, we used the same cut-off (65 μg/dL) as for non-morning fasting blood sample of children < 10 yrs, as no cut-off value is available for fasting blood samples of this age group.

Anemia and iron-status parameters

Hemoglobin (Hb)

Complete blood count (CBC) analysis was performed using an electronic Coulter Counter (Automated Hematology Analyzer, Sysmex K-1000, Kobe Japan). This included red blood cell count (RBC), hemoglobin concentration (Hb), hematocrit percent (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) measurement. Anemia was diagnosed according to the WHO cut-off values (29), which are Hb < 130 g/L for males ≥ 15 yrs, Hb <120 g/L for children of 12-14 yrs of age and non-pregnant women ≥ 15 yrs, Hb < 115 g/L for children aged 5-11 yrs and Hb < 110 g/L for children < 5 yrs. MCH values between 27-34 pg/cell and MCV values between 80-100 fL were considered normal as recommended by the IUMC laboratory.

Serum ferritin (SF)

The samples of frozen serum were transported to a private laboratory (Ferdowsi) in Isfahan city and analysed by means of an enzyme-linked immunoassay, using commercial test kits and manufacturers standards (Monobind Inc. California, USA). Values of SF < 12 μg/L (< 5 yrs) and <15 μg/L (≥ 5 yrs) were considered to indicate ID in the respective age group [30].

Total Iron Binding Capacity (TIBC)
TIBC was measured using the magnesium carbonate precipitation method [31] also at Ferdowsi Laboratory in Isfahan. A concentration of 230-440 µg/dL was considered normal in healthy individuals, as recommended by the manufacturer of the test kits (Darman Kave 2000).

**Type of anemia**

Hemoglobin amount per red blood cell (MCH) and average red blood cell size (MCV) were used together with Fe indicators to determine the anemia type. Cell size was described as normocytic (80< MCV< 100 fl), microcytic (MCV< 80 fl) and macrocytic (MCV> 100 fl) whereas the cell color as normochromic (27< MCH< 34 pg/cell), and hypochromic (MCH < 27 pg/cell). Based on the morphologic changes the type of anemia was defined as a) macrocytic-normochromic anemia (mainly related to Folate or Vitamin B₁₂ deficiency), b) microcytic-hypochromic anemia (often due to iron-deficiency, thalassemia, sideroblastic or chronic diseases), or c) normocytic-normochromic (which could be related to chronic disease and acute blood loss). IDA was defined as iron deficiency concurrent with anemia.

**C-reactive protein (CRP)**

The concentration of CRP, as a measure of acute infection or inflammation, was determined in serum samples using an immunoturbidometric assay (Atomatic analyzer 902, Hitachi, Japan). Values of ≥ 10 mg/L were taken to indicate the presence of inflammation or infection as recommended by the manufacturer (Darman Kave 2000).

**Statistical evaluation**

The Kolmogorov-Smirnov test was used to determine whether the values of any given variable were normally distributed. SZn and TIBC concentrations were normally distributed in both communities. The distributions of Hb concentration, height and weight were also considered normal within each age group of the two sample populations. These data are presented as means ± SDs. The data for age, BMI and SF were normal for each age group after transformation into natural logarithms. They are presented as geometric means ±1 SD. Since the CRP data were skewed, even after logarithmic transformation, the median range is reported. One-Way analysis of variance (ANOVA) was used to test for differences in the means of SZn, SF, TIBC and Hb concentrations among age groups and genders. The independent sample t test was applied to compare sample means between the two communities. The Chi Square test was performed for prevalence analysis and to test whether there were significant differences in categorical variables between rural and suburban populations. For those age groups with small sample size, the Fisher Exact test was used instead of the Chi
results. To evaluate correlations between biochemical parameters, the data of all participants in the two communities were pooled in order to have a statistically meaningful sample size. The Pearson’s correlation coefficient was used to examine the relationship between SZn, logSF, TIBC and Hb. Logistic regression analysis was used to investigate relationships between Zn deficiency prevalence and potential predictors, and adjusted odd ratios (OR) were calculated for age, gender and community with 95% confidence interval (CI). To assess the relationships between SZn concentration and potential predictors, multiple linear regression was employed. Differences were considered statistically significant in case of \( p<0.05 \).

Samples that were hemolyzed or with a CRP value \( >10 \) were excluded from the statistical analysis of SZn and TIBC. For the statistical analysis of SF, only values with CRP \( >10 \) were excluded while for CRP, BMI and age, all samples were taken into account. Statistical analyses were performed using SPSS version 16 (SPSS Inc., Chicago, IL, USA).

**Results**

**Subject’s height and weight**

Overall, there were no significant differences in Zn and Fe status between the rural and suburban communities and only small differences in anthropometric and CRP measurements. The results of the anthropometric measurements are shown in Table I. The average height difference between men and women was larger in Rooran (mean difference (MD) =15.9 cm, \( p<0.001 \)) than in Khomeini Shahr (MD=10.8 cm, \( p=0.007 \)). The adult female participants tended to be taller (\( p=0.03 \)) and heavier (\( p=0.001 \)) in suburban Khomeini Shahr than in rural Rooran and had significantly higher BMIs (\( p=0.04 \)).

The prevalence of overweight and obesity in the adult study subjects is shown in Table II. The prevalence of overweight (BMI 25- 30) and obesity (BMI \( \geq 30 \) Kg/m\(^2\)), was higher in suburban men and women than in their rural counterparts although it was only significantly higher (\( p=0.002 \)) in men. About 46% (13/28) of the suburban men were overweight and 18% (5/28) obese while in the rural area, compared to only 24% (9/38) overweight and 2.6% (1/38) obese in the suburban area.

**Inflammation**

The prevalence of inflammation or infection, as indicated by elevated CRP concentrations, was also higher in Khomeini Shahr than in Rooran for all age groups except for men \( \geq 15 \) yrs. Overall, the prevalence of inflammation or infection was 6.6% in Rooran and 10.9% in
Khomeini Shahr (p=0.2). The highest frequencies of elevated CRP were found in men with a prevalence of 14.3% in Khomeini Shahr and 16.2% in Rooran. The median CRP values ranged from 5.3 to 6.8 mg/L, with minimum 25% quartile of 2.7 mg/L and maximum 75% quartile of 16.6 mg/L.

**Socioeconomic status**

The social and economic status of the study subjects is shown in Table III. The rural participants were more homogeneous in economic status than the suburban group, with the vast majority being classed as lower middle to middle class. The suburban group contained a broader spectrum of classes, with more subjects in the deprived and semi wealthy classes and 4 subjects from different families being classed as wealthy.

**Zinc status**

The SZn concentrations in the different age groups are shown in Table IV and prevalence data on Zn deficiency in Table V. The mean SZn concentration in the entire study population was 91.1 ± 15.5 µg/dL. This translates to a moderate prevalence of Zn deficiency (5-10%) in the two communities as based on the criteria of International Zinc Consultative Group (IZiNCG) [14]. Only 6.4% (16/249) of the population had SZn concentrations below the respective cut-offs with similar prevalence rates in the rural (5.9%) and suburban (7.2%) communities. The average SZn concentration, however, was significantly lower in Rooran than in Khomeini Shahr (89 ± 14.3 µg/dL vs. 94 ± 16.9 µg/dL, p=0.02). On average, the SZn concentration was lower in women (87.9 ± 13.9 µg/dL) than in men (92.7 ± 16.2 µg/dL) (p=0.095), whereas the prevalence of Zn deficiency in men (14.0%) exceeded that for women (6.8%) (p=0.02).

The SZn values did not significantly differ among age groups in Rooran (p=0.46), but did so in Khomeini Shahr (p=0.007), primarily due to a significant difference between women (86.8 ± 14.1 µg/dL) and school-age children (98.8 ± 15.3 µg/dL) (p=0.01). Only rural men were found to be moderately more Zn deficient (16.7%).

**Iron status**

Based on the SF values (Table IV), 28.7% (86/300) of all participants were Fe deficient (Table V). As with Zn deficiency, there was no significant difference between Khomeini Shahr (30.7%) and Rooran (27%). According to the Fisher Exact test, the differences in ID prevalence among age groups were significant in both Khomeini Shahr (p=0.02) and Rooran (p<0.001). Overall, women had the highest prevalence of ID (46.6%) followed by school-age
children (30.3%). ID affected 23.1% of the preschool children. Men showed the lowest frequency of ID with only 2.2%. Using TIBC as an ID indicator resulted in fewer subjects being identified as iron deficient. The TIBC values indicated an ID prevalence of 13.0% in Rooran and 17.9% in Khomeini Shahr (p=0.3). As the TIBC is reported to be less sensitive than SF for detecting ID (32), only SF values were used for classifying IDA.

**Prevalence of anemia**

The prevalence of anemia was relatively high in all population groups ranging from 17.9-54.5% with a mean of 28.6% (94/329). The prevalence of anemia was higher in Rooran (33.5%) than in Khomeini Shahr (22.7% (p=0.04)). Anemia was observed in 43.5% of the preschool children, 24.2% of the school-age children, 33.0% of the women and 27.7% of the men. The differences in anemia prevalence between population groups were not statically significant (p> 0.05).

**Anemia etiology**

Based on SF and Hb values, IDA was prevalent in 11.2% (37/329) of the whole study population (Table V). Among different age groups, 4.3% of preschool children, 11.1% of school-age children, 18.2% of women and 4.5% of men (p=0.04) were affected by IDA. The prevalence of IDA was 10.7% in Khomeini Shahr and 11.7% in Rooran. ID appeared to be the most common cause of anemia in women from both study sites. Forty four percent of the anemic women in Rooran and 72.7% in Khomeini Shahr were also iron deficient. ID was also the most common cause of anemia in the school age children of Khomeini Shahr (56.5%). In contrast, none of the 13 anemic men from Rooran was iron deficient whereas three in five of the anemic men in Khomeini Shahr had ID.

In Table VI, anemia in the different population groups is classified as IDA, or into 5 other categories according to cell size, serum ferritin, normal or elevated CRP; hypochromic or unidentified. The most common type of anemia in the 94 subjects with anemia was microcytic-hypochromic anemia, (71 subjects, 75.5%). Anemia in 37 of these subjects was associated with ID (low SF) and thus 39.4% of the anemia was classified as IDA. Thirty four anemic subjects had normal iron stores (SF>15µg/L) and 36% of the anemia was thus classified microcytic-hypochromic with normal iron stores among which only 5 had elevated CRP, 1 had missing CRP and 28 had normal CRP values. About 4% of anemia (4 subjects) was of normocytic-normochromic type, which was only found in the adult participants of the rural community. The remaining 20% of the anemia (19 subjects) could not be classified mainly due to lack of
MCH and MCV data from 19 rural participants although 16 of these 19 subjects had normal iron stores. Twenty percent of the anemia was thus classified as unidentified but not IDA. Among these anemic participants, 2 had elevated CRP, 1 had missing CRP and the remaining 16 had normal CRP levels.

The type of anemia could not be classified in most of the preschool children and some of the school age children of the rural area due to lack of serum ferritin data. None of these children had elevated CRP. There was no macrocytic-normochromic anemia, which is often caused by folate or vitamin B₁₂ deficiency, in our study populations.

**Relationships between Zn deficiency, ID and anemia**

One fourth (4/16) of all participants, who had low SZn concentrations were also diagnosed as iron deficient. This ratio did not significantly change when TIBC values were used to determine the iron status (3/16). Low Fe status alone was not associated with reduced SZn concentration (average SZn concentration of 90.6 µg/dL in ID participants vs. 91.3 µg/dL in those with normal SF and the correlation analysis showed no significant relationship between the concentration of SZn and the two Fe status indicators (Table VII). Of the participants with Zn deficiency, 37.5% (6/16) were also anemic and the SZn concentration was significantly lower in subjects with anemia than in those without anemia (mean SZn of 85.7 ± 13.1 µg/dL vs. 93.0 ± 16.0 µg/dL, p=0.001). The concentration of SZn and Hb showed a significant positive correlation. Iron deficient anemic participants, had the lowest SZn concentration with an average of 84.5 ± 11.9 µg/dL as compared to all other participants (91.8 ± 15.8 µg/dL, p=0.02). Hemoglobin was significantly correlated with the two Fe indicators, SF and TIBC.

**Zinc and phytic acid intake**

The results of the dietary survey are shown in Table VIII. The average daily phytic acid and Zn intakes were 951 mg and 9.0 mg in Rooran and 892 mg and 7.8 mg in Khomeini Shahr. The average daily PA:Zn molar ratio was similar in both populations (10.7 in Rooran and 12.4 in Khomeini Shahr). Although the average phytic acid and Zn intakes were higher in the rural population, the difference was not significant. Three different International Institutions have suggested values for the Estimated Average Requirement (EAR) of zinc. These are the WHO [27], the IZiNCG [14] and Food and Nutrition Board/Institute of Medicine (FNB/IOM) [33]. FNB/IOM has the highest Zn EAR values followed by IZiNCG and WHO. Zn deficiency prevalence was 41.4% using the FNB/IOM EAR values, 33.5% using the IZiNCG EAR values, and 18.3% using the WHO EAR values (Table IX). The WHO prediction of 18.3% low Zn
intake agreed more closely with the measured Zn deficiency of about 6% (Table V). The proportion of the study subjects below EAR was similar in the rural and suburban communities but was higher in children <9y (10/23) and men > 19y (13/61) than in older children, adolescents or adults.

The proportion of Zn intake coming from the different food groups is shown in Figure 1. Equal amounts of dietary Zn (24-25%) came from bread and red meat even though on a weight basis these foods provided only 15% and 4% of daily food intake (Figure 1a). Dairy (milk, yoghurt and cheese) and rice provided 13% and 12% of the daily Zn intake, respectively. Animal source foods (red meat, chicken, milk, yoghurt, cheese and eggs) provided in total 47% of the daily Zn intake. The main zinc containing foods were red meat, chicken, dairy, bread and rice (Figure 1b). They provided 81% of the Zn intake but only 57% of the daily per capita consumption on a weight basis (Figure 1a). The remaining 19% of the Zn intake came mostly from eggs, fruits, vegetables and pulses. The dietary survey additionally showed that neither Zn or Fe supplements were frequently consumed by the study population and that daily tea consumption averaged 276 g per individual. Tea was frequently consumed directly after food.

Discussion

The mean prevalence of Zn deficiency based on SZn was relatively low (6-7%) and similar in both suburban Khomeini Shahr and rural Rooran. The prevalence of Zn deficiency in our study subjects ranged however from 0-17.5 % in the different population groups with suburban children and rural men having somewhat higher levels of Zn deficiency. Previous reports of Zn deficiency in Iran have varied widely. A recent study by Dehghani et al. [10] reported a similarly low prevalence of Zn deficiency (8%) in children in Shiraz. In Tehran, Zn deficiency was reported to be 28% [5], 30.1% [6] and 85.5% [8] in three different studies and Fesharakinia et al. [7] reported that Zn deficiency in Khorasan to be 28.1%. There are several reasons for these differences and Dehghani et al. [10] suggested that they could be due to the use of different SZn cut-off values, different soil Zn levels influencing the Zn content of locally produced foods, the wide prescription of Zn supplements by pediatricians in recent years, and the balance of plant and animal source foods in the diet.

In relation to the SZn cut-off values for Zn deficiency, we used 65-74 µg/dL as recommended by Hotz and Brown [14]. In the Zn status surveys reported above SZn cut off values ranged from 70-100 µg/dL. The mean SZn concentrations measured in our study subjects were of the same order as reported in other Iranian studies. In our study, the mean SZn ranged from 87-102 µg/dL with a mean of 91.1 ± 15.5 µg/dL. Farzin et al. [34] found an
average Szn of 89 ± 16 µg/dL in 115 healthy volunteers (60 males and 55 females aged 6-62 yrs) living in Tehran; and Khalili et al. [9] reported an average Szn concentration of 89.9 ± 14.8 µg/dL in 100 Iranian males (20-43 years of age), In the latter study, 8% of the subjects were Zn deficient, taking 67 µg/dL as the cutoff value. Some studies have reported slightly higher mean Szn concentrations than found in our current study. In a survey of 600 healthy males (20-69 yrs) from 5 Iranian provinces (Tehran, Mashhad, Shiraz,Tabriz and Boushehr), the average Szn concentration reported was 92.15 ± 35.15 µg/dL [35]. Some 30.1% of the men had Zn concentrations <75 µg/dL, 56.8% between 75-120 µg/dL and 13.1% >120 µg/dL. In an earlier survey in Isfahan conducted in 2000, the average Szn concentrations of 100 healthy male and female children and adolescents (2-18 yrs) was reported to be 92.26 ± 23.7 µg/dL. In this group, 29% had Zn concentrations below 80 µg/dL, 62% between 80-120 µg/dL and 9% above 120 µg/dL [36].

With regard to the soil Zn concentration in Isfahan province, it would seem unlikely that this would be a major risk in the etiology of Zn deficiency in the rural villages, which grow wheat and rice. Karami et al. [37] reported that the total soil micronutrient concentrations (including Zn) in the provinces of Isfahan, Fars and Khozestan, were all in the normal range, and that 84% of the surveyed fields had acceptable levels of DTPA (diethylenetriaminepentaacetic acid) extractable Zn indicating good Zn bioavailability for the crops. Additionally, only 20% of the wheat grain samples grown in the province of Isfahan were found to have critically low Zn concentration (< 24 mg kg-1 dry matter) [37].

As Zn supplements were not routinely taken by our study subjects, the most likely explanation of the low prevalence of Zn deficiency in Khomaini Shahr and Rooran was an adequate intake of bioavailable Zn from the normal diet. The PA:Zn molar ratios for the whole diet ranged from 5-15 (Table IX), indicating a modest Zn bioavailability diet [27]. The 18.3% prevalence of low Zn intakes, according to WHO EAR values, is little higher than the observed 6-7% Zn deficiency in blood, but this is still much closer than when the FNB/IOM and IZiNCG EAR values are used to compare measured Zn intakes. Using the IZiNCG values predicts a 33.5% and the FNB/IOM values 41.4% prevalence of low Zn intakes. Our results thus show that the FNB/IOM and IZiNCG EAR values overestimated the prevalence of low Zn intakes for our study populations.

The high level of adequate Zn intakes in the Iranian communities studied was unexpected and results from a good intake of animal source foods that provided almost 50% of Zn intake. Dairy products were widely consumed and provided a useful source of Zn. Although red meat was little consumed (4% by weight), its high Zn content makes it an excellent Zn source. Red
meat provided some 25% of the Zn intake in our populations. The high intake of animal source foods presumably results from the reasonably good socio-economic status of the rural and suburban populations studied. Only 5% of our study subjects were classed as deprived and most were classed as lower middle class, middle class or semi wealthy. Red meat is expensive and failure to consume red meat by our study subjects would put many more at risk of Zn deficiency.

In addition to the wide provincial variations, socio-economic conditions cause basic differences in the food consumption pattern of the populations living in different parts of Iran [38]. The low socio-economic classes found mostly in the southern, southeastern and some northern provinces, may not be able to consume animal protein and red meat products because they are very expensive [38]. In these provinces, bread is the main food for rural dwellers and poor urban residents. Therefore, these groups of people may be in danger of higher Zn deficiency.

Anemia was a serious public health problem in all population groups with the prevalence ranging from 17.9-54.5%, with a mean of 28.6% (Table V). Iron deficiency explained 50% of the anemia in older children and women but was not a major cause in younger children and adult men. Women of childbearing age have higher iron requirements due to menstruation, and older children need more iron during the adolescent growth spurt for the increased blood volume. Additionally, low dietary iron absorption can be expected due to the high phytate bread-based diet, the frequent consumption of dairy foods and the habit of consuming tea with meals [39]. Other recent Iranian studies have reported similar anemia prevalence values in women (25.8%) in Northern Iran [40] and 14-20y old girls (21.4%) in Western Iran [41]. In these studies, ID affected some 24% of the girls and 36% of the women and, as in our present study, ID explained only about 50% of the anemia.

The etiology of the anemia, which is not associated with ID, is unknown. Most of these anemic subjects (33/57) in our study had microcytic hypochromic anemia (Table VI). The remaining anemia could not be classified, largely due to lack of analytical results. Three prominent reasons given for microcytic-hypochromic anemia, other than ID, are chronic disease, thalassemia and sideroblastic anemia [42]; where the major cause of the latter is lead or Zn poisoning [43, 44]. Only 5 of the subjects with microcytic hypochromic anemia had elevated CRP levels indicating that inflammatory disorders such as overweight and obesity or chronic disease were not a major cause of the low hemoglobin values in our study population. The prevalence of overweight and obesity in our adult study population was nevertheless
relatively high, especially in the suburban area, although similar to the combined 43% national estimate for obesity and overweight prevalence, which has been reported earlier [45].

The prevalence of thalassemia varies in different parts of Iran. It is more prevalent in the northern (Caspian Sea coast) and southern (Persian Gulf and Oman Sea coasts) areas of the country but the central parts are reported at low prevalence of thalassemia [46]. The overall prevalence ranges approximately from 3 to 100 patients per 100,000 people in different provinces [46].

Nowadays, the main source of environmental lead contamination, especially in large crowded Iranian cities such as Tehran, Mashhad, Shiraz, Isfahan and Tabriz is cars. Painters and the traditional tile workers are potentially at risk of lead poisoning. In the past two decades, cases of lead exposures have been reported among the opium addicts [47]. A few sources of lead (Pb) around the city of Isfahan may cause sideroblastic anemia among the population. Use of leaded gasoline and heavy traffic are the main sources of Pb poisoning in Isfahan [47, 48]. Another source could be the Irankooh lead mine located 20 km southeast of the city. Lead water pipes, lead-containing paints may also be culprits in sideroblastic anemia.

Nutritional anemia could also be due to dietary deficiencies in folic acid, B12, riboflavin and vitamin A. The dietary pattern (Figure 1) with a high consumption of animal source foods would rule out B12 and vitamin A deficiencies and dairy products should provide adequate riboflavin. Likewise, the relatively high consumption of fruits and vegetables should provide adequate folic acid and pro vitamin A carotenoids.

There was no relationship between Zn and Fe status in our study, although there was a positive correlation between Zn and Hb. It is sometimes argued that Zn and Fe deficiencies occur together because Fe and Zn absorption is low from high-phytate plant foods; and meat provides a good source of bioavailable Fe and Zn. We found however much higher prevalence of ID than Zn deficiency. One exception therefore might be populations such as ours, which consume a high proportion of dairy foods that provide a useful source of Zn, but are low in Fe and additionally might inhibit the absorption of Fe from other foods [49]. The high tea consumption would also be expected to decrease Fe absorption [50] but not Zn. The role of Zn in the production of red blood cells [51 – 53] might explain the positive correlation between Zn and Hb. As there was no correlation between SF and SZn, it is possible that Zn deficiency be an independent cause of anemia. Although this has been previously suggested by Idei [54], the low number of Zn deficient subjects (16/249) in our study, means it needs further investigation.

In conclusion, our study demonstrated a relatively low level of Zn deficiency (6-7%) in the mostly middle class rural and suburban communities studied. Neither Zn supplements nor soil
Zn would be expected to influence the Zn status of our populations. The relatively good Zn status could be explained by an adequate supply of dietary Zn from dairy products and animal tissue foods, which supplemented the bread and rice staples. Only 18% of the study subjects consumed less Zn that their estimated requirements. Anemia and ID were more common affecting 28% and 29% of the total population, respectively. ID was more common in women and older children and could be explained by their higher Fe requirements, the high phytate diet and frequent tea consumption. ID however, explained less than half of the anemia whose etiology remains unknown. The lack of correlation between Zn and iron deficiencies could be due to the frequent consumption of dairy products and tea.

Acknowledgements
We greatly appreciate the cooperation of the people of Khomeini Shahr and Rooran and the grant provided by the Swiss Development Cooperation, SDC. We also appreciate the cooperation by the Isfahan University of Medical Sciences, and the Isfahan Cardiovascular Research Center. We thank Dr. Ghasem Yadegarfar for his help with statistical analysis.

The author’s responsibilities were as follows – NR collected the data, designed the study, performed the analysis, and prepared the manuscript; RH formulated the research question, checked data quality, helped with data interpretation and preparation of the manuscript; RW helped with manuscript preparation; RS formulated the research question, helped with preparation of the manuscript. None of the authors declared a conflict of interest.

References
Table I: Anthropometric measurements including height, weight and BMI together with age and number of the participants in Khomeini Shahr (Kh) and Rooran (R)

<table>
<thead>
<tr>
<th>Age group</th>
<th>N</th>
<th>Age (yrs)</th>
<th>Height (cm)</th>
<th>Weight (Kg)</th>
<th>BMI (Kg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kh</td>
<td>R</td>
<td>Kh</td>
<td>R</td>
<td>Kh</td>
</tr>
<tr>
<td>&lt; 5 yrs</td>
<td>13</td>
<td>14</td>
<td>4.3 (3.8, 4.8)</td>
<td>4.1 (2.6, 6.4)</td>
<td>103.8 ± 3.3</td>
</tr>
<tr>
<td>5-14 yrs</td>
<td>68</td>
<td>89</td>
<td>8.0 (5.6, 11.2)</td>
<td>9.2 (6.9, 12.1)</td>
<td>129.51 ± 18.9</td>
</tr>
<tr>
<td>F ≥ 15 yrs</td>
<td>47</td>
<td>44</td>
<td>25.0 (17.3, 36.1)</td>
<td>30.8 (19.9, 47.6)</td>
<td>160.4 ± 6.2</td>
</tr>
<tr>
<td>M ≥ 15 yrs</td>
<td>28</td>
<td>38</td>
<td>32.8 (21.1, 51.0)</td>
<td>34.5 (22.2, 53.6)</td>
<td>171.23 ± 9.7</td>
</tr>
</tbody>
</table>
Table II: Prevalence of overweight and obesity in adult females (F ≥ 15 yrs) and adult males (M ≥ 15 yrs) in Khomeini Shahr (Kh) and Rooran (R).

<table>
<thead>
<tr>
<th>Age group</th>
<th>Overweight (%)</th>
<th>Obesity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kh</td>
<td>R</td>
</tr>
<tr>
<td>F ≥ 15 yrs</td>
<td>27.1</td>
<td>17.4</td>
</tr>
<tr>
<td>M ≥ 15 yrs</td>
<td>46.4</td>
<td>23.7</td>
</tr>
</tbody>
</table>

Overweight: 25 ≤ BMI ≤ 30, Obesity BMI > 30
Table III: Socio-economic status\(^1\) of subjects in Khomeini Shahr (Kh) and Rooran (R)

<table>
<thead>
<tr>
<th></th>
<th>Wealthy</th>
<th>Semi-wealthy</th>
<th>Middle</th>
<th>Low-middle</th>
<th>Deprived</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kh</td>
<td>4</td>
<td>28</td>
<td>64</td>
<td>43</td>
<td>12</td>
<td>151</td>
</tr>
<tr>
<td>R</td>
<td>0</td>
<td>5</td>
<td>93</td>
<td>81</td>
<td>6</td>
<td>185</td>
</tr>
<tr>
<td>Total</td>
<td>4</td>
<td>33</td>
<td>157</td>
<td>124</td>
<td>18</td>
<td>336</td>
</tr>
</tbody>
</table>

\(^1\) Socio-economic status was estimated based on monthly expenditure, profession, ownership of house, car etc., residential area, household size and education as explained in the Socio-economic section of Subjects and Methods.
Table IV: Hemoglobin (Hb), serum ferritin (SF), total Iron binding capacity (TIBC) and serum Zn (SZn) concentrations of different age groups in Khomeini Shahr (Kh) and Rooran (R).

<table>
<thead>
<tr>
<th>Age group</th>
<th>$^1$Hb (g/dL)</th>
<th>$^2$SF (µg/L)</th>
<th>$^3$TIBC (µg/dL)</th>
<th>$^4$SZn (µg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kh</td>
<td>R</td>
<td>Kh</td>
<td>R</td>
</tr>
<tr>
<td>&lt; 5 yrs</td>
<td>11.3 ± 0.9</td>
<td>11.0 ± 1.7</td>
<td>15.6 (7.5, 32.4)</td>
<td>29.4 (22.3, 38.7)</td>
</tr>
<tr>
<td>5-14 yrs</td>
<td>12.1 ± 0.8</td>
<td>12.3 ± 1.5</td>
<td>21.2 (10.8, 41.3)</td>
<td>20.2 (10.8, 37.6)</td>
</tr>
<tr>
<td>F ≥ 15 yrs</td>
<td>12.3 ± 0.8</td>
<td>12.1 ± 1.2</td>
<td>17.8 (7.0, 44.8)</td>
<td>17.6 (5.4, 57.8)</td>
</tr>
<tr>
<td>M ≥ 15 yrs</td>
<td>13.7 ± 1.7</td>
<td>13.7 ± 1.6</td>
<td>58.7 (23.0, 149.6)</td>
<td>63.5 (30.1, 133.9)</td>
</tr>
</tbody>
</table>

$^1$ Arithmetic mean ± SD.
$^2$ Geometric mean (-1 SD, +1 SD).
<table>
<thead>
<tr>
<th>Age group</th>
<th>Anemia (%) (n/N)</th>
<th>Iron deficiency (ID) (%) (n/N)</th>
<th>Iron deficiency anemia (IDA) (%) (n/N)</th>
<th>Zn deficiency (%) (n/N)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kh/R</td>
<td>Kh/R</td>
<td>Kh/R</td>
<td>Kh/R</td>
</tr>
<tr>
<td>&lt; 5 yrs</td>
<td>33.3 (4/12)</td>
<td>8.3 (1/12)</td>
<td>27.3 (3/11)</td>
<td>12.5 (1/8)</td>
</tr>
<tr>
<td></td>
<td>54.5 (5/11)</td>
<td>0.0 (0/11)</td>
<td>0.0 (0/7)</td>
<td>0.0 (0/6)</td>
</tr>
<tr>
<td>5-14 yrs</td>
<td>21.2 (14/66)</td>
<td>6.1 (4/66)</td>
<td>3.1 (19/61)</td>
<td>7.3 (3/41)</td>
</tr>
<tr>
<td></td>
<td>26.4 (23/87)</td>
<td>14.9 (13/87)</td>
<td>28.9 (24/83)</td>
<td>1.3 (1/78)</td>
</tr>
<tr>
<td>F ≥ 15 yrs</td>
<td>25.0 (11/44)</td>
<td>8.2 (8/44)</td>
<td>43.9 (18/41)</td>
<td>5.9 (2/34)</td>
</tr>
<tr>
<td></td>
<td>40.9 (18/44)</td>
<td>18.2 (8/44)</td>
<td>47.6 (20/42)</td>
<td>7.7 (3/39)</td>
</tr>
<tr>
<td>M ≥ 15 yrs</td>
<td>17.9 (5/28)</td>
<td>10.7 (3/28)</td>
<td>8.3 (2/24)</td>
<td>7.1 (1/14)</td>
</tr>
<tr>
<td></td>
<td>35.1 (13/37)</td>
<td>0.0 (0/37)</td>
<td>0.0 (0/31)</td>
<td>17.2 (5/29)</td>
</tr>
<tr>
<td>Total</td>
<td>22.7 (34/150)</td>
<td>10.7 (16/150)</td>
<td>11.8 (21/179)</td>
<td>7.2 (7/97)</td>
</tr>
<tr>
<td></td>
<td>33.0 (59/179)</td>
<td>30.7 (42/137)</td>
<td>27.0 (44/163)</td>
<td>5.9 (9/152)</td>
</tr>
</tbody>
</table>

**Table V:** Prevalence of Zn deficiency, Iron deficiency (ID), anemia and Iron deficiency anemia (IDA) by age group in Khomeini Shahr (Kh) and Rooan (R)

- **n:** Number of participants below cutoff
- **N:** Total number of the participants
Table VI: Prevalence of different types of anemia in different age groups in Khomeini Shahr (Kh) and Rooran (R)

<table>
<thead>
<tr>
<th>Anemia type</th>
<th>% (n/N)</th>
<th>&lt; 5 yrs</th>
<th>5-14 yrs</th>
<th>F ≥ 15 yrs</th>
<th>M ≥ 15 yrs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Kh</td>
<td>R</td>
<td>Kh</td>
<td>R</td>
</tr>
<tr>
<td>IDA</td>
<td>25.0 (1/4)</td>
<td>0.0 (0/5)</td>
<td>28.6 (4/14)</td>
<td>56.5 (13/23)</td>
<td>72.7 (8/11)</td>
</tr>
<tr>
<td>Microcytic-hypochromic;</td>
<td>50.0 (2/4)</td>
<td>20.0 (1/5)</td>
<td>57.1 (8/14)</td>
<td>4.3 (1/23)</td>
<td>27.3 (3/11)</td>
</tr>
<tr>
<td>normal SF; normal CRP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microcytic-hypochromic;</td>
<td>25.0 (1/4)</td>
<td>0.0 (0/5)</td>
<td>14.3 (2/14)</td>
<td>0.0 (0/23)</td>
<td>0.0 (0/11)</td>
</tr>
<tr>
<td>normal SF; elevated CRP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unidentified; normal SF;</td>
<td>0.0 (0/4)</td>
<td>80.0 (4/5)</td>
<td>0.0 (0/14)</td>
<td>30.4 (7/23)</td>
<td>0.0 (0/11)</td>
</tr>
<tr>
<td>normal CRP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unidentified; normal SF;</td>
<td>0.0 (0/4)</td>
<td>0.0 (0/5)</td>
<td>0.0 (0/14)</td>
<td>8.7 (2/23)</td>
<td>0.0 (0/11)</td>
</tr>
<tr>
<td>elevated CRP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normocytic-normochromic</td>
<td>0.0 (0/4)</td>
<td>0.0 (0/5)</td>
<td>0.0 (0/14)</td>
<td>0.0 (0/23)</td>
<td>0.0 (0/11)</td>
</tr>
</tbody>
</table>

n: Number of participants below cutoff  
N: Total number of the participants  
Anemia: Hb < 130 g/L for males ≥ 15 yrs, Hb <120 g/L for children 12-14 yrs and non-pregnant women ≥ 15 yrs, Hb < 115 g/L for children 5-11 yrs and Hb < 110 g/L for children < 5 yrs.  
Iron deficiency (ID): SF < 12 μg/L (< 5 yrs) and <15 μg/L (≥ 5 yrs)  
Iron deficiency anemia (IDA): ID concurrent with anemia.  
Normocytic- normochromic: normal MCV (80-100 fl) and normal MCH (27-34 pg/cell) but lower than cutoff Hb  
Microcytic- hypochromic: Low MCV (< 80 fl), low MCH (< 27 pg/cell) and lower than cutoff Hb  
Elevated CRP: CRP ≥ 10 mg/L
Table VII: Correlation coefficients between hemoglobin (Hb), serum Zn (SZn), serum ferritin (SF), and total iron binding capacity (TIBC) of the whole study population

<table>
<thead>
<tr>
<th></th>
<th>SZn</th>
<th>TIBC</th>
<th>SF (ln)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TIBC</td>
<td>-0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SF (ln)</td>
<td>0.02</td>
<td>-0.45**</td>
<td></td>
</tr>
<tr>
<td>Hb</td>
<td>0.31**</td>
<td>-0.13*</td>
<td>0.34**</td>
</tr>
</tbody>
</table>

** Correlation is significant at the 0.01 level (2-tailed)
* Correlation is significant at the 0.05 level (2-tailed)
Table VIII: Average Zn and PA intake and PA:Zn molar ratio in different population groups in the two study locations and the prevalence of Zn intakes below the EAR

<table>
<thead>
<tr>
<th></th>
<th>&lt;4 y</th>
<th>4-9 y</th>
<th>9-14 y</th>
<th>14 y ≤ M &lt; 19 y</th>
<th>14 y ≤ F &lt; 19 y</th>
<th>M ≥ 19 y</th>
<th>F ≥ 19 y</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rooran</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zn intake (mg/day)</td>
<td>2.7</td>
<td>4.6</td>
<td>8.9</td>
<td>8.0</td>
<td>9.0</td>
<td>11.7</td>
<td>8.1</td>
<td>9.0</td>
</tr>
<tr>
<td>PA intake (mg/day)</td>
<td>256</td>
<td>360</td>
<td>1035</td>
<td>909</td>
<td>774</td>
<td>1260</td>
<td>878</td>
<td>951</td>
</tr>
<tr>
<td>PA:Zn</td>
<td>8.4</td>
<td>8.7</td>
<td>12.2</td>
<td>9.3</td>
<td>10.3</td>
<td>11.2</td>
<td>11.1</td>
<td>10.7</td>
</tr>
<tr>
<td>1EAR (mg/day)</td>
<td>3.4</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
<td>5.8</td>
<td>4.1</td>
<td></td>
</tr>
<tr>
<td>2n &lt; EAR</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>4</td>
<td>17</td>
</tr>
<tr>
<td>% below EAR</td>
<td>80.0</td>
<td>37.5</td>
<td>25.0</td>
<td>0</td>
<td>0</td>
<td>13.2</td>
<td>11.1</td>
<td>17.0</td>
</tr>
<tr>
<td><strong>Khomeini Shahr</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zn intake (mg/day)</td>
<td>2.8</td>
<td>5.0</td>
<td>8.1</td>
<td>0</td>
<td>7.8</td>
<td>8.9</td>
<td>7.8</td>
<td>7.8</td>
</tr>
<tr>
<td>PA intake (mg/day)</td>
<td>164</td>
<td>585</td>
<td>793</td>
<td>0</td>
<td>836</td>
<td>1196</td>
<td>875</td>
<td>892</td>
</tr>
<tr>
<td>PA:Zn</td>
<td>8.0</td>
<td>11.7</td>
<td>10.7</td>
<td>0</td>
<td>12.6</td>
<td>13.8</td>
<td>12.2</td>
<td>12.4</td>
</tr>
<tr>
<td>1EAR (mg/day)</td>
<td>3.4</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
<td>5.8</td>
<td>4.1</td>
<td></td>
</tr>
<tr>
<td>2n &lt; EAR</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>8</td>
<td>3</td>
<td>18</td>
</tr>
<tr>
<td>% below EAR</td>
<td>33.3</td>
<td>28.6</td>
<td>0</td>
<td>0</td>
<td>17.4</td>
<td>34.8</td>
<td>12.0</td>
<td>19.8</td>
</tr>
<tr>
<td>Total % (n/N(^3))</td>
<td>62.5</td>
<td>33.3</td>
<td>7.7</td>
<td>0.0</td>
<td>13.8</td>
<td>21.3</td>
<td>11.5</td>
<td>18.3</td>
</tr>
</tbody>
</table>

1 The EARs are given for moderate bioavailability diet as recommended by WHO (2006) based on the average PA:Zn molar ratios of 5-15.
2 n: Number of participants with Zn intakes below the EAR.
3 N: Total number of participants in each age group.
Table IX: Suggested EAR values for zinc (mg/d) by WHO, IZiNCG and FNB/IOM and the prevalence of Zn deficiency based on these references.

<table>
<thead>
<tr>
<th>Age</th>
<th>WHO</th>
<th>IZiNCG</th>
<th>IOM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-3 y</td>
<td>3.4</td>
<td>2</td>
<td>2.5</td>
</tr>
<tr>
<td>4-8 y</td>
<td>4.0</td>
<td>3</td>
<td>4.0</td>
</tr>
<tr>
<td>9-13 y</td>
<td>4.0</td>
<td>5</td>
<td>7.0</td>
</tr>
<tr>
<td>M 14-18 y</td>
<td>4.0</td>
<td>8</td>
<td>8.8</td>
</tr>
<tr>
<td>F 14-18 y</td>
<td>4.0</td>
<td>7</td>
<td>7.3</td>
</tr>
<tr>
<td>M ≥ 19 y</td>
<td>5.8</td>
<td>10</td>
<td>9.4</td>
</tr>
<tr>
<td>F ≥ 19 y</td>
<td>4.1</td>
<td>6</td>
<td>6.8</td>
</tr>
<tr>
<td>Zn deficiency</td>
<td>18.3%</td>
<td>30.4%</td>
<td>41.4%</td>
</tr>
</tbody>
</table>

WHO EAR values are given for moderate bioavailability diet (PA:Zn molar ratio: 5-15). IZiNCG values are given for mixed diet (PA:Zn molar ratio:4-18).
Figure 1. a) Diet composition: The amount of each food group is reported as percent of total diet weight for whole population, b) Zinc intake for individual food groups: The amount of Zn consumed for each food group is reported as percent of mean total Zn (8.7 mg/day) for whole population.
CHAPTER 4

Modeling dietary zinc intake in Central Iranian population groups

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Abstract

Objective: To develop a model for the evaluation of different intervention scenarios for abating human zinc (Zn) deficiency by enhancing dietary Zn intake and demonstrate its applicability for test populations in central Iran.

Method: The model determines dietary Zn intake for different user-defined population groups using the molar phytate:Zn ratio as an indicator of Zn bioavailability and taking account of uncertainty in the input data by means of Monte Carlo simulation. Based on data from dietary surveys in two sample populations in the province of Isfahan, Iranian national statistics and other available sources, the model was used to assess the risk of Zn deficiency in the study population and to compare how different scenarios influence its future development.

Results: The Zn intake of almost one third (31%) of the study population were below the respective thresholds of gender- and age-specific Estimated Average Requirements (EAR). The uncertainty associated with this estimate due to uncertainty in the Zn concentrations of the consumed foods was between (28 – 35%), demonstrating the need to take uncertainties into account in such studies. The scenario analysis predicted that it would take up to 60 years until 97.5% of the Iranian population met their Zn EAR’s if the consumption of major food items continued to increase at the current rate. With fortification of wheat flour, this goal could be reached within 15 years.

Conclusion: The Zn intake model proved a useful tool for the analysis of possible future trends and intervention scenarios such as fortification. The dataset on which the scenario analysis was based shows that uncertainty analysis is crucial in such studies.

Introduction

Zinc (Zn) deficiency in humans was first detected in Iran in 1961 (Prasad 1963). Since then, many surveys based on laboratory and clinical tests, including Zn supplementation trials, have reported widespread prevalence of Zn deficiency in human populations (Caulfield and Black 2004). Zn deficiency can occur particularly in developing countries, where populations depend on plant-based foods with low concentrations in bioavailable Zn, and have insufficient dietary intake of Zn from animal products. Bioavailable Zn refers to the fraction of Zn intake that is absorbed from the digestive tract and thus available for physiological functions (Lönnerdal, 2000). Phytic acid (PA) is the principal dietary factor known to impair Zn bioavailability (Lönnerdal 2000, Miller et al. 2007, Hambidge et al. 2008). It is present in high concentrations in cereal grains and legume seeds, but not in animal foods (Schlemmer et
Zn deficiency can be exacerbated in arid regions, where Zn uptake by crops is often affected by low Zn availability in the soil (Cakmak et al. 1999).

Foods with naturally high content of absorbable micronutrients are considered the best means for preventing or abating micronutrient deficiencies (Tontisirin et al. 2002). In communities where supplies of such foods are unavailable, micronutrient intake can be increased by the traditional interventions of fortifying staple foods or condiments, or by taking supplements as pharmacological doses (Hambidge 2000). As recent alternative intervention is the biofortification of the edible parts of crop plants (Lönnerdal 2003). The choice of intervention depends upon the availability of resources, technical feasibility, and societal factors.

Models designed to predict the outcome of different food consumption scenarios on Zn intake could be helpful tools in evaluating policies or intervention strategies. Models based on substance flow analysis are particularly well suited to describe trace element intake with food consumption, as was recently shown by Malde et al. (2011), who studied fluoride intake with food and drinks in young children in Ethiopia. Substance flow models are transparent, versatile, easily extendable (e.g. to include more substances) and can be readily linked to mass flow models describing connected flow and transfer processes. A food consumption model for example may be linked to a model describing food processing and meal preparation.

The objectives of this study were to develop a stochastic Zn intake model describing the intake of bioavailable Zn from different food sources by user-defined population groups, and to demonstrate the potential use of the model by performing a scenario analysis of possible future trends on Zn intake and intervention strategies to abate Zn deficiency in the population of central Iran. The modeling approach adopted here is based on that of Keller and Schulin (2003), who developed a flexible stochastic mass transfer model to assess regional heavy metal fluxes as a basis for a sustainable management of agricultural soils. The model accounts for parameter uncertainty, which is an important aspect to be considered in using model predictions as the basis of decision-making.

**Methods**

**Model**

The model calculates average Zn and PA intake rates for user-specified scenarios of meals \( m \) considered representative for the members of a selected population group. A meal \( m \) is defined by the quantities \( Q_{gmi} \) of the ingredients consumed by a person of group \( g \) and the
concentrations of Zn, Z_i, and PA, P_i, in each food item i. The latter are assumed to depend only on the type of food items and thus to be the same for all meals and groups. The total intake of Zn and PA of a member of group g with meal m are:

\[ Zn_{gm} = \sum_i Q_{gm}Z_i \]  
and

\[ PA_{gm} = \sum_i Q_{gm}P_i \]

respectively. The model assumes that all food items consumed during a meal are well mixed in the intestine, that no carry-over occurs from one meal to the next, and that the bioavailability of Zn is a group-specific fraction of the total Zn intake determined by the overall molar ratio of PA and Zn in the meal. The intake of bioavailable Zn by a member of group g with meal m is then calculated as:

\[ BZn_{gm} = k_{gm}Zn_{gm} \]

The function used in this study to specify the dependence of the bioavailability fraction \( k_{g,m} \) on the PA:Zn molar ratio,

\[ k_{gm} = f(PA_{gm} \cdot Zn_{gm}) \]

is given in Table 1. Average rates of daily total Zn, PA and bioavailable Zn intake are then calculated by averaging over all meals consumed per person of group g over a chosen period of time D:

\[ TZn_g = \frac{1}{D} \sum_m Zn_{gm} \]

\[ TPA_g = \frac{1}{D} \sum_m PA_{gm} \]

\[ TBZn_g = \frac{1}{D} \sum_m BZn_{gm} \]
In order to account for uncertainty and variability, the Zn and PA concentrations of ingredients are treated as random variables in the model. In this study we used log-normal distributions to describe their probability density functions (Table 2). Using Monte Carlo simulation with Latin Hypercube sampling, the model propagates the randomness of the respective input parameters into probability distributions of the model outputs. In the present study, 500 equally probable sets of realizations of the random input parameters were used in each simulation to estimate the probability distribution of the computed Zn intake rates. The 2.5% (lower bound) and 97.5% (upper bound) quantiles were taken to quantify the 95% prediction uncertainty (95PPU) interval.

**Model parameterization**
For the case study of scenarios presented here, the parameters (Q, Z, P) were determined for a rural and a suburban population from Isfahan Province in central Iran. The target populations were grouped by age and gender in accordance with the classification used to define gender- and age-specific Estimated Average Requirements (EARs). The EAR values are Zn intake rates at which half of the respective populations meet their requirement (IZiNCG, 2004). The International Zinc Consultative Group (IZiNCG, 2004), the Institute of Medicine (IOM, 2002), and the World Health Organization (WHO, 2006) published different Zn EAR values. We used those of IZiNCG, because they are almost as conservative as those of IOM and differentiate between types of diets. The age groups in our study were: (< 4 yrs), (4-8 yrs), (9-13 yrs), (14-18 yrs), and (≥19 yrs).

**Food consumption data (meal scenarios)**
The food consumption data defining the meal scenarios for the different age and gender groups were obtained from two dietary surveys, one performed in the village of Rooran and the other in the suburban community of Khomeini Shahr. The village was chosen on the assumption that, with agriculture as the main sector of occupation, the population would consume more local products, such as vegetables, dairy, rice and home-made bread than a (sub)urban community, while the suburban population would consume larger varieties of such food commodities.

The surveys included 25 households with a total of 91 individuals in Khomeini Shahr and 28 households with 100 individuals in Rooran. Using the method of three-day-weighed-food records, all food intakes were recorded for each participant on three consecutive days, including 2 week-days and one weekend day, during which the households were asked to
maintain their usual food habits. We weighed the total food prepared for consumption, the proportions consumed by the individual household members, as well as the uneaten remains. Further details and results of the surveys are presented elsewhere (Roohani et al., 2011a, b).

**Zn and PA concentrations of the food items consumed in the meals**

Concentrations of Zn and PA in crop plants vary with soil and climate conditions as well as with cultivation practices (Cakmak et al. 1999). Zn levels in animal foods mainly depend on species, age, gender, and feeding regime (Giuffrida-Mendoza et al. 2007; Mioč et al. 2009; Park 1998). In addition, notable differences have been found for the concentration of Zn between individual meat cuts (Gerber et al. 2009). The Zn and PA concentrations in the final diets also depend on food processing and meal preparation procedures. The variations in Zn and PA concentrations among ingredients were the main source of uncertainty in our analysis. Zn concentrations in dairy products, chicken, and red meat, and Zn and PA concentrations in bread and rice were determined from the analysis of food samples collected during the dietary surveys (Roohani et al., submitted). These foods were the major sources of Zn in the diet. For food items that had not been sampled and analyzed, such as snacks, fruits and vegetables, tea and other beverages, and preserved foods, we used published food composition tables, including those of the USDA ([http://www.nutrisurvey.de/](http://www.nutrisurvey.de/)), FAO, Germany, Egypt, Kenya, India, Mexico ([http://www.nutrisurvey.de/](http://www.nutrisurvey.de/)), and PA data reported by IZiNCG (2004). Published food composition data were also used to determine the variances of the probability distributions for the Zn and PA concentrations of ingredients for which the number of samples was too small in our surveys (Table 2). In the case of red meat, the probability distribution covers the variability in Zn concentration of different cuts of different types of red meat including lamb, mutton, veal, and beef.

**Sensitivity analysis**

We performed a sensitivity analysis to identify the importance of different food items in Zn intake. The quantities of bread, rice, chicken, red meat, poultry, and dairy consumption were varied one at a time by ±5% and ±10% to examine their influence on the calculated Zn intake.

**Scenario analysis**

The scenario analysis was based on the meal composition and consumption data collected in the two surveys. Given that we found only few differences in the dietary patterns of the two
communities and that these did not lead to significant differences in Zn intake rates, we pooled the data of the two surveys. Starting from the status quo as represented by the survey data, past trends in food consumption changes were extrapolated into the future unchanged or with variations due to potential interventions and hypothetical changes in the future socio-economic development of the country.

In our survey bread, dairy, rice, red meat, and poultry constituted 46% of the total food consumed and provided about 80% of the Zn intake. Data published by the FAO (2010) show that the per capita consumption of poultry, fish, fruits and vegetables has strongly increased in recent years, while the consumption of red meat, dairy products, and rice has remained rather stable, and the consumption of wheat, although still the main staple, has started to decrease over the last decade (Figure 1a). Based on the Zn concentrations found in these items in our survey, we estimate that these changes have led to an increase in the average daily per capita Zn intake from around 7 mg to 12 mg (Figure 1b).

The average annual rates of increase or decrease in the consumption of bread, dairy, rice, red meat, and poultry in the past two decades were assumed to hold for our study population. The rationale for this assumption is that (a) good agreement between the FAO averages of per capita food consumption and our survey results (Figure 2a) and (b) our sample populations were very similar in their distributions by age and gender groups to the total population of Iran as reported by the US Census Bureau in 2009 (Figure 2b). The assumption that the annual changes in the consumption of the major food items observed over the past 20 years would continue for the next 15 years provided the “baseline scenario” (Scenario B) of our analysis (Table 3). The annual rate of change in egg consumption was maintained at 4% increase from year to year, while the consumption of other food items was assumed to be constant due to their negligible influence on the dietary Zn intake of the study population.

Based on the baseline scenario B, three “dietary diversification scenarios” were defined. In Scenario D1 we evaluated the effects of increasing the average annual change in bread consumption from -0.2% in the past two decades to 1.5% and in dairy consumption from zero to an annual increase of 5%, while the consumption of rice and poultry remained stable (Table 3). In Scenario D2 we assumed that the consumption of bread would decrease by 1.5% compared to the rate of the preceding year, while that of red meat would increase by 5%, but poultry consumption would not further increase (Table 3). This scenario indicates an improving economic status of the people. In D3, the past trends of annual changes were kept the same as in the baseline scenario for the consumption of bread, rice and poultry, while they were increased to 14% for dairy and 12% for red meat. The idea behind this scenario was to
show what kind of changes would be necessary to achieve a situation within the next 15 years at which the population would meet their EAR in average with a certainty of >97.5%.

Furthermore we defined two “bread fortification scenarios” (F1 and F2), in which food consumption rates were changed in the same way as in the baseline scenario, and a combined “fortification/dietary change scenario” (DF). In Scenario F1 the average Zn concentration of bread consumed by the study population was increased from 11.7 mg/kg to 33.9 mg/kg of fresh weight, based on a study by Khoshgoftarmanesh et al. (2010) in which they increased the Zn concentration of wheat flour by 30 mg Zn/kg using Zn sulfate as fortificant. In Scenario F2 the Zn concentration of bread was only doubled (from 11.7 to 23.4 mg/kg). This scenario covers the potential increase in bread Zn that was achieved by means of biofortification in a study by (Cakmak et al. 2010). Scenario DF combines fortification as in F1 with increased red meat and dairy consumption (5% and 13% annual increase relative to preceding year, respectively), while all other trends were assumed to follow the baseline scenario.

Results
Sensitivity and uncertainty analysis
The sensitivity analysis revealed that bread had the largest effect on total Zn intake followed by dairy and rice (Figure 3a). Comparison between Figures 3a and 3b reveals that ratio between bioavailable and total Zn was lower in bread than in rice, suggesting a larger inhibitory effect of PA on Zn bioavailability in bread due its the higher PA concentration.

In a next step, before embarking on the analysis of the scenarios, we used the model to investigate how the uncertainty in food Zn concentrations affected the estimated Zn intake rates. Using the Monte Carlo uncertainty propagation procedure described before, we ran simulations for 500 realizations of the random model parameters from which we derived probability distributions of Zn and PA intake for each participant of the dietary surveys. These allowed us to construct cumulative frequency distributions of the quantiles of the estimated individual probability distributions. As Figure 4 shows, there was close agreement between the cumulative distributions of measured and predicted median Zn and PA intakes. The average difference between predicted and measured medians was around 3% for Zn and 15% for PA. From these results we concluded that 500 Monte Carlo simulations were sufficient to produce adequate simulations of dietary Zn and PA intake by the participants of the dietary surveys during the 3-days period of recording. The narrow 95PPU bands bracketing the experimental data show that uncertainty due to analytical errors and true
variability in the Zn concentrations of the consumed food was small. The relative uncertainty was larger for PA than for Zn, in agreement with the larger deviation between predicted medians and measured values.

As can be inferred from Figure 5, bioavailable Zn averaged between almost one half and one third of the total Zn intake in the various groups. The ratio between bioavailable and total Zn intake showed a clear trend to decrease with group age, in line with an increasing average PA:Zn molar ratio in the diets of groups. The upper uncertainty bounds of the average PA:Zn molar ratio were below 18 in all but the group of 14-19 years old adolescents. Also in the latter group, the median PA:Zn molar ratio was clearly below this critical threshold. These results reflect a predominance of mixed diets in all population groups. Still, the uncertainty range in Zn intake rates overlapped with the group-specific EAR values proposed by IZiNCG (2004) for many individuals in all groups. As a result the estimated prevalences of inadequate Zn intake showed quite large uncertainty ranges. Using a Zn intake rate below the respective group-specific EAR threshold as indicator of Zn deficiency risk, the estimated average risk of Zn deficiency based on individual median values of Zn intake was 31% across the entire study population, with an uncertainty interval of (28%-35%). Comparing group-specific risks, the maximum was found with 45% (38-52%) in the adult males, and the minimum was in the children of age 9-14, who had no Zn deficiency. The latter were the only group in which the estimated prevalence of inadequate Zn intake did not exceed the 25%-threshold considered critical for public health concern by IZiNCG (2004).

Scenario analysis
The scenario analysis shows that, if the present trends in dietary changes would continue (Scenario B) and thus also the current trend of increasing Zn intake, the risk of Zn deficiency in the study population due to inadequate Zn intake would tend to decrease to below 25% within 12 years even without any intervention (Figure 6). But it would take about 60 years to reach a level at which 97.5% of the population would meet their EAR requirement. If dairy and bread consumption would increase (instead of rice and poultry consumption) as in Scenario D1, the time required to drop the average population risk of Zn deficiency to 25% would be shortened by around 5 years to 7 years, and the expected risk of Zn deficiency would be below 20% within 15 years (Figure 6).

The important role of bread as a dietary Zn source is particularly well illustrated by Scenario D2, in which we assumed an accelerated increase in the consumption of read meat, approximately compensating a stop in a further increase in poultry consumption, and an
accelerated decrease in bread consumption. In this scenario, the increase in dietary Zn intake would be even slightly less than in the baseline scenario, and the expected prevalence of inadequate Zn would just reach the critical level of 25% within 15 years (Figure 6). If the goal would be to drop the population risk of inadequate Zn intake to 2.5% within the 15 years by increasing red meat and dairy consumption, e.g. in response to increased cattle husbandry, then annual growth rates in the consumption of these products substantially higher than 10% would be required, as illustrated by the example of Scenario D3 (Figure 6). According to this scenario the risk of Zn deficiency would drop to 24% already within 3 years.

Implementing Zn fortification of wheat flour or biofortification of wheat grains would have an immediate effect, depending on the resulting level of Zn enrichment in the food items produced with these ingredients, in particular bread. The simulations run for scenarios F1, F2 and DF are based on the hypothetical condition that this could be achieved immediately and that the (bio)fortified bread would be consumed by the entire target population. Under this premise, (bio)fortifying bread would immediately decrease the risk of Zn deficiency in the study population from 30% to 10% in Scenario F1 and DF, and to 16% in Scenario F2. Thereafter, Zn intake rates would further increase gradually according to the assumed rates of change in the diets consumed. If the past trends continue as assumed in Scenario F1 and F2, the risk of Zn deficiency would decrease to 7% and 12% within 15 years, respectively. The simulation for the DF scenario illustrates that the goal of decreasing the prevalence of inadequate Zn intake to 2.5% within 15 years could be reached with substantially lower growth rates in red meat consumption than in Scenario D3, if this increase would be combined with Zn fortification of bread (or flour).

Interventions such as Zn fortification of flour have to take into account that excessive intake rates of Zn can lead to adverse health effects. Figure 7 shows that the Zn intake rates predicted to be reached within 15 years are well below the critical limits in all scenarios for adolescents and adults. For the group of 9-14 year old children the range of within-group variability (including uncertainty in food Zn concentrations) extends beyond all three upper limits of recommended or tolerable Zn intake in Scenario D3 and DF, while for younger children only the WHO limit of safe Zn intake would be exceeded by a fraction of the group in these scenarios, but not those proposed by IZiNCG and IOM. While all these limits include a margin of safety, these results suggest that Scenario D3 and DF are close to what is maximally possible also from the food safety point of view.
Discussion

The baseline scenario B shows that the current trend of rapid increase in poultry consumption is not sufficient to drop the prevalence of inadequate Zn intake substantially below 25% of the population, the level defining the threshold of public health concern according to IZiNCG (2004). Scenario D1 reflects a change to a healthier consumption pattern. Dairy products are popular among Iranians and the assumption of a 5% increase is realistic. Increased consumption in bread would also increase the intake of PA, but in Scenario D1 this would be compensated at least partially by a decreased growth rate in rice consumption and increased consumption of dairy, which does not contain PA. The D2 scenario shows a situation in which meat consumption shifts from white to red meat and the consumption of staple food from bread to rice. Such a trend could result from increased economic wealth and prosperity of the population and promotion of cattle husbandry and beef production in Iran.

The latter is also the rationale underlying Scenario D3 and DF, although in these two scenarios the increased consumption of red meat is in addition to the continued trend of increasing poultry meat consumption. Without the latter an even higher growth rate in red meat and/or dairy consumption would have been required to achieve the goal of decreasing the prevalence of inadequate Zn intake to ≤2.5% in the study population. Such a scenario is rather unrealistic, however, because increasing red meat consumption by 14% each year is neither affordable for most households, even if the economic situation should rapidly improve, nor healthy. Considering increasing white meat instead of red meat consumption to reach the same goal is not more realistic. Given that Zn intake was less sensitive to the same relative change in the consumption of white meat than in red meat, as demonstrated also by our sensitivity analysis, white meat consumption would have to increase by about 35% each year relative to the level of the year before to achieve the same level of Zn adequacy after 15 years as with the 12% growth rate in red meat consumption in Scenario D3.

Fortification of bread seems to be the most practical and effective intervention to achieve a rapid and substantial improvement in the Zn status of the Iranian population. Bread is consumed in rather stable and predictable amounts by a large proportion of the Iranian population. Following the WHO guidelines for food fortification (WHO 2006; 2009), the fortificant would be added to the flour, which is centrally produced, processed, and then distributed to the bakeries via local distribution networks. Conventional fortification could also be supplemented or even replaced by biofortification. This is another way to enrich the grains of staple crops such as wheat and rice with essential micronutrient elements such as
Zn, using genetic (e.g. breeding) or agronomic (e.g. fertilization) methods (Graham and Welch 1996; Graham et al. 2001; Welch 2003; Cakmak, 2009; Schulin et al. 2009).

In discussing the scenarios analyzed here, it should be noted that they were chosen simply to illustrate the potential use of the model. There are many more alternative scenarios one could think of and many of these may be more appropriate than the ones chosen here. A systematic scenario analysis covering a wider range of alternatives, however, was beyond the scope of this study. But independent of the choice of scenarios, there are some general assumptions implicitly underlying the approach taken here that require discussion. The first of these assumptions is that the food consumption data collected in the survey are indicative of the average Zn intake rates in the study population over longer periods of time, because the Zn status of the human body and thus the risk of Zn deficiency does not vary substantially over periods of a few days or weeks. The fact that the average food consumption rates of our study population were comparable to those published by the USDA for the entire Iranian population indicates that the 3-day food records also gave a good indication of the foods consumed on average by the study population over longer time periods. Seasonal variations did not seem to have a strong effect, as indicated by the fact that the surveys were performed during summer in the rural and during winter in the suburban community. While this supports the assumption that our data provide a valid basis for the estimation of average Zn intake rates in the population as a whole and likely also for the age and gender groups selected here, the estimation of Zn deficiency risk is based on the distribution of these rates among individuals within the respective population groups. If these would fluctuate around the group mean over a period of weeks or months, then the variation of rates between individuals determined by means of a 3-days food consumption record may overestimate the variability in long-term average rates and thus over- or underestimate the prevalence of Zn deficiency, depending on whether the group mean lies above or below the critical threshold.

In addition, the implicit assumption underlying the scenario analysis was that the same trends in food consumption equally apply to all subgroups in the population, regardless of social or economic status. For example, it may be expected that absolute rates of meat consumption will increase more on average in more affluent parts of the population than in groups with low income who already cannot afford to consume as much meat as people with higher income. By defining the trends in terms of relative rates of change, i.e. percentage increase or decrease in the consumption of a certain food item relative to the year before, and not in terms of absolute rates of increase or decrease, we strived to account at least to some degree for such differences among sub-groups. In absence of more detailed data, we consider
this a plausible first approach. But a more detailed study on recent trends in the dietary habits of the Iranian population and their variability among groups accounting for the influence of socio-economic and other factors would certainly be a warranted sequel to this study.

Despite these reservations, the study illustrates how our model can be used to predict the outcome of different scenarios of food consumption trends and interventions in combating Zn deficiency. Consideration of parameter uncertainty is crucial in doing so as demonstrated by the wide ranges of uncertainty in the estimates of Zn deficiency risks in the study population.

References


Table 1. Values of the Zn bioavailability index \( k \) used in this study. The values are based on IZiNCG (2004).

<table>
<thead>
<tr>
<th>Group</th>
<th>( 4 \leq \text{PA:Zn} \leq 18^a )</th>
<th>( \text{PA:Zn} &gt; 18^b )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female ≥ 19 yrs</td>
<td>0.34</td>
<td>0.25</td>
</tr>
<tr>
<td>Male ≥ 19 yrs</td>
<td>0.26</td>
<td>0.18</td>
</tr>
<tr>
<td>Children &lt; 19</td>
<td>0.31</td>
<td>0.23</td>
</tr>
</tbody>
</table>

\( ^a \) mixed or refined vegetarian diet  
\( ^b \) unrefined, cereal based diet
Table 2. Parameters (means and standard deviations) of the lognormal probability distributions used in this study to model the uncertainty in food Zn concentrations (mg/100 g). The distributions include the variability among different types and varieties of the respective food items consumed in Iran.

<table>
<thead>
<tr>
<th>Food item</th>
<th>Mean (mg/100g)</th>
<th>Standard deviation</th>
<th>Data source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice</td>
<td>0.41</td>
<td>0.21</td>
<td>Roohani et al. (submitted)</td>
</tr>
<tr>
<td>Egg</td>
<td>1.53</td>
<td>0.4</td>
<td>Roohani et al. (submitted)</td>
</tr>
<tr>
<td>Meat</td>
<td>4.45</td>
<td>1.50</td>
<td>Roohani et al. (submitted)</td>
</tr>
<tr>
<td>Chicken</td>
<td>1.42</td>
<td>1.06</td>
<td>Roohani et al. (submitted)</td>
</tr>
<tr>
<td>Dairy</td>
<td>0.67</td>
<td>0.5</td>
<td>Roohani et al. (submitted)</td>
</tr>
<tr>
<td>Bread</td>
<td>1.17</td>
<td>0.27</td>
<td>Roohani et al. (submitted)</td>
</tr>
<tr>
<td>Snacks</td>
<td>0.76</td>
<td>0.52</td>
<td>USDA, Mexico, German, Egypt, Kenya, India, FAO*</td>
</tr>
<tr>
<td>Fruits</td>
<td>0.13</td>
<td>0.08</td>
<td>USDA, Mexico, German, Egypt, Kenya, India, FAO*</td>
</tr>
<tr>
<td>Vegetables</td>
<td>0.40</td>
<td>0.60</td>
<td>USDA, Mexico, German, Egypt, Kenya, India, FAO*</td>
</tr>
<tr>
<td>Juices</td>
<td>0.11</td>
<td>0.14</td>
<td>USDA, Mexico, German, Egypt, Kenya*</td>
</tr>
<tr>
<td>Tea</td>
<td>0.11</td>
<td>0.27</td>
<td>USDA, German*</td>
</tr>
<tr>
<td>Nuts</td>
<td>2.72</td>
<td>1.02</td>
<td>USDA, Mexico, German, Egypt, Kenya, FAO*</td>
</tr>
<tr>
<td>Seeds</td>
<td>6.09</td>
<td>1.56</td>
<td>USDA, Mexico, German, Egypt, India*</td>
</tr>
</tbody>
</table>

*http://www.nutrisurvey.de/*
Table 3. Nutrition scenarios analyzed in this study (see Section 3.3).

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Change in consumption rate relative to previous year (in %)</th>
<th>Bread Zn concentration (mg kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B Baseline</td>
<td>Change in consumption rate relative to previous year (in %)</td>
<td>Bread Zn concentration (mg kg⁻¹)</td>
</tr>
<tr>
<td>D1 (↑Bread) &amp; (↑dairy)</td>
<td>1.5 0 0 0.4 5 11.7</td>
<td></td>
</tr>
<tr>
<td>D2 (↓Bread) &amp; (↑red meat)</td>
<td>-1.5 1.5 0 5 0 11.7</td>
<td></td>
</tr>
<tr>
<td>D3 Lower Zn deficiency &lt; 2.5%</td>
<td>-0.2 1.5 6.5 12 14 11.7</td>
<td></td>
</tr>
<tr>
<td>F1 Fortification of bread</td>
<td>-0.2 1.5 6.5 0.4 0 33.9</td>
<td></td>
</tr>
<tr>
<td>F2 (Bio)fortification of wheat</td>
<td>-0.2 1.5 6.5 0.4 0 23.4</td>
<td></td>
</tr>
<tr>
<td>DF (↑red meat &amp; ↑dairy) + fortification</td>
<td>-0.2 1.5 6.5 5 13 33.9</td>
<td></td>
</tr>
</tbody>
</table>
Figure 1. (a) Trends in the consumption of different food groups by the Iranian population from 1961 to 2007 according the FAO (2010). The broken lines belong to the secondary axes on the right. (b) Trend in average Zn intake resulting from the FAO consumption data and Zn concentration data found in this study.
Figure 2. (a) Comparison of the consumption of major foods (red meat, chicken, egg, rice, bread and dairy) found in this study (boxplots) with average consumptions estimated by FAO in 2007 (triangles) for the population of Iran. No value was given for bread in the FAO food balance sheets. The consumption of wheat, which includes bread consumption, is much larger than bread alone. (b) Population distribution by age groups of the test population in this study in comparison to the total Iranian population as reported by the US Census Bureau (2009).
Figure 3. (a) Sensitivity of Zn intake to variations in the consumption of different food groups. (b) Changes in the intake of total and bioavailable Zn as a function of bread and rice consumption.
Figure 4. Measured and simulated Zn (a) and PA (b) intake in the study population. The shaded region shows the 95% prediction uncertainty (95PPU) interval.
Figure 5. Median values and ranges (intervals between 2.5% and 97.5% quantiles) of (a) individual daily intake of total and bioavailable Zn and the Estimated Average Requirements (EAR) of different age/gender groups, and (b) average PA:Zn molar ratio and prevalence of Zn deficiency in the different age/gender groups of the study population, based on 500 Monte Carlo simulation runs to account for uncertainty in the Zn concentration of the consumed food items.
Figure 6. Predicted total Zn intake (a) and Zn deficiency (b) in the study population over the 15-year period from 2009 to 2024 for the food consumption scenarios defined in Table 3.
Figure 7. Expected Zn intake rates (group means and 95% ranges of within-group variability) in the different age/gender groups distinguished in the study population after 15 years for the 7 scenarios defined in Table 3. Also shown are the upper limits of safe intakes by IZiNCG, IOM, and WHO.
CONCLUSIONS AND PERSPECTIVES

All five objectives of this thesis were achieved (see introduction). Knowledge of the nutrient content of foods is essential for many types of nutrition research and applied nutrition projects, including the interpretation of food consumption studies, the nutritional assessment of food supplies, and the planning of nutritionally adequate diets. Appropriate nutrient databases are not always readily available for these activities. We thus hope that our analysis of major basic foodstuffs and composite meals consumed in the two surveyed communities provides information on the Zn, Fe and PA concentrations of common Iranian foods that is useful beyond the immediate scope of this study.

Many scientific studies have included the measurement of nutrients in foods, and reports of these nutrient values are found throughout the scientific literature. However, assembling the available data, evaluating their reliability, and summarizing them into meaningful tabulations are an important research task. Such work is quite essential and it is especially missing for Iran. Our results show that using food composition tables from other countries is very problematic. We recommend a further, more comprehensive and nationwide studies of food composition in Iran.

Although soils of Iran are reported to be often deficient in Zn, the Zn concentrations of rice and bread were not very low in our study. The PA analyses, and the calculated PA:Zn molar ratios, indicated low Zn absorption from common flat breads (PA:Zn molar ratio 20-22) but higher absorption from composite dishes containing cereals and legumes with meat. These dishes had PA:Zn molar ratios ranging from 4-13. The analyses of dairies and cheese, of raw and cooked rice, of raw and cooked legumes, and of flour and bread indicate that processes used in food manufacture and kitchen preparation can have a major influence on Zn concentrations in the final product. Here may be a large potential to improve micronutrient nutrition. Thus, the impact of food manufacturing and preparation processes on micronutrient concentrations and bioavailability in foods should be further explored systematically in width and depth.
Based on the measured serum Zn concentrations, Zn deficiency was indicated in only 6-7% of the two studied communities. As Zn supplements were not routinely taken by our study subjects, the most likely explanation is an adequate dietary intake of bioavailable Zn. The PA:Zn molar ratios for the whole diet ranged from 5-15, indicating a modest Zn bioavailability diet. The 18.3% prevalence of estimated inadequate Zn intake based on the 3-days food records of the study populations was little higher than the 6-7% estimate of Zn deficiency based on the blood samples. This estimate was obtained using the WHO EAR values as critical thresholds. Using the FNB/IOM and IZiNCG EAR values the estimated frequency of of inadequate Zn intake would have been even higher. Using the IZiNCG values predicted a 33.5% and the FNB/IOM values 41.4% prevalence of low Zn intake. Our results thus suggest that the FNB/IOM and IZiNCG EAR values may overestimate the prevalence of inadequate Zn intake for our study populations. Determining Zn intake rates from 3-day food consumption records may have resulted in over-estimated variability among individuals. On the other hand, it has to be considered that limitations do not only apply to the assessment of Zn intake rate but also to measurement of serum Zn level as indicator of Zn sufficiency or deficiency.

The Zn intake model developed here is generic and can be applied wherever the respective input data on food consumption rates and food composition are available or can be generated. The bioavailability is accounted for in the current version of the model by multiplying total Zn intake per meal by a bioavailability coefficient based on the overall PA:Zn molar ratio of the respective meal. However, it is easily possible to implement also other approaches such as the absorptivity model based on an explicit formulation of the biochemical Zn-PA interaction as proposed by Miller et al. (2007). Of the scenarios simulated, it seemed like flour fortification is the most effective and safest strategy to combat Zn deficiency. It lowers the Zn deficiency from about 30% to below 7% in 15 years without causing Zn toxicity in the population. Also, biofortification could be another effective measure in lowering Zn deficiency to 12% after 15 years.

A more systematic scenario analysis covering a wider range of alternatives deserves more study and investigation in the future. Also, assessing and including other possible sources of uncertainty such as the uncertainty in the quantity of food consumed in the Zn intake of the population is recommended. Some limitation in the representativeness of the scenario analysis may be due to variability in the dietary patterns not accounted for in the surveys, which covered
only 3 days per household and only a rather small sample size of participants. Only longer surveys repeated in different seasons on larger numbers of participants can show how relevant the limitations of shorter singular surveys of smaller population samples actually are. Irrespective of present shortcomings and potential further improvements, the study demonstrates that the model can serve as very useful tool to assist designing and to evaluate appropriate and efficient intervention strategies.