Zinc Adequacy in the UK Population

Farzad Amirabdollahian

A thesis submitted in partial fulfilment of the requirements of
London Metropolitan University
For the degree of
Doctor of Philosophy

October 2008

Abstract

Zinc (Zn) is an essential trace element with wide range of biochemical and physiological functions. Human Zn requirements are estimated by a consideration of the physiologic Zn requirement, coupled with an estimate of the proportion of Zn absorbed from the usual diet.

An accurate assessment of Zn status in a population is difficult because there is no reliable and globally accepted biomarker and/or index of adequacy. An initial analysis of the National Diet and Nutrition Survey (NDNS) showed that in the UK population average Zn intake ranged from 4.18 mg/day to 10.56 mg/day; and that, based on the current UK Zn Dietary Reference Values (DRVs), a substantial proportion of population sub groups appear to have inadequate Zn intake.

UK DRVs have not been revised since publication, in 1991; however, since then both the knowledge about Zn requirements and the characteristics of the UK population (e.g. average body weight) have substantially changed. In light of this, there is a need to re-consider the UK DRVs. Following the approaches used by recent national and international expert committees, this thesis has developed new estimates for the physiologic Zn requirements of the UK population. In order to develop DRVs, based on these, new estimates of Zn absorption were also needed, to take into account up-to date data on the average phytate and Zn intakes of the population.

There is little data on the phytate content of UK foods so tables of the phytate content of foods were constructed from the best available international data. These were used with the average weight of foods consumed in the NDNS to estimate the median phytate intake in the UK population (ranging from 463 mg/day in toddlers to 948 mg/day in adults). In order to validate the phytate tables for use in the UK, an experimental protocol using Ion Pair High Performance Liquid Chromatography (HPLC) was used. It was found that the method was not precise, linear or accurate for the measurement of the phytate content of foods. The validation of these tables remains a priority for further research.

The validity of two recently published models to estimate Zn absorption in the UK population was investigated. Although there was evidence to support validity of the most recently developed mathematical model, it proved unable to estimate proportion of Zn absorption, using parameters for the UK population. A logit regression model was found to be valid and this, used together with estimates of dietary Zn and phytate intake, gave estimates of Zn absorption for the different subgroups of UK population.

Using the new estimates of physiologic Zn requirement and Zn absorption determined in the thesis, a revised version of UK DRVs was developed. The application of these revised DRVs to NDNS data demonstrated that the risk of inadequate zinc intake for many subgroups of the UK population was higher than estimates of risk made on the basis of the existing DRVs. 'Inadequate Zn intake' was associated with a poorer quality diet and the selection of a diet of low Zn density. The health and nutritional status of the population classified with 'inadequate zinc intake' was considered; biomarkers indicated that they had poorer Zn status and that they had significantly more CHD risk factors than those classified with 'adequate Zn intakes'.

However, these findings must be treated with caution, as higher Zn DRVs would lead to a larger proportion of the UK population being considered Zn inadequate and there is little independent data to suggest that this is a problem. Additionally, the study did not take account of the possibility of adaptation to low zinc intakes, the role of other factors in the absorption process, the validity of phytate data for the UK and the robustness of the studies used in the theoretical model of Zn requirements.

Current results suggest that UK DRVs need to be revised. There is a need for systematic national review of the current UK zinc DRVs.

To:

My family. Dad and Mum, for teaching me to be a man of principle and value before being a man of success. Nastaran and Nazanin, for making me smile, even at the darkest moments of my life and to my dear brother Farshid, for his constant and unconditional love and support.

To:

My body, for surviving studying at days and working at nights.

And to:

People who are at risk of zinc deficiency.

Acknowledgements

The following people and organisations deserve my gratitude for their involvement in conducting this research and/or preparation of this thesis:

Firstly, I would like to thank Dr. Ruth Ash for supervising this work, for her advice, guidance and encouragement from the very early stage of this research and in particular for being a great friend and for her invaluable moral support. I am indebted to Ruth more than she knows.

Thanks to my dear teacher Dr. Angela Madden at University of Hertfordshire, for carefully reading and commenting on my thesis, for her friendship and advice and for teaching me about the design concepts of a research project. Angela's scientist intuition has made her my role model and has inspired, and enriched my growth, as a student, a researcher and as the scientist want to be.

Grateful thanks to my second supervisor Dr. David McCarthy, as well as Dr. Soma Mitra, for their constructive comments on this thesis. I am thankful that in the midst of all their very busy academic activities, they accepted to read my thesis and helped me to improve my work. I would also like to thank Dr. Ken White for reviewing Chapter 6 and providing positive feedback and Dr. Simon Dryden for his support and encouragement.

Thanks to Dr. Brian Bointon, the Dean of the Faculty of Life Sciences, for his constant support and for providing financial assistance for my conference presentations throughout my studies.

For the proof reading of the thesis, I would like to especially thank my dear friend; Thomas Kealy. From the other side of the Atlantic, Tom stayed awake for many nights, helped me to meet my deadlines, and never gave up on correcting my endless grammatical errors. Thanks also to my friends Dimple Radia and Silvia Loureiro for reviewing my English and for their moral support.

This research would not have been possible without support of the UK Data Archive. Substantial part of this research was based on the National Diet and Nutrition Surveys 1992-2001. I would like to acknowledge Food Standard Agency, Ministry of Agriculture, Fisheries and Food, The Department of Health and The Data Archive, University of Essex as related responsible organisations of the surveys.

I would like to thank Prof. Donald Oberleas at Texas Tech University and Prof. Barbara Harland at Howard University for their advice, support and guidance in using HPLC for the assessment of phytate content of foods.

For providing part of the data on phytate content of foods, I would like to thank: Mark Roe and Paul Finglas at Food databanks, Institute of Food Research; Dr Anne-Louise Heath at University of Otago; Dr Torbjörn Lind at Umea University Hospital; Steve Wearne and John Dixon at Food Standard Agency; and the World Food Dietary Assessment System at Food and Agriculture Organisation of the United Nations.

Grateful thanks to Dr Kathryn Pinna and Professor Janet King at University of California and in particular, to Dr Leland Miller at University of Colorado for providing data, advice, and guidance regarding modelling of zinc absorption.

For their laboratory expertise, I would like to thank Brigitte Awamaria, Karlene Marsh, Pramila Haria, Dr. Phil Baillie and in particular, Wimal Dissanayake. I would also like to thank Dr. Mikis Stasinopolous for reviewing part of my statistical analysis and discussing it with me.

Many thanks to my dear friends: Dr. Kris De Meyer, Dr. Outi Remes, Dr. Robin Wolf, Dr. Maeve Cosgrove, Rui Loureiro, Anna-Maria Hartikainen, Dulcidio Coelho, Stefan Jansen, Yu Ling Du, Mana Golesorkhi, Mark Thatcher, Nick Karagiannis, Lars Evje, Mary Nobakht and especially to Ahmad Reza Laleh, for their friendship and their moral support.

And most importantly, my greatest thanks go to my family; Mum, Dad, Farshid, Nastaran and Nazanin. They supported me emotionally and financially through these years and have always been source of invaluable love and constant inspiration.

Table of contents

List of Tables

List of Figures

Chapter 1: Introduction to the Thesis

1.1 Aim

The aim of this thesis is to consider the current UK zinc Dietary Reference Values (DRVs) and to assess the risk of inadequate zinc intake in the UK population.

1.2 Objectives

- To review the current knowledge about the role of zinc in human nutrition.
- To assess the zinc intake and status of the UK population using the available theoretical measures (i.e. current DRVs and cut-off points of serum zinc concentration) in order to provide a picture of the UK zinc status and to identify vulnerable groups.
- To incorporate the latest evidence into the theoretical model of zinc requirement, in order to derive an updated version of the estimated physiologic zinc requirements.
- To review the current models that estimate zinc absorption as a function of dietary zinc and phytate intake, and to assess the validity of these models.
- To produce a database on the phytate content of foods consumed in the UK.
- To investigate if Ion Pair HPLC is a valid method for the assessment of phytate in food sources and to validate the tables of phytate content of foods for the UK.
- To estimate dietary phytate intake and the dietary phytate: zinc molar ratio of the UK population.
- To estimate the proportion of zinc absorption in the UK, using updated information on dietary intake of zinc and phytate.
- To revise the UK zinc estimated average requirement (EAR) and to derive a revised set of zinc DRVs for the UK population.
- To assess the adequacy of dietary zinc intake of the UK population using the revised DRVs, in order to provide a better picture and understanding of the UK nutritional zinc status.

- To identify the high-risk groups who can be targeted by public health interventions and characterise the health and nutritional status of these vulnerable groups.

1.3 Hypotheses and research questions

1.3.1 Hypotheses

1. Zinc intake of a large proportion of the UK population is less than LRNI.
2. The Ion Pair HPLC is a valid method for the assessment of phytate content of foods.
3. The mathematical model of zinc absorption as a function of dietary zinc and phytate is a valid model for the estimation of proportion of zinc absorption in the UK population.

1.3.2 Research questions

4. Are the current UK zinc DRVs based on the latest knowledge of physiologic requirement?
5. Are the current UK DRVs based on up-to-date estimate of the proportion of zinc absorption, allowing for gender and age differences?
6. Using revised zinc DRVs, will the picture of the UK zinc adequacy and vulnerable groups to inadequate zinc intake be different from the current picture?

Flowcharts summarising the conceptual framework and outline of the thesis are presented in Figure 1.1 and Figure 1.2.

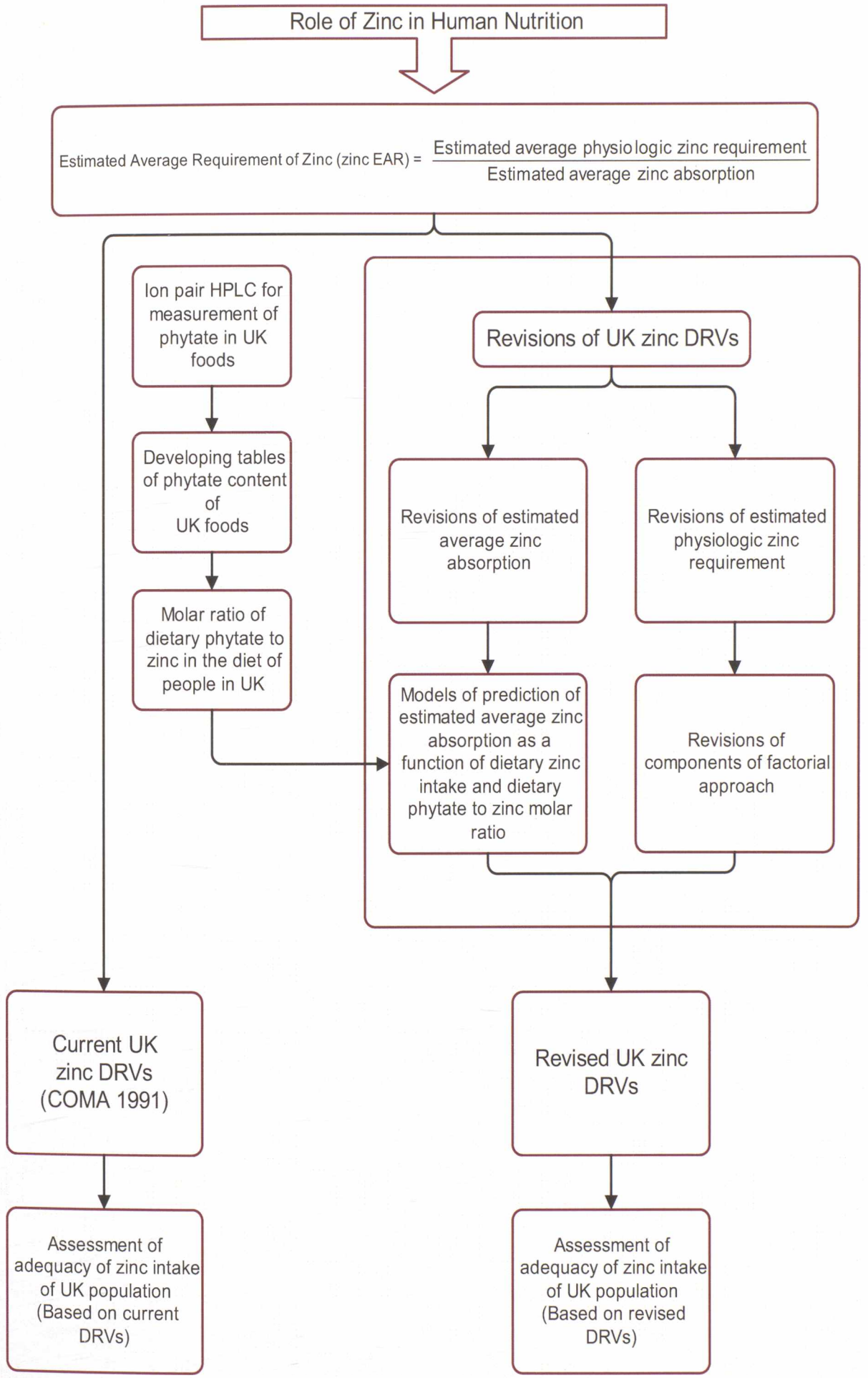


Figure 1.1 Flowchart summarising the conceptual framework of this thesis.

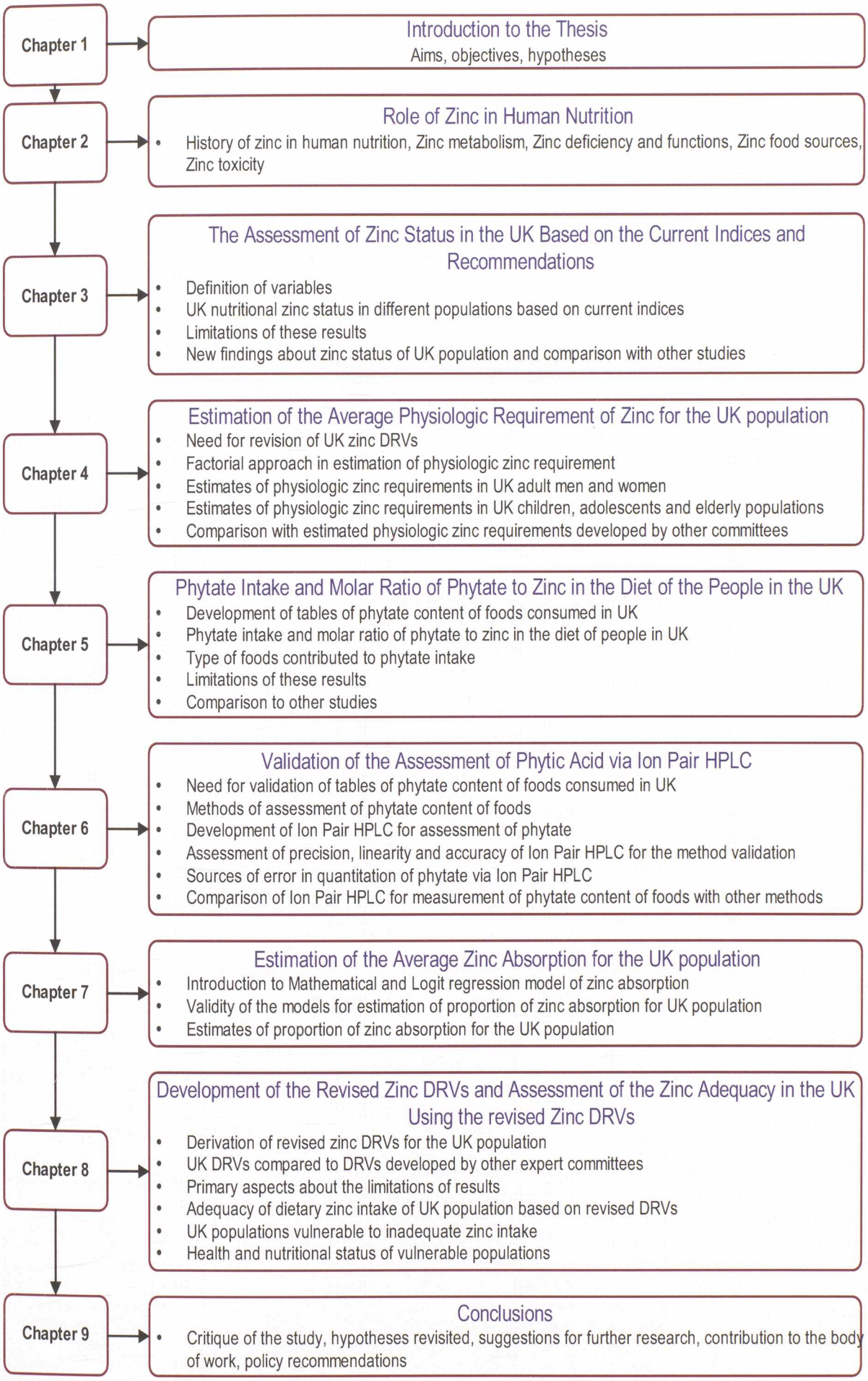
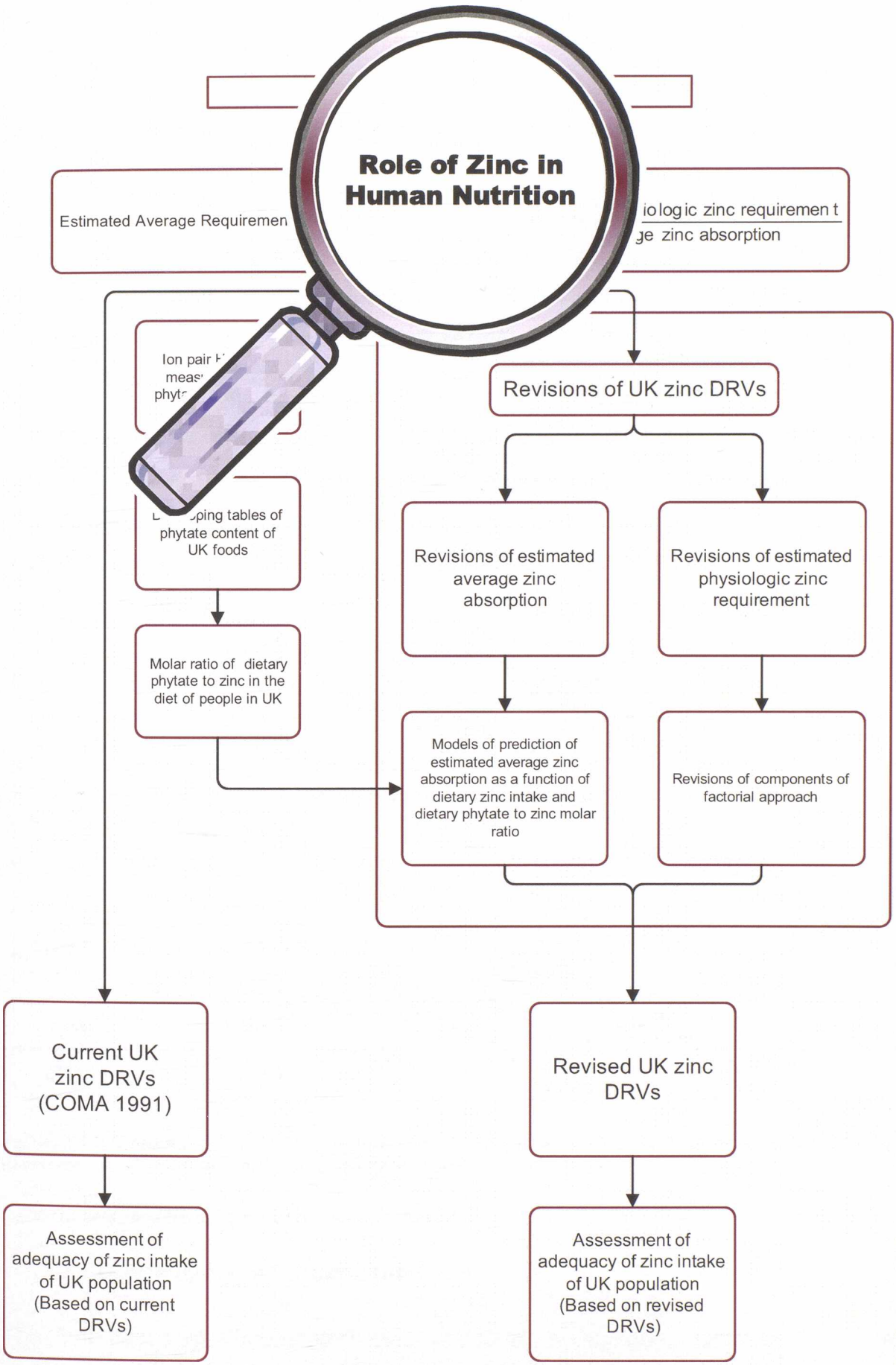


Figure 1.2 Flowchart demonstrating the structure of this thesis.

Chapter 2: The Role of Zinc in Human Nutrition



The magnifier symbol shows where you are in the conceptual framework of the thesis.

2.1 Historical aspects

2.1.1 Background

Although Zinc was established as a distinct element in 1509 [1], it's believed that centuries before that, Romans used its ores to make brass. Marco Polo, described the manufacture of zinc oxide in Persia and how the Persians used to prepare Tutia (a solution of zinc vitriol) for healing sore eyes [2]. Both metal and zinc oxide were produced in the twelfth century in India and later on in China. Paracelsus was the first scientist to state clearly that 'Zincum' was a new metal with distinct properties [2].

The essentiality of zinc for microorganisms, plants and animals has been known for more than a century. In 1934 Todd and his colleagues established that zinc is essential for the growth of rats [3]. Major research that has been conducted in microorganisms, plants and animals, is summarized in Table 2.1. Later on, these studies made a notable impact on the discovery of zinc essentiality for humans.

Researcher	Year	Summary
Raulin	1869	1st evidence of zinc essentiality for the growth of Aspergillus Niger
Bertrand & Javillier	1911	Confirmation of Zn role in the growth of Aspergillus Niger
Sommer & Lipman	1926	Evidences of Zn deficiency in onions and potatoes as the first establishment of Zn role for higher forms of life
Bertrand & Benson	1922	Unsuccessful attempts for the demonstration of Zn essentiality in animals
McHargue	1926	
Hubbel & Mendel	1927	
Todd *et al*	1934	First evidence that Zn is essential for the growth of rats
Nishimura	1953	Report of clinical manifestations of Zn deficiency in suckling mice
Tucker & Salmon	1955	Report of role of Zn in prevention and treatment of Parakeratosis in swine
O'Dell *et al*	1958	Evidence of Zn essentiality for the growth of birds
Miller & Miller	1960	Confirmation of features of Zn deficiency in experimental deficiency of Zn in calves
Blamberg *et al*	1960	Report on clinical consequences of Zn deficiency in the diet of breeding hens

Table 2.1 Summary of the main studies in microorganisms, plants and animals before the discovery of zinc essentiality of humans in 1961.

Table is adapted from the text of reference [4].

2.1.2 Zinc deficiency in humans (males)

The first cases of documented zinc deficiency in humans, dates back to the autumn of 1958, at Saadi Hospital in Shiraz, Iran. Dr Ananda Prasad visited a 21-year-old male patient who appeared like a 10-year-old boy. Besides severe growth

retardation and dwarfism, the patient was suffering from hepatosplenomegaly, rough and dry skin, mental lethargy, geophagia (clay eating) and iron deficiency anaemia. The patient's unusual diet was mostly unleavened bread and very restricted in animal proteins. Although his intake of energy and protein (which was mostly cereal based) was adequate, he consumed 0.5 kg of clay daily [4].

In the next three months, 10 patients with similar characteristics were visited in the same hospital. The fact that growth retardation and testicular hypofunction was not observed in iron deficient animals, refuted the theory that iron deficiency was responsible for the symptoms.

Later on and in the Nile Delta of Egypt, when Prasad visited patients with similar clinical features, he started searching for a common cause of the problem. The Iranian patients had a history of geophagia (which could have explained their more pronounced hepatosplenomegaly). Although the Egyptian cases were missing this symptom, they had a high prevalence of Schistosomiasis and hookworm infections. The dietary pattern of the Egyptian patients was similar to that of the Iranians; very carbohydrate-based (bread and beans in particular) and included negligible amounts of animal proteins [4-6].

In 1961, Prasad and his colleagues provided a detailed description of the clinical syndrome in Iran. At that time, they had no data to certainly document the cases as zinc deficient, but he speculated that zinc deficiency could have caused growth retardation, gonadal failure, skin change and mental lethargy [7].

2.1.3 Zinc deficiency in humans (females)

As all the cases of syndrome seen in Iran and Egypt were males, it was thought that the syndrome of zinc deficiency was exclusive to males. In 1969, a 20-year-old female was admitted in Saadi Hospital in Shiraz, Iran with a chief complaint of weakness and paleness. Laboratory findings on admission revealed iron deficiency anaemia and a very low level of zinc in plasma and red blood cells. Although she had had a history of geophagia in her childhood, the female patient had no history of blood loss from any source and had never menstruated. Treatment started with a well-balanced diet of 2500 kcal/day together with 100 mg ferrous fumarate per day following by 120 mg zinc sulphate (which contains 27 mg of elemental zinc). After three months of therapy, the concentration of zinc in plasma and red blood cells became normal. Her height and weight on admission were 132 cm and 40 kg respectively, but after 6 months of treatment, her height

increased to 146 cm and her weight to 50 kg. On the 45th day of her treatment, she menstruated for the first time in her life. On completion of the 6-month treatment, she was in perfect health and requested a discharge from the hospital. Admission of a 19-year-old girl in the same hospital supported the findings of the first case [6].

2.1.4 Zinc deficiency in the developed world

Because of the absence of a known sign of zinc deficiency in the developed world, it was assumed that zinc disorders are limited to less developed countries. Two particular studies in industrialized countries highlighted the practical importance of zinc:

During the 1970s, Dr. Michael Hambidge conducted a series of randomised control studies in Denver, US demonstrating that marginal zinc deficiency and its consequences, particularly growth impairment, could be common in some children [8, 9]. In 1972, he showed that in children of middle and upper income families, low hair zinc concentration was associated with impaired taste acuity, poor appetite and a low growth percentile [10].

2.2 Chemistry

Zinc is the last transition element in the series of the fourth period (IIB element) of the periodic table, with a completed subshell and two additional S electrons. It has an atomic number of 30 and atomic weight of 65.37 (isotopic mean) and has a bluish-white colour in pure form. There are several isotopes of zinc. Five recognised stable isotopes of zinc and their abundance on planet earth are ^{64}Zn; 48.89%, ^{66}Zn; 27.91%, ^{67}Zn; 4.11%, ^{68}Zn; 18.57% and ^{70}Zn; 0.62% [11].

Six radioisotopes of zinc have been identified, three of them including ^{65}Zn, ^{69m}Zn and ^{63}Zn with respective half-lives of 245 days, 13.8 hours and 38 minutes. These are often used in tracer studies, which ^{65}Zn appears to be the most useful radioisotope in biomedical sciences [11, 12].

Zinc is a reducing agent, which in biological systems is usually in a divalent state. This element typically forms stable complexes with a coordination number of four with tetrahedral disposition of ligands around the metal. These complexes include a wide range of chemical components such as other ions, salts and members of the halogen family as well as carbonates, phosphates, sulphates, oxalates and phytates. In physiologic environments, these complexes are in the form of ligands

such as amino acids, peptides, proteins and nucleotides. Special affinity of zinc for thiol and hydroxy groups and for ligands containing electron-rich nitrogen as a donor, may explain the role of factors, which affect zinc absorption [11].

2.3 Metabolism

2.3.1 Zinc occurrence in the body

Although zinc is found in all organs, tissues, fluids and secretions of the body, it is primarily an intracellular ion. More than 95% of total body zinc is present within cells. Inside the cells, zinc is associated with all organelles; however, about 60% to 80% of cellular zinc is cytosolic [11, 13, 14].

Zinc is widely distributed throughout the human body and it is second most abundant after iron, among the trace elements. The body of a newborn contains approximately 60 mg zinc. This is based on an estimation of total body concentration of 20 µg/g. During growth development and maturation, the zinc concentration of the human body increases to 30 µg/g so that it is estimated that the adult human body contains 1.5-gram zinc in women and 2.5-gram zinc in men [11].

The highest zinc concentration can be found in hair, bone, the liver, kidneys and muscles. Other tissues with high zinc concentration include various parts of the eye (cornea, iris, retina and lens), glands such as the pancreas and prostate, spermatozoa, skin, and the finger and toe nails [11, 15]. Table 2.2 demonstrates the approximation of zinc concentration and content in various organs and tissues of an adult man.

As a general rule, zinc is found wherever protein is [14]. Because of the proportion and volume of the skeletal mass and bone in the body, zinc in these tissues contains the majority (83%) of whole body zinc [16]. The fundamental differences in the amount of fat-free mass tissues between males and females may explain the higher content of zinc in males.

Plasma zinc concentration is about 75 to 125 µg/dl [17]. Tissues do not easily give up their zinc so when blood level falls, frequent dietary intake is necessary [14]. Alternatively, during zinc depletion when total body zinc is decreased, zinc losses among all tissues are not uniform. Zinc levels decline in the bone, liver, testes, hair and plasma, while the brain, lung, skeletal muscles, skin and heart are insensitive to reductions (or increases) in zinc intake. It is not known why the dietary intakes

of zinc are reflected in the zinc concentration of some tissues, but not other tissues [16, 18].

Although there is no conventional tissue zinc reserve that can be released in response to dietary zinc decline, it has been suggested that bone is the passive reserve. Bone has a regular turnover of osseous tissue and some zinc may become available during this process. Bone zinc release does not appear to increase in response to the dietary inadequacy, but when the dietary intake is too low, maybe less zinc (that is released in regular bone remodelling) is re-deposited in the skeleton. The physiologic importance of this phenomenon is highlighted in growing individuals as they have a more frequent bone turnover [16].

Tissue	Zinc Concentration (mg/kg wet weight)	Total zinc content (mg)	Proportion of total body zinc (%)
Skeletal muscle	50	1400	62.9
Skeleton			
Bone	90	450	20.2
Marrow	20	60	2.7
Cartilage	11	11	0.5
Periarticular tissue	34	30	1.3
Liver	40	72	3.2
Lung	40	40	1.8
Skin	15	39	1.7
Whole blood	6	33	1.5
Kidney	50	15	0.7
Brain	10	14	0.6
Teeth	250	11.5	0.5
Hair	200	4	0.2
Spleen	20	3.6	0.2
Lymph nodes	14	3.5	0.2
Gastrointestinal tract	15	1.8	0.1
Prostate	100	1.6	0.1
Others (adipose, etc)	2	35	1.6
Total		2225	100

Table 2.2 Approximate concentration and content of zinc in some organs and tissues of an adult (70 kg) man.

Table is adapted from reference [19].

2.3.2 Zinc uptake

Like iron and some other minerals, the absorption of zinc is relatively poor. It is assumed that from an approximate daily zinc consumption of 4-14 mg, only 10% to 40% will be absorbed [12]. Although zinc absorption and excretion are homeostatically controlled, the mechanisms of this balance is still not fully clear [15, 17, 20].

Zinc is mostly absorbed throughout the small intestine. The other sites of absorption, including the stomach and large intestine, contribute only a small amount of absorption [11].

In a study published by Lonnerdal in 2000, where there was no interference from the other meal components, more than 70% of the small zinc dose (less than 3 mg) was absorbed from the small intestine [21]. The relative contribution of each part of the small intestine (duodenum, jejunum and ileum) toward overall absorption of zinc is not clear [13], but considering factors such as length and surface area of different segments of the small intestine, the transit time of digestion and, more importantly, the site of endogenous secretion of zinc in the lumen, most of this element is likely to be absorbed in the jejunum [11].

After a meal, zinc content in the lumen increases to about 1.5 to 3 times compared to the amount ingested in the distal duodenum, most likely because of the secretion of the digestive juices, which contain zinc. This appears to confirm the jejunum as the most likely site of absorption when the jejunum luminal content of zinc substantially decreases [11].

2.3.3 Digestion and absorption

Zinc is hydrolysed from most amino acids and nucleic acids prior to absorption. During the process of digestion, digestive enzymes (proteases and nucleases in the stomach and small intestine) release dietary zinc from the food matrix as well as endogenous zinc from binding ligands [13].

2.3.4 Factors affecting zinc absorption

2.3.4.1 Intraluminal factors

Free zinc can make coordination complexes with different endogenous ligands including amino acids, phosphate and other organic acids or to the exogenous material in the intestinal lumen (prior to the transcellular uptake in the distal duodenum and proximal jejunum) [11, 16]. Because of the affinity for thiol and hydroxy groups, the histidine and sulphur containing amino acids; methionine and cysteine are the preferred amino acid ligands. In the lumen, zinc-histidine and zinc-methionine ligands are absorbed more efficiently than zinc-sulphate [11].

Apart from these amino acids, there is a possibility that some other amino acids such as lysine and glycine act as possible ligands. Moreover, glutathione (a tripeptide composed of cysteine, glutamate and glycine) or products of protein

digestion are considered to be enhancer ligands. In the ligands, zinc can be attached to sulphur (e.g. cysteine and glutathione), nitrogen (e.g. histidine) or oxygen (e.g. phytate and oxalate) [13].

In general, enhancer substances have been shown to increase zinc absorption especially when inhibitors are present [13]. Zinc absorption is enhanced with consumption of glucose and lactose, as well as with soy protein (consumed alone or with beef). Red wine might increase zinc absorption (further investigations are required to confirm this finding and explore the reason). There is no parallel study about white wine [15].

Citric acid and picolinic acid previously have been considered as possible absorption enhancer ligands [13, 20], but they do not appear to enhance the absorption of zinc [11].

Increased gastric pH, is shown to decrease zinc absorption. This can be a predisposing factor of zinc deficiency particularly after taking some medications such as: antacids, H_2-receptor blockers (e.g. Zantac, Tagamet and Pepcid) or proton pump blockers (e.g. Omeprazole) [13].

In contrast with amino acids and other enhancers of zinc absorption, many constituents in food can form a complex with zinc and inhibit its absorption. phytate, oxalate, polyphenols, dietary fibres and the other nutrients including vitamins and divalent cations are examples of factors inhibiting zinc absorption (Table 2.3).

2.3.4.2 Intracellular factors

Zinc uptake across the brush border surface is operated by both a saturable carrier mediated mechanism and a non-saturable passive mechanism; however, the subcellular mechanisms of zinc uptake are not completely clear [11, 22].

At a low to normal zinc intake (less than about 5 mg/day), the main mechanism is carrier mediated, which does not require energy. At low dietary zinc intake, although the carrier affinity for zinc remains unchanged, the capacity for carrier transporters increases perhaps because of a rise in the number of receptor sites [11]. Recent discoveries about zinc transporters (ZnT), zinc and iron-regulated proteins (ZIP) and metallothionein (MT) have significantly improved the understanding of zinc uptake and homeostasis, although there are still unanswered questions about their roles [23].

When the dietary supply of zinc is high, the non-saturable mechanism predominates. This mechanism includes passive diffusion or paracellular movement (movement between mucosal cells). If the theory of passive effect of lactose and glucose polymers on zinc absorption is correct, it is based on an increase in paracellular movement of zinc [11].

After entering into the enterocyte, there are several possibilities for zinc: 1) the cell can use zinc for zinc-dependent processes; 2) zinc may be bound to metallothionein and held within the cell; and 3) zinc can pass through the cell and across the basolateral membrane into the plasma [13]. Intracellular zinc can be used within the enterocyte. In this case, its use is for zinc-dependent processes, similar to the other cells of the body.

Enhancers of zinc absorption	Inhibitors of zinc absorption
• A protein rich diet (e.g. soy protein consumed alone or mixed with beef) [15] • Physiologic situation (pregnancy and lactation [15, 22, 24, 25], low zinc status [13, 22]) • Glucose and lactose [15] (though lactose also mentioned with no enhancing role [26]) • Picolinic acid [13, 27, 28] and citric acid [13] (also mentioned with no enhancing role [11, 28]) • Histidine, Cysteine [11, 13, 29] and possibly other amino acids (lysine and glycine [13] and methionine [11]) • Glutathione [13] • Red wine [15] also mentioned with no enhancing role [30-32] • Vitamin D [12]	• Phytate [12, 13, 15] and phytate in presence of calcium [13] • Divalent cations [13, 33] (e.g. calcium [13, 17], (also reported with no inhibitory effect [29]), iron [11, 13] (also reported with no inhibitory effect [29]), copper [13, 15] and cadmium [15] • Complexing agents [13] (tannins [15], oxalate [34] and polyphenols [30] are also reported with no inhibitory role) • Dietary fibre [13, 14] (insoluble dietary fibre; major inhibitory role [35], soluble dietary fibre; minor [35] or no inhibitory effect [36, 37]) • Intestinal diseases [15, 17, 38] (e.g. Crohn disease [15, 39, 40]) • Casein [14, 21] • Clay (mixed mineral soil fragment particularly important in geophagic (pica) cases [6, 7, 12] • Folic acid [13, 15] also reported with no inhibitory role [41]

Table 2.3 Factors affecting zinc absorption.

Table is produced to summarise current knowledge about factors affecting zinc absorption. Factors show the current controversy about the enhancing or inhibitory roles.

Cysteine-rich intestinal protein (CRIP) is thought to have a role in zinc absorption, but its role in zinc transcellular movement is still not elucidated [11]. Zinc is also tightly attached to metallothionein (MT). Metallothionein gene expression is positively correlated to zinc intake. When synthesis of the MT increases in response to dietary zinc intake, zinc absorption declines. Among the many theories about the role of MT, one is that MT can act as a cellular zinc pool to limit free zinc concentration within a limited range [11, 13, 22].

Zinc trapped as metallothionein in the enterocytes is normally lost in the faeces during the regular course of mucosal cell turnover. The amount of zinc, which is not bound to MT or utilized within the enterocyte, is carried across the basolateral membrane. A number of zinc transporters (ZnTs) have been thought to be involved in zinc cellular transport. ZnT-1 which can be found in different tissues including the basolateral membrane of the enterocytes, does not need ATP or sodium to transport zinc out of the duodenal and jejunal intestinal cells [11, 13].

2.3.5 Zinc transport and turnover

Albumin, as the main transporter of zinc in the bloodstream, is loosely bound to zinc to carry it from the intestinal cells to the liver. The quantity of zinc transported in the blood depends on the availability of both zinc and albumin. Thus, any physiologic or pathologic condition that alters the level of serum albumin concentration can have a secondary negative effect on serum zinc concentration. For example, during pregnancy, because of an expansion in plasma volume, a decline in serum albumin concentration results in a subsequent decline in serum zinc concentration. Other examples are aging and protein energy malnutrition where serum zinc concentration decreases with hypoalbuminemia [14-16].

Zinc, which is transported to the liver, is released back into the bloodstream for delivery to other tissues. About 3 mg of zinc is usually circulating in the plasma. Albumin transports up to 60% of plasma zinc [11, 42]. Some other compounds including α_2-macroglobulin, transferrin and immunoglobulin G (IgG) are thought to be responsible for the remaining blood zinc transfer. Histidine and cysteine as the two amino acids with a loose bond to zinc in blood, are also suggested to be associated with 2-8% of zinc transport [13]. This amino acid bind fraction determines the amount of zinc filtered by the kidneys [11].

Zinc circulating in the bloodstream is transported into several tissues at different rates as various tissues have different rates of zinc turnover. For example, uptake

of zinc by the central nervous system and the bone is slow and zinc in these tissues stays strongly bound for a long time. Bone zinc is not usually available for metabolic use. Hair zinc is also not available to the tissues and is lost when the hair is shed [18].

The pancreas, liver, kidney and spleen have the quickest rates of zinc accumulation and turnover, whilst red blood cells and muscles have a relatively slower zinc uptake and turnover [11, 18].

As the total amount of zinc in plasma is much smaller than the total amount present in the major tissues, minor variations in tissue zinc concentration can have a major impact on plasma zinc concentration. For instance, in order to enhance zinc retention, a 1% increase in liver zinc concentration could cause a 40% decrease in plasma zinc concentration [11].

Part of the released zinc can go back into the enterocytes and to the intestinal lumen. The fraction of albumin bound zinc in plasma (ready to be taken up by tissues), appears to have a role in the regulation of zinc absorption [13].

There is still much to discover about the mechanisms of zinc uptake by tissues. Two phases have been suggested for the entry of zinc into cells; the first phase is including a saturable rapid uptake, which is likely to be carrier mediated. The second phase appears to be slower and passive [18].

2.3.6 Zinc homeostasis regulation

The adjustments of intestinal absorption and endogenous intestinal excretion, are only partial and the primary means of zinc homeostatic regulation.

When luminal zinc concentration increases, fractional zinc absorption decreases; however, the actual zinc absorption in total increases. Regulation of zinc absorption at high dietary zinc intake allows a 'coarse control' of body zinc. Changes in zinc absorption are slow, but able to respond to a large range of changes in dietary zinc intake [11, 42].

Alternatively, an increase in endogenous zinc excretion via faeces provides the 'fine control', which is required for zinc balance. Endogenous zinc excretion quickly changes in response to changes in dietary zinc intake just above or below optimal levels. Thus, endogenous faecal zinc excretion, which can increase several fold in response to high dietary zinc intake, together with adjustment of intestinal zinc absorption, have a major role in zinc homeostatic regulation [11, 42].

When dietary zinc intake is very low or in chronic marginal zinc intake, the second means of homeostatic zinc adjustment can complete the gastrointestinal adjustments. Secondary adjustments in homeostatic zinc regulation include changes in urinary zinc excretion, plasma zinc turnover rates and the retention of zinc released from tissues (i.e. bone) to other tissues [42].

2.3.7 Storage

There is no specific tissue as a zinc 'store' [11]. The zinc content of most tissues including skeletal muscles, brain, lungs and heart are relatively constant. Therefore, when zinc intake is low, these soft tissues do not respond or equilibrate with other zinc pools to release zinc. Zinc in the bone, is part of apatite, and as bone release minerals slowly, it cannot be relied upon as a zinc supply during a period of dietary zinc inadequacy. Thus, when the dietary zinc intake is not adequate, plasma zinc containing enzymes and metallothionein are the most likely resources to supply zinc. In this situation, liver metallothionein and zinc containing metalloproteins (enzymes) can be metabolized and zinc can be redistributed to supply the critical requirements of the tissues [13].

Metallothionein is available in most tissues of the body and in particular in the liver, pancreas, kidneys, intestines and red blood cells. When the dietary intake of zinc decreases, the metallothionein bound zinc in the liver and red blood cells diminishes. Based on this observation, metallothionein is suggested as one of the indices of zinc status and stores [13].

2.3.8 Excretion

The faeces are the major route of zinc excretion. The studies administrating either oral or intravenous tracer doses of zinc, showed that only 2-10% of zinc is recovered in the urine and the reminding is excreted via faeces [18].

The source of zinc in faeces is from unabsorbed dietary zinc and endogenous zinc secretions. These endogenous zinc secretions are mainly from the pancreatic secretions. Zinc also can be originated from biliary and gastroduodenal secretions, transepithelial flux of the mucosal cells and removal of the old mucosal cells into the gastrointestinal tract [11].

After eating a meal, pancreatic secretion of zinc increases, but much of the zinc secreted into the gut is absorbed and returned to the body. Therefore, maintenance of zinc enteropancreatic circulation has a role in zinc homeostasis.

There is a relationship between the amount of zinc in the diet and the amount of zinc secreted into the gut. In humans, endogenous faecal zinc excretion may range from less than 1 mg/day with very low intake, to more than 5 mg/day with very high intake [11].

Daily urinary zinc excretion is usually minor (300-600 µg) compared with faecal zinc losses. Only extremely low and extremely high dietary zinc intake can have an impact on urinary zinc losses. Up to 95% of filtered zinc is usually reabsorbed in the distal parts of the renal tubule. The quantity of urinary zinc losses, is strongly correlated with the creatinine excretion and urine volume. Factors such as muscle catabolism occurring during severe burns, major surgery, trauma and starvation cause a dramatic raise in urinary zinc excretions [11, 18].

The other sources of body zinc excretion include surface losses (skin, hair and sweat), semen and menstrual secretions [16].

2.4 Zinc deficiency and functions

Zinc, as the most abundant intracellular trace element [11], is crucial for several biological functions. These biological roles can be categorised into three classes; catalytic, structural and regulatory [43, 44]. Zinc is essential for: 1) the activity of many enzymes, 2) being a part of biomembranes, 3) necessary for the stabilisation of RNA, DNA and ribosomes, 4) stabilises some hormone-receptor complexes and 5) might have a regulatory part in tubuline polymerisation. Given this wide range of biological functions of zinc, it is not surprising that zinc has a significant impact on various physiologic functions including physical growth, immune functions, sexual maturity and neurobehavioral developments [11, 16]. A great part of present knowledge about physiologic and biochemical roles of zinc is based on findings and observations of zinc deficiency and functions in humans and experimental animals.

2.4.1 Biochemical aspects

Over 200 (also cited as over 300 [15, 45-47] and several hundreds [29, 43]) enzymes need zinc for their activity [47, 48]. These enzymes which are from all six classes of the International Union of Biochemistry (IUB), can have a catalytic (e.g. carbonic anhydrase), structural (e.g. Cu-Zn superoxide dismutase) and regulatory (e.g. fructose bis phosphatase) role in biochemical systems [11, 13]. Some of these zinc-dependent enzymes are listed here:

Zinc is an integral part of carbonic anhydrase and acts as a carbon dioxide carrier particularly in erythrocytes. In removal of carbon dioxide from the cells, zinc catalyses the reaction to take up carbon dioxide and combine it with water to form carbonic acid. Zinc subsequently helps to release carbon dioxide from the capillaries into the alveoli of the lungs.

$$CO_2 + H_2O \xrightarrow{\text{carbonicanhydrase}-Zn^{+2}} H_2CO_3 \xrightarrow{\text{dissociation}} H^+ + HCO_3^-$$

The H^+ disconnected from carbonic acid reduces oxyhemoglobin as oxygen is released to the tissues. The bicarbonate goes into the plasma to take part in buffering reactions. This role of carbonic anhydrase in the maintenance of acid-base balance is also observed in renal tubule cells, mucosal cells and glands of the body [13, 17].

The amount of zinc associated with carbonic anhydrase being carried by the erythrocytes is almost 8-9 times as much as that distributed to tissues in plasma. The high affinity of this enzyme for zinc and the chemical structure of the ligands and the surrounding hydrogen of the zinc-binding site of the enzyme [46] show how zinc can have a major, indirect role in cell respiration and acid-base balance.

Carboxypeptidase A, which is a protein-splitting enzyme, is another example of zinc-dependent metalloenzymes. Carboxypeptidase A, is secreted by the pancreas into the duodenum to remove amino acids from the C-terminal (carboxy=COOH) in the process of the digestion of proteins. Zinc is a cofactor of this enzyme. The enzyme activity decreases with zinc deficiency; thus zinc has a key indirect role in protein digestion [13, 17, 49].

Alcohol dehydrogenase is an enzyme necessary for the conversion of alcohols to aldehydes. Examples include the conversion of retinol to retinal for the visual cycle and night vision [13] or in the metabolism of alcohol in the liver [50].

Zinc in proteins, usually either participate directly in chemical catalysis or has a role for maintaining protein structure and stability [46]. However, molecules of alcohol dehydrogenase contain four ions of zinc, two required for catalytic activity and two for structure and protein conformation [13]. Zinc supplementation increases the activity of alcohol dehydrogenase in the liver and subsequently suppresses alcohol-induced oxidative stress [50]. The role of zinc-dependent retinol alcohol dehydrogenase in oxidative conversion of retinol to retinal is mentioned as one of the reasons that zinc status can influence the metabolism of vitamin A [51].

Alkaline phosphatase (ALP) is another important example of zinc-dependent metalloenzymes. ALP is a broad term referring to a family of non-specific phosphomonoesterases that catalyze the hydrolysis of phosphate esters with optimal activity in alkaline pH. ALP is present in all human tissues. For example within osteoblasts, ALP has a major function in the calcium deposition in bone diaphysis [47, 48].

Several investigations have shown that ALP activity decreases with zinc deficiency. These reports suggest serum or erythrocyte ALP as an index of zinc status as the activity of enzyme strongly depends on zinc availability [11, 40, 49, 52, 53]. Zinc also is a component of the structure of ALP [13, 47].

Superoxide dismutase (SOD) enzymes catalyze the removal of superoxide radicals (O_2^-) by breaking it down into hydrogen peroxide and water. Thus, superoxide dismutase has a key role in protection against oxidative damages [13, 54].

$$2O_2^- + 2H^+ \xrightarrow{\text{CuZn-SOD}} H_2O_2 + O_2$$

Conformity, stability and activity of superoxide dismutase is dependent on zinc and the role of zinc in protection of cell membranes in vessel wall [55], airway epithelium [56] and the skin epidermis [57] may be because of its role in this antioxidant enzyme.

Many enzymes in the metabolism of DNA and RNA are zinc dependent. DNA and RNA polymerase (in synthesis of nucleic acid) and deoxythymidin kinase (in conservation of thymine in DNA metabolism) are both zinc metalloenzymes. Catabolism of RNA might be regulated by the impact zinc has on ribonuclease activity [13].

The role of zinc in the structure and function of biomembranes is a critical function of zinc. A reduction in biomembrane zinc concentration is suggested as the reason behind some of the disorders associated with zinc deficiency, as loss of zinc from biomembranes can enhance susceptibility to oxidative damage, structural strain and alteration in specific receptor sites and transport systems [11].

Zinc has a role in the structure and function of biomembranes as it stabilises and protects thiol groups and phospholipids. Although zinc does not directly interact with oxidant components, zinc occupies the sites that can alternatively be occupied with transitional metals having redox potential. Prevention of the interaction between chemical moieties and iron to form free radicals (e.g. in

Fenton reaction) is an antioxidant property of zinc; however, zinc also protects the biomembranes from free radical oxidative damages by maintaining an adequate level of metallothionein; as MTs are reported to be free radical scavengers [11, 58].

As an intracellular ion, zinc is critically involved in maintaining genetic stability and in gene expression. Several mechanisms are suggested for the role of zinc in gene expression. For example, zinc has an important role in gene transcription as it interacts with nuclear proteins that bind to the promoter sequence of particular genes. These DNA binding proteins, known as transcription factors, control proliferation, differentiation and cell death and they contain zinc fingers [13, 59].

The term 'zinc fingers' originates from the shape of these proteins (which are similar to fingers) and the attendance of zinc as the main mineral bound to these proteins. In the structure, cysteine and histidine containing domains are repeated. They are twisting and coiling and bound to the tetrahedral configuration of zinc at the base of protein. This structure enables polypeptides that are generally too small to fold by themselves, to fold through firm stabilisation by bound zinc [11, 13, 58].

Several transcription factors contain zinc finger regions. Many of the nuclear hormonal receptors, particularly for steroids (e.g. nuclear receptors of oestrogen, testosterone and vitamin D) are zinc finger proteins [11, 58, 60]. The Cys_2His_2 zinc finger domain is just one of the more than 10 classes of zinc fingers that have been discovered and biochemically characterised. Over 4000 such domains are found in more than 700 proteins in humans [11, 61]. Zinc mediates DNA-protein, protein-protein and protein-lipid interactions as part of the structure of zinc fingers, in transcription factors and DNA repair proteins [62, 63].

2.4.2 Physiologic aspects

2.4.2.1 Zinc and growth

The role of zinc in growth and development is probably the most well known of its functions. As a symptom of zinc deficiency, growth retardation has been observed and reported in a wide range of investigations in humans, as well as the experimental animals [49, 64-67].

The development of zinc deficiency seems to be different from most other nutrient deficiencies. Usually inadequate dietary intake of nutrients firstly leads to

mobilisation of stores and functional reserves, then a decline in tissue nutrient concentration and finally some specific metabolic function that depends on the decline of nutrients. Growth retardation in this instance seems to be the secondary manifestation of the nutrient deficiency.

On the contrary, when the dietary zinc intake is inadequate, the first response is a reduction in growth, by growing organisms, and a decline in endogenous zinc excretion to conserve tissue zinc. The crucial function of zinc in the gene expression and the maintenance of the transcription factors is the probable reason for the strong conservation of zinc in tissues [11].

Zinc has a major influence on growth at many levels (Table 2.4):

2.4.2.1.1 Biochemical level

Understanding the biochemical functions (i.e. catalytic, structural and regulatory actions) of zinc in hundreds of enzymes and proteins, zinc participation in biological membranes and the role of zinc in gene expression via its actions in zinc finger regions provides a good background for an understanding of the role of zinc in growth.

2.4.2.1.2 Hormonal level

Synthesis and secretion of growth hormone (GH) is totally zinc dependent. Growth hormone stimulates the production and release of insulin-like growth factor-1 (IGF-1) in liver and zinc has been shown to control this process. Zinc deficiency reduces the synthesis of IGF-1. Receptor resistance to both GH and IGF-1 is also seen in zinc deficiency [68-73]. In addition to GH and IGF-1, several other growth-related hormones such as insulin, thyroid hormones and testosterone are affected by zinc status [72].

2.4.2.1.3 Food intake

Zinc has a role in the regulation of food consumption as it is involved in the physiological pathways of appetite, smell and taste. Appetite is influenced by zinc as zinc can directly alter the hypothalamic receptor responsiveness to the neurotransmitters of the central nervous system (e.g. neuropeptide Y). Alterations of smell and taste are reported with zinc deficiency and the situation can be accompanied by anorexia and weight loss [72].

Role of zinc in growth		
Regulation of food consumption [74-77]		
Appetite regulation [78, 79]	Neurotransmitters (e.g. Neuropeptide Y and Galanine [80-83] Leptin [84-88])	
Taste and smell regulation [47, 72, 89]	Proteins and enzymes (e.g. Gustin and alkaline phosphatase) [90-92]	
Biochemical functions		
DNA replication [47, 93]	Proliferation of chondrocytes in diaphysis and epiphysis [67, 94]	
	Differentiation of chondrocytes and osteoblasts [59, 95-97]	
Gene transcription and protein synthesis [59, 98, 99]	(Further research is required) examples: Alkaline phosphatase [57], Osteocalcin [100, 101], Collagen [102, 103] and maybe IGF 1 [104, 105]	
Metabolism of proteins, lipids and carbohydrates [47, 106-108]	Many enzymes (e.g.→)	gustin and carboxypeptidase [47, 109-113]
		lipoprotein lipase [114]
		fructose 1,6-bisphosphatase [23, 115]
Hormonal functions		
Growth hormone [72, 93, 116]	Structure [117], synthesis [70], secretion [118] and circulating level [119]	
	Stimulation of the secretion of hepatic IGF-1 [70, 104, 120]	
	Activation of IGF-1 in bone cartilage [47]	
	Direct growth effects in response to GH [121]	
Insulin like growth factor-1 [47, 93]	Synthesis [104, 122]	
	Secretion in response to GH [123, 124]	
	Potentiation of the action [125]	
	Circulating level [69, 126]	
Other hormones [127]	Insulin, testosterone, thyroid hormones [47, 93, 128, 129]	

Table 2.4 Role of zinc in growth

Table is produced to demonstrate how zinc can affect growth. References show that the role of zinc in growth is well documented.

2.4.2.2 Zinc and cognitive development

Cognition is an area of thought processes by which a person analyses information via skills of perception, thinking, memory, learning and attention [130]. The role of zinc in children's cognitive development has been the subject of many studies. Present knowledge about this role is firstly based on animal and human studies suggesting that zinc deficiency may lead to delays and disorders in cognitive development and secondly, studies that assessed the response to supplementation in populations thought to be zinc deficient.

The exact mechanism of zinc's functions in cognitive development is not clear. There is a high concentration of zinc in the brain as zinc binds to both structural and functional proteins in the brain. Some structural brain malformations such as anencephaly, microcephaly and hydrocephaly and behavioural disorders such as reduced activity and short term memory loss have been associated with zinc deficiency [131, 132].

Apart from the regulatory, structural and catalytic functions of zinc in many proteins and enzymes associated with brain function, zinc also has an additional role as a neurosecretor or a cofactor in the central nervous system (CNS). Zinc participates in production of precursors of neurotransmitters and these zinc-dependent neurotransmitters have a role in memory function.

Ten to fifteen percent of brain zinc is concentrated inside synaptic vesicles of a special group of glutamatergic neurons called 'zinc containers'. Zinc in these glutamatergic terminals is in a free or loosely bound state and is released with neuronal activity or depolarisation for the psychological functions [72, 133, 134].

The presence of a high level of zinc in synaptic vesicles of zinc containing neurons, in the anterior brain, together with the role of zinc in myelinisation and the release of neurotransmitters such as glutamate and γ-amino butyric acid (GABA), show how zinc may be an important modulator in neuronal excitability.

The neuronal routes of these zinc-containing neurons are associated with memory, behaviour, emotions and cognitive function. Furthermore, zinc is thought to have a key role in neurogenesis, neural migration, synaptogenesis and neurotransmission. The glutamate and zinc synapses are one of the most abundant in the mammal cerebral cortex indicating an exclusive role of zinc in cortical communications [72, 134].

Zinc deficiency is linked with disorders in motor activity, attention, social behaviour and motor development:

In animals, zinc deficiency undermines cognitive and motor development via decreasing activity and emotionality. During the infancy period in rats, severe zinc deficiency caused an increased emotionality (response to stress). In adult and in pre-pubertal rhesus monkeys, moderate zinc deficiency led to reduced motor activity and less accurate performance on measures of attention (e.g. visual-attention tasks) and short-term memory. Monkeys with severe zinc deprivation before weaning were emotionally less mature, had more difficulty with separation and protective behaviour of their mother increased. Monkeys with severe zinc deprivation after weaning had difficulties in retaining previously learnt visual discrimination problems as well as difficulties with learning new problems [132, 134].

In humans, however, a number of studies investigating the link between zinc deficiency and children's cognitive development are less conclusive. Investigations

of the effect of zinc deprivation on the cognitive development in human beings, is difficult as it is not easy to correctly evaluate the population under study (Table 2.5).

Most of the human studies are based on a positive response to zinc supplementation in vulnerable or nutritionally deprived children. Results of more than ten major trials that concentrated on behaviour and development are contrasting. The three research studies that focused on activity demonstrated that zinc supplementation was associated with more activity during play. Five studies were based on motor development in infants and toddlers. From these studies, one study demonstrated the beneficial effects of zinc supplementation among very low-birth weight infants, one showed an improvement in the quality of motor development and three studies showed that zinc supplementation had no impact on the motor development of infants and toddlers [135].

Three investigations concerning mental development of infants and toddlers, found no beneficiary impact on zinc supplementation. One study showed that after zinc supplementation, children had lower scores of mental development index (MDI) [136]. In that study, zinc was supplemented alone, in between meals, without other food or micronutrients, for a period of five months; however, in most of the studies that reported beneficial effects on growth and development, children were treated for a shorter period and zinc was administered with additional food and/or multi-micronutrient supplement. It is therefore possible that the lower scores of MDI in the zinc supplemented group were caused by an imbalance of micronutrients [136].

Assumption	Practical example
Zinc deficiency may affect children's emotionality and response to stress	Difficulty with maternal separation in early life
Zinc deficiency may affect cognitive performance via changes in attention and other aspects of neuropsychological functions	Undermined academic performance
Zinc deficiency may lead to reduced level of activity	Inactive children do not practice the existing skills and are less likely to acquire new and more complicated skills
Zinc deficiency may lead to anxiety and depression	Anorexia nervosa in adolescents with low zinc intake

Table 2.5 Summary of the association between zinc deficiency and cognitive development.

Table is produced based on the information presented in references [72, 132, 135].

In elementary school-age children, hair zinc concentration was associated with reading ability showing that zinc deficiency may influence academic performance. From three randomised trials of zinc supplementation in these children, one

research study showed no beneficial effect on cognitive performance and two trials found that zinc supplemented children have a better neuropsychological performance particularly in reasoning in comparison with the control group. These results suggested that zinc supplementation may have more of an evident impact in time-dependent challenging tasks (e.g. reasoning and attention rather than in general aspects [135].)

The relationship between zinc deficiency and cognitive development may vary by age, sex, individual differences, variation of zinc requirements, periods of intellectual development being exposed to zinc deficiency, the severity threshold of zinc deficiency, and the existence of other nutritional deficiencies. This relationship is mediated by neuropsychological functioning, activity, social context and the caregiving environment and motor development [132, 134].

2.4.2.3 Zinc and sexual development

Effects of zinc deficiency on sexual maturation can be secondary to growth retardation or directly by interfering with the regulation and function of gonad hormones. In the same way, growth retardation can be secondary to the arrest of sexual development because sexual steroids play important roles in skeletal growth, bone maturation and an increase in muscle mass during puberty [134].

2.4.2.3.1 Males

In males, zinc has important roles in gland differentiation, testicular growth, spermatogenesis, functional ability of sperms, steroidogenesis, metabolism of androgens and interaction of androgens with receptors (Table 2.6). Like other steroid hormones, the nuclear receptor of testosterone is a protein of a zinc finger type [60, 134, 137, 138].

Zinc participates in the production and metabolism of LH (luteinizing hormone) [85] and FSH (follicle stimulating hormone) [134, 139]. In males, LH controls testosterone synthesis via stimulating steroid genesis in the testicular Leydig cells. This hormone together with FSH, stimulate the process of testicular spermatogenesis.

There are several theories concerning the aetiology of hypogonadism seen in zinc deficiency, including the influence of hypothalamo-hypophyseal axis by zinc deficiency. As the circulating levels of LH and FSH are reduced in zinc deficiency, this can negatively influence the activity of Leydig cells and be the reason for

hypogonadism. Another possibility is that hypogonadism of zinc deficiency is due to the direct action of the zinc in testicular steroidogenesis rather than its indirect action through a failure in Leydig cells [134, 140].

2.4.2.3.2 Females

In females, zinc has important roles in sexual development, ovulation and the menstrual cycle [141]. A high level of gene expression of zinc finger proteins as seen in Leydig cells in the testes is also seen in the granulosa cells in the ovaries. These proteins act as transcription factors and have a major role in gonadal development [142]. Zinc also alters the affinity of sex hormone-binding globulin (SHBG) for estrogens. SHBG connects to circulating androgens and estrogens and regulates the access of these steroids to their target cells [143].

Zinc deficiency in females has been associated with impaired synthesis and secretion of FSH and LH, abnormal ovarian development, disruption of the oestrous cycle, frequent abortion, a prolonged gestation period, difficulty in parturition and low birth weights of infants (Table 2.6) [144].

Males
• Impotence [145, 146], infertility and subfertility [137, 141, 147, 148] • Hypogonadism [7, 18, 140, 149, 150] • Atrophy of seminiferous tubules, Sperm malformations (e.g. sperms with malformed tails), reduced motility of sperms, Oligospermia [148, 151, 152] • Impaired synthesis and secretion of the sexual hormones (e.g. testosterone, FSH and LH) [140, 144, 153] • Delayed sexual characteristics (e.g. no pubic and axillary hair, dwarfism) [4, 149]
Females
• Delayed secondary sexual characteristics (e.g. primary Amenorrhoea, no pubic and axillary hair, retarded linear growth [6, 154, 155] • Dysmenorrhoea (e.g. pain and cramps of the uterus particularly in first days of menstruation) [156] • Disturbed bone mineralisation [157-159] • Pregnancy complications including extended pregnancy, prematurity, malformations (e.g. cleft palate), difficulty in delivery, abortion, preeclampsia, abortion, low birth weight [24, 60, 65, 144, 160-165]

Table 2.6 Impacts of zinc deficiency on sexual and reproductive system

Table is produced to demonstrate the current knowledge about the adverse effects of zinc deficiency on the sexual and reproductive system.

2.4.2.4 Zinc and immune function

There is substantial evidence to support a major role of zinc in the functioning of the immune system. The mechanism by which zinc affects the immunity has many dimensions because of a widespread action of zinc on different enzymes,

peptides, transcriptional factors and cytokines involved in the development and activity of the immune system [166, 167].

In mammals, the immune system consists of innate and specific means [168] and zinc deficiency significantly affects both these mechanisms:

2.4.2.4.1 Zinc and innate immunity

The innate mechanisms are the first line of defence to provide a natural protection against infections. The innate immunity is not specific and responds to different antigens in the same way regardless of previous exposure. The innate mechanism includes mechanical barriers (e.g. skin) and cellular components (e.g. macrophages, neutrophils and phagocytes) [11, 13].

The efficiency of the innate immunity is disturbed by altered zinc levels. In vitro, recruitment of neutrophil granulocytes and chemotactic activity of polymorphonuclear leukocytes were directly influenced by zinc levels. In vivo, activity of natural killer (NK) cells, processing of phagocytosis in macrophages and neutrophils, generation of the oxidative burst and the quantity of granulocytes are impaired by declining zinc levels [168, 169].

Serum zinc concentration may decline in response to infection. Change in plasma zinc concentration is associated with simultaneous elevation of selected plasma proteins such as C-reactive protein (CRP) and α_1- antichymotrypsin. This predefined set of metabolic reactions to infection is known as the acute phase response. The acute phase response is mediated by cytokines such as interleukin 1 (IL-1), which in turn, stimulates secretion of interleukin 6 (IL-6) and glucocorticoids, both of which, activate hepatic secretion of metallothionein (MT) [170, 171].

Infection can induce hepatic MT and plasma and hepatic levels of MT are closely related. Radioisotope studies after injection of IL-1 in experimental animals indicated an increased uptake of zinc by liver, bone marrow and thymus and decreased uptake by bone, skin and intestine compared with control animals; therefore, it is suggested that decreased plasma zinc levels in the presence of increased plasma MT levels shows a tissue zinc redistribution caused by infection as a confounding condition, and not impaired zinc status [170, 171].

As zinc is required by both human beings and pathogens for proliferation, a decline in plasma zinc concentration during the acute phase of infection, is a defence mechanism to inhibit the reproduction of infectious agents. Decreased

zinc concentration in fungal infections with Candida Albicans [172] is an example of this mechanism.

NK cells have a major role in immunity against infections and tumours. The activity and number of these cells are dependent on serum zinc concentration. Zinc is also required by NK cells for the recognition of a specific class of molecules in inhibitory receptors of the NK cells to inhibit killing processes [168].

2.4.2.4.2 Zinc and specific immunity

B and T cells of the specific immune system have a wide range of specific receptors and can produce memory cells that react quickly and strongly to antigens that they have been previously exposed to:

B cells

Proliferation of B cells is less influenced by zinc status compared with T cells. The total number of B-lymphocytes is decreased during zinc deficiency, but this reduction is more evident in the precursors of B cells especially in pre-B and immature B cells. This may be due to the induction of apoptosis in young cells. Although immunologic memory is affected by zinc, since mature B cells are more resistant to zinc deficiency, their memory is less influenced by zinc deficiency than the T cell based primary response to neoantigens. Production of antibodies (in particular immunoglobulin G (IgG)) is also influenced by zinc status [168, 173].

T cells

Zinc has a role in development of T-lymphocytes and influences their cytolytic activity. Zinc is also a crucial cofactor for thymulin, the hormone that is produced and released by thymus. Furthermore, zinc has a regulatory role in differentiation of immature T cells in the thymus and function of mature T cells in the periphery. The number of T cells decrease during zinc deficiency and as most antigens are T cell-dependent, the body can not respond properly with antibody production particularly in response to neoantigens in zinc deficiency [168].

Zinc deficiency (even a mild deficiency) causes a thymic atrophy, decreases thymulin activity, decreases T cell proliferation and impairs the function of T-helper (TH) cells via affecting secretion of their cytokines including interleukin-2 (IL-2) and interferon (IFN)-γ [168, 174].

Apoptosis

Zinc deficiency can induce apoptosis in variety of cells in the immune system. Apoptosis among pre-T cells of zinc deficient mice was increased by 300% and this was the reason for thymic atrophy and altered host defences. Apoptosis (and autophagy) in hepatocytes, glial cells, kidney cells, monocytes, fibroblasts and testicular cells is also initiated by an insufficient level of zinc (Figure 2.1) [175, 176].

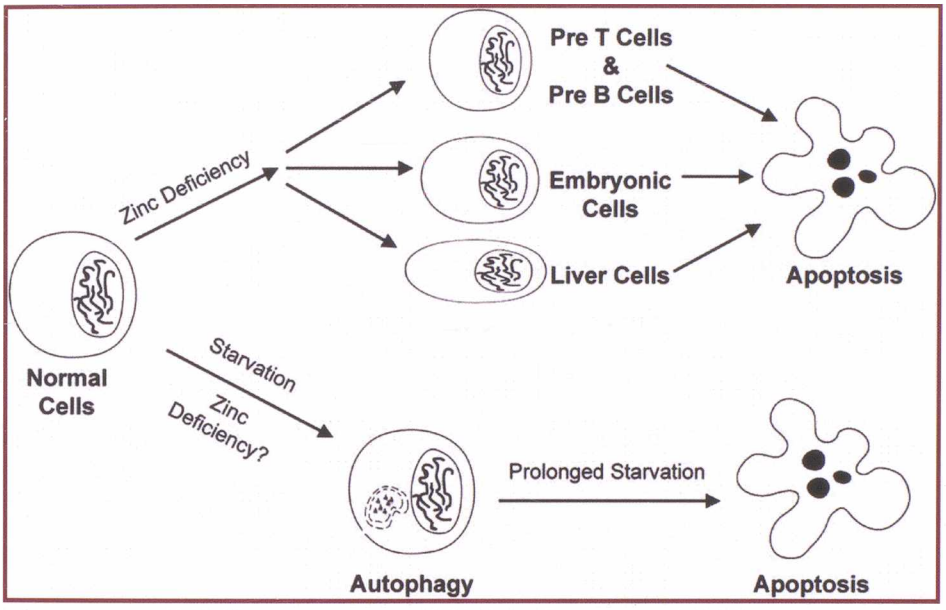


Figure 2.1 Zinc deficiency can induce apoptosis in a wide range of cells and tissues.

Pre-T cells and pre-B cells of the thymus and marrow together with other young developing cells of embryos are particularly vulnerable to this programmed cells death (PCD) during zinc deficiency. Autophagy as a new form of PCD induced by starvation or zinc deficiency, initiates formation of an autophagosome, which digest part of the organelles and proteins of the cell, and releases the required nutrients to try to deflect the process of apoptosis. Figure is adapted from reference [175]

Zinc deficiency is thought to be associated with increased risk of cancer (particularly head and neck cancers) [177, 178], progression of human immunodeficiency virus (HIV) infection [167, 179], prolonged parasitic infections (e.g. nematode infections) [180] and increased diarrhoeal and respiratory diseases and morbidity [181-183].

If an underlying zinc deficiency is present, zinc supplementation together with other micronutrients can enhance immunity [184]. It has been reported that a positive effect of zinc supplementation is a reduction in child mortality, infant infections, diarrhoea, malaria and respiratory diseases (especially pneumonia) [169, 185, 186].

Long time single nutrient zinc supplementation at high doses can have adverse effects including interactions with copper [184], impairment of immune functions (e.g. impairment of lymphocyte proliferative responses, a reduction in chemotaxis and phagocytosis [187]) and an increased risk of prostate cancers [188].

2.4.2.5 Zinc and the eyes

In most animals, there is a very high concentration of zinc in the eye. In humans, there is a substantial concentration of zinc in ocular tissue, especially in the retina, retinal pigment epithelium (RPE) and choroid [18, 189].

Experimental zinc deficiency in animals dramatically affected ocular development and caused a wide range of functional disorders but failed to produce morphological ocular changes. In humans, most of the ocular diseases that are associated with zinc deficiency have their pathogenesis related to declined serum zinc concentration or by improvement after zinc supplementation and only rarely by reduced tissue zinc concentrations. Altered vision, depression of the electroretinogram and oscillatory potentials are reported as clinical manifestations of zinc deficiency and ultrastructural changes in the retina and RPE is reported only in severe zinc deficiency [189].

Zinc is an essential trace element for visual process and an important factor to improve visual sensitivity during visual adaption. A number of mechanisms have been suggested to explain the role of zinc for the visual process. The interaction with taurine and vitamin A, modification of synaptic transmission, regulation of light rhodopsin reaction, modification of photoreceptor plasma membrane, and the role of zinc via antioxidation and metalloenzymes activities are some of these suggested mechanisms [189, 190].

Age-related macular degeneration (ARMD) is the major cause of blindness within the UK [191]. There has been significant interest about the beneficial role of zinc supplementation in patients with ARMD particularly after the initial reports which showed that 80-81 mg of oral zinc supplementation throughout a two year period reduced the risk of vision loss [189, 192].

Although further studies provided no support for the protective role of zinc supplementation [192-194], many ophthalmologists continued to prescribe zinc and the other antioxidant supplements at 100% of recommended dietary allowances for patients with some retinal degeneration [189].

2.4.2.6 Zinc and skin

The use of zinc for medical skin lotions was mentioned in the Egyptian papyri of 4000 years ago [195]. There is now significant evidence supporting the importance of zinc for skin health.

Zinc, as part of zinc protein complexes can be found in the intracellular and extracellular matrix of dermal and epidermal tissues. These basal cells are mitotically active. Subsequently, the activity of zinc dependent enzymes in the epidermis is more than the dermis particularly with DNA and RNA polymerases. The zinc content of the epidermis does not fluctuate in response to dietary zinc changes [18, 196].

The zinc concentration of the skin can be increased by a topical application of zinc supplements. A protective role of zinc against UV radiation was confirmed when a topical application of zinc chloride was reported to protect mouse skin against UV-A and UV-B induced sunburn cell formation [197].

Increased zinc concentration in response to topical application indicates a precutaneous absorption of zinc salts. A topical application of a zinc supplement induced mRNA for metallothionein (MT). Induction of MT by subcutaneous injection of $CdCl_2$ (an inducer of MT similar to zinc ion), protected skin from UVB-induced injury. It is thought that zinc ion may induce MT synthesis, to form a zinc-thiolate moiety that acts as a preferred sacrificial site for oxidant attacks. Perhaps the protective effect of zinc on photo-oxidative stress is related to zinc induction of MT [58, 196, 197].

The second mechanism of action for zinc antioxidant effects, is that zinc may replace redox reactive metals such as iron and copper at important cellular or extracellular sites: Iron and copper can transfer electrons and generate reactive oxygen species such as HO. and O_2^-.; however, at physiologic pH, zinc has a single ionization state and is redox stable. Zinc can compete with iron and copper and decrease generation of free radical at the ligand-binding site [197].

Zinc is also reported to have a role (in combination with nicotinamide) for the treatment of inflammatory skin diseases (e.g. acne vulgaris). Several potential mechanisms of action are suggested for the anti-inflammatory effect of zinc. The possible role of zinc in lysosomal enzyme release, lymphocytic transformation and bacteriostatic effect against bacteria that could cause acne, are some of these suggested mechanisms. Another possible mechanism can be based on

biomembrane accumulation of zinc via changing of the structure and function of the membrane. For example, in mast cells, zinc accumulates and obstructs receptor sites for histamine-releasing agents and this function consequently decreases the release of histamine [11, 198].

Several studies investigated the beneficial effect of zinc on wound healing [199-203]. Zinc is required for rapid cell replication and differentiation. During tissue repair, epithelialisation occurs and the collagen matrix achieves its normal strength. Zinc deficiency decreases the content of DNA and collagen of early wounds and both collagen and DNA increased when zinc deficiency was corrected [18]. Zinc supplementation, and consequently, up-regulation of metallothioneins and zinc metalloenzymes helped to understand many of the bimolecular events that happen in wound repair [196].

Alopecia, cutaneous lesions, defective collagen synthesis, poor wound healing, nail dystrophies and dermatitis, particularly around body orifices are some of the skin-related disorders of zinc deficiency. Sometimes clinical symptoms can be misdiagnosed as eczema especially in breast feeding preterm babies [13, 18, 204].

The classical route of zinc administration for therapeutic dermatic purposes is via direct topical application of zinc containing compounds to the skin. Alternatively, the dietary zinc should pass down the gastrointestinal tract, cross the intestinal barriers, reach the bloodstream and be distributed to different tissues including the skin. This process may appear longer and more complicated, but as the bloodstream continuously replenishes the skin, zinc can be distributed to the entire body and to all skin layers including the epidermis, dermis, subcutaneous fat and sebum. Thus, dietary supply of zinc is necessary for skin-related clinical situations [205].

2.4.2.7 Zinc and taste

Impaired taste acuity has been frequently reported as one of the features of zinc deficiency in children, adults and the elderly [206-209]. The exact mechanism by which zinc deficiency can affect the taste sensitivity is not well understood.

Zinc is a cofactor essential for the activity of ALP that is the most abundant enzyme in taste bud membranes. Furthermore, zinc is a structural component of gustin (an essential parotid salivary protein involved in taste perception) and is important for the development and maintenance of normal taste buds. A blockage

of pores in taste buds together with some form of dysfunction in neural mechanisms of transduction in the central nervous system is reported as a result of zinc deficiency [90, 92].

A number of the clinical situations known to cause dysgeusia or hypogeusia are also predisposing factors of zinc deficiency. Alcoholism, malnutrition, cirrhosis, malabsorption, renal failures, surgical stress, burns and some of the drugs (e.g. penicillamine and histidine) are some examples [92].

Some studies used the activity of angiotensin converting enzyme (ACE) as a zinc dependent enzyme in patients with idiopathically impaired taste acuity in comparison to zinc deficient patient with taste dysfunction and also healthy volunteers. It was proposed that zinc deficiency is a constant factor underlying hypogeusia even when levels of serum zinc concentration are within normal range [210, 211].

Other research investigated the relationship between the taste sensitivity and dietary zinc intake in healthy young adults and observed that taste was more influenced by the zinc status in males. It was concluded that zinc is important for taste acuity and gender differences must be taken into the account in investigations of taste acuity [90].

An age-related decline in the taste acuity was also reported among zinc-deficient elderly patients. This can either be a cause or an effect of zinc depletion and/or increased zinc requirements in the elderly population [212].

2.5 Zinc food sources

Zinc intake is normally associated with protein intake. Zinc in food is attached to the protein (amino acids and peptides) fraction and/or the DNA (nucleic acid) fraction [13, 15, 29].

Oysters are exceptionally rich sources of zinc providing up to 91 mg/100 g. Very good sources of zinc are red meat and seafood. The amount of zinc varies within the meat group, as in general, zinc content of the red meats is more than poultry or fish. Other good animal sources of zinc include shellfish and molluscs, liver, poultry, pork, and to a lesser extent, dairy products. Whole grains, leafy and root vegetables, and particularly legumes, represent good plant sources of zinc, but zinc in these products occurs in a less available form as inhibitors such as phytate and dietary fibre limit its absorption [11, 13, 17].

In the United States, 40-70% of zinc consumed by adults came from animal products especially from meats [13, 17]. In the United Kingdom, in adults aged 19 to 64 years, 34% of the mean daily zinc intake was from meat and meat products including 11% from beef, veal and dishes and 5% from chicken, turkey and dishes (including coated chicken). Twenty five percent of the daily zinc intake in the UK, came from cereal and cereal products mainly from white breads (6%) and breakfast cereals (5%). Milk and dairy products contributed to 17% of mean daily zinc intake. About a third (6%) of this came from cheese and another (6%) came from semi skimmed milk [213].

As animal food sources provide the major portion of daily dietary zinc intake, vegans might be vulnerable to marginal zinc deficiency. Due to the elimination of meats and increased intake of phytate-containing legumes and whole grains, zinc absorption in vegetarian diets is lower than non-vegetarian diets. No adverse health effects of this lower zinc absorption has been demonstrated with varied vegetarian diets [17, 214, 215].

The processing of food may affect its zinc content and bioavailability. For instance, in whole grain cereals, zinc is concentrated mostly in the bran and germ portions and up to 80% of total zinc is lost in the wheat milling process. Heat treatment can form zinc complexes that are resistant to hydrolysis, and therefore, unavailable for absorption. Products of Mailard reactions (amino acids-carbohydrate complexes resulting from browning) also have a negative impact on zinc bioavailability [11, 13].

Zinc fortification and enrichment can improve the zinc content of plant products. In the US, some breakfast cereals are fortified with up to 100% of the recommended dietary allowance [11]. Growing in zinc-rich soil or treatment with zinc-rich fertilizer may also enhance plant zinc concentration. Genetic modification of the plants can also improve zinc bioavailability [216].

2.6 Zinc requirements

Dietary requirements of zinc are derived from the factorial approach. In this approach, estimates of zinc requirements are derived from adding all zinc losses and approximating the dietary requirement by dividing these losses by an average fractional zinc absorption. This approach is explained in chapter 4 in detail. The current zinc dietary reference values for the UK population are shown in Table 2.7.

Age	LRNI	EAR	RNI
0-3 months	2.6	3.3	4.0
4-6 months	2.6	3.3	4.0
7-9 months	3.0	3.8	5.0
10-12 months	3.0	3.8	5.0
1-3 years	3.0	3.8	5.0
4-6 years	4.0	5.0	6.5
7-10 years	4.0	5.4	7.0
Males			
11-14 years	5.3	7.0	9.0
15-18 years	5.5	7.3	9.5
19-50 years	5.5	7.3	9.5
50+ years	5.5	7.3	9.5
Females			
11-14 years	5.3	7.0	9.0
15-18 years	4.0	5.5	7.0
19-50 years	4.0	5.5	7.0
50+ years	4.0	5.5	7.0
Pregnancy	No increment		
Lactation			
0-4 months			+6
4+months			+2.5

Table 2.7 Dietary Reference Values for Zinc (mg/day)

The table is adapted from reference [217].

2.7 Zinc toxicity

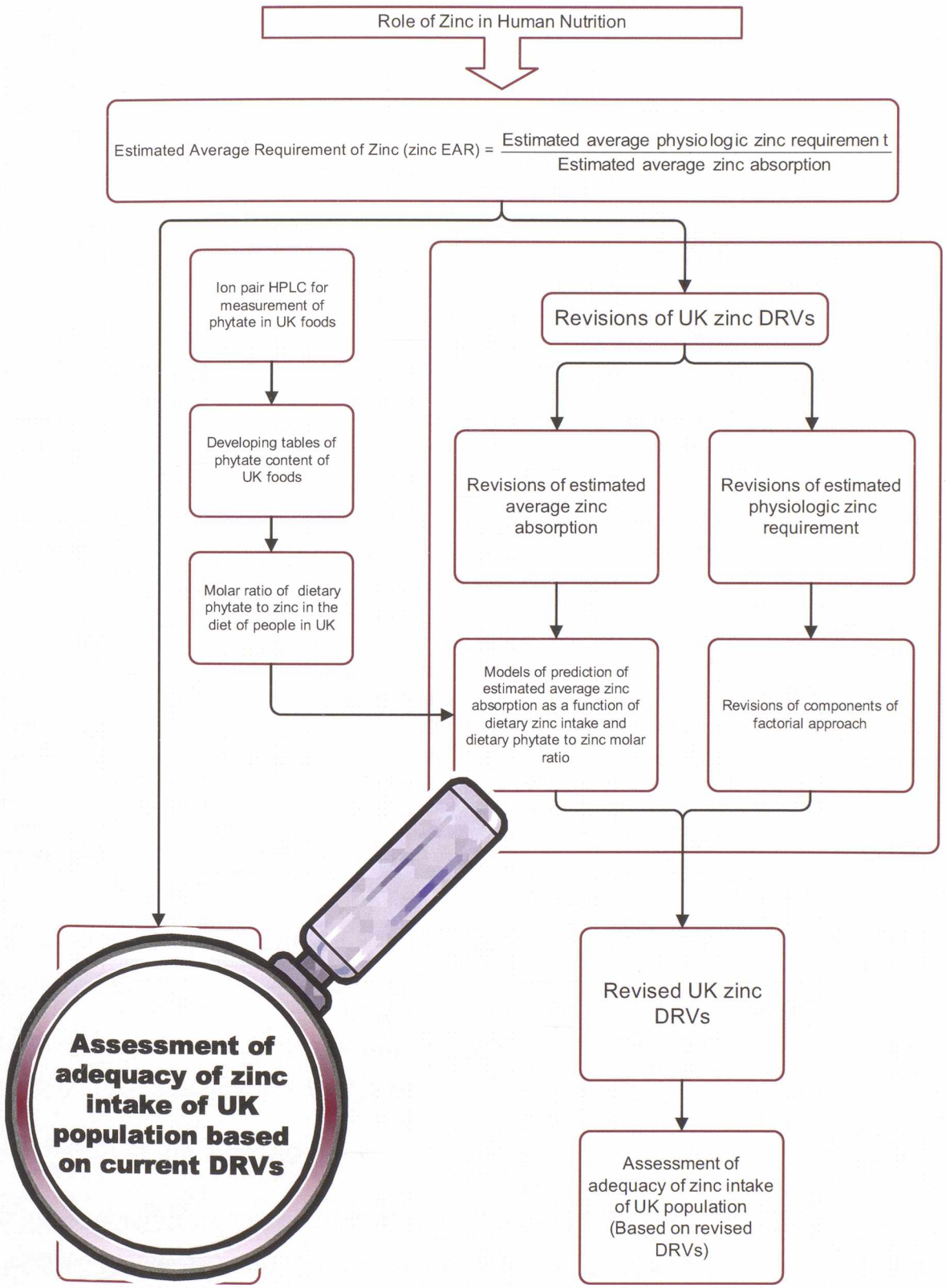
Acute zinc toxicity because of ingestion of very large doses of zinc is uncommon. Most of the cases of the acute toxicity have been in relation to food poisoning. Long storage of acidic food or drinks in galvanized containers resulted in a release of zinc from the galvanized coating and was followed by manifestations of the toxicity. The symptoms included nausea, vomiting, epigastric pain, abdominal cramps and diarrhoea (or dysentery) [1, 218].

Pharmacological zinc intake of 100-300 mg/day either because of excessive self-supplementation or in order to treat special/experimental medical conditions (such as sickle cell anaemia or celiac) developed clinical symptoms of severe copper deficiency including hypocupremai, anaemia, leucopenia and neutropenia [11, 13, 218].

The other adverse effects of chronic zinc toxicity include decreases in copper-dependent enzymes such as superoxide dismutase, cytochrome c oxidase, ceruloplasmin and alterations in immunological parameters, cholesterol and lipoprotein distribution. Some studies suggested that even moderately excessive zinc supplementation can interfere with the utilisation of copper and iron and

negatively affect high density lipoprotein (HDL) cholesterol concentrations [218, 219].

Chapter 3: The Assessment of Zinc Status in the UK Based on the Current Indices and Recommendations



The magnifier symbol shows where you are in the conceptual framework of the thesis.

3.1 Introduction

3.1.1 Background

The National Diet and Nutrition Survey (NDNS) programme is a rolling series of cross-sectional surveys of different UK population age groups. The programme was set up by the Ministry of Agriculture, Fisheries and Food (MAFF) and the Department of Health (DH) in 1990 following the success of the first survey. The programme is now jointly funded and directed by the Food Standard Agency (FSA) and the DH [220, 221].

The NDNS programme includes four separate surveys conducted at three-year intervals. Surveys of NDNS provide cross-sectional information on the dietary habits and nutritional status of a nationally representative sample of the British population. These include:

1. NDNS: Children aged 1½ to 2½ years (1992/3)
2. NDNS: People aged 65 years and over (1994/5)
3. NDNS: young people aged 4 to 18 years (1997)
4. NDNS: adults aged 19 to 64 years (2000/1)

Surveys collect detailed quantitative information on food consumption and nutrient intake, anthropometric measurements, nutritional status indices, and socio-demographic and life style characteristics. The programme provides baseline information on the diet and nutritional status of the UK population and is used by the government as a basis for the development of evidence-based nutrition policies [221].

The datasets of the NDNS are deposited at the UK Data Archive, University of Essex and have been used for this research with permission.

3.1.2 Remit of the investigation

Survey reports are published for all four of the NDNSs [213, 222-228]; however, published results only provide general information on zinc. Moreover, the overall picture of zinc status among all different age groups of the UK population was not studied in depth. In order to have a clearer picture of zinc intake and status of the UK population and to identify any vulnerable groups, there was a need for an independent analysis of the data.

There are some key benefits of the NDNS data making it suitable for this investigation:

1. Information on current zinc intake is provided by combining food consumption with the latest analysis in the nutrient data bank of the Food Standards Agency (FSA) [220].
2. Data on diet and indices of nutritional status including zinc biomarkers and related socio demographic characteristics allow analysis of the links between them.
3. The similar methodologies of the different surveys allows a broad, nationally representative, picture of zinc status in different population age groups.

3.1.3 Definition of NDNS derived variables

1. Daily zinc intake from food sources (mg).

Also called 'zinc intake from food sources' and/or 'dietary zinc intake'; is calculated from the weight of food consumed over 4 or 7 days and deriving the average for one day. This represents the daily zinc intake excluding supplements and prescribed medicines.

2. Daily zinc intake from all sources (mg).

Also called 'daily zinc intake' derived from the weight of food consumed over 4 or 7 days and calculating the mean for one day, including supplements.

3. Zinc daily density from food sources (mg/1000 kcal).

Also called 'zinc daily dietary density' was calculated as:

$$\text{daily zinc density from food sources} = \frac{\text{daily zinc intake from food sources} \times 1000}{\text{daily energy intake from food sources}}$$

4. Zinc intake as percentage of Reference Nutrient Intake (%RNI).

RNI was based on the UK Dietary Reference Values (DRVs) 1991 [217]. The variable was calculated as:

$$\text{zinc intake as percent of RNI} = \frac{\text{daily zinc intake from food sources} \times 100}{\text{zinc RNI}}$$

5. Percentage of the population with dietary zinc intake below Lower Reference Nutrient Intake (% below LRNI).

To derive this index, first LRNI for all individuals participating in the survey was defined based on the UK DRVs of 1991 [217]. Dietary zinc intake of the individuals was then compared to LRNI and population was derived to two groups:

- People who had zinc intake below LRNI.
- People who had zinc intake at or above LRNI.

The LRNI defines a theoretical cut-off point (two standard deviations below the Estimated Average Requirement (EAR), below which 97.5% of individuals are unlikely to be consuming adequate amounts of zinc. Thus, the prevalence figure exceeding 2.5% may indicate a potential shortfall in zinc intake. Although these individuals cannot be categorically defined to have 'inadequate zinc intake' because of the uncertainties surrounding individual requirements and the measurement of diet, they can be regarded as 'at risk' [229]. Therefore, this index was one of the major indices of zinc status of the population in the current investigation.

6. Plasma zinc concentration (µmol/l).

Also referred to the 'plasma zinc level' or 'serum zinc concentration', which is the level of zinc in blood plasma during fasting (or in some cases non-fasting) for the sample of individuals participating in the investigation.

Plasma zinc concentration was not measured in the NDNS for adults aged 19 to 64 years. The Food Standard Agency (FSA), was contacted for advice regarding why plasma zinc was not measured in this survey. The reply was that in this survey, to take account of technical constraints and clinical and policy relevance, there was a priority list of analytes to be measured and plasma zinc was not as high on this list, as other analytes [230, 231].

7. Percentage of population with low plasma zinc concentration (%).

To derive this index, plasma zinc concentration of the individuals was compared to the cut-off points. For instance, 10.71µmol/l was considered as the cut-off point of plasma zinc for fasting adult samples [224]. The population was divided to two groups:

- People who had plasma zinc concentration below normal.
- People who had plasma zinc concentration at or above normal.

For NDNS adults aged 19 to 64 years, this index was not available, as plasma zinc concentration was not measured.

8. Correlation of plasma zinc concentration and daily zinc intake from all sources ('r' and 'p' values).

The Pearson correlation coefficient (r) was used as a numerical measure of the amount of association between plasma zinc and zinc intake.

There are several factors that may affect the zinc status. For example, region, social class of head of household and household income could have an effect on zinc intake and status. Among all these factors, only age and gender were included into the current analysis as:

1. Gender and age were factors measured similarly in all NDNS investigations.
2. Data of the NDNS was comparable to the UK DRVs based on gender and age.
3. Results of the analysis by gender and age could be used for further steps of this research.
4. The effect of the socio-demographic factors are not presented in this thesis; however, the results of the statistical analysis of those factors are available by contacting the author.

3.1.4 Aim

The present study aimed to assess the zinc intake and status of different age groups of the UK population, using the available DRVs and cut-off points in order to provide an understanding of zinc status and to identify any vulnerable groups.

3.2 Methodology

The details of the methodologies and procedures used in the National Diet and Nutrition Surveys are described in the survey reports [213, 222-228]. A brief description of time and setting, sampling and the components of the surveys is presented here:

3.2.1 NDNS: children aged 1½ to 4½ years

This Survey was carried out between July 1992 and June 1993 in mainland Britain. A nationally representative sample was identified from a pool of addresses selected from the Postcode Address File. Only children living in private dwellings were eligible to be included and only one child per household was selected. From 1859 children whose parents or guardians completed a structured interview, a weight dietary intake was collected for 1675 and blood samples were obtained from 1003. Fifty one percent of the children provided both weighed dietary record and blood samples.

The survey included a face to face interview with the child's mother or guardian to provide information about the socio-demographic characteristics of the child's household; use of medicines; eating and drinking habits; a weighed dietary record of all food and drink (including supplements) consumed in and out of the home over four consecutive days including a Saturday and Sunday; record of bowel movements for the same period; agreeing to a dental examination, blood sampling and performing anthropometric measurements; and, giving a written consent for performing these procedures.

3.2.2 NDNS: young people aged 4 to 18 years

The survey was conducted from January to December 1997 in mainland Britain. Participants were randomly recruited only from private residences and selected from 132 postcode sectors of the survey. Of the 2127 young people interviewed for the survey, seven day weighed dietary records were obtained from 1701 (80%). Subsequently, the anthropometric measurements, urinary and blood sampling for the assessment of nutritional status was obtained from 1193 of those initially interviewed [229].

The survey included an initial face-to-face interview using computer assisted personal interviewing (CAPI) methods to collect information about the socio-demographic characteristics of a young person's household; smoking, eating and

drinking habits; use of medicine, health status and a weighed dietary records of all food and drink (including supplements) consumed both in and out of the home over seven consecutive days; a record of bowel movements for the same period; a 7-day physical activity diary over the same period as the dietary record (collected only for young people aged 7 year and over); anthropometric and blood pressure measurements; the collection of a spot urine sample; written consent for participation in each section including blood sampling; a venepuncture procedure to collect a fasting sample of blood; a short post dietary record interview to collect information about any unusual circumstances or illness that might have affected the results together with a face-to-face interview using CAPI; and, to collect information about young person's oral health and performing an oral health examination.

3.2.3 NDNS: adults aged 19 to 64 years

Fieldwork for this survey took place over 12 months between July 2000 and June 2001 in mainland Britain. The sample was selected using a multi-stage random probability design and selections were made from the Postcode Address File. As first stage units, with probability proportional to the number of postal delivery points, 152 postal sectors were selected. Thirty-eight sectors were allocated to each of four fieldwork waves and this was to take account of the need to have an almost equal number of households in each wave of fieldwork and for the sample to be nationally representative in each trimester. Finally, 40 addresses were randomly selected from each postal sector.

The participants were selected among the adults aged 19-64 years who were not pregnant or breastfeeding at the time of the doorstep sift. If there was more than one eligible adult, living in the same household, only one was selected randomly to take part in the survey.

Of the 5673 addresses issued to the interviewers, 35% were eligible for the survey mainly due to the exclusion of those aged under 19 or over 64 years. Over one third of the eligible sample (37%) refused outright to participate in the survey. Sixty one percent of the eligible sample (2251 participants) completed the dietary interview and 47% (1724 participants) completed a full seven-day dietary record [225].

Overall, 65% of the responding sample (1459 participants) provided a sample for the 24-hour urine collection [213]. At least 77% of the responding sample had at

least one of the physical measurements taken. A blood sample was obtained from 60% of the responding sample (1347 participants) [227].

The elements of the survey were: a face-to-face interview to collect information on socio-demographic and lifestyle characteristics using the CAPI method; a 7-day weighed intake dietary record of all food and drink (plus supplements) consumed over seven consecutive days in or out of the home; a record of bowel movements and a record of physical activity for the same seven days; physical measurements including height, weight, waist and hip circumferences; a blood pressure measurement; a 24-hour urine collection to assess urinary analytes; a venous blood sample, collected with the participant's written consent and analysed for a range of indices of nutritional status; and, a self-count of the number of teeth and amalgam fillings.

3.2.4 NDNS: People aged 65 years and over

In this report, people aged 65 years and over are also referred to as the 'elderly'. The fieldwork of this NDNS was carried out in four waves of 10–12 weeks each, between October 1994 and September 1995 in mainland Britain.

The free-living sample (i.e. living in the community) was identified from a pool of addresses selected from the Postcode Address File as the sampling frame. Eighty postal sectors were selected at the first stage of multiple-stage cluster sampling. To identify those households containing an eligible adult, from each sector, 375 households were randomly selected and a form was sent to them. A random sample of eligible adults was then taken and only one free-living individual per household was selected.

A sample of people living in institutions (i.e. living in nursing homes or residential homes but not people in acute hospital beds) was selected from residential and nursing homes for elderly people in the postal sectors of the free-living sample. Three individuals from each institution were then selected [223, 232].

Because of the relatively low number of men in the population aged 85 and over, the survey protocol deliberately took preference in selecting males for this group in order to obtain sufficient numbers for statistical evaluation. Weighting procedures were then applied to ensure that the published data were nationally representative according to the most recent population census [223, 233].

Of the 2172 free-living people eligible to participate, 1632 (75%) responded positively by participating at a full or partial interview and 1275 completed a full

dietary record. Fifty four percent of the eligible sample (1164 participants) co-operated with at least one of the physical measurements. The level of participation was slightly lower with the blood sample at 45%, which included 986 participants.

Of an eligible sample of 454 participants living in institutions, 428 participated in an interview and 412 completed the 4-day weighed dietary record. Eighty percent of the eligible sample (363 participants) took part in at least one of the anthropometric measurements. This co-operation level with the blood sample was 64% (290 participants) respectively.

A face-to-face interview was performed by a trained interviewer in order to obtain information about socio-demographic characteristics of a participant's household; health and life style; employment and source of income; smoking, eating and drinking habits; use of medicine; a 4-day weighed dietary record including all food and drink (plus supplements) consumed in and out of the home over four consecutive days as well as seven day records of the number of bowel movements; anthropometric measurements; measurement of blood pressure, pulse rate, and hand grip strength, obtained by a trained nurse; a 30ml fasting blood sample, obtained by the nurse for haematology and biochemical measurements; a single early morning urine sample collection for the analysis of the urinary analytes; and, an oral health interview and examination [233].

3.2.5 Statistical analysis

Values of zinc intake, density and plasma zinc are summarised by the 'mean' values to describe the population that they belong to. Where necessary variables were weighted and new variables were computed or recoded.

Analysis excluded participants who failed to complete their weighed dietary record. To analyse the associations of gender, age and their interactions with zinc intake and status, a factorial analysis of variance was applied with 'Benferroni' post hoc comparison. The results are presented by gender and age.

Subjects with low zinc intake or low plasma zinc concentrations were identified. Independent sample t-tests were then performed to compare the zinc intake of the population with low plasma zinc concentrations and those with low zinc intake with the other participants.

To investigate if the contribution of supplements was significant, a paired samples t-test was conducted to compare 'daily zinc intake from food sources' with 'daily zinc intake from all sources'.

A bivariate Pearson test of correlation was conducted with the two-tailed test of significance to investigate correlations between plasma zinc with zinc intake.

The parametric statistics were used because:

1. For large sample sizes (such as data of the NDNSs) legitimacy of the parametric tests is safeguarded by the Law of Large Numbers and Central Limit Theorem, regardless of correctness of the assumption of normality [234].
2. Other analyses of data (i.e. published reports and analyses of Chapter 8) are parametric and the results of this chapter are compared with those results.

The statistical significance for all tests was set at 0.05. All statistical analyses were conducted using the SPSS statistical package (SPSS 14.0 for windows, Release 14.0.0, 5 Sep 2005, Chicago, SPSS Inc).

3.3 Results

3.3.1 NDNS: children aged 1½ to 4½ years

The average daily zinc intake from food sources for children in this survey was 4.4 mg. The average intake increased slightly with age: the lowest was found in girls aged 1½ to 2½ years (4.18 mg) and highest for boys aged 3½ to 4½ years (4.65 mg).

When differences in energy intake were taken into account, the average daily zinc density (mg/1000 kcal) decreased with the age of the child, falling from 4.17 mg for boys aged 1½ to 2½ years to 3.67 mg for boys aged 3½ to 4½ years ($p<0.001$). This finding suggests that dietary zinc content per 1000 kcal of energy decreased as children became older (Table 3.1).

Although girls aged 1½ to 2½ years had a slightly lower average daily zinc intake compared to the boys of the same age group (4.18 mg vs. 4.40 mg and $p<0.05$), no significant gender-related differences were found after adjusting for differences in energy intake. No other variation in average daily zinc intake and density was found between boys and girls of the other age groups.

The contribution of supplements to the average daily intake of zinc in all age groups for both boys and girls was negligible. Mean daily zinc intake from food sources was less than the UK RNI for all age groups of both boys and girls. The average daily dietary intake of zinc was 87.1% of the RNI for boys and 83.3% of the RNI for girls.

Overall, 13.6% of boys and 20.9% of girls had an intake below the UK LRNIs. The highest percentage of children with intake below the LRNI was among children aged 3½ to 4½ years, those with the lowest zinc density diets.

Among boys aged 3½ to 4½ years, 18.4% had an intake below the LRNI whereas within this group, 32.0% of boys aged 4 years and over had an intake below the LRNI. The zinc intake of 28.4% of girls aged 3½ to 4½ years was below the LRNI. Within this group, 41.7% of girls aged 4 years and over was intake below the LRNI.

The level of LRNI increases from 3 mg/day for children under the age of 4 years to 4 mg/day for 4 to 6 year olds. Boys and girls aged 4 years and over had mean intakes below this level. Although dietary zinc intake increased slightly as children

became older, for many children, and in particular the ones aged 4 years and over, this increase was not sufficient to match the higher LRNI.

Overall, average plasma zinc concentration was 13.03 µmol/l for boys and 12.89 µmol/l for girls. Plasma zinc levels showed no significant variation with either age or gender. Among children, 4.7% of boys and 6.3% of girls had plasma zinc levels below 10 µmol/l.

The correlation of plasma zinc concentration with dietary zinc intake was very weak and only significant for boys aged 2½ to 3½ years ($r=-0.223$ and $p<0.05$) and for the overall population of boys regardless of age group ($r=0.131$ and $p<0.05$). No other statistically significant correlation was seen among any other subgroups.

The mean daily dietary zinc intake of children who had plasma zinc concentration below normal (4.84 mg) was slightly higher than children who had plasma zinc levels above normal (4.46 mg). This difference was not statistically significant ($p>0.05$).

There was also no significant difference between average plasma zinc concentration of children who had zinc intake below the LRNI (13.10 µmol/l) and average plasma zinc concentration of children who had intake above the LRNI (12.94 µmol/l, $p>0.05$).

Figure 3.1 shows that for children aged 1½ to 4½ years, the main sources of zinc intake were milk and milk products, meat and meat products and cereal and cereal products, respectively. Milk and milk products provided about a third of the zinc intake. Just over quarter of the zinc intake came from meat and meat products, mostly in the form of beef, veal and dishes, burgers, and kebabs, chicken and sausages. Cereal and cereal products contributed to about quarter of zinc intake. Within this group, white and wholemeal breads, breakfast cereals and biscuits were the main contributors to zinc intake.

Gender	Boys				Girls			
Age group	1½ - 2½ years	2½ - 3½ years	3½ - 4½ years	All	1½ - 2½ years	2½ - 3½ years	3½ - 4½ years	All
Average zinc intake (mg/day) from food sources	4.40	4.43	4.66	4.49	4.18	4.32	4.45	4.31
Average zinc intake (mg/day) from all sources	4.40	4.45	4.66	4.49	4.20	4.33	4.45	4.32
Average zinc density (mg/1000kcal) from food sources	4.17	3.73	3.67	3.87	4.09	3.83	3.76	3.90
Base (N)	298	300	250	848	278	306	243	827
Zinc RNI (mg/day)	5	5	5 | 6.5	NA	5	5	5 | 6.5	NA
Zinc Intake (as % RNI)	88.1	88.7	84.1	87.1	83.5	86.4	79.1	83.3
Zinc LRNI (mg/day)	3	3	3 | 4	NA	3	3	3 | 4	NA
Percentage of people with intake below LRNI	11.7	11.3	8.8 | 32.0 18.4	13.6	18.7	17.0	16.4 | 41.7 28.4	20.9
Base (N)	35	34	13+33	115	52	52	21+48	173
Average plasma zinc concentration (µmol/l)	13.39	12.88	12.87	13.03	12.88	12.58	13.22	12.89
Base (N)	91	110	99	300	83	111	108	302
Percentage with plasma zinc concentration below 10 µmol/l	3.3	4.5	6.1	4.7	6.0	8.1	4.6	6.3
Base (N)	3	5	6	14	5	9	5	19
Correlation coefficient (Pearson) between zinc intake and plasma zinc concentration	r=-0.095 p=0.376	r=-0.223 p=0.021	r=-0.061 p=0.554	r=0.131 p=0.025	r=-0.071 p=0.526	r=0.101 p=0.297	r=-0.203 p=0.038	r=-0.049 p=0.404

Table 3.1 Indices of zinc status for children aged 1½ to 4½ years

Average daily intake of zinc, average daily intake of zinc as percentage of zinc RNI, the proportion of population with zinc intake below zinc LRNI, plasma zinc concentration, proportion of population with plasma zinc level below normal and correlation coefficient for plasma zinc and dietary intake of zinc by gender and age of child.

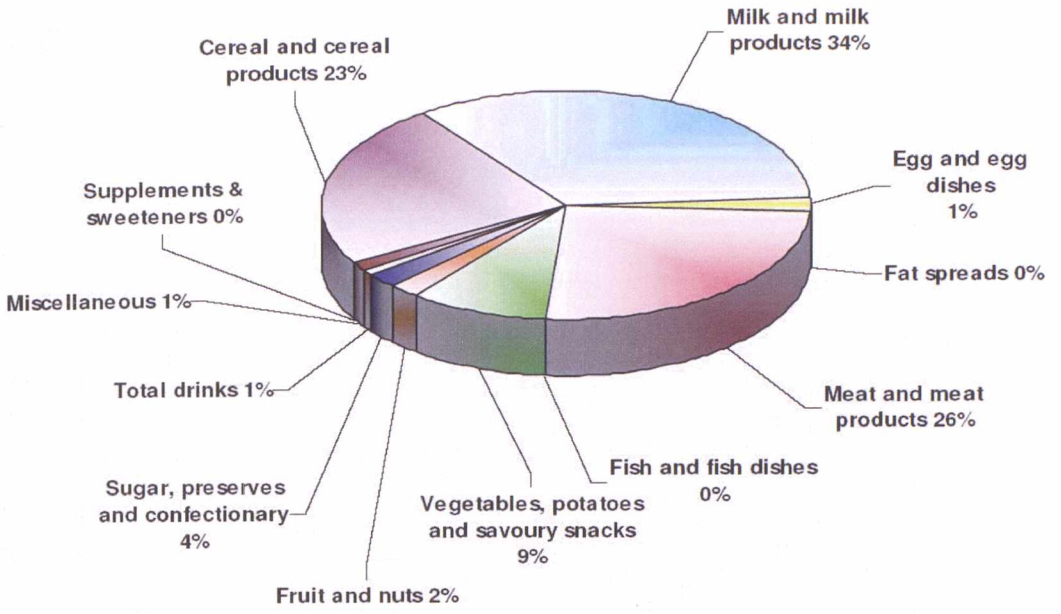


Figure 3.1 Percentage contribution of food types to average daily zinc intake of children aged 1½ to 4½ years.

3.3.2 NDNS: young people aged 4 to 18 years

In this survey, the average dietary zinc intake from food sources was 6.88 mg for boys and 5.74 mg for girls. The difference between the dietary zinc intake of boys and girls was statistically significant ($p<0.001$) among all age groups (Table 3.2).

The average daily zinc intake from food sources increased with age for both boys and girls; the increase was sometimes due to increasing energy intakes. For instance, the mean daily dietary zinc intake among boys aged 4 to 6 years increased from 5.59 mg to 6.13 mg among boys aged 7 to 10 years ($p<0.01$); but after allowing for differences in energy intake, the mean daily dietary density for these boys decreased from 3.62 mg/1000kcal to 3.46mg/1000kcal accordingly ($p<0.05$).

Among girls for the same age groups (4 to 6 years and 7 to 10 years) average daily zinc intake from food sources increased with age from 4.89 mg to 5.73 mg ($p<0.001$). After allowing for differences in energy intake, dietary zinc content per 1000 kcal energy was not significantly different for girls in those age groups ($p>0.05$).

When differences in energy intake was taken into account, both boys and girls aged 15 to 18 years had statistically higher average daily dietary zinc density than boys and girls in the younger age group ($p<0.05$). This indicated that the oldest

adolescents were consuming diets richer in zinc than those in the younger age groups.

Only 1% of the young people (7 boys and 10 girls) used zinc supplements. Supplements containing zinc made a very small contribution to average daily zinc intake of the population. The average daily zinc intake from all sources for both boys and girls was statistically more than the average daily zinc intake from food sources (6.90 mg versus 6.88 mg, $p<0.05$ for boys and 5.77 mg versus 5.74 mg, $p<0.05$ for girls); however, on a population basis (and not for the individuals), this difference was not nutritionally important. Contribution of supplements for all specific age groups of both boys and girls was negligible ($p>0.05$).

Average daily intake of zinc from food sources was less than the UK RNI for all age groups of both boys and girls. Mean daily dietary zinc intake was 86.3% of the RNI for boys and 77.7% of the RNI for girls.

When the intake of zinc from food sources was compared with UK LRNIs, overall 9.3% of boys and 20.0% of girls had an intake below this cut-off point. Among subgroups, 26.2% of girls aged 4 to 6 years and 34.5% of girls aged 11 to 14 years had an intake below the LRNI, indicating some may be vulnerable to zinc inadequacy.

Overall mean of plasma zinc concentration was 14.62 µmol/l for boys and 14.57 µmol/l for girls. Among boys, there was no significant difference in mean plasma zinc concentration for all age groups ($p>0.05$). For girls, there was a slight decline with increasing age (not necessarily significant for all age groups) as the mean plasma zinc concentration of 15.08 µmol/l among girls aged 4 to 6 years was significantly different from the mean plasma zinc concentration of 13.29 µmol/l for girls aged 15 to 18 years ($p<0.001$).

2.0% of boys and 4.8% of girls had plasma zinc concentration below 10.70 µmol/l (the common cut-off point for assessing plasma zinc concentration). Boys aged 4 to 6 years had the highest percentage of plasma zinc level that was below the cut-off value (10.3%).

The correlation of plasma zinc concentration with dietary zinc intake was weakly significant for girls aged 11 to 14 years ($r=0.207$ and $p<0.05$) and for the overall population of girls, regardless of their age group ($r=0.170$ and $p<0.01$). This correlation was not statistically significant in any other age groups ($p>0.05$).

The average daily dietary zinc intake for children who had plasma zinc concentration below normal (6.17 mg) was slightly less than children who had plasma zinc levels above normal (6.38 mg); however, this difference was not statistically significant ($p>0.05$).

In contrast, there was a significant difference ($p<0.05$) between the average plasma zinc concentration of children who had zinc intake below the LRNI (14.15 µmol/l) and average plasma zinc concentration of children who had intake above the LRNI (14.69 µmol/l).

For young people aged 4 to 18 years, the main source of zinc was from meat and meat products, mainly from beef, veal and dishes, burgers, kebabs and sausages and chicken, turkey and dishes, respectively.

One quarter of zinc intake came from cereal and cereal products. Within this group, the main contributors were white bread, high fibre and whole grain breakfast cereals, biscuits, buns, cakes and pastries, wholemeal bread, pizza and other breakfast cereals respectively.

Milk and milk products, together with vegetable, potatoes and savoury snacks contributed a further one third to mean daily zinc intake of young people aged 4 to 18 years. Figure 3.2 demonstrates the percentage contribution of food types to the average daily zinc intake of young people aged 4 to 18 years.

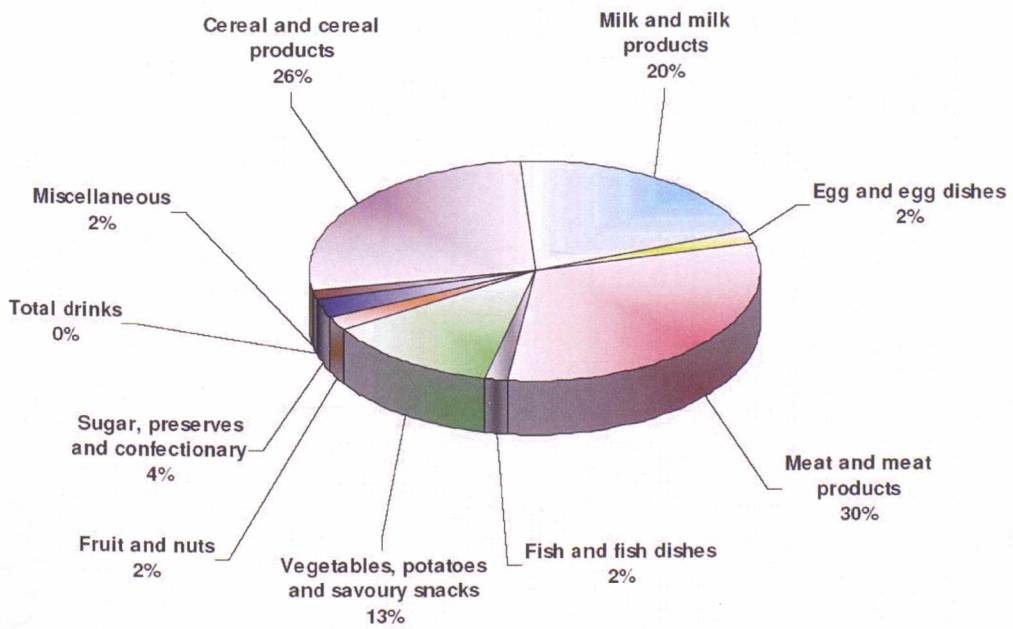


Figure 3.2 Percentage contribution of food types to average daily zinc intake of young people aged 4 to 18 years.

Gender	Males					Females				
Age group	4 - 6 Years	7-10 years	11-14 years	15-18 years	All	4 - 6 years	7-10 years	11-14 years	15-18 years	All
Average zinc intake (mg/day) from food sources	5.59	6.13	7.22	8.80	6.88	4.89	5.73	6.01	6.14	5.74
Average zinc intake (mg/day) from all sources	5.63	6.14	7.24	8.84	6.90	4.96	5.73	6.05	6.15	5.77
Average zinc density (mg/1000kcal) from food sources	3.62	3.46	3.69	3.92	3.65	3.53	3.59	3.61	3.77	3.63
Base (N)	184	256	237	179	856	172	225	238	210	845
Zinc RNI (mg/day)	6.5	7.0	9.0	9.5	NA	6.5	7.0	9.0	7.0	NA
Zinc Intake (as % RNI)	86.0	87.6	80.2	92.6	86.3	75.2	81.8	66.8	87.7	77.7
Zinc LRNI (mg/day)	4.0	4.0	5.3	5.5	NA	4.0	4.0	5.3	4.0	NA
Percentage of people with intake below LRNI	11.4	5.1	13.5	7.8	9.3	26.2	8.9	34.5	10.5	20.0
Base (N)	21	13	32	14	80	45	20	82	22	169
Average plasma zinc concentration (µmol/l)	14.22	14.96	14.52	14.67	14.62	15.08	14.72	14.62	13.95	14.57
Base (N)	39	124	150	134	447	39	98	139	145	421
Percentage with plasma zinc concentration below 10.70 µmol/l	10.3	0.8	0.7	2.2	2.0	0	1	1.4	4.8	2.4
Base (N)	4	1	1	3	9	0	1	2	7	10
Correlation coefficient (Pearson) between zinc intake and plasma zinc concentration	r=0.035 p=0.837	r=-0.095 p=0.302	r=-0.105 p=0.227	r=0.119 p=0.202	r=0.035 p=0.477	r=0.156 p=0.371	r=0.175 p=0.096	r=0.207 p=0.020	r=0.164 p=0.060	r=0.170 p=0.001

Table 3.2 Indices of zinc status for young people aged 4 to 18 years

Average daily intake of zinc, average daily intake of zinc as percentage of zinc RNI, the proportion of population with zinc intake below zinc LRNI, plasma zinc concentration, proportion of population with plasma zinc level below normal and correlation coefficient for plasma zinc and dietary intake of zinc by gender and age of young person.

3.3.3 NDNS: adults aged 19 to 64 years

In this survey, the mean daily zinc intake from food sources was 10.21 mg for men and 7.37 mg for women (Table 3.3). In all age groups, the average daily zinc intake for women was significantly lower than men ($p<0.001$).

Among men, the youngest age group (men aged 19 to 24 years) had a significantly lower mean daily zinc intake than men in all other older groups ($p<0.001$). For women aged 19 to 24 years, the average daily zinc intake was not significantly different from women aged 25 to 34 years; however, the youngest group had a zinc intake lower than women aged 35 to 49 years ($p<0.01$) and women aged 50 to 64 years ($p<0.001$).

Dietary zinc intake per 1000 kcal of energy increased with age for both men and women. The average daily zinc density of both men and women aged 19 to 24 years was significantly lower than the oldest group ($p<0.001$). As alcoholic drinks contributed to 7-9% to average daily energy intake [235], energy of alcohol was also taken into the account and 'average daily zinc density based on foods energy' was calculated to exclude the confounding effect of alcoholic drinks. After allowing for the difference in energy intake by excluding alcoholic drinks, the above findings remained unchanged. For example, it was confirmed dietary zinc intake of adults aged 19 to 24 years is less than the other age groups regardless of their energy intake.

Overall, there was a significant difference between average daily zinc intake from food sources and average daily zinc intake from all sources (including supplements) for both men and women ($p<0.01$ and $p<0.001$ respectively).

6.4% of adults (45 men and 65 women) used zinc supplements. Among men, the contribution of supplements to average daily zinc intake was most marked for those aged 25 to 34 years and those aged 50 to 64 years. Only in these two groups the average daily zinc intake from food sources was significantly different from the average daily zinc intake from all sources ($p<0.01$ and $p<0.001$, respectively).

Among women only for the youngest group, the effect of supplements to average daily zinc intake was negligible. For women aged 25 to 34 years, 35 to 49 years and 50 to 64 years, there was a significant difference between average daily zinc intake from food sources and average daily zinc intake from all sources including supplements ($p<0.05$, $p<0.001$ and $p<0.001$, respectively).

Overall, the mean daily zinc intake from food sources was more than the current UK RNI. Mean daily dietary zinc was 107.5% for men and 105.3% for women. Among men, only for the youngest group, the mean daily zinc intake from food sources fell below the RNI (at 94.7% of the RNI). For women aged 19 to 24 years and 25 to 34 years, the mean daily dietary zinc intake was 97.8% and 96.2% of the RNI respectively.

For both men and women, 3.9% of the population had an intake of zinc from food sources, which was below the current UK LRNI. Among different age groups, the highest percentage below the LRNI was among the youngest age group for both men and women (7.2% and 5.7% respectively).

Figure 3.3 shows that the main source of zinc for the respondents of this survey was meat and meat products contributing to around one third of average daily zinc intake. Within this group, beef, veal and dishes, chicken, turkey and dishes, burgers and kebabs, bacon and ham were the main contributors to the zinc intake.

Overall, cereal and cereal products contributed to around quarter of the average daily zinc intake. Within this group, the main contributors were white bread, breakfast cereals, wholemeal bread, soft grain and other breads and pizza. Milk and milk products, potatoes, vegetables and savoury snacks contributed a further 27% to average daily zinc intake of adults aged 19 to 64 years.

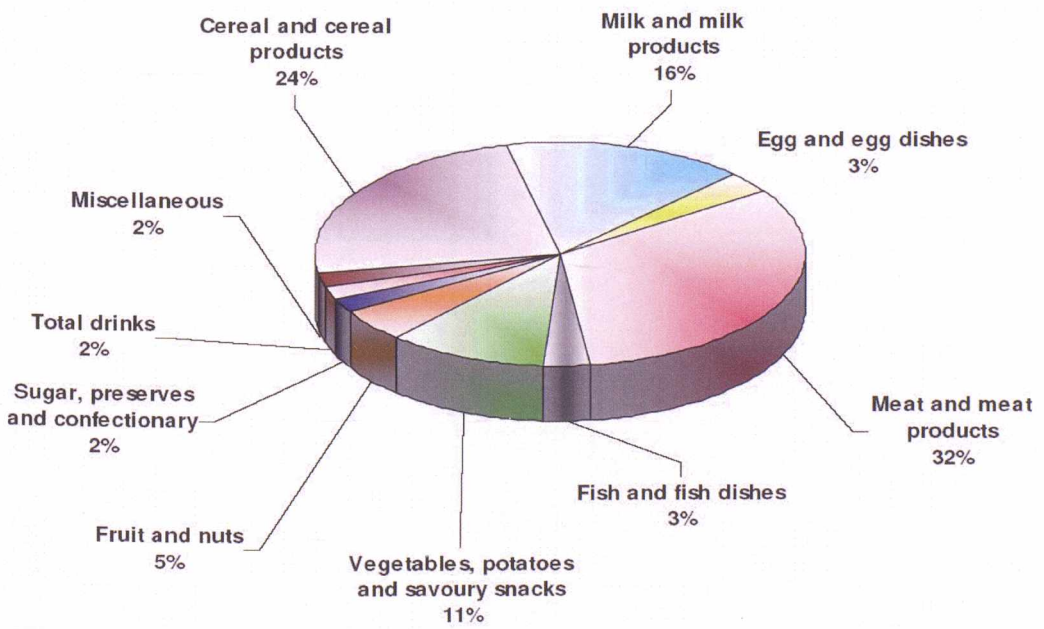


Figure 3.3 Percentage contribution of food types to average daily zinc intake of adults aged 19 to 64 years.

Gender	Males					Females				
Age group	19-24 years	25-34 years	35-49 years	50-64 years	All	19-24 years	25-34 years	35-49 years	50-64 years	All
Average zinc intake (mg/day) from food sources	8.99	10.25	10.56	10.35	10.21	6.85	6.73	7.59	7.84	7.37
Average zinc intake (mg/day) from all sources	9.17	10.65	11.37	10.84	10.73	7.11	7.12	8.22	8.59	7.94
Average zinc density (mg/1000kcal) from food sources	4.04	4.41	4.52	4.60	4.45	4.19	4.34	4.67	4.82	4.58
Average zinc density (mg/1000kcal) from food sources **Excluding energy from alcohol**	4.43	4.91	5.00	5.07	4.92	4.48	4.61	4.95	5.07	4.85
Base (N)	108	219	253	253	833	104	210	318	259	891
Zinc RNI (mg/day)	9.5					7.0				
Zinc Intake (as % RNI)	94.7	107.9	111.2	108.9	107.5	97.8	96.2	108.4	112.0	105.3
Zinc LRNI (mg/day)	5.5					4.0				
Percentage of people with intake below LRNI	7.2	2.3	4.2	3.4	3.9	5.4	4.6	3.5	3.0	3.9
Base (N)	8	5	11	9	32	6	10	11	8	34
All indices related to plasma zinc concentration	Not available					Not available				

Table 3.3 Indices of zinc intake for adults aged 19 to 64 years.

Average daily intake of zinc, average daily intake of zinc as percentage of zinc RNI, the proportion of population with zinc intake below zinc LRNI by gender and age.

3.3.4 NDNS: People aged 65 years and over

The average daily zinc intake from food sources for this survey was 8.73 mg for men and 6.92 for women. Dietary zinc intake decreased with age; for men, the average daily zinc intake was 9.02 mg in the 65 to 74 age group, compared to 8.36 mg for those aged 75 to 84 years and 8.20 mg for the men aged 85 years and over. For women, the corresponding intake was 7.09 mg, 6.92 mg and 6.62 mg (Table 3.4).

In men, when differences in energy intake were taken into account, the above results were different as the average daily zinc density (mg/1000 kcal) did not significantly decrease with age ($p>0.05$). This showed that the decrease in average daily zinc intake in older men is due to less energy consumed. In contrast, zinc content per 1000 kcal of energy reduced as women became older ($p<0.05$).

The average dietary intake of zinc was 91.9% of the RNI for men and 98.8% of the RNI for women. Women aged 65 to 74 years had an average daily dietary zinc intake above the RNI (at 101.3%). The mean daily intake of zinc from food sources was less than the UK RNI for all other age groups of both men and women.

Overall, 8.7% of men and 4.6% of women had intake below the LRNI. Elderly individuals, particularly men aged 75 years and over, seemed to be at increased risk of zinc deficiency as 12.8% of men aged 75 to 84 years and 13.2% of men aged 85 years and over had intakes below the LRNI (Figure 3.4).

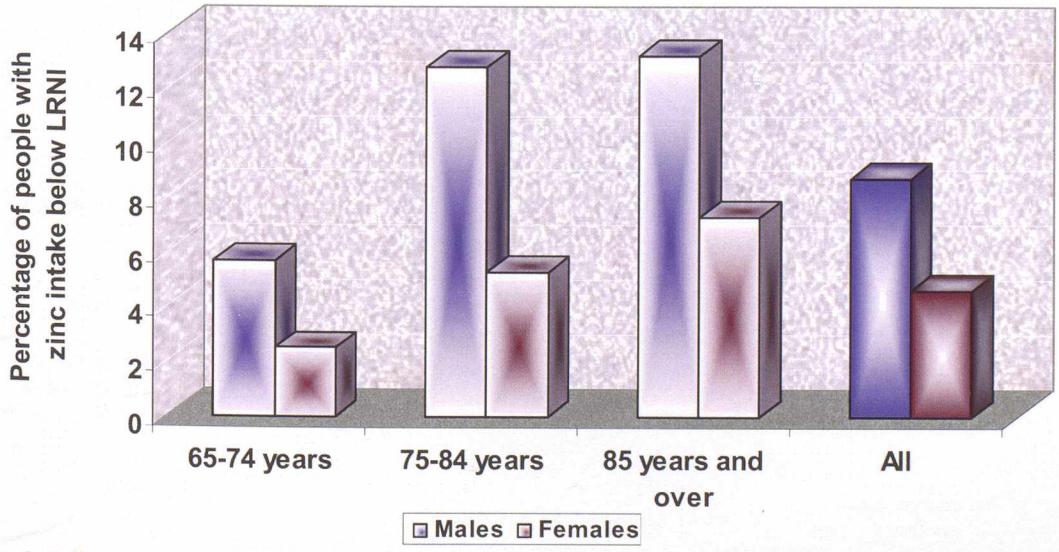


Figure 3.4 Percentage of population with zinc intake below LRNI in NDNS: People aged 65 years and over.

The mean plasma zinc concentration was 14.10 µmol/l for men and 13.92 µmol/l for women. The average plasma zinc concentration decreased with age in both

men and women. For example, men aged 85 years and over had lower plasma zinc concentration compared to the other age groups ($p<0.001$). A statistically significant trend toward lower plasma zinc concentration with increasing age was also seen among all age groups of women.

Overall, 8.2% of men and 7.1% of women had plasma zinc levels below 10.70 µmol/l. Elderly individuals aged 85 years and over had the highest percentage of plasma zinc concentration below normal, as in 24.7% of men and 16.6% of women, plasma zinc levels were below the cut-off point of 10.70 µmol/l.

Intake of zinc showed a weak positive correlation with plasma zinc concentration only in women aged 65 to 74 years ($r=0.198$ and $p<0.01$) and in overall population of elderly (i.e. aged 65 years and over) women ($r=0.110$ and $p<0.05$). There was no significant correlation between dietary intake of zinc and plasma zinc for men in any other age groups.

About a third of zinc intake of the elderly population was provided by meat and meat products mainly from beef, veal and dishes, bacon and ham and lamb and dishes, respectively. Cereal and cereal products contributed to about one quarter of zinc intake. High fibre and whole grain breakfast cereals, wholemeal breads and white breads were the main contributors within this group. Milk and milk products and vegetables potatoes and savoury snacks contributed a further 28% to zinc intake for the elderly population (Figure 3.5).

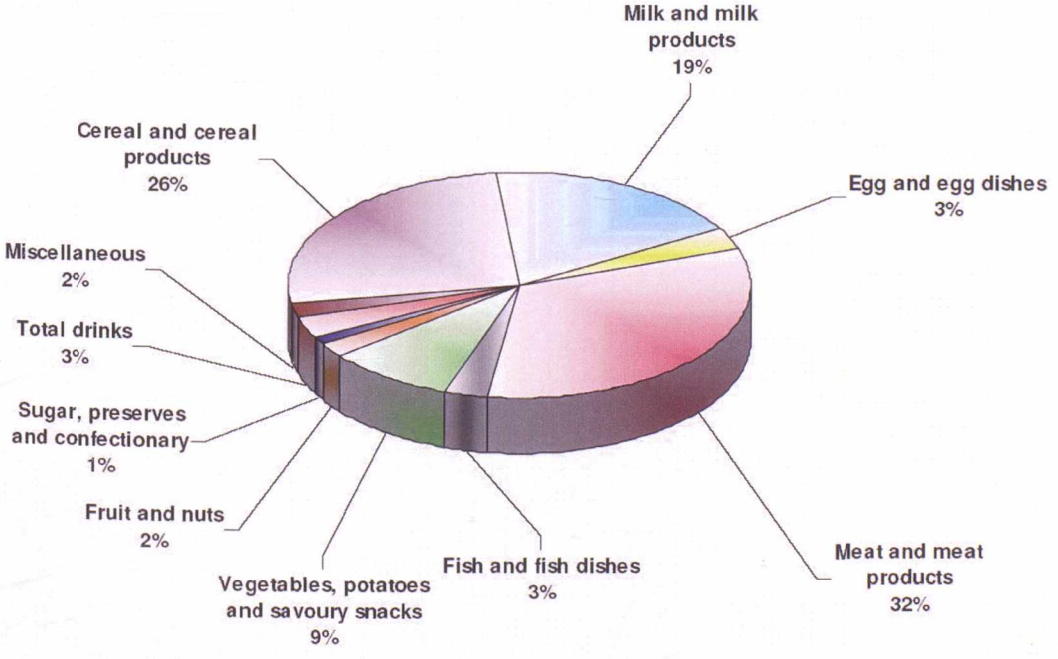


Figure 3.5 Percentage contribution of food types to average daily zinc intake of adults aged 65 years and over.

Gender	Males				Females			
Age group	65-74 years	75-84 years	85 years and over	All	65-74 years	75-84 years	85 years and over	All
Average zinc intake (mg/day) from food sources	9.02	8.36	8.20	8.73	7.09	6.92	6.62	6.92
Average zinc intake (mg/day) from all sources	9.10	8.43	8.24	8.80	7.17	7.05	6.75	7.03
Average zinc density (mg/1000kcal) from food sources	4.70	4.56	4.55	4.64	4.97	4.74	4.37	4.75
Base (N)	371	200	62	633	434	368	251	1054
Zinc RNI (mg/day)	9.5				7.0			
Zinc Intake (as % RNI)	94.9	88.0	86.4	91.9	101.3	98.8	94.6	98.8
Zinc LRNI (mg/day)	5.5				4.0			
Percentage of people with intake below LRNI	5.7	12.8	13.2	8.7	2.5	5.3	7.3	4.6
Base (N)	21	26	8	55	11	19	18	48
Average plasma zinc concentration (µmol/l)	14.46	14.00	12.31	14.10	14.53	13.70	12.88	13.92
Base (N)	241	125	41	406	276	218	117	611
Percentage with plasma zinc concentration below 10.70 µmol/l	7.2	4.6	24.7	8.2	1.2	9.4	16.6	7.1
Base (N)	17	6	10	33	3	21	19	43
Correlation coefficient (Pearson) between zinc intake and plasma zinc concentration	r=0.090 p=0.208	r=-0.068 p=0.318	r=0.097 p=0.326	r=0.062 p=0.157	r=0.198 p=0.009	r=-0.057 p=0.474	r=0.108 p=0.202	r=0.110 p=0.016

Table 3.4 Indices of zinc status for adults aged 65 years and over.

Average daily intake of zinc, average daily intake of zinc as percentage of zinc RNI, the proportion of population with zinc intake below zinc LRNI, plasma zinc concentration, proportion of population with plasma zinc level below normal and correlation coefficient for plasma zinc and dietary intake of zinc by gender and age.

3.4 Discussion

3.4.1 NDNS: children aged 1½ to 4½ years

3.4.1.1 NDNS: children aged 1½ to 4½ years, New findings

The published results of this NDNS were based on considering all children aged 1½ to 2½ years as a single group and all children aged 2½ to 3½ years as another group. Boys and girls aged 3½ to 4½ years were then separated into two different groups [222]. All data were regrouped to 'all aged under 4 years' and 'all aged 4 years and over' and comparisons with RNI and LRNI were also presented based on these groups.

The reason behind this was that the zinc UK DRVs of boys and girls are the same for all boys and girls aged less than 4 years, and the presentation of results with these categories is more concise.

The reanalysis of the data separated boys and girls of all age groups to present a more detailed result. For example, in the published results of the NDNS, the lowest average daily zinc intake was reported among children aged 1½ to 2½ years (4.3 mg compared with 4.7, as the highest intake for boys aged 3½ to 4½ years). Reanalysis of the data showed that the lowest average daily zinc intake is 4.18 mg for the girls 1½ to 2½ years (Table 3.1).

Only UK DRVs categorises all children aged under 4 years in the same group [217]; although, all other national and/or international dietary references (e.g. WHO, US FNB/IOM and IZiNCG) are based on smaller and more detailed age groups [16, 236, 237].

In this current report, the comparison with DRV cut-off points (including RNI and LRNI) are based on boys and girls of all three age groups of the survey (Table 3.1) and therefore, a clearer picture of zinc status is presented.

3.4.1.2 NDNS: children aged 1½ to 4½ years, Comparison with results of the other studies

3.4.1.2.1 United States

In the United States, in phase one of the third National Health and Nutrition Examination Survey (NHANES III), the average daily zinc intake for both boys and girls aged 1 to 2 years and 3 to 5 years were higher than for the children in the

NDNS. Table 3.5 summarises the average daily zinc intake in phase one of NHANES III [238].

Gender	Age groups	Mean (mg)	Median (mg)	Base (N)
Boys	1 to 2 years	6.96	6.57	601
	3 to 5 years	8.43	7.53	744
Girls	1 to 2 years	6.37	5.71	630
	3 to 5 years	7.64	6.76	803
Total	1 to 2 years	6.67	6.04	1231
	3 to 5 years	8.04	7.20	1547

Table 3.5 Mean and median zinc intake (mg/day) by age and gender in phase one of NHANES III, United States, 1988-1991.

Table was extracted from reference [238].

Briefel *et al* (2000) used the current estimates of dietary and total zinc intake in both phases of the NHANES III to indicate population groups with whom zinc status may be a concern. Among Non-Hispanic White children aged 1 to 3 years, average daily zinc intake was 6.6 mg for boys and 6.6 mg for girls [239]. These intake levels were higher than the zinc intake of boys and girls aged 1½ to 2½ and/or 2½ to 3½ years in the current NDNS.

The higher zinc intake of children aged 1 to 4 years in the US has been previously reported. For instance, according to the Continuing Survey of Food Intake in Individuals (CSFII), the 75th percentile of zinc intake in the US is 7.74 mg/day, which is higher than the tolerable upper limit for zinc in US [237, 240].

The results of the 2002 Feeding Infants and Toddlers Study also confirms that in general there is a high level of zinc intake among children and toddlers in the US. In that study, among toddlers aged 12 to 24 months, zinc intake of supplement users and supplement nonusers were 8.1 mg and 6.5 mg respectively. The mean zinc intake of these two groups were respectively 68% and 38% higher than the tolerable upper intake level of zinc [241].

There are some fundamental differences in design concepts, structure and the methodology of NHANES III and NDNS that must be taken into account when comparing the results of the two surveys. NHANES III is an important source of periodic information on the dietary, nutritional and health status of the whole US population that was designed to collect information on the population aged 2 months and older, over a 6-year period. The NDNS survey targeted the 1½ to 4½ year old age group over a year.

NHANES estimated nutrients using food and drink intake data from 24-hour dietary recalls [239]. The principle benefits of the 24-hour dietary recall, including its speed and ease of administration, allows a large number of subjects to be interviewed and this is particularly suitable for very large scale studies such as NHANES III. Its limitation is that it does not provide a reliable estimate of an individual's intake because of day-to-day variation [242, 243].

The 'flat-slope' syndrome (i.e. overestimating low intake and underestimating high intake toward reporting a good diet) is another major limitation of the 24-hour dietary recall [206, 242]. Thus, the 24-hour recall may be a method 'not suitable for assessing usual food and/or nutrient intake of individuals', particularly children [206, 242]. In contrast, NDNS used a 7-day weighed dietary record which was reported to be 'the most precise method available for estimating usual food and/or nutrient intake of individuals' [206].

Different methodology for the assessment of nutrient intake in NHANES III and NDNS may explain part of the differences in average daily zinc intake. When zinc intake of a small group of American children aged 1 to 4 years was measured via weighed dietary records as part of a balance (homeostatic) study, the average daily zinc intake was reported to be 5.0 mg [240]. This supports the assumption that the method of the assessment may in part explain the higher daily zinc intake of NHANES III compared with NDNS.

3.4.1.2 2 United Kingdom

In England, a sub study from the Avon Longitudinal Study of Pregnancy and Children (ALSPAC) known as Children in Focus (CIF), provided detailed dietary information on over a thousand 18-month old children residing in Southwest England. Results of the current study were compared with the most appropriate figures available from the CIF. Whilst only the results of children aged 1½ to 2½ years participating in NDNS were chosen for comparison, the mean age of children in CIF was less than children in this study [244].

In CIF, the average daily zinc intake was 5.1 mg for boys and 4.8 mg for girls (Table 3.6). Although the mean intake of zinc was slightly (but not significantly) higher for boys, after allowing for energy differences, there was no significant difference between the genders in zinc density. This was also seen in the current study.

In another study, based on the ALSPAC population, nutrient intake of children who participated in CIF was estimated at the age of 43 months (≈3½ years). Data was compared to the average zinc intake of children at age 18 months and to average daily zinc intake of children aged 3½ to 4½ years in the NDNS (Table 3.6).

When zinc intake per unit of energy was calculated, there was no significant difference between boys and girls confirming the previous results of CIF for 18-month olds and the results of the current NDNS.

	Mean (SD) dietary zinc intake (mg) at 18 months from ALSPAC (CIF)	Mean (SD) dietary zinc intake (mg) in 1½ to 2½ years from NDNS	Mean (SD) dietary zinc intake (mg) at 43 months from ALSPAC	Mean (SD) dietary zinc intake (mg) at 3½ to 4½ years from NDNS
Boys	5.1(1.3)	4.4(1.4)	5.3(1.5)	4.7(1.4)
Base (N)	563	298	488	250
Girls	4.8(1.2)	4.2(1.3)	4.9(1.4)	4.5(1.5)
Base (N)	463	278	375	243

Table 3.6 Comparison of average daily zinc intake in the UK ALSPAC and NDNS (present study).

The mean and standard deviation (SD) daily dietary zinc intake (mg/day) estimated from dietary records kept at 18 and 43 months of age in boys and girls in two sub-samples of the ALSPAC investigation compared with two age groups of NDNS: children aged 1½ to 2½ years and aged 3½ to 4½ years. Table was developed using references [244, 245] and the current study.

In CIF, the mean daily dietary zinc intake was just slightly above the RNI; however, for 3-year old boys, the median was below the RNI. For girls who participated in CIF, the mean and median daily dietary zinc intake were below the RNI confirming there is a risk of inadequate zinc intake in these children. In the NDNS in all age groups for both boys and girls, the mean and median daily dietary zinc intake was below RNI (results of the median were not presented here).

Although there were some differences in the design and structure of the NDNS and CIF, the main findings regarding zinc intakes were in agreement [244, 245].

3.4.2 NDNS: young people aged 4 to 18 years

3.4.2.1 NDNS: young people aged 4 to 18 years, New findings

Thane *et al* (2004) assessed the adequacy of zinc and vitamin A intakes [246] by analysing NDNS. Most of the results of that study are in agreement with the current findings; however, there are some differences.

In general, zinc intake was reported as adequate for most of the young people aged 4 to 18 years based on:

- Consideration of likely error of underreporting in participants [247].
- The fact that 'low' plasma zinc concentration was detected in only very small minority of participants.

However,

a) Underreporting was not considered to be significant except for a subgroup of girls (those aged 15 to 18 years) in the NDNS. The results of the feasibility study included estimates of the energy expenditure by a 'doubly labelled water' technique indicated that there was overall sufficient validity for adopting the methodology of the main stage survey [224].

b) Plasma zinc is a poor measure of marginal zinc deficiency. The level of zinc in plasma is homeostatically controlled and in marginal zinc deficiency it may remain within the normal range [206, 248].

c) Several confounding factors can affect plasma zinc levels. For instance, plasma zinc concentration may vary according to time of the day, proximity of meals, inflammation and infection, stress and occurrence of recent exercise [170, 206, 249, 250]. In the NDNS, these factors were not necessarily controlled particularly for the measurement of plasma zinc concentration. For example, 88 samples (8%) were collected from young people who had consented to the fasting procedure who had eaten or drank something in the morning before the blood sample was obtained [224].

d) Poor zinc status was defined as below 10.71 µmol/l for a fasting sample or below 9.95 µmol/l for a non-fasting sample [224, 246]. These cut-off points of zinc status were based on a study from Pilch and Senti (1984) using a reference population of the second NHANES [251]. 10.71 µmol/l is a value approximately two standard deviations below the mean plasma zinc concentration for adults. Since 1984, knowledge about zinc status has significantly improved. For example, a recent study by Hotz *et al* (2004) demonstrated that age and gender were significant confounders of serum zinc concentrations; therefore, separate cut-off points should be derived for children, adolescents and adult males and females. She suggests that the 2.5 percentile of morning serum zinc concentrations for fasting adolescents aged 10 years and over should be 74 ± 0.5 µg/dL (11.31 ± 0.07 µmol/l) [252]. More recent cut-off points based on age and gender would change the definition of 'low' plasma zinc concentration and suggest a larger population at risk of zinc deficiency.

e) 80% of the young people aged 4 to 18 years were reported to have dietary zinc intake below the RNI, while 13% were reported to have intake below the LRNI. 26.2% of girls aged 4 to 6 years and 34.5% of girls aged 11 to 14 years had an intake below the LRNI [224]. This implies that the dietary intake of considerable proportion of British people aged 4 to 18 years is likely to be inadequate for these subgroups [253].

3.4.2.2 NDNS: young people aged 4 to 18 years, Comparison with results of the other studies

3.4.2.2.1 United States

In the United States, the mean daily dietary zinc intake for all age groups was higher than their British counterparts were (Figure 3.6). The overall mean daily dietary zinc intake for young people aged 4 to 6 years, 7 to 10 years and 11 to 18 years were 7.7 mg, 9.1 mg and 10.4 mg respectively [239]. These values are more than 30% higher than the average intake of young people aged 4 to 18 years in the UK, as identified in the current investigation. Similar to this investigation, the NHANES III data showed that the dietary zinc intake of young people increased with age. Zinc intake of adolescent males was also higher than females [239].

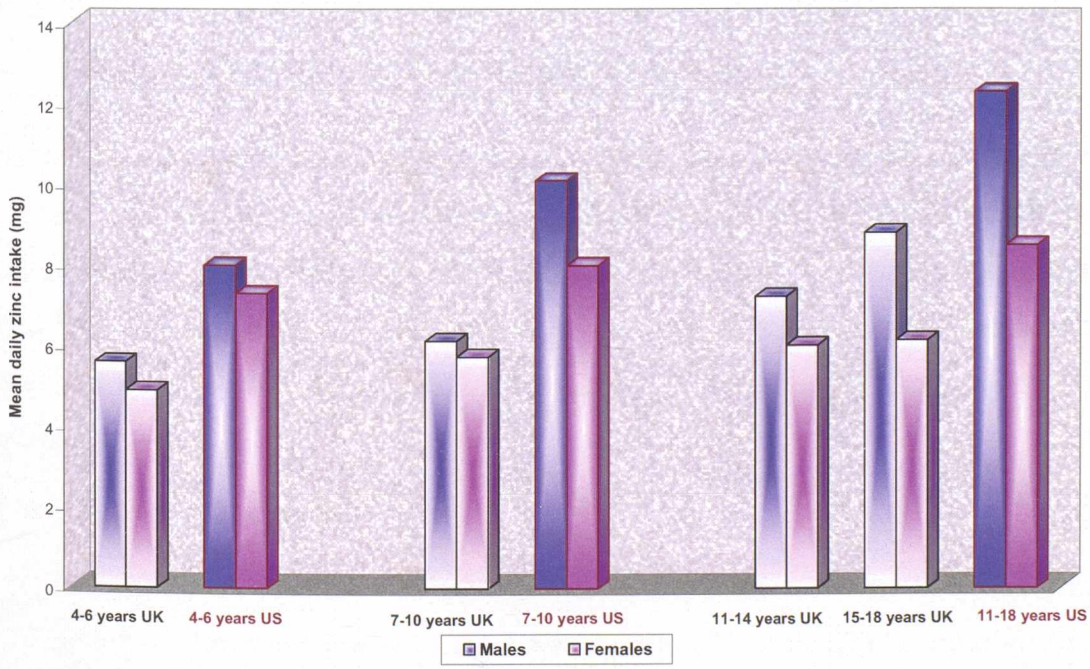


Figure 3.6 Mean daily zinc intake (mg/day) by age and gender in non-Hispanic white young people in NHANES III, US [239] compared with young people in NDNS, UK (present study).

Because the data on zinc density and standard deviation of zinc intake were not presented for non Hispanic white young people who participated in NHANES III, US [239], this graph is not adjusted for energy and error bars are not added.

3.4.3 NDNS: adults aged 19 to 64 years

3.4.3.1 NDNS: adults aged 19 to 64 years, Limitation of the study-The potential for non response bias

Despite fieldwork to improve response, response over the whole survey was low. With a high level of non-response, the potential for bias in the remaining data increases because there is a possibility that more data are collected from a particular group within the population. This may result in differential non-response where some groups are more likely to be represented in the data than the others.

Because of concerns about the potential impact of non-response and non-response bias, the survey authorities conducted an independent investigation to assess the usability of the data from this NDNS. This evaluation undertaken by the University of Southampton, concluded that there was no evidence to suggest serious non-response bias in this NDNS, even though the dietary characteristics of the total refusal and non-contact cases may be different from those of respondents. Thus, any survey estimates must be treated with care [254].

A population-based weighting procedure by gender, age and region was recommended. For example, men and the youngest age group were underestimated. To make up for this limitation, the data was weighted.

3.4.3.2 NDNS: adults aged 19 to 64 years, New findings

According to the published results of the NDNS adult men aged 19 to 64 years, the effect of supplements were most marked for those aged 35 to 49 years, because intake of zinc from all sources was 8% higher than from food sources alone. This approach was based on a percentage of the difference between the mean zinc intake from all sources (11.4 mg) and mean zinc intake from food sources (10.6 mg) [213].

This is misleading because:

1. The mean as an index of central tendency, is affected by the existence of outliers. Then, the variance and distribution of data must be studied prior to analysis.

The mean daily zinc intake from all sources for men aged 35 to 49 years is affected by the zinc intake of three participants whose zinc intake were 65.25 mg, 83.86 mg and 126.33 mg. These very high zinc intakes, from supplements, skewed the mean for the whole group.

2. A paired sample t-test can be used in order to take the variance of the data into the account.

A paired sample t-test examining differences between food and total zinc intake revealed that, among men aged 35 to 49 years, the mean daily zinc intake from food sources was not significantly different from the mean of daily zinc intake from all sources (10.6 mg versus 11.4 mg, $p>0.05$).

Figure 3.7 compares the average daily zinc intake from food sources to that from all sources, among different age groups in adult men. This figure demonstrates that any differences in mean values can be misleading, due to the variance of the groups.

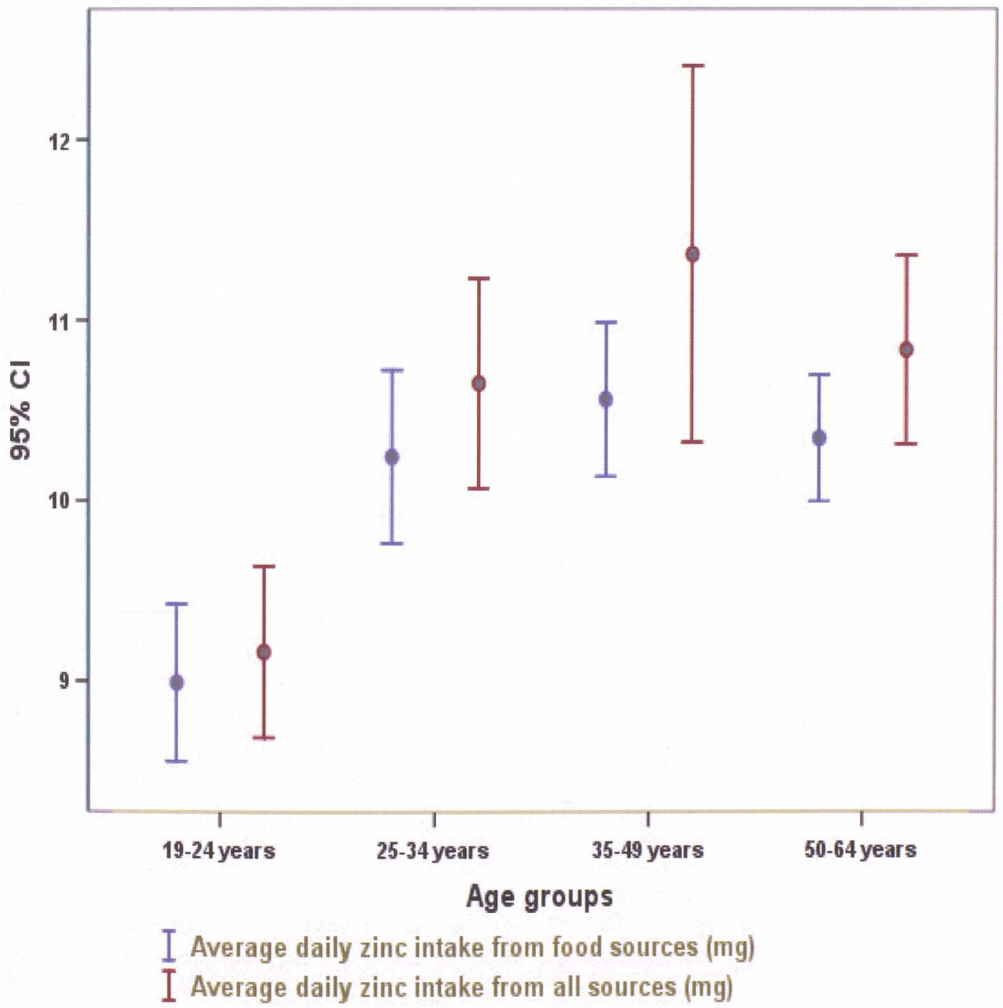


Figure 3.7 Zinc intake from food sources and all sources (including supplements) among different age groups of men in NDNS: adults aged 19 to 64 years.

There is an apparent difference between mean daily zinc intake from food sources and all sources among men aged 35 to 49 years but this difference is not statistically significant (p>0.05).

Similarly, among women aged 19 to 64 years, although the mean daily zinc intake from all sources including supplements was reported to be 4% higher than the

mean daily zinc intake from food sources, this difference was not, in fact, statistically significant (6.8 mg versus 7.1 mg, $p>0.05$).

In men, the contribution of supplements to daily zinc intake was significant only for those aged 25-34 years and those aged 50-64 years ($p<0.01$ and $p<0.001$, respectively).

Therefore, use of a simple mean to evaluate differences in nutrient intakes can be misleading, as it may be skewed due to effect of outliers. For this reason, the use of paired t-test (for between-group comparisons) should be the approach of choice [255].

3.4.3.3 NDNS: adults aged 19 to 64 years, Comparison with results of the other studies

3.4.3.3.1 United States

A valid comparison of the results of the NDNS with other surveys is difficult due to variations in the dietary assessment method and different age and anthropometric population characteristcs. The mean daily zinc intake of adults in the National Health and Nutrition Surveys 1999-2000 was higher than the mean daily zinc intake of UK adults [256].

Survey	Age	Zinc intake (mg/day)		Energy (kcal/day)		Zinc density (mg/1000 kcal)	
		Men	Women	Men	Women	Men	Women
UK NDNS 2000-2001	20-39 yrs	10.1	6.9	2131	1513	4.7	4.6
	40-59 yrs	10.5	7.9	2102	1597	5.0	4.9
US NHANES 1999-2000	20-39 yrs	14.8	10.1	2825	2028	5.2	5.0
	40-59 yrs	13.9	10.1	2590	1828	5.4	5.5

Table 3.7 Zinc intakes of US adults compared with UK adults.

Median daily zinc intake of US adults men and women as was reported from US NHANES 1999-2000 [256] was higher than UK adults. The American adults also had a higher energy intake than their UK counterparts. This difference was taken into account by expressing zinc intake per 1000 kcal consumed energy. After allowing for energy differences, US adults still had higher zinc intake compared with UK adults.

The average daily energy intake of the US adults was also higher than those of the UK adults, but higher energy and quantity of foods consumed cannot completely explain the higher zinc intake of American adults. After allowing for energy differences, the mean daily zinc density per 1000 kcal was still higher for the US population (Table 3.7).

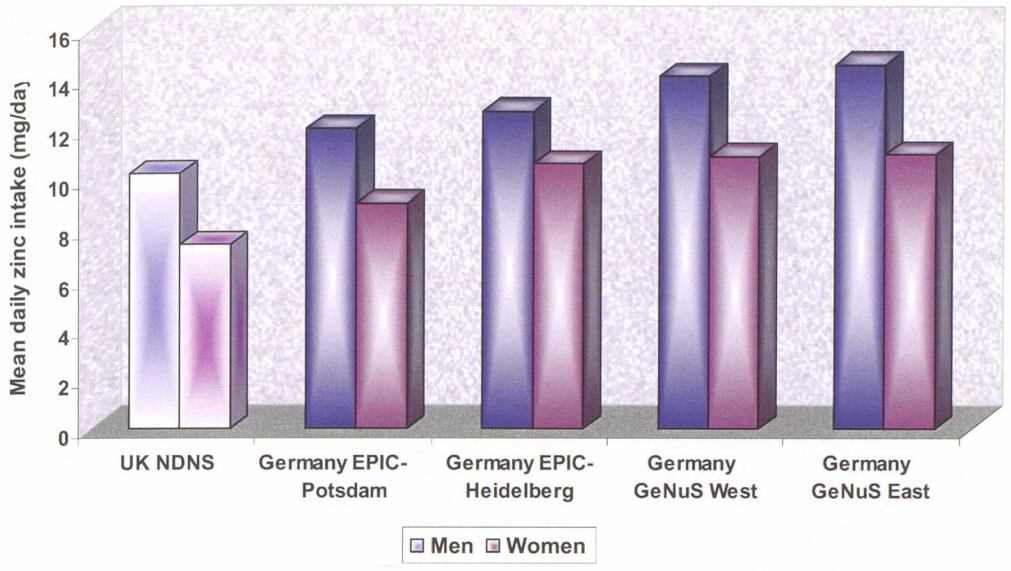
3.4.3.3.2 Germany

The German Nutrition Survey (GeNuS 1998) was conducted as a part of the German National Health Interview and Examination Survey (GNHIES) and assessed the nutritional status of German adults aged 18-79 years. Assessment of food consumption and nutrient intake in this survey was based on food frequency questionnaires [257].

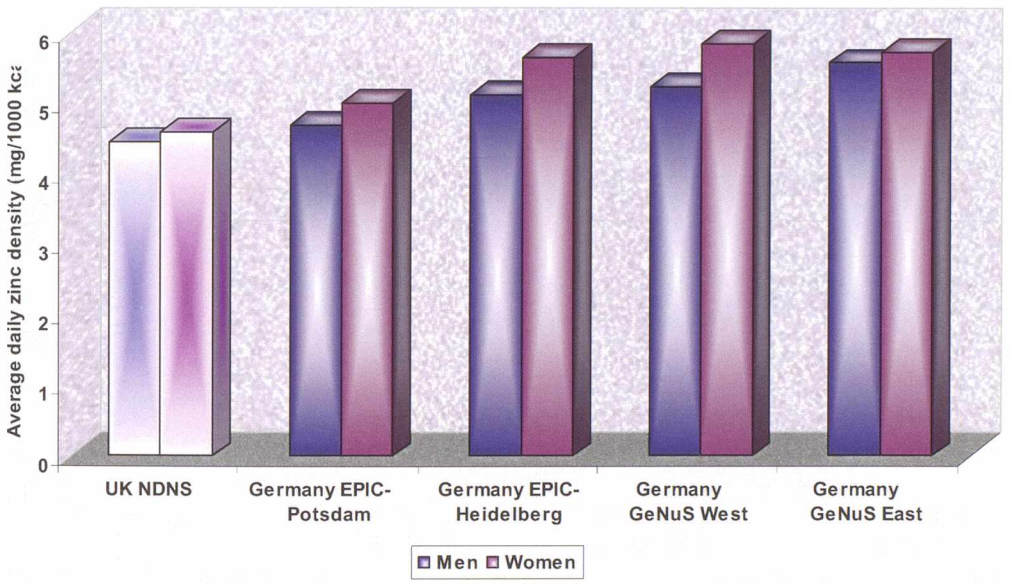
Most findings of GeNuS were in agreement with the results of two previous cohorts conducted in Potsdam and Heidelberg. These two cohorts were the German parts of the European Prospective Investigation into Cancer and Nutrition (EPIC) that described the intake of micronutrients (including zinc) assessed by 24 hour dietary recalls [258].

The average daily zinc intake for German adults in the West and East GeNuS and in both German cohorts were higher than those of UK adults. When differences in energy intake were taken into account, German men and women still had higher zinc intake per 1000 kcal energy consumption compared with their British counterparts (Figure 3.8).

A previous comparsion of the food intake of German and British adults showed that Germans comsume higher amounts of meat products and sausages, bread and pastries, eggs, cheese and cottage cheese than the British. Most of these foods are good sources of zinc. At the same time, it is not clear if higher intake of foods provides more zinc for Germans as they consumed less meat, fish and fish products, milk and dairy products, pasta, rice and cereals compared with UK adults [259]. These foods are also good sources of zinc.



A



B

Figure 3.8 Zinc intakes of German adults compared with UK adults.

Mean zinc intake of men and women in Germany based on two German cohorts conducted as part of European Prospective Investigation into Cancer and Nutrition (EPIC) in Potsdam and Heidelberg [258] and the German Nutrition Survey (Genus) in the east and west Germany [257]. Average daily zinc intake in Germany was higher than the UK for both men and women (A). German adults also had higher energy intake than UK counterparts. This difference was taken into account by expressing zinc intake per 1000 kcal consumed energy. After allowing for energy differences, German adults still had higher zinc intake compared with UK adults (B).

3.4.4 NDNS: People aged 65 years and over

3.4.4.1 NDNS: People aged 65 years and over, Limitation of the study

The NDNS of people aged 65 years and over was particularly affected by low energy reporting. For instance, the study of Cook *et al* (2000) revealed that in the free-living elderly population who participated in this survey, 48% of women and 29% of men were low energy reporters. Participants who underreported their energy, had significantly lower intake of macronutrients and micronutrients

(including zinc) than participants who normally reported their energy intake [232]. Several possible reasons were mentioned for this underreporting:

Although the weighed food record that was used in this survey is considered to be an accurate method for assessment of food intake, the completion of weighed food diaries require greater effort (compared with other methods) which can be a burden particularly for the elderly population. Increased health consciousness and reluctance to report consumption of foods known to be unhealthy could be another reason contributing to the underreporting energy and nutrient intake observed in this survey [232]. This underreporting food and nutrient intake was a significant limitation of the survey; thus, care must be taken in the interpretation of the dietary data, including the values of zinc intake reported here.

3.4.4.2 NDNS: People aged 65 years and over, New findings

Unlike the report of this survey [223], the results reported here are not separated for free-living and institutionalised participants. The characteristics of these two groups are analyzed and presented as one group for the sake of simplicity and to make the results comparable with those of other age groups and other countries.

Two previous investigations suggested that zinc status of the UK elderly population is satisfactory [233, 260]:

In the study conducted by Bailey *et al* (1997), plasma zinc concentration of all free-living men and women was higher than the cut-off point (10.71 µmol/l); however, the study was not representative of the UK population because the participants were a free-living elderly population from the city of Norwich only. Some subjects had zinc intake below the UK LRNI but had normal plasma zinc concentration and no evidence of zinc deficiency [260].

In the current investigation at least 7% of the UK elderly population had a plasma zinc concentration below 10.71 µmol/l. Bailey *et al* (1997) reported that 2% of elderly men and 1% of elderly women had zinc intake below the LRNI [260]; I found 8.7% of men and 4.6% of women had zinc intake below the UK LRNI.

The current findings are in agreement with a supplementary analysis of the NDNS by Bates *et al* (1999) [233]. Bates *et al* reported that 7% of the free-living elderly population and 8% of elderly participants living in institutions had intake below the LRNI. They concluded that the nutritional zinc status of the UK elderly population is adequate because only 2% of free-living and 9% of the institutionalised elderly population had plasma zinc concentration below normal.

However, plasma zinc concentration, although commonly used, is not a specific and selective index of zinc status [206]. The current results indicated that the percentage of people with zinc intake below the LRNI and the percentage of people with a zinc concentration below normal increases with age. For instance, one in eight men aged over 75 years had zinc intake below the LRNI and one in four men aged 85 years and over had a plasma zinc concentration below a normal level.

3.4.4.3 NDNS: People aged 65 years and over, Comparison with results of the other studies

3.4.4.3.1 United States

In the United States, average zinc intakes reported from the NHANES III and Continuing Survey of Food Intake by Individuals (CSFII) were higher than the values found in the present study:

The mean daily dietary zinc intake of American people aged 71 years and over who participated in NHANES III was 10.9 mg/day for men and 8.0 mg/day for women [239]. These findings were in agreement with findings of the CSFII with mean daily dietary zinc intake of elderly men and women were 11.5 mg/day and 7.6 mg/day respectively [261].

There is no direct investigation available to compare the dietary zinc content of the US and UK data. It was assumed that higher energy intake (and perhaps variety of foods) of the US elderly population is the underlying reason for the higher zinc intake; however, energy intake reported from the elderly population in NHANES III was not very different from the energy intake of the elderly in NDNS [235]. Table 3.8 demonstrates that the energy intake of the elderly men and women in the US cannot take account of their higher zinc intake.

Gender	Age	US NHANES III Energy (kcal/day)	UK NDNS Energy (kcal/day)
Men	60-69 years	2109.9	1993.1
	70-79 years	1880.0	1897.3
	80 years and over	1769.5	1827.4
Women	60-69 years	1571.6	1454.1
	70-79 years	1429.7	1477.1
	80 years and over	1323.8	1515.4

Table 3.7 Energy intake of elderly men and women who participated in US NHANES III and UK NDNS.

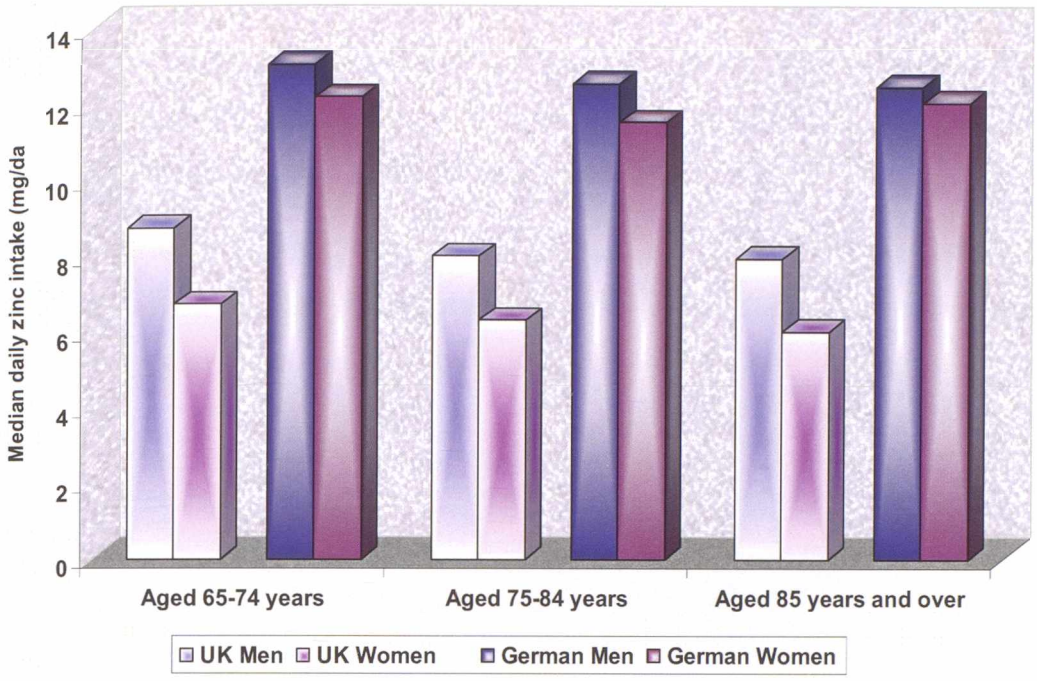
Table shows that different energy intake does not account for higher zinc intake of the US elderly population [235] compared with the UK elderly population.

In the US, major contributors to dietary zinc intake for older adults included beef, ground beef, legumes, poultry, ready to eat and hot cereals, pork, hotdogs, sausages, pasta, fish, lunch meats, yogurt, fast food milk shakes, eggs, cheese and cheese products [261]. The main food sources of zinc for the UK elderly population was meat and meat products (including beef, veal and dishes), cereal and cereal products followed by milk and milk products.

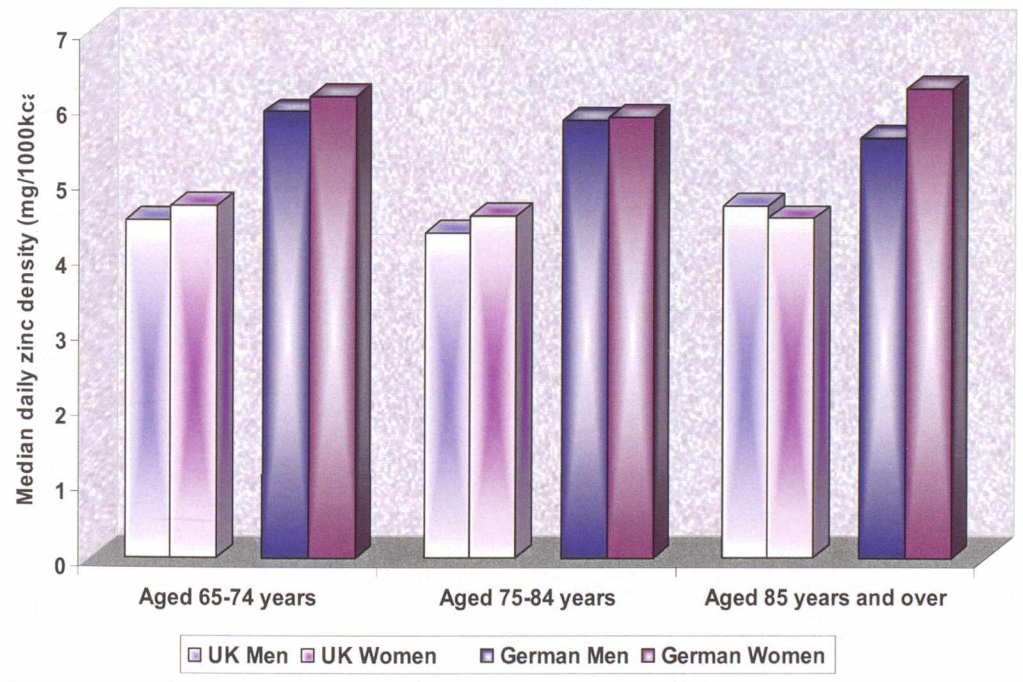
3.4.4.3.2 Germany

In Germany, a nationwide, cross-sectional survey of elderly people living in private households, provided representative and reliable data about energy and nurient intake of the German elderly population [262]. For all age groups of men and women, the median daily zinc intake of the UK free-living elderly population was lower than their German counterparts (Figure 3.9). Detailed comparisons between the two studies is difficult because of differences in dietary assessment methods and nutrient databases and designs.

In the survey that was conducted in Germany, dietary assessment was performed using a 3-day estimated dietary record. The fact that a high proportion of participants completed the dietary record (88.5%, without differences in age, health status and level of education) confirmed that it was a suitable method of dietary assessment among the elderly population. In the UK, 59% of the free-living elderly population completed a full four day dietary record and nutrient intake of elderly population was underreported because completion of weighed food diaries requires greater efforts and can be difficult for elderly people [232, 262]. It may be that 3-day estimated dietary record should be validated to be used in UK, in order to boost underreporting and incompletion seen in NDNS.



A



B

Figure 3.9 Zinc intake of German elderly compared with UK elderly.

Median zinc intake of elderly men and women in Germany [262] was higher than the UK for all age groups (A). The German elderly also had a higher energy intake than UK counterparts. This difference was taken into account by expressing zinc intake per 1000 kcal consumed energy. After allowing for energy differences, the German elderly still had a higher zinc intake compared with the UK elderly (B). Except for the oldest age group, the higher zinc intake of men compared with women was because of higher energy intake. This was persistent for the German and UK elderly population.

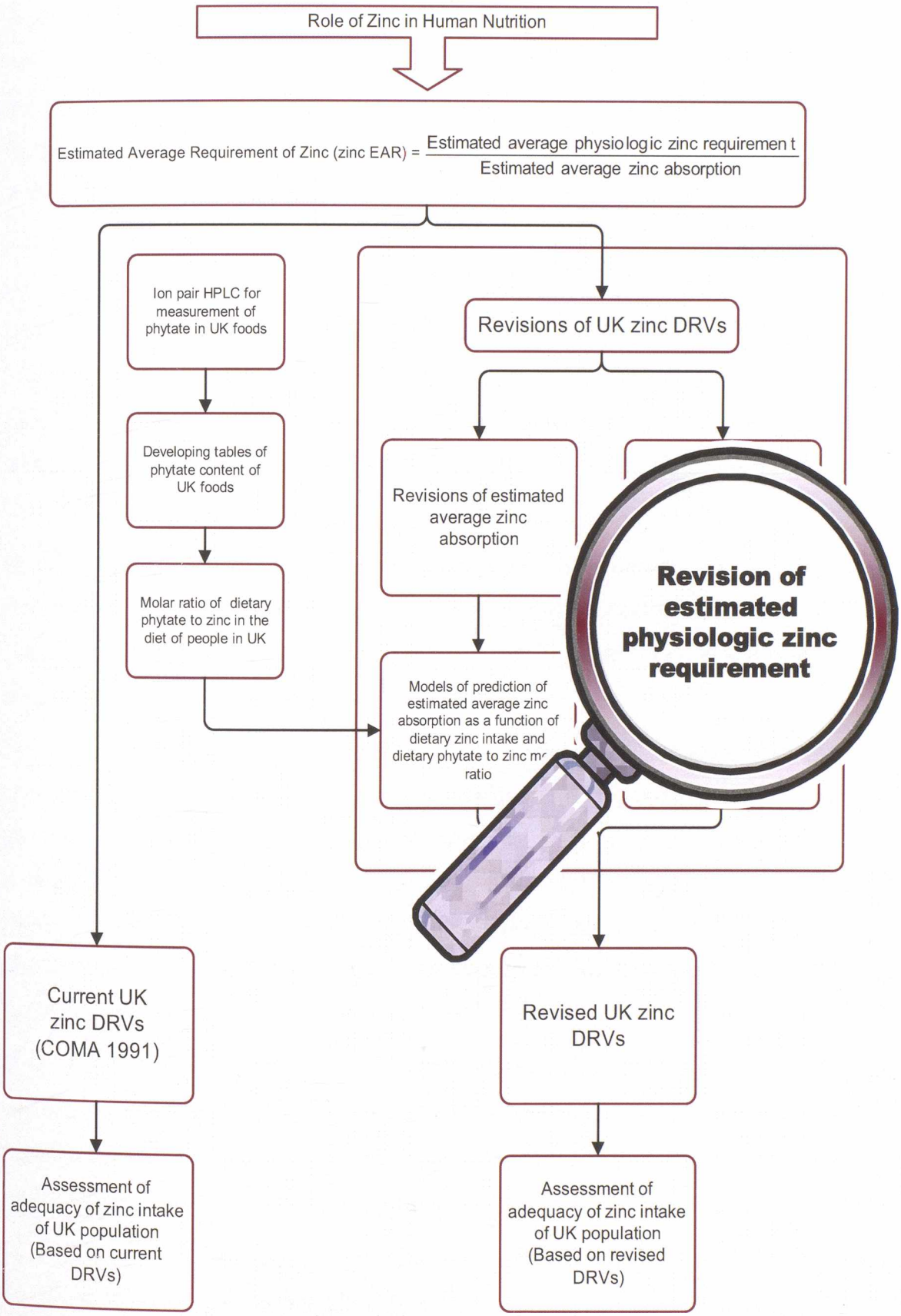
3.5 Conclusion

This purpose of this chapter was to examine zinc intake and status of different age groups in the UK population based on the current UK zinc DRVs (COMA 1991 [217]). Average zinc intake from food sources for the UK population ranged from

4.18 mg/day for toddlers to 10.56 mg/day for adults. The highest proportion of the population with zinc intake below LRNI was seen among children aged 3½ to 4½ years, young people (particularly girls) aged 4 to 6 and 11 to 14 years and elderly population (particularly men) aged 75 years and over.

These vulnerable groups exposed to inadequate zinc intake will be compared with vulnerable groups identified in Chapter 8 in order to provide a better understanding of groups who need to be targeted by nutrition or health interventions.

Chapter 4: Estimation of the Average Physiologic Requirement of Zinc for the UK Population



The magnifier symbol shows where you are in the conceptual framework of the thesis.

4.1 Introduction

4.1.1 Background

There is usually inadequate information to set a precise distribution of nutrient requirement for a group of individuals, but if we assume a normal distribution, it gives us a notional mean requirement, which is identical to the Estimated Average Requirement or EAR [217]. EAR represents the mean of dietary nutrient requirement at which 50% of individuals would meet their physiological needs [16].

For almost all of the micronutrients, the theoretical model of requirements has been approached on the basis of micronutrient turnover. These models try to estimate the requirements based on basal body losses in different age and physiologic groups. For example, in the model of physiologic zinc requirements in adults, a factorial method estimates the average physiologic requirements from the amount of zinc that must be absorbed to offset the amount of endogenous zinc lost from both intestinal and non-intestinal sites.

In this model for growing children and pregnant women, the amount of zinc retained in newly grown tissues is taken into account for total physiologic requirements. The amount of zinc transformed to breast milk is also factored into physiologic requirement of lactating women [16].

There is a difference in the calculated zinc physiologic requirement (based on conceptual balance) and dietary requirements. The physiologic requirement gives the amount of zinc, which must be absorbed, to counterbalance the sum of endogenous zinc lost through all routes of excretion plus the amount of zinc retained in newly grown tissues [16]. However, there is not 100% absorption for zinc in the body, so there is a need to translate the physiologic requirement of zinc to reflect zinc bioavailability.

Zinc EAR is an estimate of the average zinc requirement and not a recommendation for intake by individuals or groups. As the EAR represents the midpoint of dietary requirement for half of the population, it can be used to predict a theoretical criterion for zinc adequacy.

In the normal distribution, two notional standard deviations (SD) above this midpoint, is assumed as the zinc Reference Nutrient Intake (zinc RNI) and intake above this point is assumed almost certainly adequate. In contrast, two notional standard deviations below the zinc EAR, is defined as the zinc Lower Reference

Nutrient Intake (zinc LRNI). In theory, intake below this cut-off is assumed almost certainly inadequate. Therefore, any changes in zinc EAR will change our understanding of zinc adequacy and zinc status in the population [217].

A zinc EAR must take into account both the physiologic requirement for zinc and the proportion of zinc in the diet that is absorbed by the intestine. Dividing the mean physiologic requirement for zinc by the estimated average absorption of zinc derives zinc EAR.

$$\text{Zinc EAR} = \frac{\text{Mean physiologic requirement for zinc}}{\text{Estimated average absorption of zinc}}$$

Equation 4.1 Zinc EAR

Therefore, to set an EAR for zinc in any age and physiologic group, we need an estimation of the physiologic requirement and an estimate of proportion of absorption. The outline of the calculations of EAR in the factorial approach is:

1. Calculation of non-intestinal endogenous zinc excretion.
2. Calculation of intestinal endogenous zinc excretion.
3. Calculation of a relationship between intestinal zinc excretion and the amount of absorbed zinc.
4. Determination of the minimal zinc absorption required to replace total endogenous zinc excretion (determination of zinc physiologic requirement).
5. Determination of the average dietary zinc intake required to achieve absorption of the amount of zinc necessary to match total zinc excretion.

4.1.2 Remit of the investigation

Many countries and organizations have issued dietary recommendation for zinc appropriate to their individual circumstances. In the United Kingdom, the basic British standard is the zinc Dietary Reference Values (DRVs) including LRNI, EAR and RNI. The Committee Of Medical Aspects of Food Policy (COMA) established these reference values in 1991, incorporating the most recent research finding at that time [217].

In COMA (1991), the assessment of zinc requirement was based on determining basal losses with a consideration of only three references [18, 263, 264]. The report used data on basal faecal, urinary, integument, semen and menstruation losses and on a factorial basis, the report estimated that minimal losses are 2.2

mg/day in men and 1.6 mg/day in women. Based on the scientific knowledge available at the time, COMA assumed a 30% absorption efficacy for zinc and translated these physiologic requirements to give an EAR of 7.3 mg/day for men and 5.5 mg/day for women [217].

Since the mid 1990s, different organizations have put together Expert Committees to revise and develop estimates of human zinc requirements. The World Health Organization, Food and Agriculture Organization and International Atomic Energy Association (WHO/FAO/IAEA) of the United Nations as well as the Food and Nutrition Board (FNB) of the US Institute Of Medicine (IOM) have reviewed the scientific evidence to propose dietary zinc intake that is needed to meet physiologic requirements. All of these expert committees used the theoretical model of zinc requirement to estimate the physiologic requirement, and then reviewed the dietary sources of zinc in individuals to estimate the proportion of absorption in the diet that is absorbed by the intestine.

Most recently, the Steering Committee of the International Zinc Nutrition Consultative Group (IZiNCG) has considered a large number of studies to estimate the average physiologic zinc requirement and to suggest dietary recommendations. The technical document of IZiNCG has developed a new method for estimating the prevalence of inadequate zinc intake using the presence and the bioavailability of zinc in the regional food supply [16]. This document has been used as a reference in recent publication of the World Health Organisation [265].

Although all of these committees used the same conceptual framework as the UK COMA, their reports are much more recent and detailed. The latest UK 'Review of Zinc', published by the Expert Group on Vitamins and Minerals Secretariat [266], compared the DRVs published in the UK, US and Norway, but did not revise the UK DRVs or look at new studies. However, a number of studies published in the last decade have changed our understanding of both endogenous zinc losses and zinc bioavailability.

4.1.3 Aim

This chapter aims to incorporate the latest evidence into the theoretical model of zinc requirement in order to derive a revised version of the estimated physiologic zinc requirements for the UK.

The new estimates of the physiologic requirements are then used to produce dietary reference values. Based on these reference values, the available data of the National Diet and Nutrition Surveys (NDNSs) are reanalyzed in order to reconsider the vulnerable groups exposed to zinc inadequacy (Chapter 8).

4.2 Estimation of the physiologic zinc requirement in adults

The estimated physiologic requirement of zinc is the amount of zinc that must be absorbed to offset the sum of endogenous zinc lost through all routes of excretion in addition to the amount of zinc retained in newly grown tissue [16].

4.2.1 Estimation of non-intestinal zinc excretion in UK adult men

Non-intestinal losses of endogenous zinc are via the urine, the integument and to less extent via semen and menstrual secretions:

4.2.1.1 Urinary zinc excretion

In severe dietary zinc inadequacy, urinary zinc excretion may decrease significantly [267]; however, over a wide range of the dietary zinc intake (4 to 25 mg) urinary zinc excretion is not related to zinc intake. This was confirmed by 17 previously published studies (i.e. [267-283]) reviewed by FNB/IOM. Based on these studies the FNB/IOM estimated that the mean urinary zinc loss of adult men is around 0.63 mg/day [237].

The lower estimate of urinary excretion published by the WHO (0.3 mg/day) was based on just two studies [263, 276]. In those studies zinc intake was very low (0.8 to 3.6 mg/day) and the amount of urinary zinc was inflated by 40% to allow for the reduction in urinary zinc excretion in response to low zinc consumption [236].

The IZiNCG agreed with estimates of the FNB/IOM committee as it was derived from a larger number of studies; these studies provided detailed documentation of the analytic process; and more importantly, included only studies where zinc intake was within the usual range, in which the urinary zinc loss is stable and likely to include the true physiologic requirement [16].

The current investigation estimated the mean urinary zinc excretion to be 0.51 mg/day, based on the amount reported in 47 studies from 16 published articles (i.e. [267, 268, 270-276, 278, 280-285]) that measured urinary zinc excretion (Figure 4.1). The information derived by the present investigation: (1) reviewed almost all previous studies; (2) included the studies in which urinary zinc excretion

was measured when intake of zinc was accompanied with dietary factors affecting zinc absorption, because this is more likely to represent usual daily zinc nutrition; (3) excluded the studies that used unusual diets or the values that were statistically found to be outliers of normal distribution of data [269, 276]; and, (4) provided the most recent currently available studies.

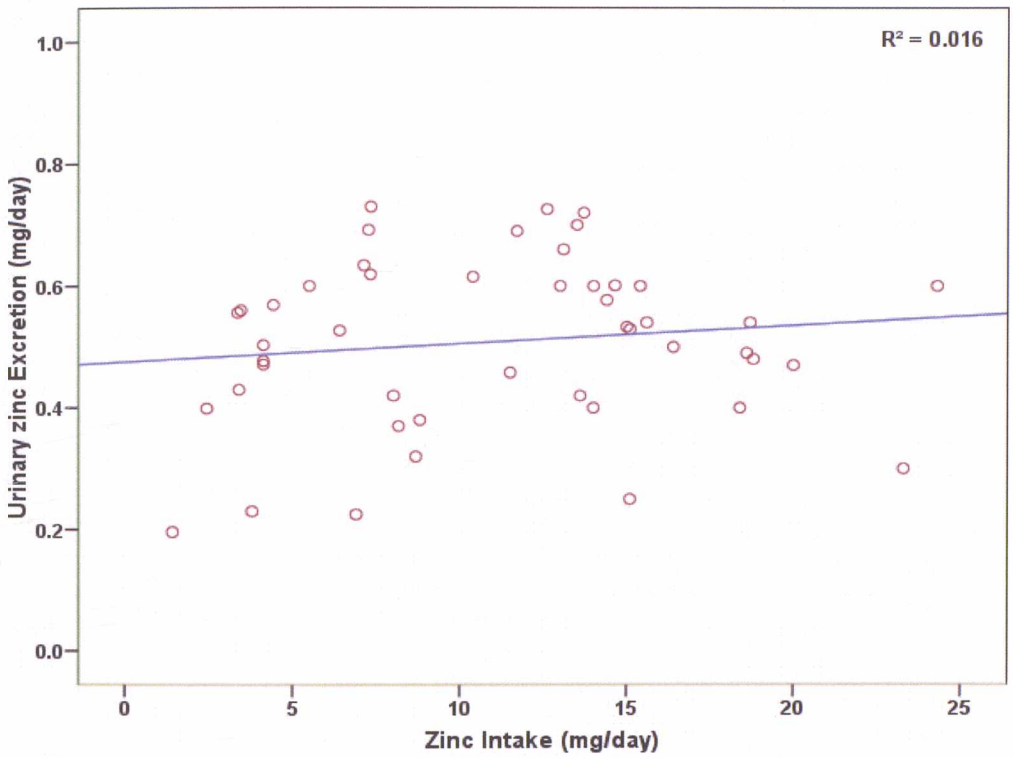


Figure 4.1 Lack of significant correlation between zinc intake and urinary zinc excretion in adult men.

The urinary zinc excretion was not related to zinc intake over a wide range of dietary zinc intake in published results (p>0.05). The 47 data points represent mean data points from 16 previously published studies [267, 268, 270-276, 278, 280-285] that investigated the relationship between dietary zinc intake and urinary zinc excretion in adult men.

4.2.1.2 Zinc excretion via the skin

The FNB/IOM committee suggested that mean integumental and sweat losses of zinc is 0.54 mg/day [237]. This assumption was based on one study of integumental and sweat zinc excretion, conducted in 11 adult men whose average zinc excretion (0.54 mg/day) did not alter in response to several levels of dietary zinc intake ranging from 1.4 to 10.3 mg/day through a period of 28 to 35 days [273].

The corresponding figure published by the WHO referred to a single former study of eight adult male volunteers in whom skin zinc excretion decreased from 0.49 mg/day to 0.28 mg/day, when their daily dietary zinc intake declined from 8.3 mg/day to 3.6 mg/day [276].

The IZiNCG steering committee concluded that the information derived by the FNB/IOM committee was more reliable and should be used until more information becomes available. IZiNCG also suggested that skin zinc excretion should be adjusted for body size. The IZiNCG applied a figure per kilogram body weight (i.e. 6.5 µg/kg) derived from reference [273] and suggested that the amount of zinc loss via integument for a 65 kg adult man is 0.42 mg/day [16].

The current investigation considered both studies [273, 276] together with one former study [286] and concluded that zinc losses via the skin should be 0.50-0.53 mg/day. The mean weight of British men in the current NDNS was 84 kg [227]. The current investigation applied the figure per kg body weight as it was recommended by the IZiNCG. For an 84 kg adult British man, the amount of zinc lost via the integument and sweat is 0.55 mg/day.

4.2.1.3 Zinc excretion in semen

Although the WHO did not include an estimate of seminal zinc excretion, the FNB/IOM committee referred to two papers [273, 287] which reported seminal zinc concentration and ejaculated volume of adult men. The zinc concentration from semen (0.11 mg/day) did not alter with restricted zinc consumption, and the ejaculated volume only declined at the lowest level of zinc intake (1.4 mg/day); therefore, the FNB/IOM committee applied a single figure of 0.10 mg zinc loss per day in semen, based on a mean ejaculate volume of 2.8 ml and a mean number of 2.45 ejaculations per week [237].

The IZiNCG agreed with estimates of the FNB/IOM, but explained that more information was required from a broader range of individuals, particularly on the average daily volume of semen. While awaiting for additional information to become available, the IZiNCG steering committee agreed with the figure of 0.10 mg/day as the average seminal zinc excretion [16].

The present research reviewed the body of knowledge available at the time (including two papers used by the FNB/IOM committee) and concluded that the 0.1 mg/day estimate of seminal zinc loss is realistic because: (1) seminal zinc loss was almost constant through a wide range of zinc intake; (2) the mean ejaculate volume of 0.28 ml was confirmed by a more recent research [288]; and, (3) although more recent evidence indicated that frequency of ejaculation for adult men aged 20 to 29 years average 3.5 ejaculations per week [289], Mean of 2.45

ejaculations per week could be realistic as it takes into account the less frequent ejaculations of older age groups.

The figure of 0.10 mg/day average zinc loss in semen was accepted for this investigation.

4.2.2 Estimation of non-intestinal zinc excretion in UK adult women

Although the general conceptual framework of the theoretical model of zinc requirement applies to women as well as men, specific figures used for the estimation of non-intestinal zinc losses of women are described in the following sections:

4.2.2.1 Urinary zinc excretion

The report of the WHO committee was based on the result of just one study of women who were consuming very limited zinc intake [290]. The results were also inflated by 40%, as described for men. The FNB/IOM committee reviewed the results of 10 published papers (i.e. [37, 270, 271, 291-297]) and concluded that the mean of the urinary zinc excretion for adult women is 0.44 mg/day. For the same reasons described in the estimation of urinary zinc excretion of men, the IZiNCG committee accepted the figure proposed by the FNB/IOM committee.

The current investigation examined the result of 10 published studies (i.e. [37, 270, 271, 292-296, 298, 299] to calculate a mean urinary zinc excretion of 0.40 mg/day for adult females (Figure 4.2). This investigation: (1) reviewed and included some more recent studies; (2) expectedly found no relationship between zinc intake and urinary zinc excretion; and, (3) confirmes the findings of the FNB/IOM committee.

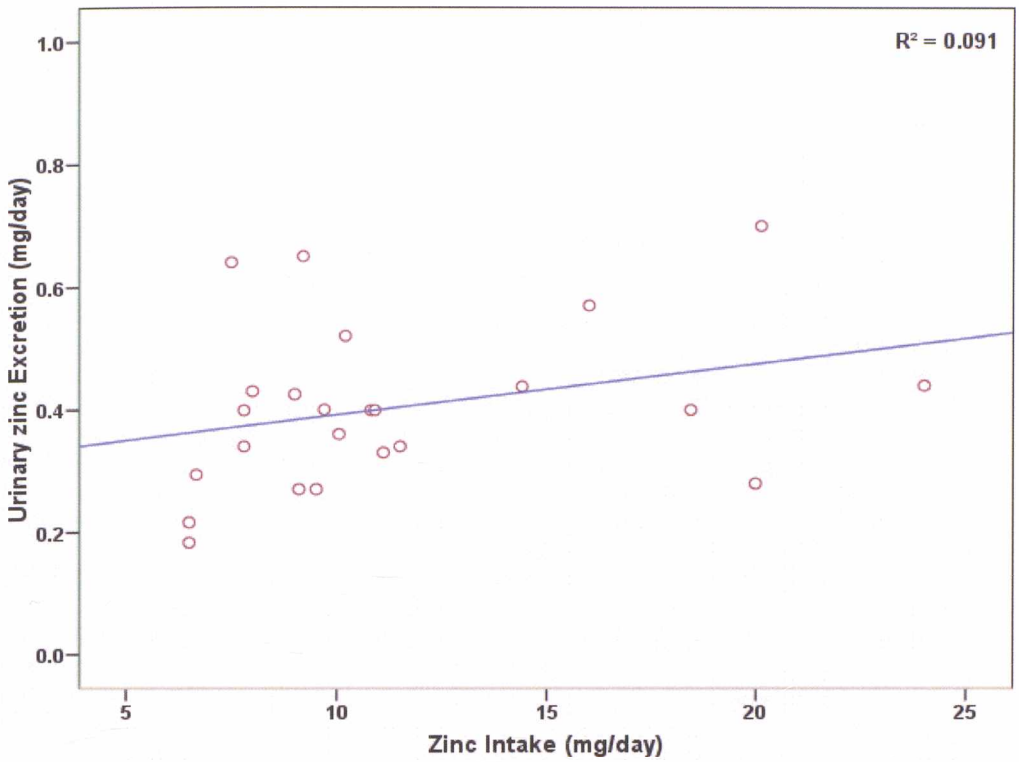


Figure 4.2 Lack of significant correlation between zinc intake and urinary zinc excretion in adult women.

The urinary zinc excretion was not statistically related (p>0.05) to zinc intake over a wide range of dietary zinc intake in published results. The 24 data points represent the mean data obtained from 10 previously published studies [37, 270, 271, 292-296, 298, 299] that investigated the relationship between dietary zinc intake and urinary zinc excretion in adult men.

4.2.2.2 Zinc excretion via the skin

Based on the assumption that there was no report about zinc excretion via the skin in women, each of the earlier expert committees estimated zinc excretion via the skin in females by adjusting for difference in body surface area extrapolated from the information available for males. However, integumental zinc excretion of young women has been studied in one investigation:

Integumental zinc losses of women under conditions of very low zinc intake (0.17 mg/day) were 0.68 mg/day [300]. The current investigation did not use this result and used the figures extrapolated from the adult men data because: (1) subjects of the study had an unusual low intake of zinc; (2) many potential sources of errors and contaminations were reported during the collection and measurement of the integumental zinc; (3) the magnitude of the potential errors was reflected by the wide range of values obtained from the subjects and blanks; and, (4) studies that investigated the dietary zinc intake of adult men were more recent and provided a more extensive documentation of the analytical process.

The mean weight of a British woman in the current NDNS was 69 kg [227]. Therefore, when adjusted figures for body size were adopted for British adult females (i.e. 0.0065 mg zinc/kg body weight/day × 69 kg = 0.4485 mg zinc /day), for a 69 kg adult British woman, the amount of zinc lost via the integument and sweat is estimated to be 0.45 mg/day.

4.2.2.3 Zinc excretion in menstrual fluid

There is very limited information on endogenous zinc excretion in menstrual fluids. The WHO committee did not account for menstrual fluids as an output of endogenous zinc [236]. The IZiNCG steering committee also assumed that zinc excretion via this route is negligible and can be ignored [16], but the FNB/IOM committee estimated that average menstrual zinc excretion is 0.10 mg/day [237].

Estimates of the FNB/IOM were based on one study in which the average loss of zinc via menstrual fluids was about 5 µg/day [300]. The current investigation assumed that the estimate of the FNB/IOM (0.10 mg/day) is based on misinterpretation of the unit of assessment (µg/day). This value seemed to be miscalculated as 5 µg/day is negligible and static zinc excretion via menstruation was ignored.

4.2.3 Estimation of intestinal zinc losses in UK adult men and women

The WHO committee used the findings of one study to estimate the intestinal zinc excretion: in six young adult men who consumed 0.28 mg zinc per day for 4-9 weeks, total faecal zinc excretion was 0.5 mg/day [267]. This level of faecal zinc excretion was thought to represent the minimal amount that could be excreted in response to a very restricted diet. The WHO committee inflated the figure for faecal zinc excretion by 40%; however, the basis for this adjustment was not clear in the WHO report. The WHO committee estimated endogenous faecal zinc losses in adult men to be 0.8 mg/day [236].

The FNB/IOM applied a different conceptual approach to estimate the intestinal excretion of endogenous zinc:

Unlike the other routes of endogenous zinc excretion, the amount of endogenous zinc excreted through the intestine is directly related to the amount of zinc absorbed over a wide range. This was confirmed in 10 set of balance data from 7 European and North American studies that was reviewed by the FNB/IOM committee [263, 271, 272, 274, 279-281].

In a homeostatic situation, absorption must match the sum of non-intestinal and intestinal endogenous zinc losses. In the FNB/IOM approach, the sum of non-intestinal endogenous zinc losses (0.63+0.54+0.10=1.27 mg/day) was added to the 2.57 mg/day estimated intestinal endogenous zinc excretion. Based on this approach the physiologic requirement in adult men was estimated to be 3.84 mg/day; therefore, it would be necessary to replace a total of 3.84 mg/day of endogenous excretion of zinc [237].

The IZiNCG steering committee expanded the database used by the FNB/IOM and included all available studies of apparently healthy men and women regardless of their age, gender and nationality. The studies in which zinc supplements or exogenous phytate were added to the diet were excluded. The IZiNCG also excluded the studies that manipulated several nutrients and/or isolated food components simultaneously. When the relationship between total absorbed zinc versus endogenous faecal zinc excretion was plotted, the slope and intercept of the best-fit line was close to the best-fit line generated by the FNB/IOM committee.

The current investigation included the studies used by both the FNB/IOM and the IZiNCG committee, reviewed the details and technical documentations of all studies and added more recent studies or the studies that were excluded from the analysis (Table 4.1). Some discrepencies were noticed, investigated and reported (Appendix A).

The relationship between the total zinc absorption and faecal endogenous zinc excretion for this combined data set is presented in Figure 4.3. Based on this set of information, 2.13 mg/day of endogenous zinc would be excreted in faeces when the quantity of zinc absorption is equivalent to the total outputs of endogenous zinc from all sources, and the physiologic requirement for adult men is 3.29 mg/day. The calculation for endogenous zinc excretion included 0.51 mg/day from urine, 0.55 mg/day from the integument and sweat, 0.10 mg/day from semen, and 2.13 mg/day from faeces.

The current investigation concluded that in adult women the amount of intestinal excretion of endogenous zinc is 1.76 mg/day when the amount of zinc absorption is just enough to counterbalance the sum of all sources of endogenous zinc excretion (2.61 mg/day). The calculation included endogenous zinc excretion of 0.40 mg/day from urine, 0.45 mg/day from the integument and sweat and 1.76

mg/day from faeces. Thus, for adult women the physiologic requirement is 2.61 mg/day to replace the equal amount of zinc excreted (Figure 4.3).

Source of data	Diet description	Base (N)	Total zinc intake (mg)	Intestinal losses of endogenous Zn (mg/day)	Total absorbed zinc (mg/day)
References used by the FNB/IOM and the current investigation					
Lee *et al* [274]	Soy protein based, 6 months	8 men	4.1	1.8	2.6
Taylor *et al* [263]	Semi-purified formula	5 men	5.7	1.9	2.2
Taylor *et al* [263]	Semi-purified formula	5 men	0.9	0.8	0.8
Turnlund *et al* [279]	Purified formula	6 men	15.4	3.8	5.1
Turnlund *et al* [280]	Semi-purified formula	4 men	15	2.7	5.1
References used by the IZiNCG and the current investigation					
Knudsen *et al* [301]	High fibre, mixed	5 men and 3 women	10.7	2.6	3.1
Hunt *et al* [296]	Lacto-ovo-vegetarian	21 women	9.1	0.8	2.4
Hunt *et al* [296]	Mixed, high fibre	21 women	11.1	1.4	3.7
Hunt *et al* [302]	Low meat content	14 women	6.7	0.4	2.0
Hunt *et al* [302]	High meat content	14 women	13.0	0.9	3.6
Sian *et al* [303]	Mixed	10 women	8.1	2.3	2.7
Sian *et al* [303]	Plant-based	10 women	5.2	1.3	1.6
Hunt *et al* [271]	Mixed	14 women	7.8	2.0	2.3
Lowe *et al* [304]	Mixed	6 women	7.0	2.0	2.0
References used by FNB/IOM, IZiNCG and the current investigation					
Lee *et al* [274]	Hospital	8 men	12.6	4.3	5.5
Jackson *et al* [272]	Mixed	1 man	7.1	3.0	3.3
Hunt *et al* [271]	Mixed	14 men	14	1.6	3.1
Wada *et al* [281]	Mixed	6 men	16.4	1.9	4.1
Wada *et al* [281]	Mixed	6 men	5.5	1.7	2.7
References added and used only by the current investigation					
Lowe *et al* [305]	Mixed	6 women	7.2	1.9	2.1
Turnlund *et al* [294]	Semi-purified formula	4 men	11.8	1.3	3.0
Turnlund *et al* [294]	Semi-purified formula	4 men	9	3.0	2.8
Kim *et al* [299]	Low-phytate	7 women	6.5	1.8	3.2
Kim *et al* [299]	High-phytate	7 women	6.5	1.8	1.7

Table 4.1 Summary of the studies used by the FNB/IOM, IZiNCG and the current investigation to estimate the relationship between the total absorbed zinc and intestinal zinc excretion.

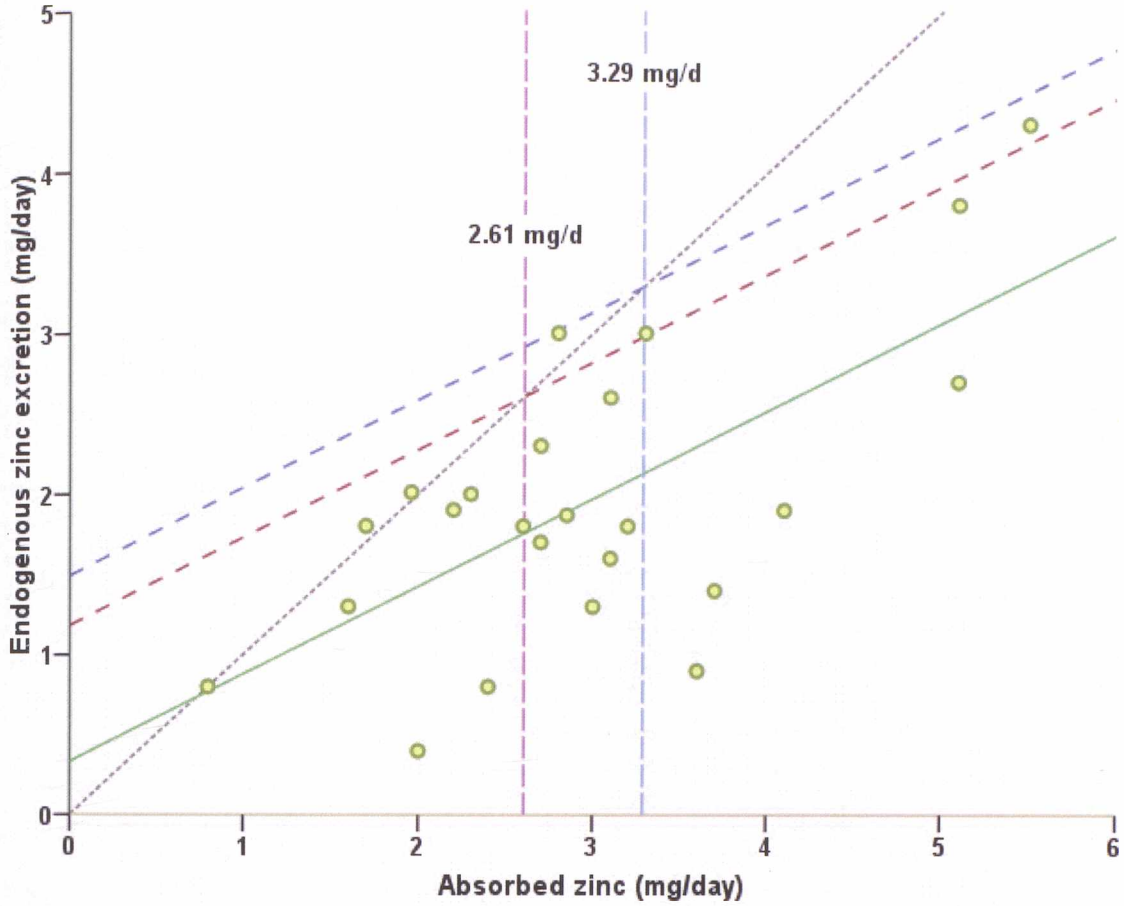


Figure 4.3 Graph of the model used to estimate physiologic zinc requirement.

The 24 data points represent the mean data from 15 published studies of zinc absorption and intestinal endogenous zinc excretion in adult men and women. The regression line ( ) of the data points demonstrates the relationship between total zinc absorption and intestinal endogenous zinc excretion. The parallel lines (men women) show the total endogenous zinc excretion after adding the static excretions via urine, integument and semen. The line of perfect agreement ( ) represents where total endogenous zinc excretion would be equal to the amount of zinc absorbed. The vertical lines (men, women) are to show the points where total endogenous zinc excretion crosses with the line of perfect agreement.

4.2.4 Estimated physiologic zinc requirement of adults as developed by several expert committees

Table 4.2 demonstrates estimates of the physiologic zinc requirement in adult men and women as developed by expert committees of the COMA, the WHO, the US FNB/IOM and the IZiNCG compared with the current investigation.

The current investigation provides a robust estimate of the physiologic zinc requirement for British adults based on: (1) a large number and the most recent studies; (2) the average weight of the British adults to estimate zinc excretion via the skin [227]; and, (3) , reanalysed information and corrected miscalculations of other expert committees (i.e. COMA, WHO, FNB/IOM, and IZiNCG).

Endogenous zinc losses (mg/day) in adult men and women, by source of loss	Source of estimated physiologic requirement				
	COMA 1991	WHO 1996	FNB/IOM 2002	IZiNCG 2003	Current revisions
Men					
Reference body weight (kg)	74	65	75	65	84
Urinary excretion	NA*	0.30	0.63	0.63	0.51
Integument	NA	0.30	0.54	0.42	0.55
Semen	NA	---	0.10	0.10	0.10
Total non-intestinal excretion of endogenous zinc	NA	0.60	1.27	1.15	1.16
Intestinal excretion of endogenous zinc	NA	0.80	2.57	1.54	2.13
Total endogenous losses	**2.20**	**1.40**	**3.84**	**2.69**	**3.29**
Women					
Reference body weight (kg)	60	55	65	55	69
Urinary excretion	NA	0.30	0.44	0.44	0.40
Integument	NA	0.20	0.46	0.36	0.45
Menstrual fluids	NA	---	0.10	0	≈0
Total non-intestinal excretion of endogenous zinc	NA	0.50	1.00	0.80	0.85
Intestinal excretion of endogenous zinc	NA	0.50	2.30	1.06	1.76
Total endogenous losses	**1.60**	**1**	**3.30**	**1.86**	**2.61**

Table 4.2 Estimated physiologic requirement for absorbed zinc in adult men and women, as developed by expert committees of the COMA [217], WHO [236], FNB/IOM [237], IZiNCG [16] and as reviewed by the current investigation for British adults (**NA*=Not Available**).

4.3 Estimation of the physiologic zinc requirement in children and adolescents

The factorial approach for an estimate of the physiologic zinc requirement has been used by all expert committees. These committees estimated the physiologic zinc requirement of children and adolescents by extrapolating from the data used to estimate endogenous zinc excretion of adults. These extrapolations were based on the assumption that direct measurements of the endogenous zinc excretion are not available for children and adolescents [237].

Although the current report has identified some investigations that conducted direct measurements of zinc loss in children and adolescents (Table 4.3), this information is yet to be conclusive. Thus, for the sake of consistency, the estimates of the current report is based on the extrapolations of the adult data.

Source of data	Diet description	Base (N)	Zinc intake (mg/day)	Intestinal zinc excretion (mg/day)	Total absorbed zinc (mg/day)
Griffin *et al* [306]	Mixed (high zinc)	16 girls	12.3	1.8	3.3
	Mixed (low zinc)	16 girls	4.4	1.1	1.3
Griffin *et al* [307]	Mixed	7 girls	11.5	1.7	NA
Griffin *et al* [240]	Mixed	14 boys and 16 girls	5.0	1.0	1.6
McKenna *et al* [308]	Mixed (low calcium)	13 girls	5.5	NA	0.8
Manary *et al* [309]	Mixed (high phytate)	2 boys and 3 girls	9.0	1.5	2.2
Manary *et al* [309]	Mixed (reduced phytate)	3 boys and 1 girls	6.6	1.5	1.6
Manary *et al* [310]	Mixed (high phytate)	10 children	5.4	1.1	1.3
Sheng *et al* [311]	Mixed	19 boys and 24 girls	1.9	0.7	0.6
Chujian *et al* [312]	Mixed (pre-test diet)	6 boys and 5 girls	5.4	NA	1.6
Chujian *et al* [312]	Mixed (post-test diet)	6 boys and 5 girls	7.1	NA	1.8

Table 4.3 Summary of the studies that directly measured zinc loss in children and adolescents.

The table demonstrates that there are several studies measuring endogenous zinc excretion in particular among children and adolescents. These studies are still not conclusive because their settings are too different. For instance, there is a very wide range of zinc intake in these studies, there are not enough studies for different age groups and not all required variables were measured in all studies. Weight specific endogenous faecal zinc excretion directly measured among 1 to 4 year old US children, exceeded the value that the FNB/IOM assumed for this age group [240].

4.3.1 Estimation of non-intestinal zinc excretion in UK children and adolescents

The FNB/IOM and the IZiNCG estimated that endogenous zinc excretion of children and adolescents via non-intestinal routes (i.e. urinary and skin losses) is 0.014 mg/kg/day. This included urinary losses of 0.0075 mg/kg/day and skin losses of 0.0065 mg/kg/day. Only for the male adolescents aged 15 to 18 years, an additional 0.10 mg/day was included in the estimated physiologic requirements to account for seminal zinc excretion [16, 237].

There is still not enough information about the direct measurements in children and adolescents; however, the author identified the related studies to be used in further investigations. At least 11 published articles [35, 240, 306-309, 312-316] measured urinary zinc excretion and only one study successfully measured integumental zinc excretion of children and adolescents aged 1 to 18 years using radio or stable isotope techniques [317].

The calculations of the current investigation were based on the extrapolation from the adult data per unit body weight. The amount of endogenous zinc excretion via non-intestinal routes was thought to be 0.013 mg/kg/day.

4.3.2 Estimation of intestinal zinc excretion in UK children and adolescents

The FNB/IOM estimated that faecal excretion of endogenous zinc for children aged 1 year and over is 0.034 mg/kg/day as extrapolated from the adult data [237]. The IZiNCG followed the approach used by the FNB/IOM committee, but calculated their own estimates of the intestinal losses of endogenous zinc. The figure derived by the IZiNCG estimated intestinal endogenous zinc excretion to be 0.020 mg/kg/day in children aged 1 year and over [16].

On the basis of extrapolations from adults per kilogram body weight, the current investigation estimated that faecal excretion of endogenous zinc is 0.025 mg/kg/day.

4.3.3 Estimation of zinc requirements for growth in UK children and adolescents

The FNB/IOM committee estimated the amount of zinc required for growth to be 0.020 mg/g of tissue gained. Then the figures for endogenous zinc excretion and zinc content of newly grown tissues were respectively multiplied by the reference body weight and the expected rate of weight gain at different ages [237].

The IZiNCG followed the approach used by the FNB/IOM, but used the NCHS/CDC/WHO reference body weights for their calculations. The current investigation followed the same method and reviewed a number of documents to choose a reference body weight representative of the UK population.

The panel of the COMA that previously calculated the dietary reference values of the UK, used information obtained from several surveys to produce a reference body weight table for each calculation. However, since publication of the previous dietary reference values (i.e. year 1991) [217], several surveys have been conducted in the UK and provided more recent information for this population.

The current investigation based its calculations on the weight obtained in the latest NDNS [224] because: (1) the prevalence of overweight and obesity among British children and young people is rising [318-320] and the table of reference body weight used by COMA is no longer representative of the population of British children and adolescents; (b) the NDNS provided a representative data for the UK population; and, (c) the zinc related variables of the data of these surveys was also analysed in other parts of this investigation and using the weight of the same population for estimation of their physiologic requirement was rational.

4.3.4 Estimated physiologic zinc requirements of children and adolescents as developed by several expert committees

The requirement of absorbed zinc for boys aged 4 to 6 years is demonstrated in Table 4.4. This is an example of the calculations used for the estimation of the physiologic requirements of UK children and adolescents explained in this chapter.

Urinary and skin zinc excretion	0.013 mg/kg/day×21 kg	= 0.273
Faecal zinc excretion	0.025 mg/kg/day×21 kg	= 0.525
Requirement for growth	6.85 g/day×0.020 mg/g	= 0.137
Required absorbed zinc	= 0.935	≈ 0.93 mg/day

Table 4.4 Estimation of the required amount of absorbed zinc in boys aged 4 to 6 years in the UK.

The current report did not differentiate the physiologic zinc requirement of boys and girls of all groups aged less than 15 years because: (1) the difference between the estimated physiologic zinc requirements of boys and girls was neglible (Table 4.5), and (2) only in boys aged 15 and over, the seminal zinc excretion is considered a significant source of endogenous zinc loss.

Age	Estimated requirement for absorbed zinc		
	Boys	Girls	Children
1 to 3 years	0.65	0.63	≈ 0.64
4 to 6 years	0.93	0.87	≈ 0.90
7 to 10 years	1.36	1.40	≈ 1.38
11 to 14 years	2.10	2.11	≈ 2.10

Table 4.5 Estimated required amount of absorbed zinc for children aged less than 15 years in the UK.

The estimated required amount of absorbed zinc for boys and girls was close. Differences between the mean values suggested for boys and girls aged less than 15 years were negligible.

WHO 1996		FNB/IOM 2002		IZiNCG 2003		Current revisions	
Age and Gender	Physiologic requirement (mg/day)	Age and Gender	Physiologic requirement (mg/day)	Age and Gender	Physiologic requirement (mg/day)	Age and Gender	Physiologic requirement (mg/day)
1-3 yrs	0.83	**1-3 yrs**	0.74	**1-3 yrs**	0.53	**1-3 yrs**	0.64
3-6 yrs	0.97	**4-8 yrs**	1.20	**4-8 yrs**	0.83	**4-6 yrs**	0.90
6-10 yrs	1.12					**7-10 yrs**	1.38
10-12 yrs, M	1.40	**9-13 yrs**	2.12	**9-13 yrs**	1.53	**11-14 yrs**	2.10
10-12 yrs, F	1.26						
12-15 yrs, M	1.82						
12-15 yrs, F	1.55						
15-18 yrs, M	1.97	**14-18 yrs, M**	3.37	**14-18 yrs, M**	2.52	**15-18 yrs, M**	2.87
15-18 yrs, F	1.54	**14-18 yrs, F**	3.02	**14-18 yrs, F**	1.98	**15-18 yrs, F**	2.33

Table 4.6 Estimated physiologic requirements for absorbed zinc during childhood by age group and gender as developed by expert committees of the WHO [236], US FNB/IOM [237], IZiNCG [16], and as reviewed for the UK population by the current investigation.

Source of data	Diet description	Base	Zinc intake (mg/day)	Intestinal endogenous zinc excretion (mg/day)	Total absorbed zinc (mg/day)
Wood *et al* [321]	Mixed	18 women	17.5	NA	2.3
Kim *et al* [299]	Mixed (high phytate)	10 women	6.3	7.0	1.5
Kim *et al* [299]	Mixed (low phytate)	10 women	6.3	6.0	2.4
Turnlund *et al* [279]	Purified formula	6 men	15.4	1.5	2.7
Turnlund *et al* [279]	Purified formula	6 men	15.5	1.9	2.8

Table 4.7 Summary of some studies that directly measured the zinc losses in elderly subjects.

The table demonstrates that there are a limited number of studies measuring the endogenous zinc excretion of elderly people and these studies are still not conclusive.

4.4 Estimation of the physiologic zinc requirement in the elderly population

As the reported values of the balance studies for the elderly have been quite variable, there was not enough evidence to estimate the physiologic zinc requirements for the elderly (Table 4.7). The former expert committees included the elderly population as an age group of the adults assuming that there is no concrete evidence that physiologic zinc requirements of the elderly were higher than those of the younger adults.

This possible difference in zinc homeostasis of the elderly and younger adults merits further investigation; however, pending the availability of additional information, the current study followed the approach taken by the former expert committees modifying it to be used for the UK population.

Thus for the present study, extrapolations from adults per unit body weight was used together with the average weight of the elderly population obtained from the NDNS people aged 65 years and over [223]. Table 4.8 demonstrates estimated physiologic requirements of absorbed zinc for people aged 65 years and over in the UK.

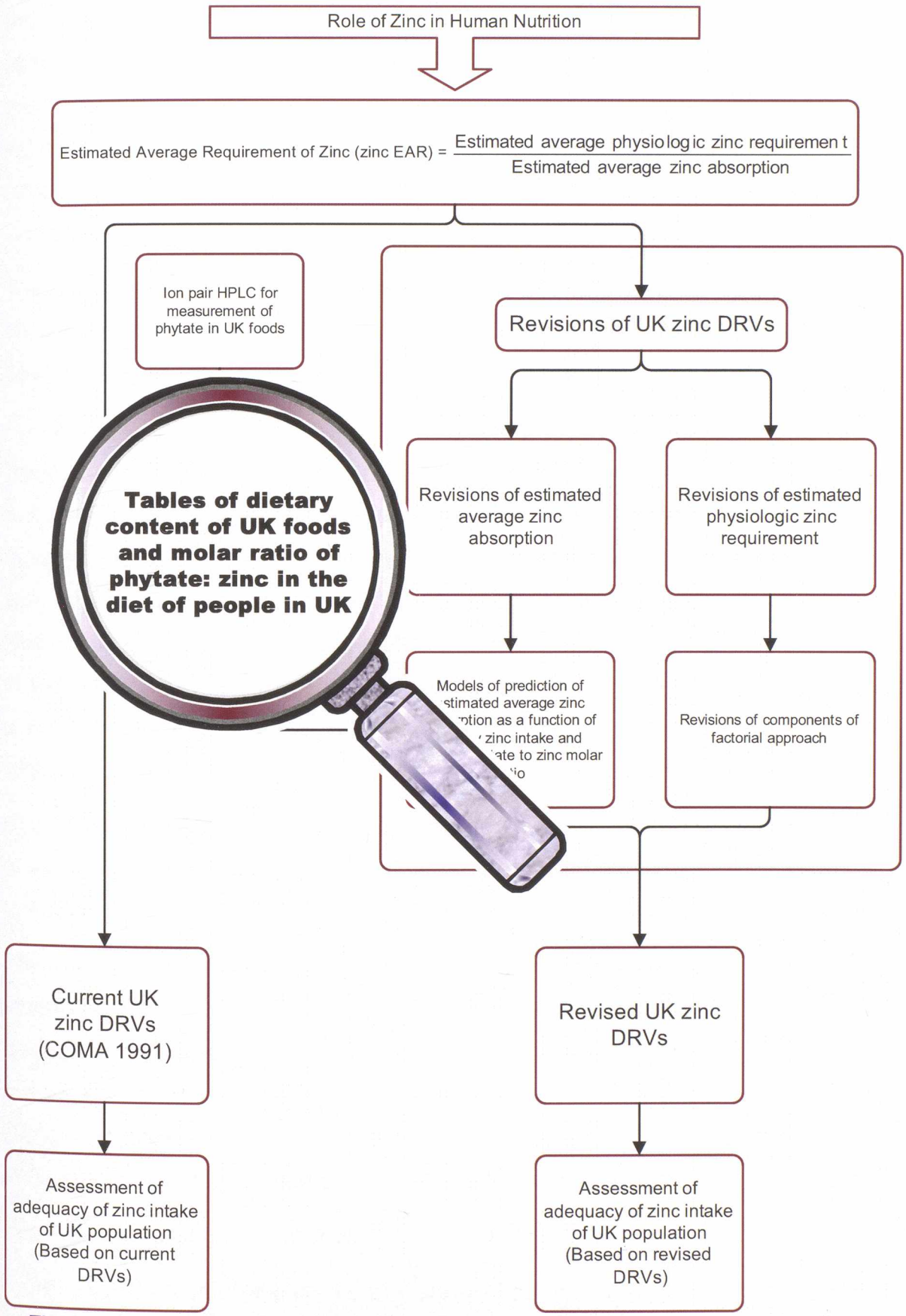
Gender	Reference body weight (kg)	Estimated requirement for absorbed zinc (mg/day)
Men	75	2.85
Women	63	2.39

Table 4.8 Estimated required amount of absorbed zinc for adults aged 65 years and over in the UK.

4.5 Conclusion

This chapter reviewed the latest information on the estimation of the physiologic zinc requirement and developed an updated estimate for the UK population. In the next chapters, these new estimates of physiologic zinc requirements will be used to update the existing UK zinc DRVs.

Chapter 5: Phytate Intake and Molar Ratio of Phytate to Zinc in the Diet of the People in the UK



The magnifier symbol shows where you are in the conceptual framework of the thesis.

5.1 Introduction

In order to develop a zinc EAR, it is necessary to have an estimate on the physiologic zinc requirement together with an estimate of the proportion of zinc absorbed. The models of zinc absorption (discussed in chapters 7) predict the proportion of zinc absorption from the dietary intake of zinc and phytate. The extent to which phytate affects the absorption of zinc in the UK diet has been difficult to estimate because information on the phytate content of foods consumed in the UK was not readily available.

There are many studies on the phytate content of various foods but these studies do not constitute an extensive database on the phytate content of foods. The development of such a database is difficult for several reasons:

Firstly, there is no universally accepted method to assess the phytate content of foods. Large differences were found in direct comparisons of results obtained from a variety of methods; therefore, a large variation in the content of phytate has been reported [16, 322]. Secondly, many factors such as growing conditions, maturity, type of soil, variety and mill fraction of grain, genetics, environmental changes, location, irrigation, year and application of fertilisers can affect the phytate content of foods [323, 324], adding to the variability in reported values. Finally, conducting a food survey that includes a representative sampling from all food groups is technically difficult.

In the UK, there have been very little and inconclusive data on the phytate content of foods consumed [325-330], and data on the phytate intake of the UK population is scarce.

The dietary intake of phytate and its meal distribution pattern was assessed on 76 students and staff at the Robert Gordon Institute of Technology [331]. Findings of that study cannot be generalised to the UK population because the study was conducted on a very limited number of participants more than 20 years ago. Dietary phytate intake of the UK population was also commented on in 1982, although details of the investigation were not published [332].

The current investigation aims to:

1. Provide a database on the phytate content of foods consumed in the UK.

2. Apply the database to the data of the National Diet and Nutrition Surveys to evaluate the phytate intake of the UK population and determine variations associated with gender and age groups.
3. Evaluate the phytate: zinc molar ratio of the population and estimate representative values for gender and age groups to be used for the calculation of zinc absorption and a revised EAR.

5.2 Material and methods

5.2.1 Selection of data

This investigation was a retrospective study among the British population based on the data obtained in the NDNS programme. The four most recent surveys of the NDNS were used. These surveys included:

1. National Diet and Nutrition Survey; Children aged 1½ to 4½ years (surveys performed in 1992-3).
2. National Diet and Nutrition Survey; people aged 65 years and over (survey performed in 1994-5).
3. National Diet and Nutrition Survey; young people aged 4 to 18 years (survey performed in 1997).
4. National Diet and Nutrition Survey; adults aged 19 to 64 years (survey performed in 2000-1).

The design and sampling procedure of these surveys are described in the survey reports [213, 222-228].

5.2.2 Phytate content of foods

Data on the phytate content of different foods are not available in the UK food composition tables [333]. Phytate values were therefore derived from published ([322-324, 326-330, 334-346]) and unpublished ([325, 347, 348]) data. Because these values were to be used for analysing NDNS data, the tables were produced to reflect the food types and food groups used for the dietary assessments in the NDNS.

Foods were divided into 12 main food types including:

1. Cereal and cereal products
2. Milk and milk products

3. Eggs and egg dishes
4. Fat spreads
5. Meat and meat products
6. Fish and fish dishes
7. Vegetables, potatoes and savoury snacks
8. Fruit and nuts
9. Sugar, preserves and confectionary
10. Total drinks
11. Miscellaneous
12. Dietary supplements and artificial sweeteners

Each of the food types consisted of one or more food groups expressed as integers (e.g. 1=main group of Pasta, Rice and Other Miscellaneous Cereals). Foods consumed in the UK were classified into 57 main groups. Food groups were then divided to 115 subsidiary food groups that were expressed as integers with an alphabetical suffix (e.g. 1A= Subsidiary food group of Pasta).

300 foods, commonly consumed in the UK, were selected and numbered as examples of foods within the subsidiary food groups. Each of these foods were allocated to one of 115 subsidiary food groups (e.g. 1A1=Noodles, dry form was allocated to subsidiary food group of 1A; Pasta). The phytate content of these foods was then estimated from the published and unpublished data. These values in turn were aggregated into the phytate values for 57 main groups.

The phytate content of foods in the literature was usually a mean value from a number of different samples and studies; hence, there was considerable variability associated with most values. In these cases, the range of the values in the literature was mentioned, citing the references. However, the values judged the most representative were used in aggregating to a single value on the phytate content of the food group.

A number of factors were considered when collating phytate content of the various sources. Firstly, the data source and its applicability to UK foods were taken into account, and secondly, the analytical sample numbers and analytical method were used. When there was insufficient information to evaluate the suitability of the phytate values, the mean of the values was used after excluding the obvious

outliers. If there was doubt regarding specific foods, advice was sought from the researcher of the original investigation. This was the approach used in the development and modification of the Meal Based Intake Assessment Tool (MBIAT) software [349] in the Institute of Food Research, (Norwich Research Park, Colney Lane, Norwich NR4 7UA, UK) and endorsed by the experts in the field [347].

Table 5.1 is an example of estimates on the phytate content of foods, for food groups. A complete table of the phytate content of subsidiary food groups, with examples of the phytate values of the foods included in each subsidiary food group, is presented in Appendix B.

No	Code	Food	Phytate mg/100g	Reference
56	Nuts and seeds			
	56R	**Nuts and seeds**	**850**	
Includes fruit and nut mixes, salted peanuts, peanut butter, tahini, Bombay mix				
223	56R1	Peanuts (roasted)	680-2008	[328, 334, 339, 344]
224	56R2	Peanuts and raisins	440	[328]
225	56R3	Peanut butter	443-1252	[337, 339, 347]
226	56R4	Almonds	970-2111	[328, 335, 336, 339, 344]
227	56R5	Cashews (roasted)	937-1229	[328, 335, 336, 339, 344]
228	56R6	Macadamia nuts	290-340	[328, 344]
229	56R7	Walnuts	580-1977	[328, 335, 336, 344]
230	56R8	Brazil nuts	1320-1799	[328, 335, 336]
231	56R9	Chestnuts	10-47	[328, 335, 336]
232	56R10	Pistachio nuts	340	[328]
233	56R11	Bombay mix	577	[347]
234	56R12	Sunflower seeds	3000	[328]
235	56R13	Sesame seeds	1380	[328]

Table 5.1 Estimates of phytate content of nuts and seeds.

Table shows how the phytate content of main and subsidiary food groups were estimated. Characteristics of the food groups were Food types=Fruit and nuts, Food group=56: Nuts and seeds and Subsidiary food group=56R: nuts and seeds.

5.2.3 Phytate intake of the individuals

Information on the amount of subsidiary foods consumed over a four or seven-day period is available on participants that completed food diaries during the NDNS survey. The phytate values of the subsidiary food groups were added to the nutrient databank of the NDNS and the estimated phytate intake from these groups was then calculated.

Total phytate intake from the main food groups was then calculated by aggregating the phytate intake of the related subgroups. The phytate intake from all foods was then aggregated and divided by the number of days that food diaries were recorded. This generated the estimated average daily phytate intake of the individuals.

5.2.4 Statistical analysis

Normal probability plots, $Z_{Skewness}$ and $Z_{Kurtosis}$, Kolmogorov-Smirnov and Shapiro-Wilk tests were used to investigate whether variables followed a normal distribution. The mean, median and quartiles range were used to express the dietary intake of phytate and the molar ratio of phytate to zinc, as these variables were not normally distributed.

In order to eliminate any difference, owing to energy intake between genders and age groups, the phytate intake was also expressed as density per 1000 kcal energy intake. The phytate to zinc molar ratio for the diets of the individuals were calculated as follows [16, 350]:

$$\text{phytate : zinc molar ratio} = \frac{\frac{\text{mg phytate}}{660}}{\frac{\text{mg zinc}}{65.4}}$$

The nonparametric Mann-Whitney test and Kruskal-Wallis (with post hoc comparison based on Benferroni correction) tests were used to compare the differences between gender and age groups. For all no nparametric tests, the p-value of the Monte Carlo 2-tailed exact significance was reported based on the number of samples and a 95% confidence interval.

5.3 Results

5.3.1 Phytate intake in children aged 1½ to 4½ years

The median daily intake of phytate for boys in the survey was 509 mg and for girls significantly lower at 481 mg ($p<0.05$). No variation in the phytate intake between boys and girls was found after adjusting for differences in the energy intake ($p>0.05$).

The median phytate intake slightly increased with age, being lowest for children aged 1½ to 2½ years (464 mg), and highest for children aged 3½ to 4½ years (510 mg, $p<0.05$). As with zinc intake, the phytate intake decreased with age when

differences in energy intake were taken into account, falling from 453.9 mg/1000 kcal for the youngest age group to 426.7 mg/1000 kcal for the oldest age group ($p<0.05$).

Overall, the median molar ratio of phytate to zinc was 11.84. There were no significant gender and age differences in the phytate: zinc molar ratios of the children ($p>0.05$). The mean, median and quartiles of the phytate intake, phytate density and molar ratio of phytate to zinc are presented in Table 5.2.

In children aged 1½ to 4½ years, the main source of phytate were cereal and cereal products, miscellaneous foods and vegetables, potatoes and savoury snacks. Just less than half of the phytate intake came from cereal and cereal products. This was mostly in the form of breakfast cereals (mainly whole grain and high fibre), biscuits, breads and pasta.

Vegetables, potatoes and savoury snacks contributed a further 20% to the mean daily intake of phytate, mainly from chips, crisps and savoury snacks, baked beans and potato salads and dishes. A further 25% of phytate intake came from commercial toddler foods and drinks and other miscellaneous foods including beverages and soups, although data on subgroups of miscellaneous foods were not available for this survey. Figure 5.1 shows the percent contribution of food types to the average daily phytate intake of children aged 1½ to 4½ years.

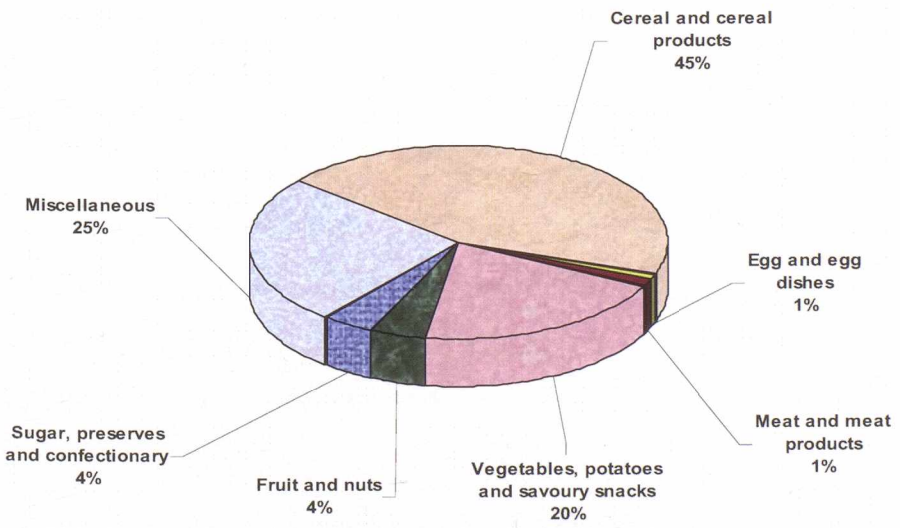


Figure 5.1 Percentage contribution of food types to average daily phytate intake of children aged 1½ to 4½ years.

5.3.2 Phytate intake in young people aged 4 to 18 years

The median daily intake of phytate was 674 mg for boys whereas in girls this was significantly lower at 566 mg ($p<0.001$). After allowing for the variation in intake of energy, the median intake of phytate per 1000 kcal energy in boys was not significantly different from girls (365.4 mg/1000 kcal versus 362.5 mg/1000 kcal, $p>0.05$).

The median daily phytate intake increased with age for boys from 576 mg for subjects aged 4 to 6, to 780 mg for 15 to 18 year olds ($p<0.001$). For girls, there was a similar increase with age only among girls aged less than 15 years. The median daily phytate intake increased from 494 mg among girls aged 4 to 6 years to 574 mg among 11 to 14 year olds ($p<0.001$).

After allowing for variations in intake of energy, the median intake of phytate per 1000 kcal energy for boys and girls was not significantly different between the different age groups ($p>0.05$).

The median molar ratio of phytate to zinc was 10.35. No variation in the phytate: zinc molar ratio was observed between boys and girls. The age related difference in the median values of the phytate: zinc molar ratio was significant only in boys, with the oldest group of boys having had a significantly lower phytate: zinc molar ratio compared to those aged 4 to 6 years ($p<0.001$), 7 to 10 years ($p<0.001$) and 11 to 14 years ($p<0.01$).

The mean, median and quartiles of phytate intake, phytate density and phytate: zinc molar ratios are presented in Table 5.3.

More than half of the phytate intake came from the consumption of cereal and cereal products. Within this group, the main contributors were breakfast cereals (mainly whole grain and high fibre breakfast cereals), white breads, biscuits, rice and pasta. Over one quarter of phytate intake came from vegetables, potatoes and savoury snacks. The main contributors within this group were chips, crisps and savoury snacks, baked beans and potatoes, potato salads, and dishes respectively.

A further 9% of the phytate intake came from miscellaneous food type (mostly beverages such as chocolate, cocoa, etc) followed by a 5% contribution from fruits and nuts (mainly from apples and pears following by nuts and seeds). Figure 5.2 shows the percent contribution of food types to the average daily phytate intake of young people aged 4 to 18 years.

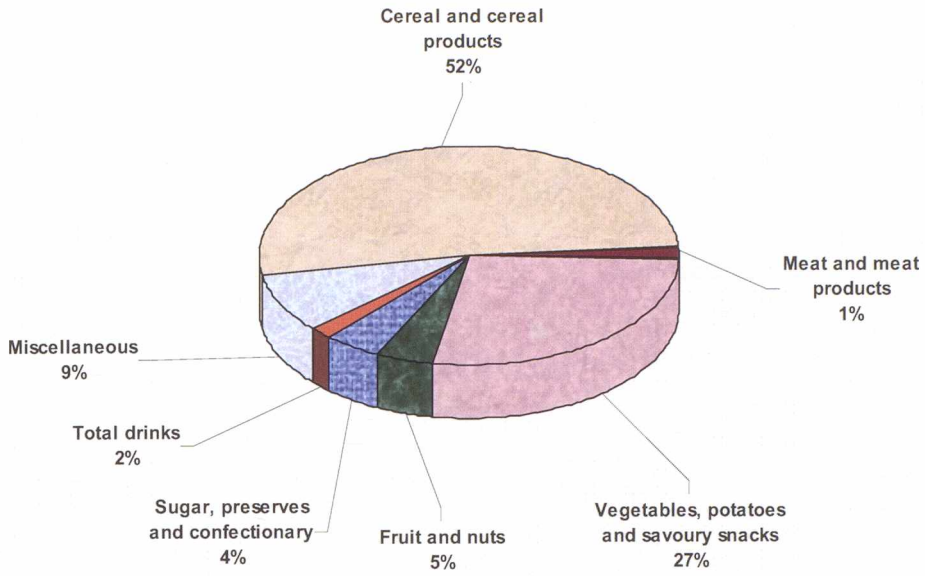


Figure 5.2 Percentage contribution of food types to average daily phytate intake of young people aged 4 to 18 years.

Gender and age groups	Base (N)	Phytate intake (mg/day)		Phytate density (mg/1000kcal)		Phytate: zinc molar ratio	
		Mean	Median (P25-75)	Mean	Median (P25-75)	Mean	Median (P25-75)
Boys aged 1½-2½ yrs	298	601	**465** (353-733)	570.4	**449.2** (334.0-706.2)	14.13	**11.50** (8.08-17.37)
Boys aged 2½-3½ yrs	300	636	**515** (408-718)	536.9	**443.4** (337.2-591.1)	14.77	**12.41** (9.47-16.92)
Boys aged 3 ½-4½ yrs	250	605	**526** (406-725)	474.4	**428.8** (323.4-550.3)	13.36	**11.84** (9.03-15.83)
All boys	848	615	**509** (379-724)	530.2	**440.2** (333.0-595.9)	14.13	**11.94** (8.92-16.92)
Girls aged 1½-2½ yrs	278	615	**463** (332-695)	616.5	**461.7** (313.5-690.3)	15.39	**11.90** (8.10-17.18)
Girls aged 2½-3½ yrs	306	577	**483** (337-688)	510.0	**429.3** (332.9-614.0)	13.74	**11.90** (8.94-15.78)
Girls aged 3½-4½ yrs	243	566	**497** (379-680)	486.8	**426.7** (328.7-603.4)	13.25	**11.58** (9.10-16.27)
All girls	827	587	**481** (347-687)	539.0	**435.5** (329.5-626.0)	14.15	**11.78** (8.76-16.40)
All boys and girls	1675	601	**496** (368-707)	534.6	**439.1** (331.5-616.5)	14.14	**11.84** (8.85-16.56)

Table 5.2 The mean, median and quartiles of the phytate intake, phytate density and molar ratio of phytate to zinc in children aged 1½ to 4½ years.

Gender and age groups	Base (N)	Phytate intake (mg/day)		Phytate density (mg/1000kcal)		Phytate: zinc molar ratio	
		Mean	Median (P25-75)	Mean	Median (P25-75)	Mean	Median (P25-75)
Boys aged 4-6 yrs	184	640	**576** (435-770)	418.7	**382.3** (310.1-473.3)	11.57	**10.90** (8.99-13.08)
Boys aged 7-10 yrs	256	733	**627** (519-831)	414.0	**365.8** (303.7-455.9)	11.94	**10.61** (9.08-13.50)
Boys aged 11-14 yrs	237	792	**714** (540-929)	402.4	**369.7** (294.0-463.0)	11.08	**10.39** (8.15-13.10)
Boys aged 15-18 yrs	179	855	**780** (616-1010)	383.1	**351.6** (295.3-437.3)	9.84	**9.34** (7.26-11.79)
All boys	856	755	**674** (521-885)	405.3	**365.4** (301.8-456.9)	11.18	**10.32** (8.41-13.03)
Girls aged 4-6 yrs	172	564	**494** (369-657)	406.1	**363.5** (290.0-463.1)	11.57	**10.54** (8.39-13.50)
Girls aged 7-10 yrs	225	644	**566** (461-740)	402.1	**354.2** (292.8-460.3)	11.30	**10.02** (8.42-12.90)
Girls aged 11-14 yrs	238	657	**594** (480-789)	393.5	**365.5** (309.8-453.2)	11.05	**10.60** (8.25-12.76)
Girls aged 15-18 yrs	210	674	**574** (459-829)	421.2	**372.5** (297.5-478.7)	11.21	**10.19** (7.90-13.91)
All girls	845	639	**566** (448-761)	405.2	**362.5** (298.0-463.7)	11.26	**10.36** (8.30-13.17)
All boys and girls	1701	697	**615** (480-831)	405.3	**364.6** (300.1-459.4)	11.22	**10.35** (8.35-13.07)

Table 5.3 The mean, median and quartiles of the phytate intake, phytate density and molar ratio of phytate to zinc in young people aged 4 to 18 years.

5.3.3 Phytate intake in adults aged 19 to 64 years

The median daily phytate intake was 886 mg for men, and significantly lower for women (754 mg, $p<0.001$). In both men and women, the youngest age groups had significantly lower phytate intake compared with older age groups. This difference was associated with different energy intake between the age groups; however, even after an adjustment for variation in energy intake, the youngest groups in both genders still had a significantly lower intake of phytate per 1000 kcal energy ($p<0.001$).

The median molar ratio of phytate to zinc was 8.82 for men, and significantly higher for women at 10.28. For men, there were no significant differences by age in the phytate: zinc molar ratios.

Women in the youngest age group had a significantly lower phytate: zinc molar ratio than women in any other age group (35 to 49 years: $p<0.05$, all others $p<0.01$). The mean, median and quartiles of the phytate intake, phytate density and phytate: zinc molar ratios are presented in Table 5.4.

Figure 5.3 shows that the main source of phytate for the respondents of the survey was from cereal and cereal products, contributing to approximately half of the average phytate intake. Within this group, whole grain and high fibre breakfast cereals, breads, rice and pasta were the main contributors to the phytate intake.

Overall, vegetables, potatoes and savoury snacks contributed to more than quarter of the average daily phytate intake. Within this group, the main contributors were potatoes, potato salads and dishes, baked beans, vegetables (not raw) and chips. Total drinks (including coffee, tea and fruit juice), miscellaneous foods (including beverages and soups) and fruits and nuts provided a further 8%, 7% and 6% of average daily phytate intake respectively.

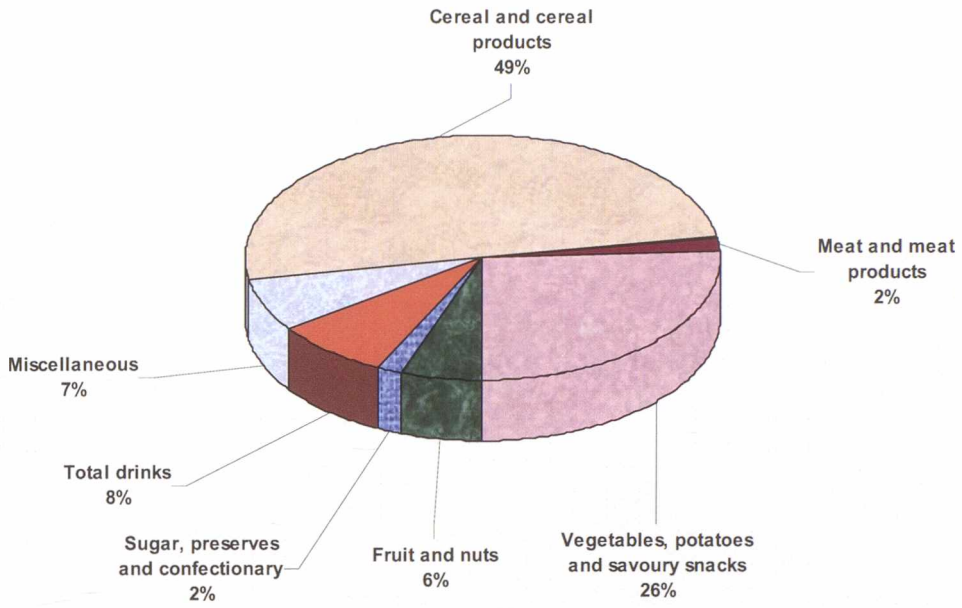


Figure 5.3 Percentage contribution of food types to average daily phytate intake of adults aged 19 to 64 years.

5.3.4 Phytate intake in people aged 65 years and over

The median daily intake of phytate for men was 727 mg, and for women, significantly lower at 572 mg ($p<0.001$). This gender difference was largely associated with difference in energy intake between the genders, as adjusting for this variation resulted in no significant difference in phytate intake per 1000 kcal energy intake. No variation in the phytate intake and density was found between different age groups of men and women.

The median molar ratio of phytate to zinc was 8.70. There were no significant differences in the phytate: zinc molar ratios between men and women or between different age groups. The mean, median and quartiles of the phytate intake, phytate density and phytate: zinc molar ratios are presented in Table 5.5.

Three fifths of the average intake of phytate came from the consumption of cereal and cereal products. Breakfast cereals, breads, biscuits and buns, cakes and pastries were the main contributors within this group.

Vegetables, potatoes and savoury snacks contributed to 23% of the average daily phytate intake. Within this group, the main sources of phytate were potatoes, potato salads and dishes, chips, boiled vegetables and baked beans.

Fruits and nuts contributed a further 5% to the average daily intake of phytate (mainly apples and pears, followed by other fruits including plums, grapes and apricots, which were either raw, stewed, dried or in form of fruit salads). Figure 5.4 shows the percent contribution of food types to the average daily phytate intake of people aged 65 years and over.

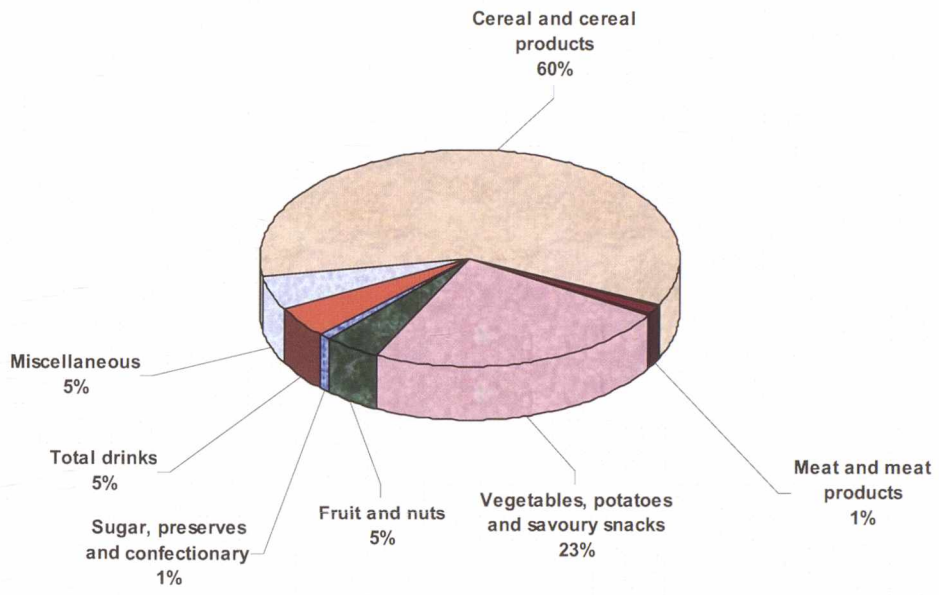


Figure 5.4 Percentage contribution of food types to average daily phytate intake of adults aged 65 years and over.

Gender and age groups	Base (N)	Phytate intake (mg/day)		Phytate density (mg/1000kcal)		Phytate: zinc molar ratio	
		Mean	Median (P25-75)	Mean	Median (P25-75)	Mean	Median (P25-75)
Males aged 19-24 yrs	108	817	**762** (565-940)	404.7	**365.7** (303.0-421.7)	9.23	**8.21** (6.82-10.30)
Males aged 25-34 yrs	219	1010	**904** (659-1132)	474.9	**432.8** (357.5-527.9)	9.76	**9.11** (7.31-11.47)
Males aged 35-49 yrs	253	993	**903** (670-1262)	465.1	**430.5** (326.8-555.5)	9.57	**8.80** (6.58-11.65)
Males aged 50-64 yrs	253	1094	**948** (679-1314)	522.5	**449.7** (357.7-607.3)	10.48	**9.27** (7.24-12.23)
All males	833	1005	**886** (657-1178)	477.2	**419.1** (335.7-545.5)	9.85	**8.82** (7.02-11.43)
Females aged 19-24 yrs	104	650	**645** (438-790)	420.5	**398.9** (320.6-502.2)	9.70	**9.28** (7.00-12.11)
Females aged 25-34 yrs	210	756	**714** (486-910)	510.2	**473.9** (370.7-589.0)	11.17	**10.50** (8.20-13.23)
Females aged 35-49 yrs	318	868	**792** (568-1071)	556.8	**505.4** (382.7-668.6)	11.63	**10.27** (8.00-14.03)
Females aged 50-64 yrs	259	928	**807** (599-1138)	605.9	**522.9** (401.5-706.7)	11.86	**10.51** (8.26-13.82)
All females	891	834	**754** (546-1013)	544.1	**482.0** (371.5-652.2)	11.36	**10.28** (7.97-13.59)
All males and females	1724	917	**809** (595-1100)	511.8	**452.0** (353.6-601.3)	10.63	**9.66** (7.47-12.55)

Table 5.4 The mean, median and quartiles of the phytate intake, phytate density and molar ratio of phytate to zinc in adults aged 19 to 64 years.

Gender and age groups	Base (N)	Phytate intake (mg/day)		Phytate density (mg/1000kcal)		Phytate: zinc molar ratio	
		Mean	Median (P25-75)	Mean	Median (P25-75)	Mean	Median (P25-75)
Males aged 65-74 yrs	371	891	**733** (509-1112)	455.4	**386.0** (267.7-549.2)	9.69	**8.70** (6.26-11.50)
Males aged 75-84 yrs	200	938	**692** (453-1145)	504.4	**378.5** (264.5-582.3)	10.96	**8.78** (6.32-12.58)
Males aged 85+ yrs	62	1059	**779** (496-1419)	569.1	**411.4** (286.8-827.5)	12.67	**8.97** (7.13-17.94)
All Males	633	923	**727** (495-1131)	482.1	**386.0** (267.9-571.3)	10.38	**8.72** (6.35-12.08)
Females aged 65-74 yrs	434	693	**630** (426-849)	476.8	**434.9** (310.5-570.4)	9.70	**8.93** (6.43-11.26)
Females aged 75-84 yrs	368	674	**549** (392-777)	466.3	**368.8** (284.7-574.4)	9.72	**8.50** (6.25-10.91)
Females aged 85+ yrs	251	712	**538** (416-772)	467.2	**336.8** (271.4-518.5)	10.62	**8.40** (6.90-11.62)
All Females	1054	690	**572** (416-813)	470.8	**387.8** (285.6-556.4)	9.93	**8.65** (6.48-11.07)
All males and females	1687	778	**629** (434-915)	475.1	**386.9** (277.6-557.8)	10.10	**8.70** (6.42-11.44)

Table 5.5 The mean, median and quartiles of the phytate intake, phytate density and molar ratio of phytate to zinc in adults aged 65 years and over.

5.4 Discussion

5.4.1 Tables of phytate content and their limitations

The tables on the phytate content of foods were extrapolated from the published and unpublished data. Inaccuracies could have arisen in the estimation of the phytate content of foods for a number of reasons: Firstly, there is a variation in the phytate content of varieties, genotypes and species of foods [351-354]. Secondly, estimates of the phytate contents of foods are dependent on the assays used, sampling procedures and environmental factors. Hence, the accuracy of the estimates will depend on the accuracy of the analyses [355, 356]. Finally, for many foods, phytate values have not been measured or were not found in the literature; in which case, the reported value has been estimated from the phytate values of foods of similar composition [349].

The author acknowledges that these tables are prone to errors but these inaccuracies were considered as the limitations of the study and were due to the difficulties associated with the assessment of nutrient intake, using the food composition tables [356].

Steps were taken to minimise the impact of error. The primary references used ([335, 336, 346, 347]) were those that had been validated, updated and often cited in the literature on the phytate content of foods. Considerable variability in the mean and range of the values quoted in the literature was taken into account and values deemed to be outliers were excluded.

Estimates of the phytate content were compared against the nutrient database of MBIAT software, developed by the Institute of Food Research [349]. The final version of the tables were consulted with the authors of MBIAT to minimise the errors [357, 358].

Opportunities to set geographic criteria or to select the investigations that used the same analytical technique were very limited. Results from studies using the anion exchange high performance liquid chromatography were selected above those obtained from other techniques; however, for at least five studies, the author had to include investigations based on methods other than anion exchange HPLC [324, 325, 340, 341, 344] to extend the range of data.

5.4.2 The use of nonparametric statistics

Nonparametric tests were used, as the assumption of the normal distribution required by parametric tests was not met. The positively skewed distribution of data was found in all four surveys. The results tables included the mean, median and quartiles; however, only medians were used for calculations in the next chapters.

Although less powerful, nonparametric tests are valid for both non-normally distributed and normally distributed data [359]. Nonparametric procedures were used in the China Nationwide Nutrition and Health Survey, in which the indices of dietary phytate were not normally distributed [360].

5.4.3 Comparison with other investigations

The average phytate intake of people in the UK is higher than those in developed countries and lower than in African and Asian countries. Figure 5.5 demonstrates the average phytate intake of UK adults estimated in this study compared to the average phytate intakes of other countries.

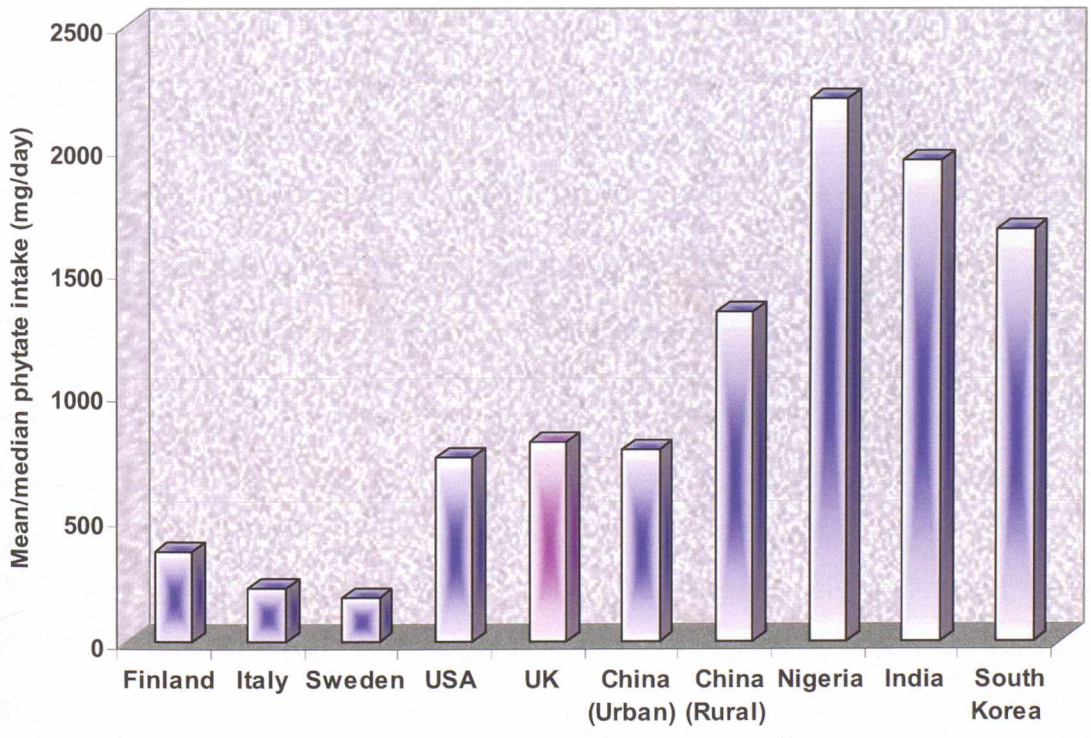


Figure 5.5 The median daily phytate intake of adults in the UK in comparison with the mean or median daily phytate intake of other countries.

Value of phytate intake for the UK is based on findings of the current investigation for adults who participated in the NDNS, while the data of the other values was derived from published studies in different countries [360-366]. The data of UK and China are based on the median and the data for the rest of the countries are based on the mean.

Comparing these findings to the other studies in the United Kingdom is difficult. The average phytate intake of adults is close to the values suggested by Davies in 1982. Davies concluded that the phytate intake of the UK population is in the range of 600-800 mg/day; although, details of the investigation was unpublished [332].

Davies also referred to an unpublished personal communication with DH Buss regarding the phytate content of some representative UK diets, which indicated an average daily phytate consumption of 806 mg/day. In this latter investigation, 70% of the phytate intake came from cereal products, 20% from fruit and the remainder from vegetables and nuts. Despite differing definitions for subsidiary food groups in the current investigation, findings from Buss agree with the average phytate intake and the percentage contribution of food types to average daily phytate intake found in the current study.

Findings of the current investigation did not agree with the range of phytate intake reported by Wise *et al*, 1987, in which they indicated that the mean phytate-phosphorous intake of students and staff at the Robert Gordon Institute of Technology ranged from 141-237 mg/day. It is important to note that these results were based on a different methodology and analytical technique (i.e. measurement of phytate phosphorous) and the study was conducted on a limited number of participants. The majority of phytate intake in that study came from breakfast cereals and breads which agrees with findings of the current investigation [331].

5.4.4 Types of foods contributed to phytate intake

Cereal and cereal products were the main sources of phytate for the UK population. For all age groups, the majority of the phytate intake from cereals came from breakfast cereals and breads. Phytate intake from these foods is high, as firstly, they contain a high amount of phytate and secondly, they are frequently consumed in the UK diet. For example, the majority of children in this survey consumed some form of bread and breakfast cereals.

Among breakfast cereals, high fibre and wholegrain breakfast cereals contained the highest amount of phytate (750 mg/100 g). Although a smaller proportion of children consumed these compared with the proportion of children who consumed other types (61% versus 66%, $p<0.05$), the mean amount of high fibre and whole

grain breakfast cereals eaten by consumers was significantly higher than the mean amount consumption of other types of breakfast cereals ($p<0.01$) [222].

Vegetables, potatoes and savoury snacks were the second largest source of phytate for the UK population. Within this group, the phytate content of the subsidiary food groups are not exclusively high, but the frequency and quantity of consumption has resulted in a high phytate intake from these foods. For example, potato chips contain 147 mg phytate per 100 g chips, and the largest proportion of young people aged 4 to 18 years (89% of boys and 88% of girls) consumed potato chips during the dietary record period. The majority of the young people population also consumed savoury snacks [224].

A possible explanation for the higher intake of phytate among the oldest group of adolescent girls (compared to younger girls) is the slightly higher consumption of rice among them. For example, rice was consumed by 44% of girls aged 15 to 18 years compared with 28% of girls aged 4 to 6 years [224].

Lower phytate intake of men and women aged 19 to 24 years could be explained by general differences in foods consumed by respondents of this group compared with older adults. The foods that were less likely to have been consumed by the youngest group of men and women were the rich sources of phytate. For example, men and women aged 19 to 24 years were less likely to consume whole meal breads, high fibre and wholegrain breakfast cereals and coffee [225].

Fruits and vegetables (together with potatoes, savoury snacks and nuts) contributed to a quarter of total phytate intake of adults. However, for nearly half of the fruit and vegetable types (i.e. peas, leafy green vegetable, cooked tomatoes, apples, pears, citrus fruits, bananas, canned fruits in juice and 'other fruits' such as plum, grapes and soft fruits), a significantly lower proportion of men and women aged 19 to 24 years had consumed these items compared with the oldest age group [225].

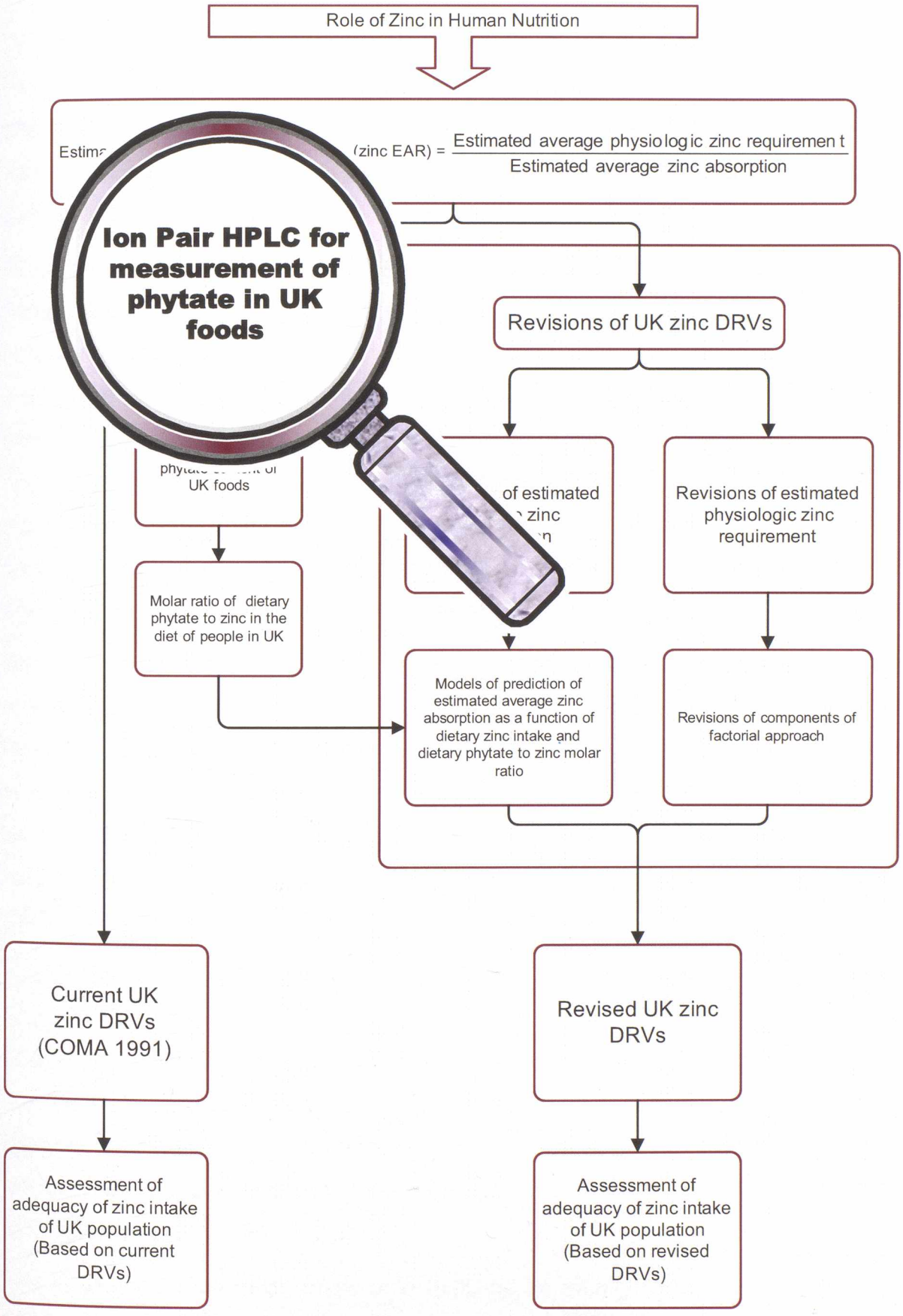
In the elderly population, boiled, mashed and baked potatoes, tea, white breads and biscuits were consumed by the largest proportion of the population. Almost all participants (95% of free-living and 98% of institution participants) drank tea. The average consumption of 24-25 cups of tea per week was greater than the 4 to 7 cups of coffees per week consumed by the elderly participants [223]. This frequent consumption of tea and coffee contributed to a large proportion of phytate intake of adults (age 19-64 years), as well as the elderly population.

5.5 Conclusion

This chapter provided tables on the phytate content of foods consumed in the UK. The variation in phytate content of foods is a possible source of error in these tables. Applying these tables to the data on average weight of foods consumed in the National Diet and Nutrition Surveys indicated that the median phytate intake of the UK population ranged from 463 mg/day for toddlers and children to 948 mg/day for adults.

Further research should focus on generation of a more complete database on phytate content of UK foods. This study has used available data on the phytate content of foods and provided an estimate on the dietary phytate: zinc molar ratio for the UK population, which will be used in forthcoming chapters.

Chapter 6: Validation of the Assessment of Phytic Acid via Ion Pair High Performance Liquid Chromatography



The magnifier symbol shows where you are in the conceptual framework of the thesis.

6.1. Introduction

6.1.1 Phytic acid: The basics

Phytic acid or myo-inositol hexakisphosphate (also known as inositol hexaphosphate), is a common component and the major storage form of phosphorous in many cereal grains, vegetables and fruits. Phytic acid also can be found in legumes, nuts, oilseeds, tubers, pollen, spores and organic soils [324, 339, 340, 367-369].

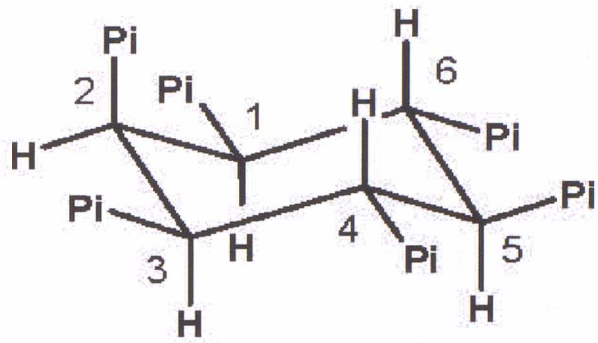
In cereals, about 1-2% of the weight of the seed is phytic acid and it can sometimes reach to 3-6%. In most plant species, 90% of phytic acid can be found in the aleuronic (external) layer and 10% is located in the embryo [323]. In grains, it usually occurs as phytin, which is a mixed calcium-magnesium salt of phytic acid and can represent 60-90% of total phosphorous; however, this level can fluctuate depending on factors such as growing conditions, maturity, type of soil, variety and mill fraction of the grain [324]. Some other factors such as genetics, environmental changes, location, irrigation, year and the way that fertilizer was applied can also affect the phytic acid content of cereal grains [323].

Because of the anionic nature of the six phosphate groups on inositol, phytic acid has a huge potential to bind cations [368]. Because of this, phytic acid was historically considered as an anti-nutrient that could bind essential dietary minerals such as calcium, iron and zinc and form salts hardly soluble in the gastrointestinal tract [367, 368]. As well as decreasing the bioavailability of micronutrients, phytic acid can react with charged groups of proteins and reduce protein bioavailability [367].

Because of the health benefits of dietary fibre, consumption of bran from a variety of grains is increasing; however, some bran may contain more than 5% phytic acid. As a result, the ingestion of large amount of dietary fibre can result in the ingestion of a greater intake of phytic acid [340]. In this situation, eating a diet marginal in essential minerals, may lead to a nutritional mineral deficiency. Thus, micronutrient deficiency can happen as a result of an excessive amount of phytic acid in the diet [323, 324].

The chemical structure of phytic acid explains its affinity to form a complex with some cations. Inositol hexaphosphate (also called IP6) is a simple, ringed, carbohydrate with six phosphate groups attached to each carbon (Figure 6.1). In the pH range of 0.5-9.0, it accepts one phosphate at carbon position two in the

axial position and five phosphates in the equatorial position and over pH 9.5 five phosphates in the axial and one phosphate in equatorial position. This structure, with 12 replaceable protons and a high density of negatively charged phosphate groups allows it to shape a very stable complex with multivalent cations [323].



$Pi = OPO_3H_2$

Figure 6.1 Structure of phytic acid.

Adapted from reference [367]. Pi, which includes phosphate group (lower formula) shows that the six highly charged phosphate groups of the molecule, gives it the ability to extend from the central inositol ring structure and react as an excellent chelator of the mineral ions.

Despite the role of IP6 in the aetiology of micronutrient deficiencies, several epidemiological and animals studies have demonstrated that IP6 has also some beneficial functions as an anti-cancer agent (e.g. in soft tissues, colon, metastatic lung cancers and mammary cancers), as an inhibitor of renal stone development and as an antioxidant [323, 367, 370].

The anti-oxidative role of phytic acid happens when IP6 occupies all of the available coordination sides of Fe (iron) and inhibits the production of the free radical hydroxide (-OH) by chelating iron [323]. Phytic acid may also have some beneficial roles in protection against heart disease and diabetes [367].

Until 1997, phytic acid was not 'generally recognised as safe' (GRAS) by the Food and Drug Administration (FDA) of the United States, however outside of the US, it was extensively used as a food additive. In 1997, sodium phytate was listed as a GRAS ingredient and since then has been used as a preservative for baking goods in the US. The anti-oxidant or iron chelating nature of phytic acid make it as versatile food preservative that can be added to meat, fishmeal pastes, canned seafood, fruits, vegetables, cheese, noodles, soy sauce, juices, bread and alcoholic drinks to protect them against discoloration and to prolong their shelf life [323].

6.1.2 Assessment of phytic acid

The potentially damaging effect of phytic acid on mineral status, as well as its benefits and usages as a food preservative and antioxidant, has led to the development of several analytical methods to measure the phytic acid content in foods. These methods differ in specificity, sensitivity and accuracy and the applicability of them are very different depending on the type of samples [339, 370].

The earliest analytical method for the measurement of the phytate (1914) was a titrametric procedure utilising standardised ferric chloride with phytate in 6% HCl [350].

The current established method of the Association Of Analytical Communities (AOAC) is based on the step gradient anion exchange technique [371, 372]; however, in practice the background assumptions of this protocol (that (a) all of the phytic acid is retained and held on the column; and (b) only IP6 is eluted from the anion exchange column) are not necessarily true and the method may overestimate the negative impact of the phytic acid on the bioavailability of the trace elements [369].

The fact that IP6 has no spectrum and can be hydrolysed into lower esters with similar chemical properties (i.e. IP5, IP4 and IP3) makes it a difficult molecule to analyze. During food processing and digestion IP6 can be degraded to partially phosphorylated isomers (including IP5-IP1 and in some cases free myo-inositol) by enzymatic or nonenzymatic means. Enzymatic hydrolysis frequently happens in the gastrointestinal tract or during food processing (including baking, malting and fermenting) by the action of intrinsic plant phytase, extrinsic microbial phytase or both. Nonenzymatic hydrolysis usually occurs when foods are heated (for example in autoclaving and canning) or treated with a strong acid. When phosphate groups get removed from the inositol rings, the mineral binding capacity declines and, as a result bioavailability of minerals improves [339, 367].

Several methods have been suggested for the assessment of phytic acid concentrations in food products and biological samples. These methods include: high performance ion chromatography (HPIC), together with a chemically suppressed conductivity detection [341]; high performance liquid chromatography (HPLC) with refractive index (RI) detection [324, 373]; or ultraviolet (UV) detection [324, 340, 374]; liquid chromatography mass spectrometry (LCMS); gas

chromatography mass spectrometry (GCMS); and inductively coupled plasma atomic emission spectrometry (ICP-AES) [323].

The assessment of phytate content of foods is hindered by the current lack of a globally accepted method and, more importantly, certified reference materials [16]. All of the suggested protocols have benefits and limitations [341], but HPLC is the most widely used method for separation and quantification of myo-inositol polyphosphates, with a satisfactory sensitivity and reproducibility to measure low concentrations in food products [339, 341, 350, 375]. There are several HPLC methods and different modifications for each method available. The most common modifications are the use of different columns, mobile phases, flow rates, extraction solvents and preparation techniques [323].

In nutritional studies, the two most common methods of HPLC are Ion Exchange HPLC and Ion Pair HPLC as these appear to be the best methods for the analysis of inositol phosphates [376]. The principle of the first method was explained by Rounds and Nielsen [377]. The method uses a column, with fixed cationic charges, to bind the highly anionic phytic acid and its lower phosphate forms. A gradient of salt is then used to elute these components separately from the column [322, 377]. Ion pair chromatography is based on the methodology of Lehrfeld [324] and uses a reversed phase chromatography, in the presence of counter ions, to change the partition coefficient of the compound [322, 324].

Ion Pair HPLC is considered a simple, rapid and accurate method to measure the phytate content of foods. One advantage is that the 5'-nucleotides, which are flavour enhancers commonly present in foods, do not interfere with this assay [340]. Chromatographic methods for the measurement of IP6, require three steps: extraction, concentration (and/or purification) and analysis [324].

Ion pair HPLC (according to the method of Lehrfeld [324]), which is discussed in this chapter was reported to shorten the extraction and concentration procedures, uses a durable polymer column for HPLC analysis and identify and quantify IP6, IP5, IP4 and IP3 [16, 324, 340, 369, 377]. The method employs ultrasonication for extraction, a commercially available silica based anion exchange column for concentration and purification, and HPLC for the analysis [340].

Several studies used ion pair HPLC for measurement of phytic acid in food sources [322, 324, 340, 369, 376]; however the complete validation study including studies of precision, linearity and accuracy is not available. The overall objective of the study was to develop and validate a method, based on Ion Pair

HPLC, for the assessment of phytic acid in UK food sources to validate tables of phytate content of foods.

6.1.3 Introduction to the validation studies

The overall objective of the validation studies was to investigate if the method is acceptable for the assessment of phytic acid in UK food sources. Identifying phytic acid and then precision, linearity and accuracy were the four dimensions taken into account. If proven to be valid, further validation studies of range, limit of detection and quantification, specificity and robustness were planned.

6.1.3.1 Identification of phytic acid

The initial aim of this study was to develop a method to identify phytic acid in food samples by Ion Pair HPLC. For this purpose, a separation assay condition was developed and phytic acid in food samples was identified based on its retention time compared with the retention time of phytic acid standard solution.

6.1.3.2 The studies of precision

The aim of the three types precision studies was to assess the degree of agreement when the procedure was applied repeatedly to multiple sampling of a homogenous sample of phytic acid [378].

The aim of the repeatability tests was to assess precision under the same operating condition, over a short period of time (i.e. in a day). Precision was measured by subsequent repetitive injections of the same homogenous sample of phytic acid and the determination of coefficient of variation (CV).

Intermediate precision tests aimed to assess the agreement of complete measurements when the same method was applied many times within the same laboratory.

The test of reproducibility (which examines the precision between laboratories and is usually determined during method transfer) was not conducted, as the researcher did not have any collaborators capable of repeating the protocol under the same conditions and in different laboratories, but is considered under future work.

6.1.3.3 The studies of linearity

The establishment of linearity is perhaps the most important part of the analytical method development. The aim of the studies of linearity was to investigate how

well the calibration plot of detector response to phytic acid versus concentration approximates a straight line [378].

Detector linearity in HPLC method development is often assumed if 1) the analyte response is plotted as a function of concentration, 2) least squares regression analysis typically generates a straight line through the data points, and 3) the correlation coefficient or coefficient of determination is 0.99 or greater [379].

Achievement of linearity in a small calibration range is easier, but in reality, the range of phytate content presented in foods is broad. The study of linearity was conducted using standard solutions, and then was repeated with a wider range of standards. The equation for the ideal linear detectors response [379, 380] is:

$$R = S\,C$$

Equation 6.1 Equation of ideal linear detector response

Where R is the Response, S is the Slope and C is the analyte concentration. This equation is true if the detector response was zero for zero analyte concentration; however, in reality, there is usually a concentration that the detector is unable to response against. As a result, the actual equation for linearity includes another term [378, 379] as in Equation 6.2:

$$R = S\,C + R_o$$

Equation 6.2 Equation of linear detector response

Where, R_o is the intercept or response at zero concentration.

6.1.3.4 The studies of accuracy

The aim of the study of accuracy was to define the closeness of the measured values of phytate to 'true' known values. In general, there are three methods for the assessment of the accuracy: 1) comparison to a reference standard, 2) recovery of the analyte spiked into a blank matrix and 3) standard addition of the analyte [378].

When the analyte is not in a complex sample matrix (like this study), the determination of the accuracy by a direct comparison to a reference standard solution is the simplest and the preferred technique. As long as the ingredient is commonly assayed, a certified standard can be obtained from an external source; however, new components like phytic acid reference standards are not available.

A 'special batch' of phytic acid was used as a standard and a certificate of analysis was obtained from the supplier. The level of the purity of the material should ideally be confirmed by several methods (e.g. mass spectrometry, nuclear magnetic resonance, gas chromatography, etc) [378]. However, in these studies, the researcher did not have the collaborator and expertise to use the other methods, so measured values of phytate were compared to the values of the certificate of the analysis. This is a major limitation of these studies.

6.2. Methodology

In this section, the main procedures of preparation of samples and mobile phases, together with HPLC features are given. Complete descriptions of the equipments, reagents and solutions, as well as the preparation procedures of all other solutions (e.g. standard solutions) are available by contacting the author. This section continues with the protocols of studies of precision, linearity and accuracy.

6.2.1 Procedures

6.2.1.1 Sample preparation

Samples analyzed for the development of the methodology were mostly commercial phytic acid solutions or simple breakfast cereals (e.g. Kellogg's cornflakes). Cereal samples to be analysed were received in solid form. The samples were milled using a blender, for 2 minutes. 5 g of milled samples were added to a metal container and transferred to a vacuum oven, which was heated at 100°C, for 6-8 hours.

Moisture content was calculated by deducting the weight of the sample after drying in the vacuum oven, from the initial weight. 500 mg of the sample was measured using a balance and added to 50 ml plastic centrifuge test tubes (28.5 ×104 mm ployallomer) containing 10 ml of 0.5M hydrochloric acid (HCl). Samples were stirred by an ultrasonic disintegrator while a microtip probe was halfway into the liquid. Each sample then was sonicated, for 2 minutes, to mix thoroughly and ensure the removal of air pockets [324].

As the laboratory was not equipped with the recommended centrifuge (i.e. Sorvel SS-3 automated centrifuge, Dupont Inst., Newtown, CT), the suspensions were centrifuged in ordinary lab centrifuges (e.g. Denley BS400 DJB purchasable from Labcare, 20 Howard Way, Interchange Business Park, New Park, Newport Pagnell, Bucks, MK16 9QS, UK) with a maximum speed (6000 rpm) for 30

minutes. If separation of precipitate and supernatant was not completed in any sample, that particular suspension was additionally centrifuged for another 5 minute at 6000 rpm with a paired test tube containing an equal weight of water.

An aliquot of 3 ml supernatant was carefully removed, weighed and diluted with 20 ml of HPLC grade water. The solution was poured onto an analytical silica based, anion exchange SAX column.

As a vacuum manifold was not available, a plunger of a phlebotomy syringe (volume: 10 ml) was attached to the column from the top and gently pressed to give a positive pressure to push the solution through the column. Excessive pressure or contact of the liquid with the plunger can lead to contamination or overflow of the liquid from the top and consequent loss of solution.

The loaded SAX column was washed with 10 ml of weak hydrochloric acid (0.05M). HCl that was used for the sample extraction and elution from the SAX column was trace element grade, as the concentration of heavy metals in analytical grade HCl is high enough to cause an insoluble phytate-metal complex after evaporation [324, 340]. The formation of a phytate-metal complex due to heavy metals in water and/or HCl can lead to underestimation of phytate.

As a vortex evaporator was not available, the resin-bound inositol phosphates were eluted with 2 ml of HCl into a round-bottom flask attached to a rotary evaporator (Buchi Rotavapor R-205, BUCHI Analytical Ltd. Manchester M24 2RH, UK) instead. Eluted samples were evaporated by a rota evaporator, which was equipped with a heating bath adjusted to 40°C, and worked with a vacuum.

The residue of evaporation was hardly visible white crystals, which were re-suspended with 1 ml H_20-TBNOH (Tetra butyl ammonium hydroxide) solution. To resolublise the precipitate, samples were sonicated in an ultrasonic bath for 5 to 10 minutes. If after solubilisation there was still some precipitate, there was a need for additional centrifugation for 5 minutes at 14000 rpm. Ependorf microcentrifuges (Eppendorf 5415D, from Eppendorf UK Limited, Endurance House, Chives Way, Histon, Cambridge CB4 9LR, UK) were used to remove any suspended material prior to injection.

6.2.1.2 Preparation of the mobile phases

Two solvent systems were used during the development of the methodology. A methanol based mobile phase was initially used; however the methanol volume decrease in this solvent was a source of error and the system was very sensitive

to slight variations in composition and pH. An acetonitrile based mobile phase was tried and found to be less viscous and more stable. A mobile phase based on acetonitrile was used in the main study.

6.2.1.2.1 Mobile Phase A (based on methanol)

The solvent was prepared by mixing 560 ml of methanol (HPLC grade) and 440 ml of 0.035 M formic acid, together with 10 ml TBNOH. The pH was adjusted to 4.3 with adding drops of sulphuric acid. The pH buffers of 4 and 7 were used for setting and calibration of the pH meter. Buffer solutions were produced to dissolve a buffer tablet in 100 ml deionised water following by 15 minutes of sonication with an ultrasonic bath.

6.2.1.2.2 Mobile phase B (based on acetonitrile)

Solvent was prepared by mixing 430 ml of acetonitrile and 570 ml of formic acid (0.035 M). Ten millilitre of TBNOH was then added. TBNOH can crystallise in slightly cool room temperatures and was sonicated in an ultrasonic bath at 40°C for 10 to 15 minutes until making sure that there were no crystals in the solution. The pH of the solvent was adjusted to 4.3 by the addition of sulphuric acid.

6.2.1.3 HPLC features

The HPLC analysis was performed using an Agilent series 1100, equipped with a 20 μl Rheodyne fixed loop injector (Rheodyne, Cotati, California, USA) which was not equipped with an autosampler. Therefore, 20 μl of sample was injected to the unit manually (with a HPLC syringe). Care was taken to take and inject the exact amount and avoid air pockets. The main system included a PRP-1 100Å Reversed Phase HPLC 5 μm Column 150 × 4.1 (Hamilton Great Britain Ltd), Agilent 1100 pump (Agilent Technologies UK Ltd), Agilent 1100 Refractive Index Detector and Variable Wavelength Detector (Agilent Technologies UK Ltd). Detector signals were processed by a Drew Scientific software of chromatography (Version Rossette, 1986, Drew Scientific Ltd).

The pH of the mobile phase, which was adjusted to 4.3, remained relatively constant as it was measured on a regular basis. Although a very wide range of detectors, wavelengths, temperatures, sensitivity, time and flow rates were employed for the development of the methodology, the final settings of the system were as close as possible to the major references [324, 340, 369]. The following HPLC setting is the one that most of the results of this report are based on:

The mobile phase was pumped through a heated 40°C PRP-1 column at a rate of 0.9 ml/min. The injection volume was 20 μl, attenuation was set at 125 and UV wavelength was set at 190 nm. The chromatography data were initially recorded for 15 minutes; however, as IP6 was detected within the first 3 minutes, the data acquisition time was changed to 5 minutes (however, the results at some stages suggested that equilibrium of the system might have required more time).

6.2.2 Method Validation Protocols

6.2.2.1 The studies of precision

6.2.2.1.1 Repeatability

This was measured by a sequential, repetitive, injection of a known sample, 3-mg/ml phytic acid, for more than 12 times in a day. The retention time, peak area and the peak height values were then averaged and the Coefficient of Variation was calculated as [378]:

$$CV = \frac{100.SD}{\bar{X}}$$

Equation 6.4 Equation of Coefficient of Variation

Where CV is the Coefficient of Variation, SD is the Standard Deviation and $\bar{X}$ is the Mean.

6.2.2.1.2 Intermediate precision

For the measurement of intermediate precision, the protocol of instrumental precision was repeated, using a range of standard solutions, with multiple preparations of samples, and on different days. 12 samples including 3-mg/ml phytic acid were injected to a HPLC system. Possible difference in purity of IP6 in different commercial phytic acid solutions were taken into the account as six of the samples were prepared from Sigma- Aldrich Ltd phytic acid solution (50% (w/w) in water, Aldrich, Catalogue Number: 593648) and the other six was prepared from Fluka Ltd Phytic acid solution (technical, ~40% in water, Fluka, Catalogue Number: 80180).

Samples were kept in the fridge to avoid any hydrolysis of phytic acid and were randomly injected into the system over a period of three days. During the whole study, serial dilutions of the standards were made from one stock solution to avoid inherent errors of independent preparation of several concentrations. But for this

section of study, different stock solutions were used to produce multiple sample preparations required in this section so the level of imprecision from the sample preparation could be determined.

The coefficient of variation was calculated for retention time and the detector's response. The detector's response was based on peak area because it is less affected by peak broadening.

6.2.2.2 The studies of linearity

Several phytic acid concentrations were injected to establish the full calibration range by plotting detector response (peak area) against the concentration.

Although linearity can be assessed by performing single measurements at several phytic acid concentrations, the average of triple measurements at several phytic acid concentrations were obtained.

The lowest concentrations were injected first, so that any possible remaining sample in the column could not influence later results. The averages of the triple results, of the detector response, were used in a scatter plot to approximate the level of linearity versus concentration. The data were processed using a linear least square regression model. The resulting plot slope, intercept and correlation coefficient were obtained.

6.2.2.2.1 The first study of linearity

1) A calibration curve of detector response versus concentration was used to demonstrate the level of linearity; 2) the linearity plot of sensitivity versus concentration was generated. If sensitivity was constant in any range of concentrations, the method was assumed linear in that working range.

6.2.2.2.2 The second study of linearity

Repeating the process with different solutions generated another set of linearity and permitted a measure of the repeatability of the linearity data. Several phytic acid concentrations were injected in the same methodology. A wider range of concentrations was used. The calibration curve and the linearity plot of sensitivity versus concentration were produced as in the first study of linearity.

6.2.2.3 The studies of accuracy

Because of the simple matrix of the samples, accuracy could be assessed via a comparison to standard solutions [378]. Quality control tests on standards

section of study, different stock solutions were used to produce multiple sample preparations required in this section so the level of imprecision from the sample preparation could be determined.

The coefficient of variation was calculated for retention time and the detector's response. The detector's response was based on peak area because it is less affected by peak broadening.

6.2.2.2 The studies of linearity

Several phytic acid concentrations were injected to establish the full calibration range by plotting detector response (peak area) against the concentration.

Although linearity can be assessed by performing single measurements at several phytic acid concentrations, the average of triple measurements at several phytic acid concentrations were obtained.

The lowest concentrations were injected first, so that any possible remaining sample in the column could not influence later results. The averages of the triple results, of the detector response, were used in a scatter plot to approximate the level of linearity versus concentration. The data were processed using a linear least square regression model. The resulting plot slope, intercept and correlation coefficient were obtained.

6.2.2.2.1 The first study of linearity

1) A calibration curve of detector response versus concentration was used to demonstrate the level of linearity; 2) the linearity plot of sensitivity versus concentration was generated. If sensitivity was constant in any range of concentrations, the method was assumed linear in that working range.

6.2.2.2.2 The second study of linearity

Repeating the process with different solutions generated another set of linearity and permitted a measure of the repeatability of the linearity data. Several phytic acid concentrations were injected in the same methodology. A wider range of concentrations was used. The calibration curve and the linearity plot of sensitivity versus concentration were produced as in the first study of linearity.

6.2.2.3 The studies of accuracy

Because of the simple matrix of the samples, accuracy could be assessed via a comparison to standard solutions [378]. Quality control tests on standards

(technical, ~40% in H2O, Fluka, Catalogue Number: 80180) and Phytic acid solution (50 % (w/w) in H2O, Aldrich, Catalogue Number: 593648) were conducted by the supplier (Sigma Aldrich Ltd) and a 'Certificate of Analysis' was obtained.

The high concentrations of phytic acid available in these commercial solutions are not found in food sources. Therefore, samples were diluted to the measurable concentration and these concentrations were still assumed standard. This was a further potential source of error.

To avoid any hydrolysis of phytic acid with time and temperature, the standards were produced in the same day and injected into the equipment immediately. The 'Standard' was supposed to produce a single well-defined peak chromatograph to be validated.

6.2.2.3.1 The first test of accuracy

The first test of accuracy was carried out using the repetitive measurements of a single concentration of phytic acid [323]. The data of this concentration were available from the studies of precision.

6.2.2.3.2 The second test of accuracy

To minimise bias in sample preparation, three repetitive measurements of three different concentrations, with different preparations were used [378]. The data for the test were obtained from the second study of linearity. For the calculation of the between days accuracy, the mean of the concentrations of both the first and second studies of linearity was used.

6.3. Results

6.3.1 Identification of phytic acid

For both food samples and standard solutions, the peak of phytic acid appeared at 1.2 to 1.3 minutes of the retention time. This constant retention time provided evidence that the method could successfully detect phytic acid among other components available in food samples.

6.3.2 Precision

6.3.2.1 Repeatability

6.3.2.1.1 Retention time

The CV for the samples' retention time was less than 1% (N=12, CV=0.94%, $\bar{X}$ = 1.26 min, SD=0.01). This repeatable retention time was observed over the whole validation study (Figure 6.2).

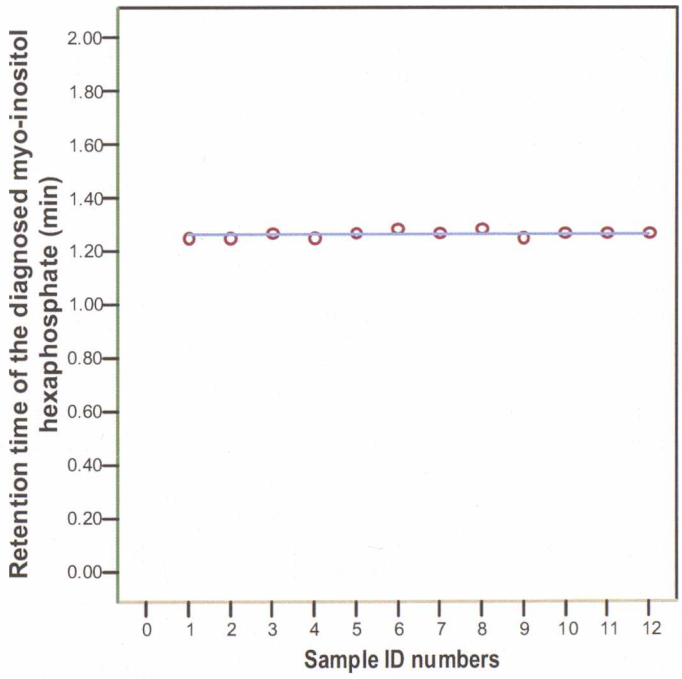


Figure 6.2 Precision of the retention time for measurements of phytic acid in the study of repeatability.

6.3.2.1.2 Peak area

An unacceptably large CV in peak area was obtained (N=12, CV=31.4%, $\bar{X}$ =63.1, SD=19.8) (Figure 6.3).

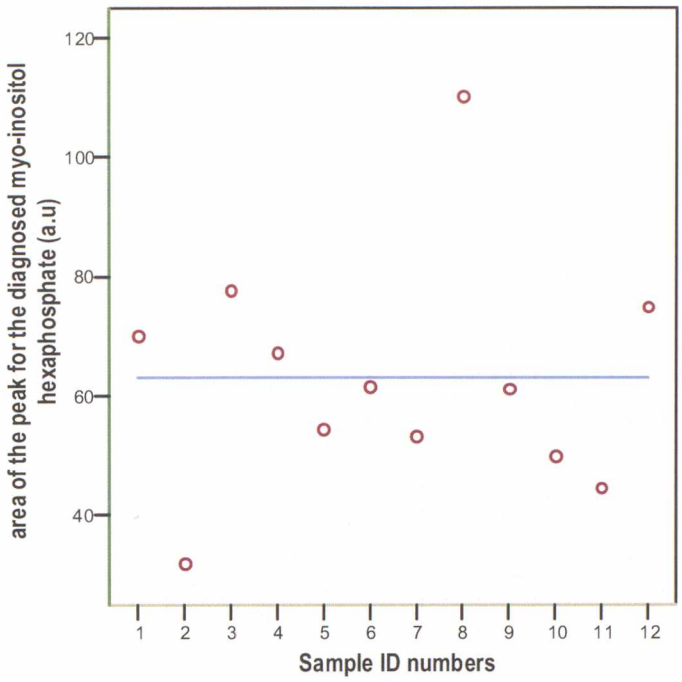


Figure 6.3 Precision of the peak area for measurement of phytic acid in the study of repeatability.

6.3.2.2 Intermediate precision

6.3.2.2.1 Retention time

A slightly higher CV was seen for the retention time of the study of intermediate precision (N = 12, CV=2.93%, $\bar{X}$ =1.25 min, SD=0.04), but this was expected because of the effect of the variables that could influence the coefficient of variation (different chemicals, different preparations and on different days).

These results were compared to the results of instrumental repeatability (N=12, CV =0.94%, $\bar{X}$ =1.26 min, SD=0.01). As the variance for the two groups were significantly unequal, a t-test for unequal variance was used. There was no significant difference between the mean of the two series of injections ($p>0.05$).

6.3.2.2.2 Peak area

An unacceptable high coefficient of variation was seen in the study of intermediate precision (N=12, CV=23.7%, $\bar{X}$ =66.7, SD=15.8). When the mean of the peak area for the study of intermediate precision was compared to the mean of the peak area for the study of instrumental repeatability, no significant difference was seen ($p>0.05$). This demonstrated that the mean of responses to similar concentrations of phytic acid in both experiments were not different and their high standard deviation pointed to the irreproducibility of the peak area.

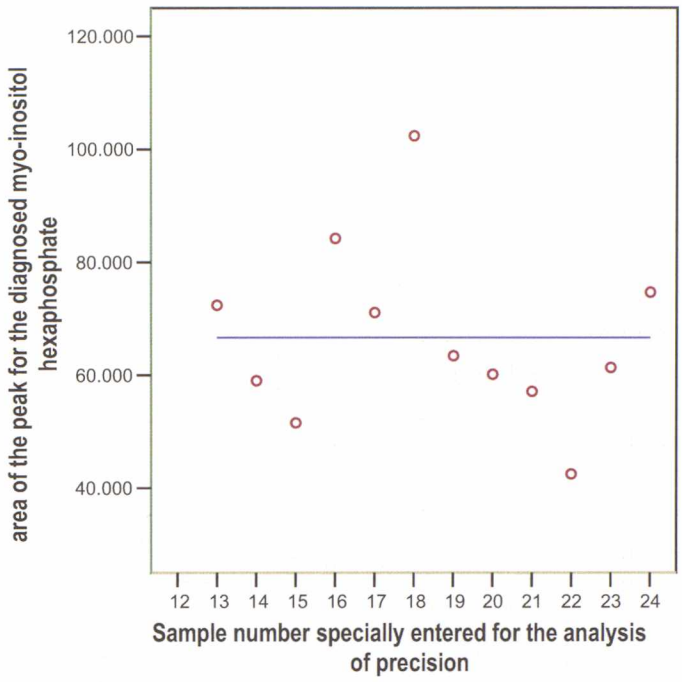


Figure 6.4 Precision of the peak area for measurement of phytic acid in the study of intermediate precision.

6.3.3 The studies of linearity

There was a very strong correlation between the peak area and peak height in the different measurement series of linearity (for example in the first study and based on the average of three times injection of the samples: $r=0.99$, $p<0.001$). Therefore, results are reported based on the peak area as the main index of the detector's response to avoid repetition.

The results presented here are based on the assumption that there is a linear relationship between the detector response and concentration of phytic acid. A further study on the characterisation of the linearity, demonstrated that based on the least square regression analysis (i.e. R^2 and comparison of the residuals of the models) no other nonlinear models could describe the behaviour of the detector better than the general linear model. The results of this study are available by contacting the author.

6.3.3.1 The first study of linearity

6.3.3.1.1 Calibration plot

Linearity of the method was assessed by performing triplicate measurements, at several phytic acid concentrations. The peak area response (R) for each injection, mean R, Standard deviation (SD) and CV are listed in Table 6.1. The mean response for each level was used to generate a linear regression (Figure 6.5). The

Pearson correlation coefficient (r) of the relationship between average peak area and concentration was 0.927 ($p<0.01$).

C(mg/ml)	Injection#1	Injection#2	Injection#3	Mean	SD	CV
	R	R	R	R	R	R
0.5	16.19	NA	2.85	9.52	9.43	99.08
1	12.93	11.17	16.64	13.58	2.79	20.54
2	38.68	35.38	24.98	33.02	7.15	21.66
3	72.36	59.00	51.54	60.97	10.55	17.30
4	210.67	158.01	109.29	159.32	50.70	31.82
5	242.43	126.59	161.80	176.94	59.38	33.56
6	218.79	107.31	120.84	148.98	60.83	40.83

Table 6.1 Detector linearity response data to phytic acid in the first study of linearity.

CV in the last column of the table was usually more than 20%. This was also noted in the studies of precision. When CV of concentration 0.5 mg/ml was excluded from the data, there was a significant, positive, correlation between concentration and CV ($r = 0.88$, $p<0.05$).

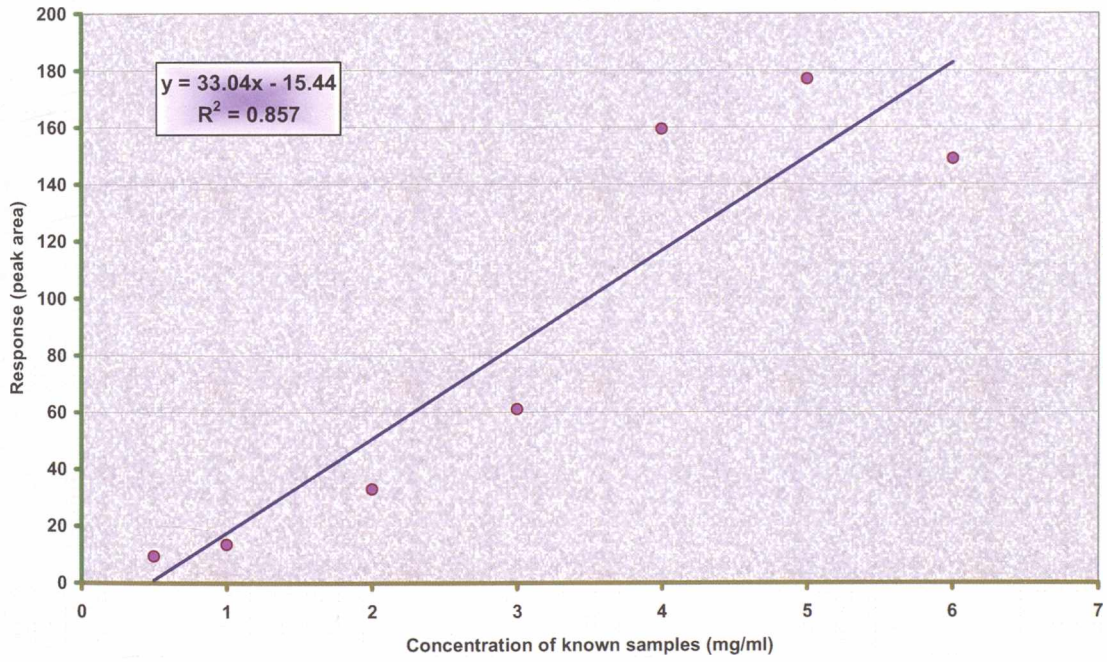


Figure 6.5 Linearity scatter plot of detector response versus concentration of phytic acid in the first study of linearity.

Least-squares regression analysis did not produce a linear curve with a desirable coefficient of determination (R^2) exceeding 0.999.

6.3.3.1.2 Linearity of sensitivity versus concentration

A superior method for determining method linearity over a range of concentration is based on determination of the response factor (RF also called sensitivity) at each measured concentration and plotting it versus analyte concentration (Table 6.2 and Figure 6.6).

If the detector could respond in an ideal linear style, the slope of the regressed line would have been zero, as all of the values of sensitivity would have stayed constant. In other words, sensitivity should ideally be independent of concentration, if the method is really 'linear' over an extensive range of concentrations [378]. To create this plot, the RF was calculated as:

$$RF = \frac{R}{C}$$

Equation 6.5 Equation of the response factor

Where RF is the response factor, R is the detector response (peak area) and C is the concentration of phytic acid.

C (mg/ml)	Mean R (Peak area)	RF (Sensitivity)
0.5	9.52	19.04
1	13.58	13.58
2	33.02	16.51
3	60.97	20.32
4	159.32	39.83
5	176.94	35.39
6	148.98	24.83

Table 6.2 Linearity data of Sensitivity and Concentration in the first study of linearity.

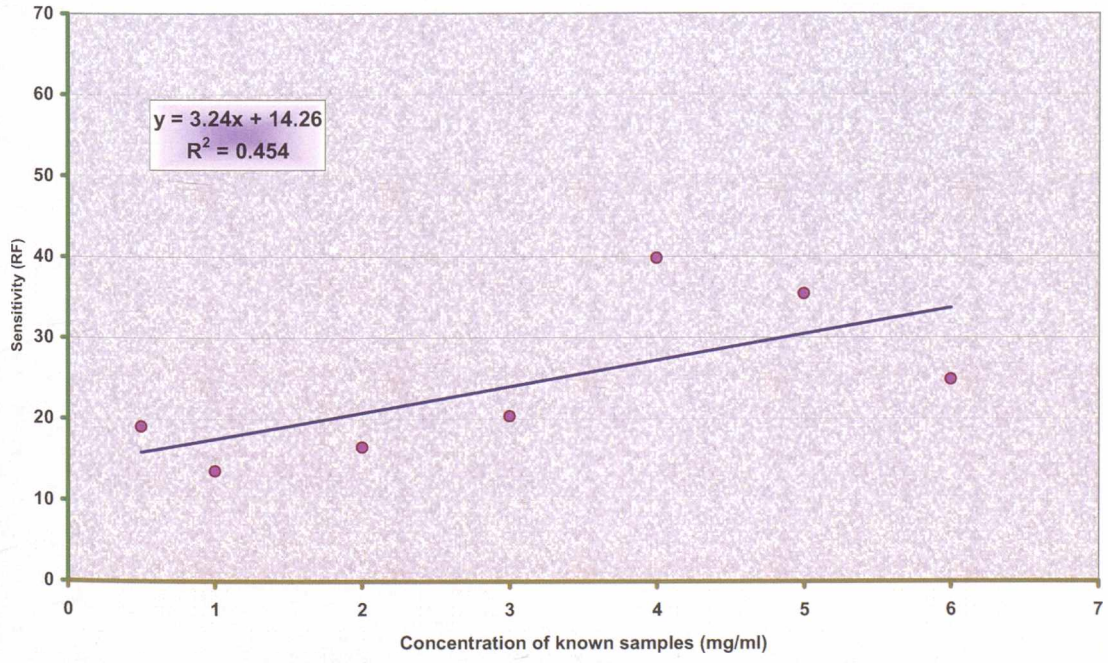


Figure 6.6 Linearity plot of sensitivity versus concentration in the first study of linearity

The line had a non-zero slope, as described by non-constant (RF) values, which points to detector nonlinearity.

6.3.3.2 The second study of linearity (repeatability of linearity)

6.3.2.2.1 Calibration curve

The mean response for each concentration was used to generate a linear regression (Figure 6.7). The detector response for the second data set of the linearity (R), mean R, SD and CV are listed in Table 6.3. The correlation coefficient of the relationship between the average peak area and concentration was 0.975 ($p<0.001$).

C (mg/ml)	Injection#1	Injection#2	Injection#3	Mean	SD	CV
	R	R	R	R	R	R
0.33	12.88	NA	NA	12.88	NA	NA
0.50	9.13	NA	NA	9.13	NA	NA
1.00	17.41	NA	NA	17.41	NA	NA
1.50	30.27	35.78	41.18	35.74	5.46	15.26
2.00	99.77	75.98	64.34	80.03	18.06	22.56
3.00	102.33	84.16	71.05	85.85	15.71	18.30
4.00	156.20	156.34	121.28	144.61	20.20	13.97
4.50	132.09	142.06	190.38	154.84	31.18	20.14
5.00	192.01	171.20	146.13	169.78	22.98	13.53
6.00	159.34	181.45	154.25	165.02	14.46	8.76

Table 6.3 Detector linearity response data to phytic acid in the second study of linearity.

The mean of the CV for the two studies were not significantly different ($p>0.05$). There was no significant correlation between CV and concentration ($r=-0.625$, $p>0.05$).

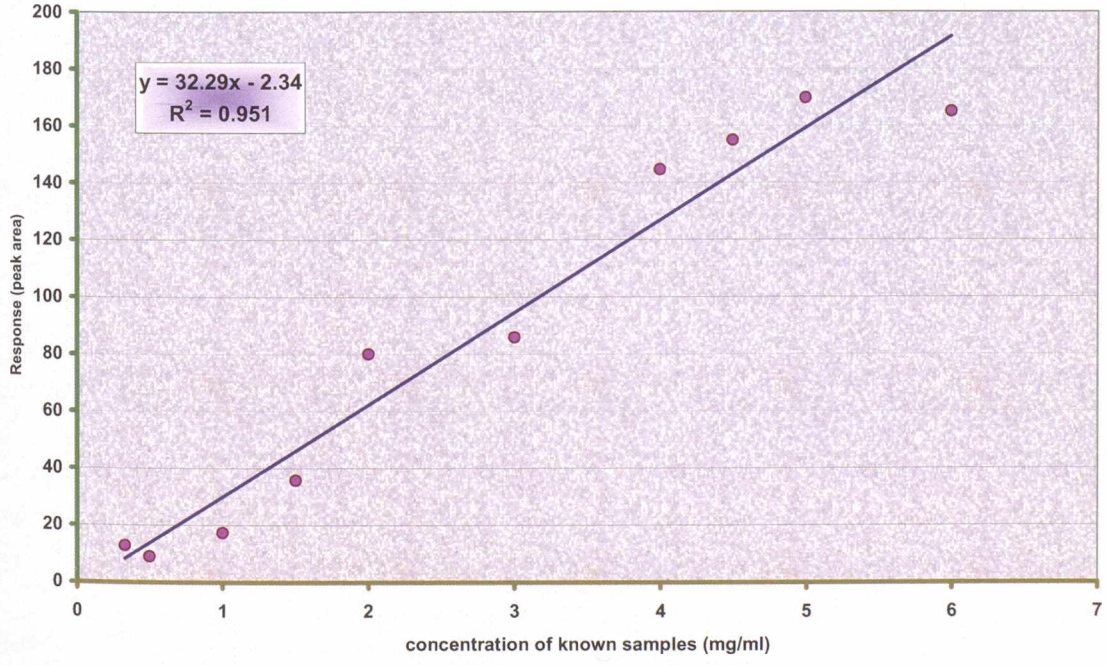


Figure 6.7 Linearity scatter plot of detector response versus concentration of phytic acid in the second study of linearity.

The least-squares regression analysis did not produce a linear curve with a desirable coefficient of determination (R^2) exceeding 0.999.

6.3.3.2.2 Linearity of sensitivity versus concentration

In the second study of linearity, RF (sensitivity) was plotted versus the concentration based on the data provided in Table 6.4. The slope of the regression line was not zero, so linearity could not be demonstrated (Figure 6.8).

C (mg/ml)	Mean R (Peak area)	RF (Sensitivity)
0.33	12.88	39.04
0.50	9.13	18.27
1.00	17.41	17.41
1.50	35.74	23.83
2.00	80.03	40.01
3.00	85.85	28.62
4.00	144.61	36.15
4.50	154.84	34.41
5.00	169.78	33.96
6.00	165.02	27.50

Table 6.4 Linearity data of Sensitivity and Concentration in the first study of linearity.

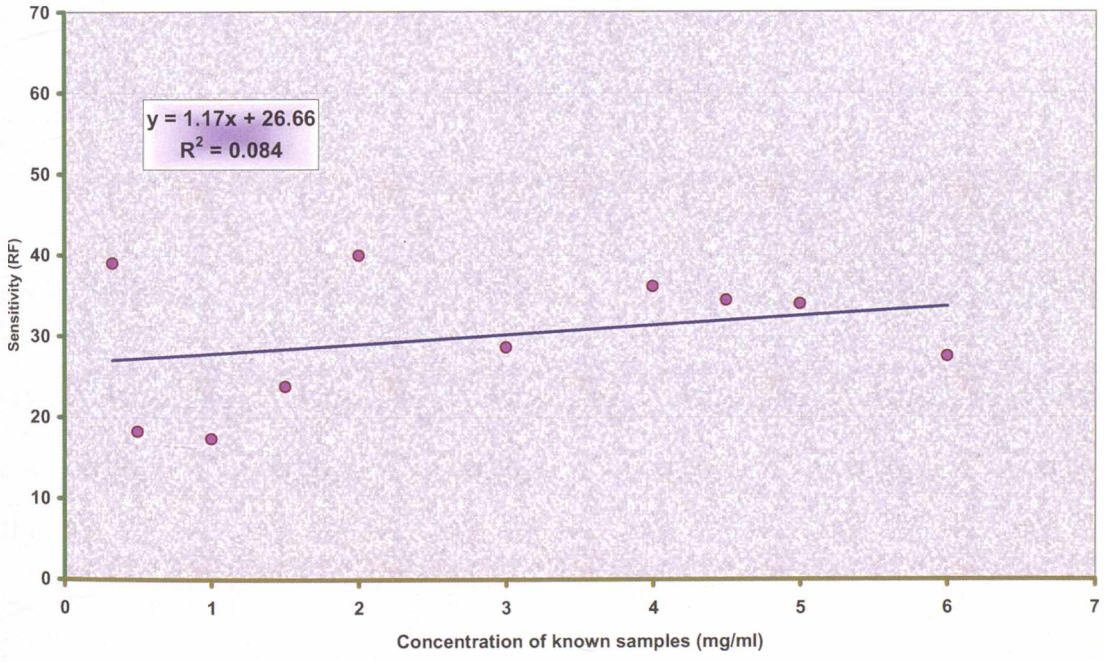


Figure 6.8 Linearity plot of sensitivity versus concentration in the second study of linearity.

6.3.4 The studies of accuracy

The first injection of standard solution (~40% in H2O, Fluka, Catalogue Number: 80180, diluted to concentration of 3 mg/ml) generated a single peak in chromatogram (Figure 6.9 A). Following injections of the same sample showed at least two peaks (Figure 6.9 B).

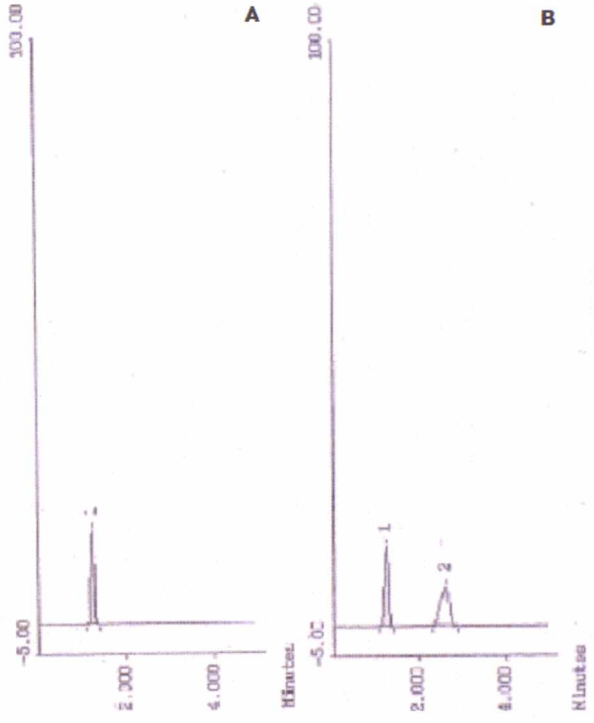


Figure 6.9 Desirable single peak of phytic acid in the study of accuracy (A) and Usual chromatogram of a commercial phytic acid solution (B).

The expected single peak in the chromatogram was not achieved in the study of accuracy.

The accuracy of the method was considered based on the absolute error (absolute error is the difference between the measured values and the actual values) [323].

6.3.4.1 The first test of accuracy

Accuracy was calculated first based on the mean of the 12 replicated injections of the 3mg/ml phytic acid standard solutions within a day and 12 separate replicated injections between the days. Accuracy was shown to be poor, with the range of the error between 28.7-32.3%, which was too high for a reliable method [323].

Period	Actual concentration: 3 mg/ml	
	Measured concentration (mg/ml)	Absolute error (%)
Within a day	2.03	32.3
Between days	2.14	28.7

Table 6.5 Accuracy of the method based on the replicated injections of one concentration

6.3.4.2 The second test of accuracy

The mean of the three replicated injections for three concentrations, from the second study of linearity was used for within the day calculations. For the between days error, the mean of the values obtained in the first and second study of linearity was used to calculate absolute error.

Period	Actual Concentrations					
	1 mg/ml		3 mg/ml		5 mg/ml	
	Measured concentration (mg/ml)	Absolute error (%)	Measured concentration (mg/ml)	Absolute error (%)	Measured concentration (mg/ml)	Absolute error (%)
Within a day	0.49	51	1.96	34.7	5.55	11
Between days	0.55	45	2.34	22	5.44	8.8

Table 6.6 Accuracy of the method based on the replicated injections of three concentrations.

Accuracy of the method in this test was very poor, with absolute error ranging between 11 to 51% and 8.8 to 45% within the day, and between days, respectively.

Summary of the main finding in the study of accuracy:

1. A single peak chromatogram of phytic acid, required for measurement of accuracy was not produced.
2. There was a high level of absolute error found in the first test of the accuracy compared with the second test.

Taken together, these results show that the method was not accurate as it was not able to measure the true known values.

6.4. Discussion

6.4.1 Methodology

6.4.1.1 Sample preparation

6.4.1.1.1 Reducing the sample size, milling and blending

Features including number of samples, sample size, volume and weight, country of origin, date, best before date (expiry date) and sample storage and preservative conditions were clearly recorded. Although storage conditions depends on the sample, in this study, cereal products were stored in a cool dry, odour free place

away from direct sunlight and once opened samples were kept in a sealed container. Standard solutions, when required, were stored in the refrigerator at 4°C. Nutritional information on the packaging gave an indication of sample composition (e.g. fat content) which was useful in determining further procedures in sample preparations.

Milling and blending was used to reduce sample particle size to obtain a more homogenous and representative sample, with greater precision and accuracy, that dissolves faster and extracts more easily because of greater surface area [378]. Depending on the method and the nature of food samples, many varieties of procedures for reducing the particle size in measurement of phytic acid in foods are reported in the literature:

In the study of Chen (2004), raw dry beans were ground in a coffee grinder to pass through a 60-mesh screen for the analysis of moisture and total dietary fibre. Black beans, red kidney beans and some other varieties of raw dry beans were soaked and cooked. The cooked beans were left to cool for 2 hours, drained and freeze-dried for 48 hours and then ground in a coffee grinder to pass a 60 mesh screen. The further analysis included the assessment of moisture content was the same as that for raw dry beans. In that study, the nuts were also ground in a coffee grinder [344]. In another study legume samples were also freeze-dried and ground to pass through a 100-mesh sieve [376].

In the recent study of Dost and Tokol (2006), prior to the extraction process, all samples apart from flour samples were homogenised. Then hard-shell samples including wheat, rye, oats, barely, rice and avocado leaf were crushed into fine particles in a mortar and pestle and then sieved from a 100-mesh standard sieve. The homemade bread samples which were made from wheat and rye flours were homogenised similarly. Carrot was sliced into fine pieces and ground in a mortar and pestle and homogenised in the extraction solution for 5 minutes with a homogeniser [323].

Germination reduces phytate content of legume seeds and is likely to increase inositol penta or tetra phosphates; therefore, in the study of Talamond *et al* (1998) a flour from germinated cowpea (Vigna unguiculata) seeds was prepared by first soaking the legumes in water for 24 hours and keeping them wet at 28°C for 48 hours while sprouting [341].

Reducing the sample particle size for the measurement of the phytic acid in infant foods was not reported [322, 370] as these samples are quite dissolvable and easy to extract.

Although passing the samples through a 40 mesh screen was mentioned in the initial methodology [324], two minutes milling of the breakfast cereals (cornflakes) with a normal kitchen blender seemed to be adequate as no problems related to the sample particle size were noted during this study.

The samples analyzed in the current study, were mostly commercial phytic acid solutions or simple breakfast cereals (in particular Kellogg's cornflakes). The food samples were milled before measurement of moisture content. For cereal grains, which consisted of toasted corn cereal, toasted wheat cereal, oat cereal and wheat bran, a sample preparation should be done according to the methodology above. However, rice bran and samples containing a large amount of fat should be treated in a different way [324, 340].

6.4.1.1.2 Fat removal

Fat influences the extractability of IP6 from food sources and should be kept under 5% or reduced before extraction [370]. The injection of samples with a high content of oil can change the chromatographic behaviour of the column and may damage it [340]. Therefore, food samples should be evaluated for fat content. Samples that are estimated with a fat amount greater than 5%, should be weighed, placed in a clean folded filter paper and the fat extracted with hexane overnight under a hood, then air dried [339].

There are different methods for fat removal from the food sources depending on the nature and composition of food and the preferred technique of chromatography. Infant food should be treated twice with Chloroform-methanol (2:1, v/v) prior to the extraction with Hydrochloric acid [370].

Brooks and Lampi (2001) studied the problems associated with measurement of infant cereals using both Ion Pair HPLC and Ion Exchange HPLC. In the Ion Pair technique, samples were extracted by 30 ml of hexane by heating in a 40°C shaking water bath, for 15 minutes, to reduce the fat content. Hexane was separated by centrifugation at 3000 rpm, for 5 minutes, and the majority of which was aspirated. The remaining solvent was removed by drying under a steam of nitrogen gas, at room temperature. For the Ion Exchange technique, samples were extracted with 35 ml of 10% ethyl ether in hexane by mixing in a rocking type

shaker for 20 hours in a 37°C incubator. Hexane was separated by centrifugation at 3000g, for 10 minutes, and most of which was aspirated. The sample was then washed with 35 ml of hexane and recentrifuged. The majority of hexane was again aspirated and the remaining solvent was removed by drying under a steam of nitrogen gas at room temperature [322].

6.4.1.1.3 Preparation of reference standard solutions

Certified reference materials for inositol phosphate are not currently available, possibly because a phytic acid standard has not been defined. Commercially available phytic acid is often used as a calibration standard, without further analysis [323, 324, 377] as recent chromatographic methods permit the quantification of most or all of the inositol phosphate species present. This allows an assessment of the purity of the IP6 in standard while performing instrumental calibration, but these methods have low limit of detection and small content of some inositol phosphates may not be diagnosed. Furthermore, most of the methods are not able to quantify the free phosphate groups that may be present in the sample [381].

Sodium phytate (dodecasodium salt hydrate) have been used to produce a calibration curve for several studies [340, 344, 350, 367, 377]. The purity and phosphorous content of sodium phytate should be determined prior to use as a standard solution. When Nuclear Magnetic Resonance (NMR) spectroscopy is available, a spectrum of the sample should be generated as it diagnoses the presence of contaminating inositol phosphate species [322, 381]. The use of ICP-AES (inductivity coupled plasma atomic emission spectrometry) for the measurement of the phosphorous content of sodium phytate has also been reported [376].

When the differentiation of the isomeric forms of inositol phosphates is important, all available isomer standards should be provided [344, 367, 382]. In this situation, an in-house reference standard solution of phytic acid is required. This can be prepared by transferring sodium phytate into a glass tube together with HCl. The tube is heated in an oven for an hour and then cooled in a water bath. The content was then dried under a stream of nitrogen and the residue redissolved by water. This solution can be stored in a refrigerator (4°C) for some months [344, 350, 367], however since the focus of the method in this study was the quantification of IP6 only, this was not the method used here.

Hydrolysis of commercial sodium phytate with phytase following the preparative enzymatic method [377], Use of the cation exchange column (2.5×18cm, AG 50W-X4) together with evaporation, dissolving to water and heating with different timings [324], and finally fractionating the inositol phosphates by using phytic acid hydrolysate, SAX columns and further retaining by sequential addition of HCl [340] all come from the protocols that have been previously reported.

Hydrolysis of myo-inositol hexaphosphate to the lower esters and the preparation of in-house reference solution were not performed for the current study as previous studies have shown that phytic acid is the major component of the commercial solutions. Lehrfeld (1994) demonstrated that inositol phosphates present in phytic acid and dodecasodium salt hydrate were 97.5% IP6 and 2.5% IP5 [340]. The aim of the development and validation of our method was to confirm the available phytate: zinc molar ratios and the role of phytate in the estimation of average zinc requirements. Since only IP6 (and to some lesser extent IP5) appear to interfere appreciably with the bioavailability of zinc, IP6 was the main phytate standard used in the method validation.

The study focused on validation of Ion Pair chromatography in the measurement of phytic acid as this is the major component and major contributor in the negative effect on zinc bioavailability. If the method was validated, there would have been a need to use phytate hydrolysate and individual myo-inositol phosphate standards or even isomeric standards together with preparation of an in-house reference standard solution, so the lower phosphoesters could be identified by comparing the individual peaks of the in-house reference standard solution. This preference in the measurement of IP6 was also reported in other studies [325, 339, 377].

Although IP4 and IP3 may contribute to a negative effect on zinc absorption with more phosphorylated inositol phosphates such as IP5 and IP6 through element binding [344, 373], in the end IP6, and to some lesser extent IP5, either singly or in combination, are still the primary trace element chelating agents [344, 368]. Partially phosphorylated inositol phosphates do not considerably interfere with the bioavailability of minerals [369], and additionally lower esters of inositol phosphates do not occur in many of foods such as grains and legumes within the detectable quantity [383] and the quantity of these lower esters are very small [324, 368, 369, 376].

Fresh standard solution was prepared for each stage of the study and was stored in the refrigerator at 4°C and was used within 3 days. A 'certificate of analyses'

was obtained from the supplier as the full process of the standardisation was not possible.

6.4.1.1.4 Solid phase extraction

There are a number of reasons for a sample preparation via solid phase extraction (SPE). When there is a large amount of interference in the original sample, separation of them from one or several analyte bands with a single HPLC is usually almost impossible. In these cases, SPE can be used to decrease or eliminate these interferences. Fats, oils and other hydrophobic components, polymeric materials and particulates which are also known as the 'column killers' can clog or disable the HPLC columns; however, SPE (in particular reversed phase SPE) can usually remove them. SPE can sometimes be used to enhance the concentration of a trace component and to consequently improve the detection sensitivity. Reversed phase SPE can also be used to desalt samples, especially before Ion Exchange HPLC [378].

In this study, the main purpose of the SPE was to decrease interference and increase detection sensitivity. SPE was also used to remove sodium ions by ion exchange chromatography, and then IP6 was permitted to perform as its own acidic catalyst [340].

SPE is a preferred technique, particularly in comparison to liquid-liquid extraction (LLE) as: 1) it has a more complete extraction of the analyte; 2) it is easier to collect the total analyte fraction and more efficient to separate and complete the removal of interference from the analyte fraction; 3) only small amounts of organic solvent are required for elution to maintain a high concentration of analyte; and finally, 4) apart from the removal of the particulates and more convenient manual procedures, SPE is more easily automated [378].

Reversed phase SPE is the most popular technique and it was used in these experiments. The SAX column, apart from the good column recovery and being fast and easy to perform, is able to retain the inositol phosphates. Washing off the inositol phosphates from the column was not difficult.

In general, instrumentation of SPE can be simple as gravity can produce the required force so that the analyte can pass through the cartridge. However, the flow through the cartridge was slow and so a plunger of a medical syringe was used to generate additional pressure and to push the sample through the column.

Pressurization with the syringe plunger was difficult. Firstly, it needed steady, continuous hand pressure over the whole period of extraction and if the plunger got slightly out of control and accidentally in contact with the liquid, there was a chance of over flow as well as external contamination. Regardless of inconsistent force of the hand to pass the sample solution through the cartridge, the flow rate should not be too fast as it can give an insufficient time of contact of the sample with the stationary phase (flow rate for most of the SPE cartridges was recommended to be less than 10 ml/min [378]). However, the syringe pressure system was easy to generate and inexpensive.

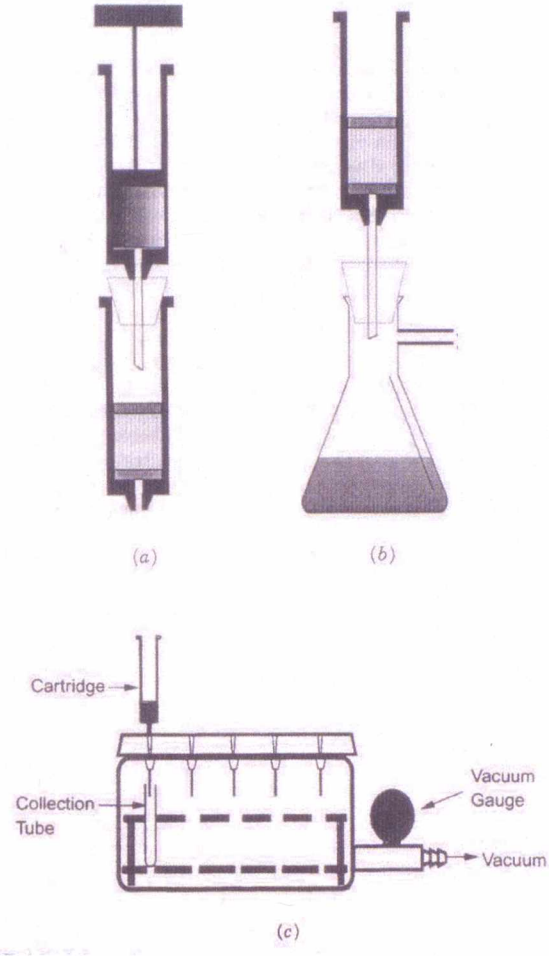


Figure 6.10 Schematics of solid phase extraction apparatus

Adapted from reference [378], (a) Pressurisation with a syringe (single cartridge). The current study used a similar system pressurizing only with a syringe plunger instead of a full syringe. (b) Employment of a vacuum flask for the single cartridge (c) Schematic figure of multicartridge vacuum manifold system.

A vacuum flask was suggested to handle one cartridge at a time; however, there was not enough detailed information for the assembly of the system. Furthermore, adjustment of the flow rate for the system was difficult and there was a chance of sample loss and/or contamination, particularly when working with small amount of sample. The alternative and preferred method was the employment of a vacuum

manifold. The vacuum manifold system can process multiple cartridges at once, and a removable rack can be located inside the vacuum manifold to keep the test tubes for eluent collection. In some of the models of the vacuum manifold, a vacuum bleed valve, a flow control valve and a gauge were incorporated to permit a better adjustment for the flow rate of the system [378]. Figure 6.10 demonstrated a schematic outline of three possible systems.

As during the validation study, most of the samples were based on commercial solutions, there was no need for reuse of the SAX columns. As a result, these columns were disposed of after use and a new column was used for each experiment. Multiple use of the SAX column for analysis and preparation of fractions were reported in another study [324]; however, the retention level of the column reduced gradually with use. If used several times, columns were washed instantly with water for neutrality, then washed with methanol and dried. In some studies, columns were reported to be in use for more than 10 times [340].

6.4.1.2 Ion Pair chromatography

6.4.1.2.1 Stationary phase

Ion pair chromatography of phytic acid has been reported in many studies [322, 324, 340, 369, 376, 384]. The Hamilton PRP-1 column has been reported as very durable and useful with a wide range of pH [324]. This series of columns maintain their performance characteristics longer than many C8 and C18 columns and therefore can be used as a replacement for them.

Some of the studies have used a C18 column instead of a PRP-1 which was used in this study. For example, in the Ion Pair chromatography of myo-inositol phosphates in Spanish legumes (Burbano *et al* 1995) a reversed phase C18 column (Spherisorb ODS 5 µm- 250×4.6 mm) was heated to 45°C and then was equilibrated with a methanol based mobile phase (similar to mobile phase A used initially in the current study but with a different solvent strength) for 1 hour [376].

Lehrfeld (1994) showed that after more than 3000 injections, the column efficiency declines to such a degree that lower inositol phosphates were no longer detectable [340]. In the present study the number of injections was less than 500 over a period of 5 months, which was too few to demonstrate any serious problem regarding durability.

In the studies of Lehrfeld (1989-1994), as the column aged, experimental parameters had to be adjusted to maintain fine resolution [324, 340]. This was not required in the current study. Furthermore, the tendency of cereal grain samples to clog the column (demonstrated by an unexpected increase of the pressure) was reported by Lehrfeld [324], but was not seen in our measurement of cornflakes.

6.4.1.2.2 Mobile phase

Acetonitrile was the preferred base for the mobile phase. The mixture of acetonitrile and water is the recommended organic solvent to be used with a UV detector, particularly at the low wavelengths (185 to 210 nm) required in the current methodology [378].

The acetonitrile-water mixture also had much lower viscosity (compared to methanol based solvents) resulting in higher plate numbers and lower column pressure [340, 378, 385]. With an acetonitrile system, a better resolution of IP3, IP4, IP5 and IP6 compared to a methanol based system was seen. This has also been previously reported [340].

Slight variations in pH and composition of the mobile phase caused problems as this altered the retention time, resolution and the shape of the peaks. This was also noted by Lehrfeld (1994) [340].

Methanol evaporation, preparing solutions by adding to volume, and variations of pH are the major causes which impact the accuracy and the precision of this method. Substantial loss of accuracy and precision in the assay due to neglecting to add phytic acid to the eluting solvent or using a less than trace element grade hydrochloric acid have been also reported [340].

These sources of error were taken into account in the development of this methodology. Most of the experiments that are discussed here were based on an acetonitrile based solvent; therefore, methanol evaporation and the volume decrease in methanol solutions was not the case of error here.

The mobile phase pH was measured to prevent any variation. However, the pH of the mobile phase was constant. The HCl used was trace element grade, so the possibility of an attraction of the phytate to the trace elements in HCl was negligible.

6.4.1.2.3 Mineral interferences

Mineral interference and sample adsorption onto the metal column of the chromatography was one of the major problems of this assay.

Non-specific adsorption of phytic acid to the stainless steel column seemed to be the major contributor of the imprecision observed in this experiment. Chelating agent characteristic of phytic acid made it vulnerable to be adsorbed to columns (including C18 silica based and PRP-1 polymeric based columns). This could be the reason why repetitive injections of the samples did not generate a similar peak.

Lehrfeld (1994) reported that the first injection of the sample, containing phytate, showed no peaks; however, subsequent injections demonstrated peaks of increasing size, until a plateau was achieved when most of the active sites were blocked by phytate. He recommended that several trial injections of phytic acid prior to any analysis is necessary [340].

In the current study, this was assumed to be one of the reasons behind the unrepeatable and irreproducible peaks of phytic acid which affected both studies of precision and linearity. The peak of phytic acid particularly in the lower concentrations disappeared and then reappeared again. However, unexpectedly, in the study of intermediate precision, the initial injections demonstrated higher peak height, but were still unrepeatable. The peak height gradually decreased with the number of injections until a plateau was reached. The running time of the assay was shorter than in the previous report [340] so this system may not have had enough time to equilibrate.

Unrepeatable detector response was a common problem and the blocking of the active sites was recommended. To solve this problem, phytic acid was injected to the system several times before analysis; however, this did not work.

Phytic acid was then added to the eluting solvent to saturate the active sites of the column, as Lehrfeld advised that the addition of phytic acid to the mobile phase (1-3 mg/L) could efficiently block all of the active sites [340]. Therefore, to minimise peak variability, different commercial solutions of phytic acid and phytate were added to the eluting solvent in a wide range of volume.

Saturation of the polymeric PRP-1 column was not seen with an addition of phytic acid (1-7 mg/L), as well as with an injection of phytic acid prior to the analysis. Therefore, the detector response remained irreproducible. A possible solution of

replacing the metal column with a plastic PRP-1 PEEK column was not practical because of budget constrains.

6.4.2 Retention time and identification of phytic acid

Retention time is the main method to chromatographic peak recognition. The CV of the retention time of the IP6, for the repeatability, was less than 1%. Phytic acid constantly and frequently appeared at a particular time of the chromatography and this confirmed that the method is able to identify phytic acid either in food samples or chemical solutions. Furthermore, this demonstrates that factors that could have affected the retention time of the measurement did not have a significant impact.

The repeatable retention time was seen all over the months that validation study was run. This may be related to the long-term reproducibility of the column. The manufacturer of the column (Hamilton Ltd) claims that the long-term reproducibility of this column is a priority, in comparison to the other columns.

The non-reproducible retention time In Ion Pair chromatography may have occurred because of the instability of temperature or the flow rate, a non equilibrated column or an over-diluted concentration of buffer [386]. Changes in retention time could also indicate a leak, pump malfunction and changes in column temperature and/or mobile phases [387].

6.4.3 Precision

The aim of the study of precision was to assess the level of agreement among the individual test results when the procedure was applied frequently to multiple sampling via measurement of a homogenous sample of phytic acid. The factors that may have affected precision and comments about the possible developments of the precision of the methodology, together with errors of accuracy are discussed in 'sources of error in quantitation of phytic acid via Ion Pair HPLC'.

Although for most of major phytate components, a method with a CV of 1-2% is required, but for the low-level impurities precision of 5-10% CV is acceptable depending on the sample complexity [378]. Precision of 5% CV was acceptable in this study because of the simple matrix of the samples and the possibility of impurity.

As the precision of the volumetric glassware is limited to about 0.5%, this is a restriction in achieving an overall good precision. To address the issue, wherever

possible the proportion of weight to volume was corrected using the solvent's density because weighing is more precise than volumetric dispensing [378, 388].

6.4.3.1 Repeatability

Achievement of highly repeatable peak area is necessary. In this study, the peak area produced a CV much higher than the accepted cut-off point.

Within the linear dynamic range of the assay, the peak area should be proportional to the amount of injected phytic acid, which is equal to the injection volume times the phytic acid concentration [389]. Figures 6.3 demonstrated that the peak area was not proportional to the concentration of phytic acid; therefore, the method is not repeatable and lacks precision.

6.4.3.2 Intermediate precision

Ideally, the measurement of reproducibility can provide a valuable index of precision, but in the current study, this experiment was not possible. The analysis should be repeated after a long time, by other people with other instruments and/or in another laboratory [385]. This study did not have any collaborator capable of repeating the protocol; however, the measurement of 'intermediate precision' as designed in this study provided the closest measure of reproducibility.

Intermediate precision refers to the agreement of complete measurements when the same method is applied many times within the same laboratory [378]. This study included the full analysis of phytic acid on different days and multiple preparation of samples, standards and various chemical reagents, but could not take the inter-laboratory precision into the account. It was previously noted that:

'There are pragmatic compromises that can be made in assessing reproducibility. What is ideally required is to carry out replicates of the complete assay and find the RSD% (CV) of the result. As well as the errors that are assessed in measuring repeatability, this gives a measure of the error in preparing sample solutions' [387].

A higher CV in measuring intermediate precision was expected as intermediate imprecision includes the errors involved in the measurement of the repeatability.

Although, the retention time of the second series of the measurements was not as repeatable and constant as shown by repeatability (CV=2.95% compared with CV=0.94% achieved in the first experiment); it was still less than 5% of the CV, as the cut-off point, indicating the precision of retention time was acceptable. The

mean of retention time of 1.25 minute achieved in the study of intermediate precision was not statistically different from the 1.26 minutes achieved as the mean of retention time for repeatability.

For the peak area, the CV for intermediate precision was unexpectedly less than CV achieved in the study of repeatability. Since the means of the two different experiments were not significantly different, the differences in the CV must be due to the variation of the standard deviation. Therefore, samples with the higher distance from the line of the mean of the peak area, may have had a common feature that meant they contributed more to the difference in the CV.

In contrast, figures of the peak area in repeatability and intermediate precision (Figure 6.3 and Figure 6.4, respectively) did not show that the samples with higher deviation from the mean had any distingushing features.

To the author's knowledge, complete precision study of measurement of phytic acid via Ion Pair chromatography including repeatability, intermediate precision and reproducibility have not been reported. The initial study of Lehrfeld (1989) stated that in samples containing more than 0.5% phytic acid, coefficient of variation was less than 3%; however, samples which contained less than 1% phytate had a CV as high as 20%. Lehrfeld suggested that the CV for these samples with such a small quantity of phytic acid could be reduced by using a more stable and sensitive detector and a larger sample [324, 369].

These current results do not confirm Lehrfeld findings about the precision of the Ion Pair HPLC. In Lehrfeld's reports, the source of the CV, the definition of repeatability and reproducibility, the number of samples and the methodology of the precision study were unclear. Furthermore, there are some fundamental differences that have to be considered while comparing the current results to the aforementioned reports:

Firstly, the sample injection in the study of Lehrfeld, was performed by autosampler, which provides more repeatable and reproducible results. Secondly, the concentration of 3 mg/ml for the samples used in this study was a usual (and not low) concentration which should have produced the precise results, even without use of the autosampler. Finally, it was not mentioned that why in low concentrations, a high CV could be acceptable.

For samples containing less phytic acid, the CV was reported to be high; therefore, the use of larger samples and more sensitive and stable detector was advised. But

in practice, the concentration of phytic acid is often unknown and must be measured. Measurement of the CV prior to deciding the initial sample size is not possible as it required several measurements of the samples for each section of the study of precision.

The other difference between the results is due to the use of a different detector. Use of the Refractive Index (RI) detector and Ultra Violet (UV) detector was mentioned in the studies of Lehrfeld [16], but there is no evidence to show that the demonstrated results of the repeatability was based on use of the UV detector.

The UV detector used in the current study is a more sensitive detector able to detect the amounts as low as 10^{-10} g/ml, however, sensitivity of the RI detector is up to 10^{-7} g/ml [390]. A UV detector with high sensitivity was employed measuring the medium concentration of phytic acid and the level of CV was still much higher than the required CV. This result differed from the points mentioned by Lehrfeld's initial studies [324, 369].

Burbano *et al* (1995) suggested that the effective extraction and purification of phytic acid may be important in achieving a better CV. The anion exchange column chromatography for the purification of the extract allows major myo-inositol poly phosphates to separate from lower inositol phosphates including IP2 and IP1.

In that study, the purification and concentration of IP6 on an AG 1X8 column (Dowex) was compared to a silica based quaternary amine column (Sax). A legume sample (lentil), which contained a significant amount of partially hydrolysed phytic acid, was analysed. The Sax column gave a CV of 17%, which was slightly less than that obtained with the Dowex column (19.4%). These results were similar to the CV reported by Lehrfeld (1989). Although, when other samples (such as the Nigerian legume samples) were measured, both columns had similar recoveries. The investigators concluded that a Sax commercial column is the preferred column as it has a less time-consuming preparation and better reproducibility; however, the precision of the methodology was not commented on [376].

Apart from the Ion-Pair chromatography, some other chromatographic methods have also studied precision:

For example, in the study of Talamond *et al* (1998) the methodology was similar to the current study; however, this study was based on HPIC performed with a 4500i Dionex liquid chromatograph with an eluent delivery pump and auto injector. The

CV of the instrument precision calculated with the values of only six injections of the phytic acid. The CV was 0.57% [341]. A larger number of the samples in this study would have been better; however, the repetitive injections of 6 samples, (as a compromise for saving time), was acceptable. This pragmatic compromise, in the calculation of instrumental precision, was also mentioned in another reference [387].

In another section of this study (Talamond *et al*, 1998), to assess the reproducibility of the method, six different samples from the same batch of ungerminated cowpea (flour) were repeatedly analysed on a daily basis for four days. The CV was 5% for the repeatability of these 24 samples and 7% for the reproducibility of the method. However, the exact definition of reproducibility in this study and the way that it was calculated was not given [341].

Subjective definitions of the reproducibility and variation in the methodology for the assessment of the reproducibility are common in precision studies for the measurement of phytic acid. For example, precision was studied as a part of the validation study of a reverse phase HPLC by Dost and Tokol [323]:

In that study, reproducibility was measured based the on peak area of the standard solution within days, between days and after a month. The CV was obtained from 10-replicated injection of the standard solution. The CV within the first day, within the second day, between days and after a month was 1.48%, 1.40%, 2.98% and 4.38%, respectively. These results, especially within a-day were very acceptable, particularly when compared to the results of the Ion Pair chromatography, including the results of the current study. The results presented as within-the-day reproducibility were more likely to be comparable to the results of the instrumental repeatability achieved in the current study. Furthermore, the results of the between the days reproducibility had a similar protocol to the study of intermediate precision in the current project.

An unacceptable level of repeatability and reproducibility was seen in this study. This was confirmed as the major problem of the method by the authorities in the field who were contacted for advice [391, 392].

6.4.3.3 Sources of error in quantitation of phytic acid via ion pair HPLC

Errors in any part of this methodology could have had an impact on accuracy and/or precision. The use of a representative sample with minimum overlap of bands, or interferences, achievement of good peak shape, accurate calibration

with purified standards, proper data handing including integration, good sample preparation technique, instrument reproducibility and injection technique, acceptable signal to noise ratio for the peak of phytic acid and finally, a useful method of quantitation and calibration are all mentioned as important factors to achieve a good level of accuracy and precision [378].

In general, the imprecision of a quantitative result can be represented as the sum of all precision errors presented as

Error! Objects cannot be created from editing field codes.

Equation 6.8 Equation of overall precision errors in the methodology

In this equation, σ^2_{tot} is the overall precision error which is called the coefficient of variation in the current study, σ^2_a, σ^2_b, and σ^2_c refer to the precision errors from various sources such as injection, volumetric error and sample preparation. The overall error in the study was higher than expected, so this section of the discussion is about the main factors that may have significantly contributed to this high total precision error of the analysis.

The primary reason for inaccuracy and imprecision in Ion Pair HPLC was likely from the homogenisation of the sample. This could be a source of error, particularly with solid samples (cornflakes and powder of sodium phytate). Samples were homogenised to ensure that they were representative of the total bulk material so the occurrence of this error was unlikely in this study.

Sample preparation of Ion Pair chromatography usually involves the preparation of a solution for injection via the series of sample dilutions. This dilution of liquid samples can, ideally, be done with a precision of better than 0.5%. This is a known source of error that usually affects the precision of studies. Care was taken to use proper glassware. For example, most of the volumetric flasks and pipettes were initially calibrated and glassware grade 'A' designed for analytical and quantitative use, was employed.

Serial dilutions of the standards were made from one stock solution to avoid inherent errors of independent preparation of several concentrations. This also minimised the number of transfers and dilutions required.

Autopipettors were used for the delivery of the small volumes as this equipment, if calibrated correctly and used properly, was supposed to have a high level of accuracy. Other references have noted that in dispensing viscous, dense or

volatile liquids, an air displacement pipettor may produce aerosol leading to errors [378, 388].

In the preparation of the higher concentration of phytic acid and the major stock solutions, an autopipettor may not have been ideal as these concentrations were prepared in larger volumes (greater than volume range of use for the autopipettor). Also there was a chance for the generation of an aerosol leading to error. Furthermore, when liquid was accidentally drawn into the barrel, the barrel needed to be cleaned before further use to prevent any cross contamination [378, 388].

The level of precision could theoretically be improved by using weighing of samples and solutions rather than volumetric transfers. Balances were used to weigh accurately how much liquid was to be dispensed; therefore, the level of accuracy in using this method also depends on the accuracy of balance. Mass was then converted to the volume using the equation of density.

Care was taken to assure that samples were completely dissolved in solvent and thoroughly mixed to assure that the dissolved sample was homogenous and representative. In this study the ultrasonic bath was often used in order to assist the complete sample dissolution.

The reproducibility of the sample injection was the major problem in this study. The sample injection with an accurate and correctly calibrated autosampler provides reproducible injections (CV<0.05%) which hardly limits the overall precision of the analysis [378]. In this study, manual (syringe) injection was used, as an autosampler was not available in the laboratory.

The coefficient of variation of the manual injection is not typically better than 2 to 3% [378]; however, in this study, the sample was injected into a holding loop to inject a specific volume (20 µl) of sample onto the HPLC column. The loop valve injector system provides a better precision and more efficient injection in comparison to the syringe and septum injector; despite the fact that the reproducibility of manual injection generally depends on the skill of the operator [385, 386]. The principle use of the injector including the loop valve injector is discussed in references [378, 385-387, 390].

A sample pre-treatment is also reported as a major source of imprecision for HPLC methods. Chemical reactions and SPE were some of the pre-treatment steps required in this study for good separation and detection. The significant

chemical reactions that can be a potential source of error are discussed in section 6.4.1.2.3 under 'mineral interferences'.

In summary, the contribution from the sample preparation and pre-treatment steps together with the sample adsorption onto the metal in chromatography contributed to the overall imprecision represented by a high CV. It is crucial to understand that in order to considerably improve the method precision, one need to first reduce the major contributors to the high level of the imprecision, and then conduct a further precision study to evaluate the method precision.

The major factors that may have contributed to the imprecision in this study are summarised in Table 6.7. These problems and suggestions of the solution for them are also discussed in references [322, 324, 340, 378].

Source	Reason	Solution	Possible impact on this study
Sampling	Careless sampling	Careful sampling	Very low
	Non representative sample	Mixing and homogenising	Very low
Sample preparation	Volumetric error	Choosing a correct method for measuring out a liquid	Low
	Operator error	Training	Low but significant
	Sample pre-treatment(e.g. errors in Solid phase extraction)	Correction and completion of sample pre-treatment (e.g. use of the correct cartridge and solvent, giving time for the elution)	Low but significant
Injection	Manual injection	Training to achieve a reproducible injection (e.g. avoiding air bubble), Use of the calibrated loop valve (good), Use of the autosampler (better)	High
Chromatographic effects	Poor shaped peaks	Use of the peak area rather than peak height	Low
	Errors in signal/noise ratio from the detector	Calibration including linearity, baseline stability, short term noise or baseline drift	Unknown
	Separation parameters including temperature, mobile phase composition, column performance and other instrumental conditions	Control of the chromatographic parameters including column thermostatting, accurate blending of solvent, stability in flow rate, column cares, change of the column when necessary	High
Data system effects	System error in filtering or averaging the short term noise	Validation and setting the system with a typical sample	Medium
	Errors in setting the data system including the point/ second data collection		

Table 6.7 Possible sources of error leading to imprecision, the solutions and their likely effects.

Table developed using points mentioned in references [322, 324, 340, 378, 386].

6.4.4 Linearity

The construction of a calibration plot, using external standards, is the most usual approach for the determination of the concentration of an unknown sample. Phytic acid standard solutions (which could also be called phytic acid calibrators) were prepared at known concentrations (in this case, concentration between 0.33 to 6.0 mg/ml) and plotted as in previous studies; these included the range of phytic acid which usually found in food sources.

As the food samples and standard solutions were prepared, injected and analysed by exactly the same method, the concentration is established graphically from a calibration plot. The calibration plot was expected to be linear with a zero intercept. As a result, calibration is a crucial part of validation studies, and only in unusual cases, where the calibration plot is not linear, the method still can be useful and the sample concentration can be measured by interpreting the results based on a nonlinear equation. It is the case where many more standards are repeated and the technique could be useful only when no other choice exists [378].

The purpose was to test that how well the calibration plot of the detector response to phytic acid versus concentration approximates a straight line. In this section, the assumption of the linearity in the assessment of phytic acid via an Ion Pair chromatography is discussed.

The presented results are based on the peak area rather than peak height. Determination of peak height of signal to measure the detector response is usually the preferred index for the trace analysis [378]. Generation of the calibration curve by using the peak area as a function of the phytic acid standard solution was previously reported by several references [322-324, 341, 369, 376, 377], although the use of peak height for this purpose was not reported in any of the references reviewed.

6.4.4.1 The first study of linearity

The confirmation of the assumption of the detector response linearity was based on the plot of phytic acid peak area as a function of phytic acid concentration, least square regression analysis and a very strong coefficient of determination.

Several phytic acid concentrations were measured in triplicate and the mean of response for each of the concentrations was used as the data points for the generation of the calibration curve. This approach was also reported in the study

of Amari (2005) in characterisation of linearity for an HPLC UV detector using a recombinant protein [379], which minimises the error in the assessment of individual concentrations.

Excluding the lowest concentration (as the outlier of the CV) a positive and significant relationship between the concentration and coefficient of variation was seen. It could have been interpreted as the higher the concentration, the less precise the linearity was; this suggests an increasing error with an increase of the concentration. The source of error was not clear; however, it was more likely to be an impurity in standard solutions, rather than an error in sample preparation or a technician error.

A similar regression plot of the concentration of phytic acid versus the peak area in the study of Lehrfeld (1989) reported a least squared regression (r^2) of 0.999 [324]. The generation of a linear standard curve (R^2 =0.99) was also reported in Ion Pair HPLC of phytic acid in the study of Brooks and Lampi (2001) by injecting 6.0 to 23.5 µg of commercially available IP6 in 20 µl, as well as by injecting 16.7 to 50.0 µg of commercially available IP6 in Ion Exchange HPLC [322].

In the study of Spanish legumes, reported by Burbano *et al* (1995), sodium phytate, was used as an external standard. A calibration curve was generated for a range of concentrations including 0.36-7.3 mg/ml versus the peak area. The correlation coefficient in this study was 0.998 [376]. The range of concentration in that study (Burbano *et al*, 1995) was very similar to the range calibrated in the current study, although there were some fundamental differences in the methodology making the comparison difficult. For instance, employment of a C18 reversed phase column, together with a refractive index detector and a methanol based mobile phase, in the study of Spanish legumes were different from the current study.

A linear detector response to the concentration of phytic acid was also reported by some other studies [323, 339, 377], but as Ion Pair HPLC was not the major method of the chromatography in those studies, those results are not discussed here.

As the linearity plot of the first study was not linear, the calibration accuracy of the method was not satisfactory enough to suggest the method as a useful method for measurement of phytic acid.

The plot of sensitivity (RF) versus the concentration was produced to explore the range of possible linearity. In theory, when the method is really linear, sensitivity (RF) should be independent of concentration over an extended range of concentration, and the slope of this plot should be equal to zero. A zero slope was not found and this suggested nonlinearity of the detector response. If there is a level of linearity in any particular range, the slope of the line in that range would have been zero and the level of sensitivity would have stayed constant.

The least square regression analysis of response as a function of concentration showed the detector behaved in a nonlinear fashion. When the full range linearity plot of sensitivity versus the concentration gave a regressed line with a slope not equal to zero and irregular values of sensitivity, this appeared to confirm that the detector did not behave in a linear manner.

Based on these results, there could be two general assumptions: 1) the assumption of a nonlinear response and, 2) the assumption of the linearity of the method but with an incidence of an error in the first study.

6.4.4.2 The second study of linearity

As a general rule, a linear method with the minimum intercept permits a quick and convenient check with one or two points to confirm calibration accuracy, but the initial results of this study showed that there was a further need to do a full recalibration process. Hence, the second study was conducted using the larger number of the samples.

An independent second series of measurement was also required to evaluate the regression model of the first study and a higher number of sample solutions were injected for the second series, better results were expected.

The calibration curve of the detector response versus the concentration was generated for the second data set. Although a linear curve with R^2 exceeding 0.99 was not obtained, the R^2 was higher compared to the first series, which meant a better linear line and one closer fitting to the data. A supplementary test of sensitivity was conducted and when the slope of the equation (Figure 6.8) for this test was not equal to or close to zero, the likelihood of nonlinear behaviour was confirmed.

6.4.5 The studies of accuracy

The study of Dost and Tokul (2006) was the only study that considered accuracy independently from reproducibility and precision [323]. In that study, the accuracy was calculated from 10 replicated injections of the standard solution containing 50 µg/ml phytic acid. The level of accuracy of the method was very good with abolute error ranging between 0.16 and 1.54% for within and between days respectively. A desired single peak chromatogram was also obtained confirming that the standard solution was pure enough [323]. The current study did not achieve such clear results.

The unacceptable level of accuracy in the current study may be due to the problems from mineral interferences, purity of standard solutions, representative sampling, contamination or system error.

6.5 Conclusion

Ion pair HPLC was not found to be the method of choice for measuring phytic acid of food sources in this study [393].

There is no certified reference phytic acid standard solution available. Sample preparation, extraction and purification of the method were very labour intensive. Mineral interference and sample absorption to the column was a major problem of the assay.

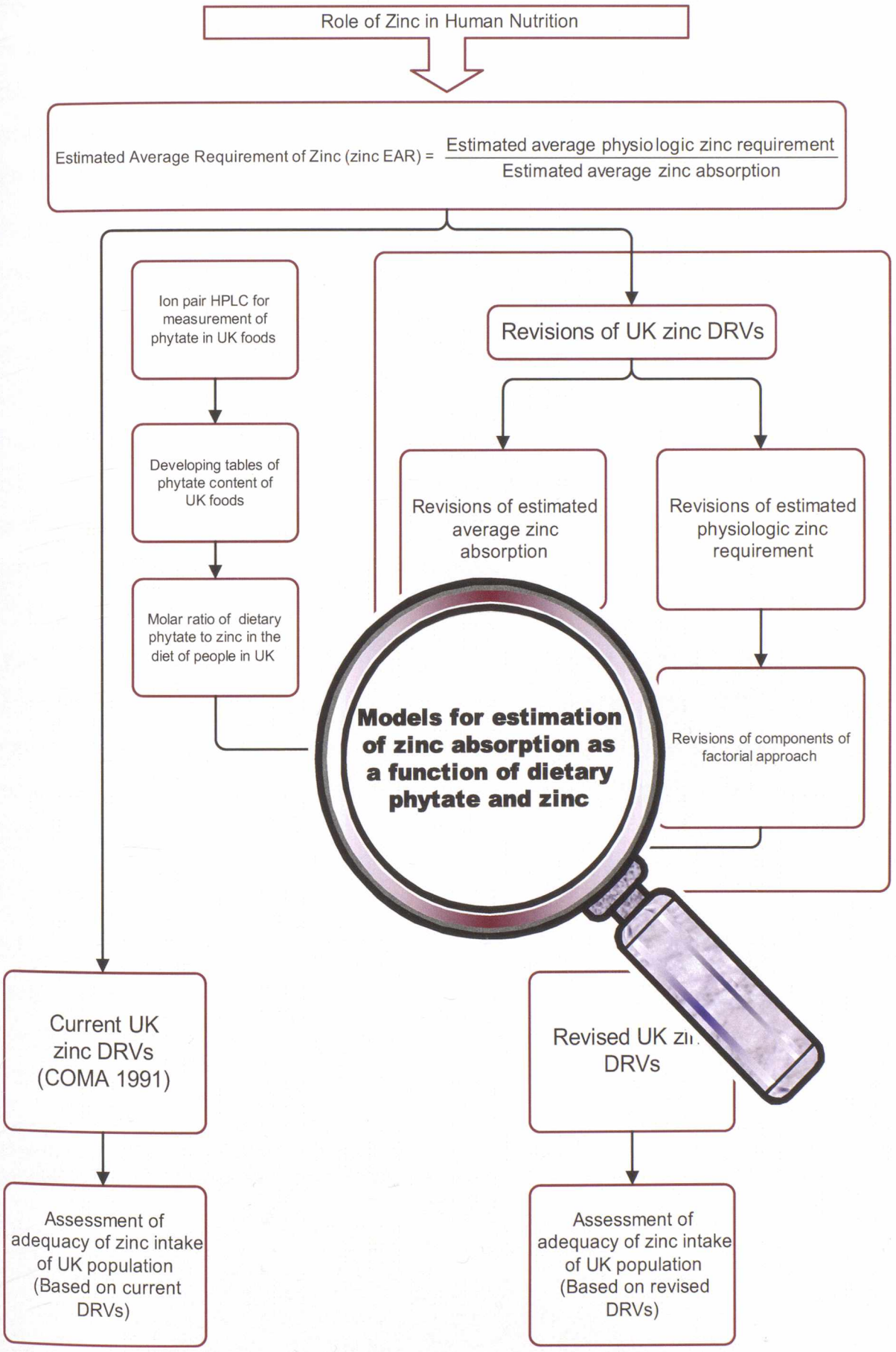
The method used was not found to be precise. The detector response to repetitive injections of one concentration was not constant, demonstrating a poor level of repeatability. Repeating the complete process on different days and with multiple reagents and preparation, showed that the method was not reproducible.

The method is not linear enough to produce a reliable calibration curve for the measurement of phytic acid. This was confirmed by several tests and the complete study was repeated more accurately with more samples. If the detector response versus concentration is really based on a model, a nonlinear model may be as good as the linear model.

The method was not accurate. A high level of inaccuracy could be because of several factors including mineral interference, contamination, and impurity of the standard solutions; however, a lack of the linearity of the method is the major reason for this inaccuracy.

Further attempts to validate Ion Pair HPLC in measuring phytic acid should be conducted with better equipment including an autosampler and vacuum manifold, and ideally, in collaboration with other research laboratories. The other chromatographic methods of measurement of phytic acid including Ion Exchange HPLC [322, 339, 377] and HPIC [341, 344, 367], may be valid and useful as the alternative techniques.

Chapter 7: Estimation of the Average Proportion of Zinc Absorption for the UK population



The magnifier symbol shows where you are in the conceptual framework of the thesis.

7.1 Background

To develop a zinc EAR, it is necessary to have an estimate of the physiologic zinc requirement together with an estimate of the proportion of zinc absorption from the diet. The methodology for the estimation of the physiologic zinc requirement is discussed in chapter 4. The current chapter is to discuss a method to estimate the proportion of dietary zinc absorption.

Bioavailability of zinc depends on many factors. Interaction with other nutrients (e.g. iron, calcium, copper and proteins) or food components (e.g. phytic acid) influences zinc bioavailability and consequently the dietary zinc requirement. To date, a globally accepted algorithm to predict zinc bioavailability and the proportion of absorption, based on the presences of these nutrients and food components, has not been established. The ideal algorithm for estimating dietary zinc bioavailability would include the dietary content of phytate, protein, zinc, and preferably calcium, iron and copper; however, zinc and phytate content have the largest effect and models currently available, focus on these two dietary factors [16, 237, 394, 395].

Two models are currently used to predict zinc absorption. The first model is an algorithm developed by the IZINCG expert committee in 2004 based on calculations of published studies available at the time [16]. The equation to predict proportion of absorbed zinc using the dietary content of zinc and phytate: zinc molar ratio is:

$$\text{Logit (Fraction of absorbed zinc)} = 1.1365 - 0.6129 \times \ln(\text{mg zinc}) - 0.3164 \times \ln(\text{phytate : zinc molar ratio})$$

And

$$\text{Fraction of absorbed zinc} = \frac{\exp(\text{logit(fraction of absorbed zinc)})}{1 + \exp(\text{logit(fraction of absorbed zinc)})}$$

Equation 7.1 The prediction equation for proportion of absorbed zinc, using the dietary phytate: zinc molar ratio and zinc content suggested by the IZiNCG expert committee.

The second model was developed by Miller *et al* in 2007 [394]. The algorithm of the model is:

$$TAZ = 0.5 \cdot \left(A_{max} + TDZ + K_R \cdot \left(1 + \frac{TDP}{K_p} \right) - \sqrt{\left(A_{max} + TDZ + K_R \cdot \left(1 + \frac{TDP}{K_p} \right) \right)^2 - 4 \cdot A_{max} \cdot TDZ} \right)$$

Equation 7.2 The mathematical model of zinc absorption in humans as a function of dietary zinc and phytate.

This model was developed and validated by Miller et al [394].

TAZ represents total daily absorbed zinc, A_{max} represents maximum absorbed zinc, TDZ represents total daily dietary zinc, K_R represents the equilibrium dissociation constant of a zinc-receptor binding reaction, TDP represents the total daily dietary phytate and K_P represents the equilibrium dissociation constant of a zinc-phytate binding reaction [394].

The aim of this chapter is to estimate the average level of zinc absorption in the UK population using a valid and applicable algorithm. This chapter includes two sections: the first section studies the validity and applicability of the available methods to estimate the proportion of zinc absorption and the second part applies the method and estimate the average zinc absorption in different subgroups of the UK population.

7.2 Selection of a model for estimating average zinc absorption

7.2.1 The validity of the mathematical model to estimate average zinc absorption of the UK population

7.2.1.1 Background

The biochemical basis for the model and the derivation of a mathematical model from the biochemical aspects were developed by Miller *et al* [394]. In this model (Equation 7.2), there are two independent variables; TDZ and TDP. The dependent variable is TAZ representing total daily-absorbed zinc and all these variables are in the unit of millimoles. The model has three parameters including A_{max}, K_R and K_P.

When the model was modified to use phytate: zinc molar ratios (R_{pz}) instead of TDP, the resulting equation for the model became [394]:

$$TAZ = 0.5 \cdot \left(A_{Max} + TDZ + K_R \cdot \left(1 + \frac{R_{pz} \cdot TDZ}{K_p} \right) - \sqrt{\left(A_{Max} + TDZ + K_R \cdot \left(1 + \frac{R_{pz} \cdot TDZ}{K_p} \right) \right)^2 - 4 \cdot A_{Max} \cdot TDZ} \right)$$

Equation 7.3 A version of the mathematical model of zinc absorption using phytate: zinc molar ratio (R_{pz}) instead of total daily dietary phytate (TDP).

This model was selected for the validation because:

1. Uniquely, it was developed from a biochemical and physiological understanding of the absorption process [16].
2. As zinc absorption is a saturable carrier mediated process [394, 396], the relationship between absorbed zinc and dietary zinc content is nonlinear. The negative effect of phytate on zinc absorption is also supposed to be nonlinear [394]. Analyzing these relationships with nonlinear regression is preferred compared with data transformation and linear regression as used by the model of IZiNCG [16].
3. Part of the data in the model validation of Miller *et al* (2007) did not meet the criteria of the selection of data, but was still used to extend the range of the phytate zinc molar ratio beyond that of the IZiNCG data [394]. This data was excluded in the current model validation because additional data have become available to extend the range of the phytate: zinc molar ratio without selecting invalid data.

This sub study aim to assess mathematical model's validity by fitting it to selected data from literature, estimating parameters of the model and predicting the dietary zinc needed to meet the estimated physiologic zinc requirement of the UK population.

7.2.1.2 Materials and methods

This chapter followed the conceptual approach suggested by Miller *et al* [394] in order to present the results comparable to the previous finding.

7.2.1.2.1 Selection of data

This mathematical model was validated using selected data from literature. The analysis used the selection criteria used by the IZiNCG technical document [16]. These criteria were: (1) radio or stable isotope studies that estimated true zinc absorption from total diets by correcting for intestinal losses of endogenous zinc; (2) studies of typical mixed vegetarian or normal cereal based diets, but not those

that were based on a semi-purified or supplemental zinc, and (3) studies conducted among healthy male or female adults, with no geographical restrictions.

The data from nine published studies [271, 281, 296, 301-304, 397, 398] provided the main body of the data used for this analysis. Fifteen data points from these studies were previously used in the development of models by the IZiNCG [16] and Miller *et al* [394]. In addition to these data points, the current investigation included 2 data points from the study conducted by Kim *et al* (2007) [299].

The 6 data points from two published studies [280, 399] that were used by Miller *et al* [394], were excluded from this analysis because these data did not meet the second criterion of the data selection. Fortunately, the additional two data points from the study conducted by Kim *et al* [299] made up for the excluded data points and also extended the range of the dietary phytate beyond that of the previous analyses.

The data used in the analysis were a mean of the findings from five to 21 adult subjects who participated in individual studies. The data only included the mean data of subjects who were apparently healthy and in normal zinc status and no data from the studies of children, elderly, pregnancy and/or lactation were included.

129 subjects, including 47 males and 82 females, participated in the 10 studies. The amount of TAZ in all of the studies was assessed based on radio- or stable isotope techniques as specified in the first selection criterion. In most of the studies, the amount of TDZ was determined based on chemical analysis of the diets; however, in some studies zinc intakes were calculated from the dietary records. The amount of TDP was calculated from the dietary records by the authors of the studies. When this value or the value of R_{pz} was not available, the calculations of the IZiNCG [16] was used or the R_{pz} value was calculated by the author of this report from the available variables.

7.2.1.2.2 Data Analysis

In order to validate the model, the model was fitted to the data with a nonlinear regression analysis using the DataFit Programme (Version 8.2.79, Oakdale engineering, Oakdale, Pennsylvania, US). The data was not weighted or log-transformed prior to the regression analysis. The regression statistics, estimating the parameters and 3-dimensional graphing of the data versus the model were performed using the DataFit programme. The additional statistical analysis were

conducted by SPSS (SPSS 14.0 for windows, Release 14.0.0, 5 Sep 2005, Chicago, SPSS Inc), and the two-dimensional graphing for the prediction of TAZ from TDZ and R_{pz} was performed using Microsoft Excel (Microsoft ® Office Excel 2003).

In order to assess the regression assumptions of the normality, constant variance and independence (lack of correlation) of the residuals, additional statistical tests were performed.

The distribution of the residuals was assessed based on the Kolmogorov-Smirnov and Shapiro-Wilk normality tests. The constant variance was tested by assessing the Spearman rank correlation between the absolute values of the residuals and the TAZ values. The assumption of independence was assessed by using the Durbin-Watson test for the serial correlations as it was previously conducted by Miller *et al* [394]. The level of statistical significance was set at 0.05 for all tests.

7.2.1.3 Results

The model was successfully fitted to the data and the nonlinear regression analysis of the equation was converged to the least square-fit. Figure 7.1 demonstrates a graphical representation of the model fitted to the data. In this figure, the data points are represented by round symbols and the model is represented by a multicolour surface. The vertical 'stems' of the data points represents the distance between each datum to the model (i.e. residuals).

When the equation of the model was solved by fitting it to the data, the parameters were estimated to be:

$$A_{max}=0.101,\ K_R=0.061 \text{ and } K_P=0.966$$

From these estimates, prob(t), which examined the null hypothesis that the parameter value is actually zero, were 0.002, 0.214 and 0.307 for A_{max}, K_R and K_P respectively.

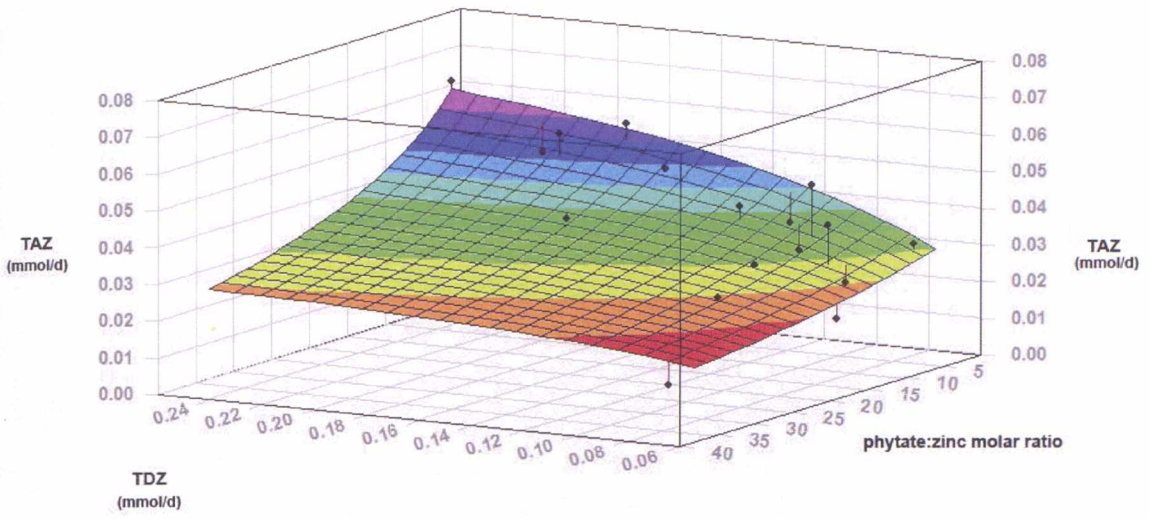


Figure 7.1 The 3-dimensional plot of the mathematical model fitted to the data.

The coefficient of multiple determination (R^2) was 0.77 and the adjusted version (R_a^2) was 0.74. The sum of the squared residuals (SS_R) was 0.0007. Generation of the residual normal probability plot (Figure 7.2) demonstrated a normal distribution.

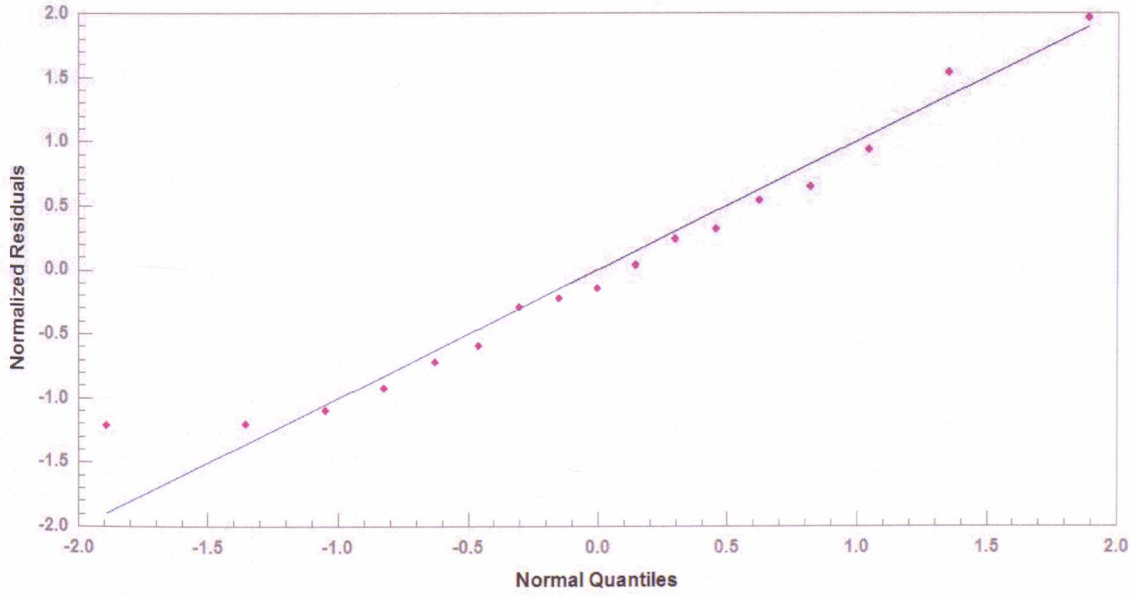


Figure 7.2 The residual normal probability plot from fitting the mathematical model.

The points roughly lied on a straight line demonstrating that there is an adequate normal distribution among the residuals.

The Kolmogorov-Smirnov and the Shapiro-Wilk normality tests were conducted to examine whether the distribution of the residuals is significantly different from the normal distribution. The p-value for Kolmogorov-Smirnov and Shapiro-Wilk tests

were 0.200 and 0.383 respectively. These non-significant values confirmed that there was no deviation in normal distribution of the residuals.

The Spearman rank correlation indicated that there is no significant correlation between the absolute values of the residuals and the values of TAZ ($p=0.54$). This confirmed the assumption of constant variance of the residuals indicating that the data points are equally reliable and by using the model, the TAZ is equally predictable from the values of the predictor.

A Durbin-Watson test value of 2.07 indicated that there is no serial correlation between adjacent residuals, confirming the assumption of independence. This simply showed that there is no serial correlation between the errors of the model.

The graphs of the residuals relative to TDZ, R_{pz} and predicted TAZ were generated to demonstrate that the assumptions of constant variance and independence are valid (Figure 7.3 A-C).

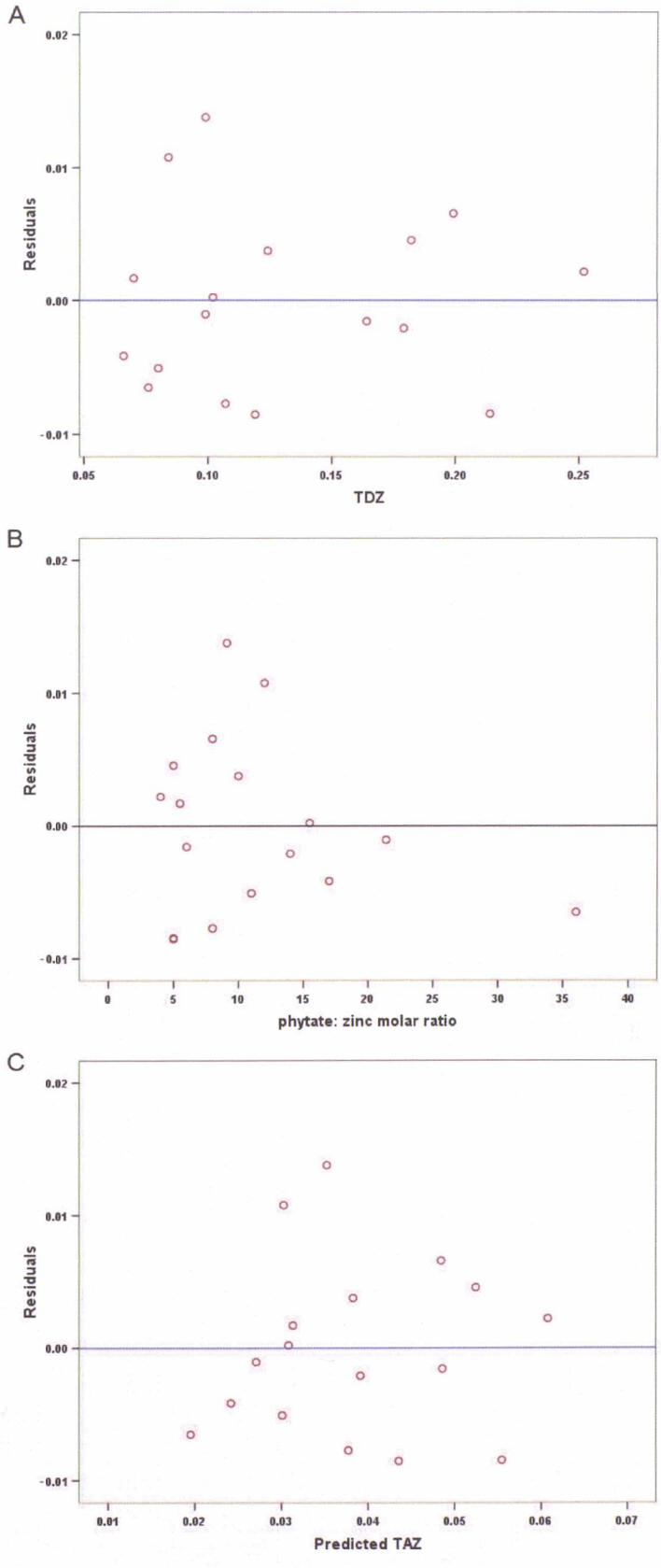


Figure 7.3 Residuals from the model compared with (A) total daily dietary zinc intake, (B) phytate zinc molar ratio, and (C) predicted TAZ.

The residuals demonstrated no evidence of nonconstant variance. Moreover, no perceptible relation was found between the residuals and any of these variables. The only possibility of a nonconstant variance was observed in figure B; however, this was perhaps attributable to the clustering of the data in the low range of phytate: zinc molar ratio.

Using the obtained values of the parameters, it is possible to predict the value of TAZ for any dietary intake of zinc and phytate in healthy adults. A series of curves predicting TAZ from TDZ and R_{pz} is illustrated in Figure 7.4.

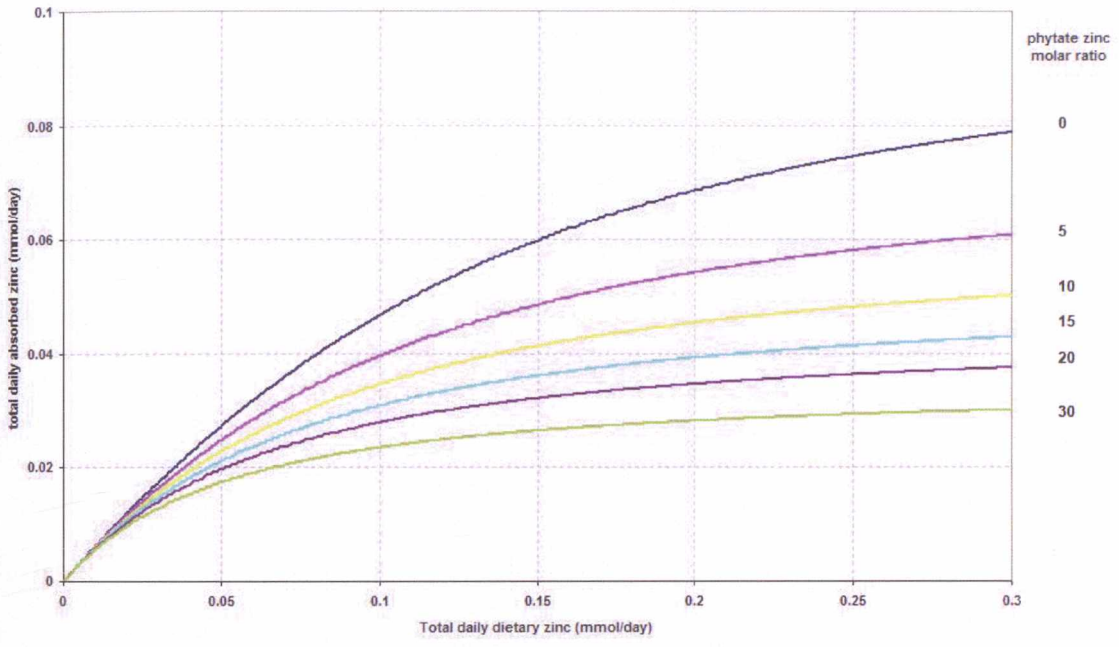


Figure 7.4 Absorption curve, demonstrating prediction of the TAZ by the model versus TDZ for selected phytate: zinc molar ratios between 0 and 30.

When the residuals were standardised by dividing them into an estimate of their standard deviation, one was flagged as a possible outlier. Although this datum was not removed for fitting the model, the assessments of the model residuals were repeated without this discrepant point. Table 7.1 demonstrates the main findings of the evaluation of the model when the possible outlier was removed.

General statistics
Coefficient of multiple determination R^2=0.83
Adjusted coefficient of multiple determination R_a^2 =0.81
Residual sum of the squares (absolute) SS_R=0.00048
Estimate of the parameters: A_{max}=0.114, K_P=1.313, K_R=0.093
Prob(t) for parameters: Prob(t) for A_{max}=0.005, Prob(t) for K_P=0.224, Prob(t) for K_R=0.154
Assumption of normally distributed residuals
Kolmogorov-Smirnov P-value=0.200, Shapiro-Wilk P-value=0.800
Assumption of homoscedasticity of the residuals
Spearman correlation rank P-value=0.58
Assumption of Independent residuals
Durbin-Watson value=2.259

Table 7.1 General statistics, resulting parameters estimates and evaluations of the model residuals when the analyses were repeated without the discrepant point.

7.2.1.4 Discussion

The mathematical model of zinc absorption as a function of dietary zinc and phytate is a basic demonstration of the absorption process that does not take all aspects of this sophisticated process into account. For example, the role of the passive absorption route, the effect of other dietary minerals on parameters of the model, and the validity of the assumption that almost all phytate content available in the process of absorption is unbound, requires further investigations [394]. At this stage, there is a limited knowledge of the absorption process and a few available (and reliable) data to validate all aspects of the model or to incorporate a more detailed knowledge of the absorption mechanisms [394, 400].

The statistical analyses of the model included the evaluation of the goodness of fit to the data and the assessment of the model accuracy from the generalisation and diagnostics aspects:

The value of R^2 was supportive of goodness of fit to the data, showing that 0.77 of the variance in TAZ is explained by the model. The cross-validation of the model as reflected in the adjusted R^2 (R_a^2) of 0.74 is supporting the validity of the model, indicating that 74% of the variance in TAZ would be accounted for if the model had been derived from the data used. This aspect provided a degree of accuracy in predicting the same TAZ in different data.

To draw a conclusion about the generalisation aspect of the validity, several assumptions underlying the regression analysis were examined. These assumptions are that residuals must exhibit a normal distribution, constant variance and independence.

An evaluation of normal distribution of the residuals indicated that the residuals of the model are random and normally distributed variables with a mean of zero. Both the Kolmogorov-Smirnov and Shapiro-Wilk tests of normality in addition to visual examination of the normal probability plot confirmed that the residuals are normally distributed. It was concluded that the difference between the model and the data were most frequently zero or close to, zero and a difference much higher than zero may happen only occasionally.

The assumption of constant variance of the residuals was also confirmed demonstrating that the standard deviation of the residuals are constant and did not depend on the values of TDZ and R_{PZ}. This means that the probability distribution

for each value of TAZ has the same standard deviation regardless of the values of TDZ and R_{PZ}.

An assessment of the assumption of independence was necessary to determine non-random behaviour of the residuals to see if there is any systematic deviation of the model using the data. The test of Durbin-Watson confirmed that this assumption is true as there was no serial correlation between the residuals.

Although residuals passed all assumptions of the generalisation aspect, from the diagnostics point of view, one datum that marginally deviated from the model caused some concerns. When normal residuals were standardised, with 95% confidence interval, this datum could be categorised as an outlier. After excluding this outlier, all indices of goodness of fit and generalisation were improved and the model was still valid.

The results of the current investigation are more reliable than the previous validation of the model [394] because:

1. The data points from the studies that used a liquid formula or supplemental diets were excluded and replaced by data that are more reliable.
2. The indices of the model validation and estimate of the parameters improved with this additional replaced data.

The six data points that were included in the previous validation of the model did not meet the second criterion of the selection of data. Contribution of these data points in the mode validation could be a source of error because a) the type of diets that these results were obtained from, do not represent a typical diet consumed by the population, and b) the absorption of zinc from these diets are expected to be higher than solid food matrices [16].

Although most theoretical aspects of the model validation were satisfactory, in practice, the model was unable to predict the critical level of zinc absorption for the population. Figure 7.5 is an example demonstrating how the model is unable to predict the level of zinc absorption for American adult men.

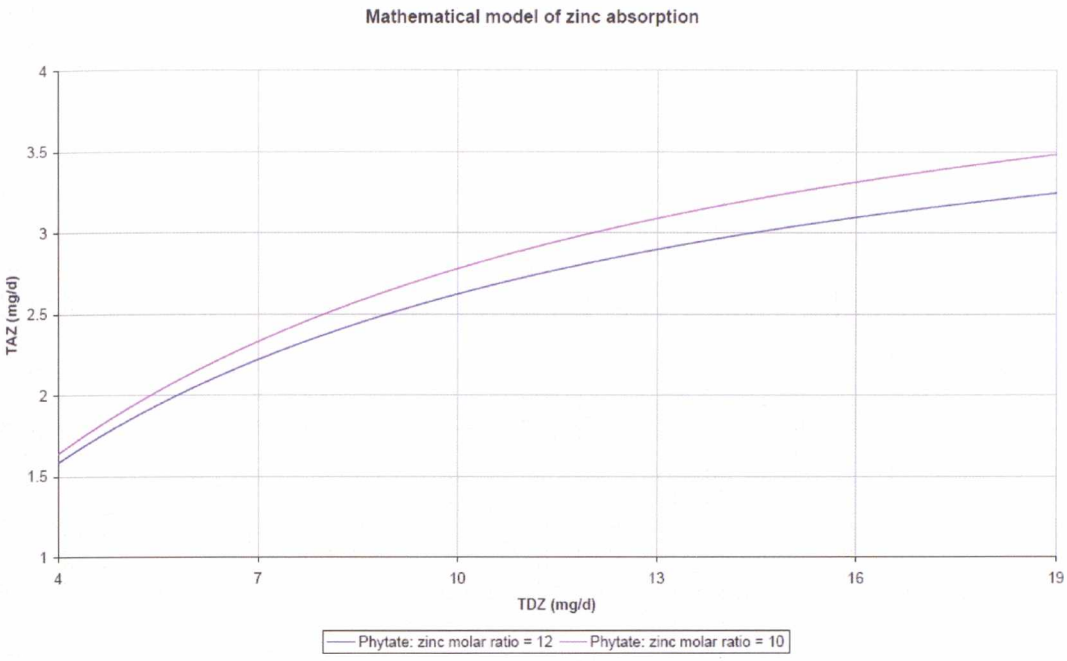


Figure 7.5 Limited use of mathematical model of zinc absorption for predicting the critical level of zinc absorption for American adult men.

A curve generated with specifics and parameter estimates of the mathematical model of Miller et al (2007) [394] to predict the level of zinc absorption for the diet of American adult men. Using this curve and the estimated physiologic requirement for absorbed zinc of American adult men (=3.84 mg/day [237]), if we assume usual phytate: zinc molar ratio of American omnivorous diets (=10 [401]), the model cannot predict a corresponding total dietary zinc intake within the reasonable range.

This example was selected on the assumption that the estimates of the physiologic zinc requirement and the dietary composition of the diets are well established for the US data. Nevertheless, the curve using UK data and estimates by the present study produced neither a better result. For instance, the curve in Figure 7.6B is unable to predict a dietary intake value for the TAZ value of 3.29, which is the estimated physiologic zinc requirement for UK adult men.

The model predictions are inconsistent with the current understanding of physiologic zinc requirements and phytate: zinc molar ratio of normal diets. The magnitude of this error is more explicit for corresponding values of TDZ, when TAZ is over 3 mg/day.

In recognition of this error, the developer of the mathematical model was informed about it [402]. The existence of the error was acknowledged [403] and possible explanations and solutions were reviewed [404, 405]:

Based on the assumption that our present knowledge about the estimates of the physiologic zinc requirement and/or dietary zinc and phytate contents of the

populations are not erroneous, the explanation of the error must be found in the mathematical model or in the data used to estimate the model parameters [403].

First, the assumption that 'it is not the estimates of physiologic zinc requirement and/or knowledge of diet composition that are in error' may not be correct. Miller VL; the developer of the mathematical model, argued that the majority of the data that he used for the initial validation of the model are from studies of normal or typical diets, usually in the US or Europe. However, from these studies, only about 20% showed zinc absorption higher than 3 mg/day. He questioned if the majority of participating subjects were in reality absorbing inadequate amounts of zinc. He also argued that the reliability of the dietary phytate data is questionable, considering that among the phytate data used for model validation, some phytate values were from laboratory analyses and some from food composition databases and findings from analyses and databases were not always in agreement [404].

Regarding the values of the parameters, only for A_{max} could it be concluded that the value is certainly not zero (prob(t)=0.002). The estimated value of A_{max} (0.101 mmol/day) was comparable to the values of 0.11 and 0.13 mmol/day obtained by the FNB/IOM [237] and Miller *et al* [394] studies respectively.

The level of uncertainty around the two other parameters (K_R and K_P) was large as it could not definitely concluded that these parameters are not zero (prob(t)<0.05). This large uncertainty in estimates of the parameters was also reported in the previous application of the model validation [394].

An uncertain estimate for K_P is thought to be responsible for the troublesome predictions observed. When the data of the IZiNCG was fitted to the mathematical model, K_P was estimated to be 1.82, indicating a lower phytate-zinc binding affinity than the initial estimates (1.2) [403] and the estimate of the present study (0.97).

The validity of the mathematical model of zinc absorption is limited by the available data that was fitted to the model. Further studies are required to estimate all parameters of the model with statistically acceptable levels of certainty, while maintaining satisfactory goodness of fit and behaviour of the residuals.

For example, a recent study of Hunt *et al* (2008), substantially added to the limited data on human zinc absorption. The study indicated that subjects absorbed zinc more efficiently from low zinc diets. After consuming low zinc, low phytate diets for several weeks, subjects adapted to a further increased level of zinc absorption; however, with higher phytate diets, there was no evidence of this physiologic

adaptation [400]. When the results were fitted to the mathematical model of zinc absorption, the estimates of the parameters (A_{max}=0.11, K_R=0.06 and K_P=1.46) were in agreement but more precise (prob(t)<0.05 for all three parameters) than those originally published [394, 400].

It was very interesting to see that estimates of two out of three of the model parameters (A_{max} and K_R) were almost identical to the estimates reported in the present report.

7.2.2 Logit regression model and its applicability to estimate average zinc absorption of the UK population

The only model comparable to the mathematical model is the logit regression model developed by the IZiNCG expert committee [16]. The current predicted values of TAZ from the mathematical model were almost perfectly correlated with those of the IZiNCG model (r=0.99, p<0.001). At the same time, caution should be exercised in interpreting this strong correlation because the data is clustered in the low range of the predictors (especially R_{pz}).

At a high range of TDZ, the predicted values of the two models diverge. Where the divergence happens, the value of TDZ varies inversely with R_{pz}. This divergence of the models at higher levels of TDZ was firstly reported by Miller *et al* [394]. Figure 7.6 demonstrates a comparison between the predicted TAZ of the mathematical model and the predicted TAZ of the model of IZiNCG. The curves are generated for the phytate: zinc molar ratios of 11 and 24, because in the modelling of the IZiNCG, these two values represent the diet types of the population (value of 11 for mixed or refined vegetarian diets and value of 24 for unrefined cereal based diets) [16].

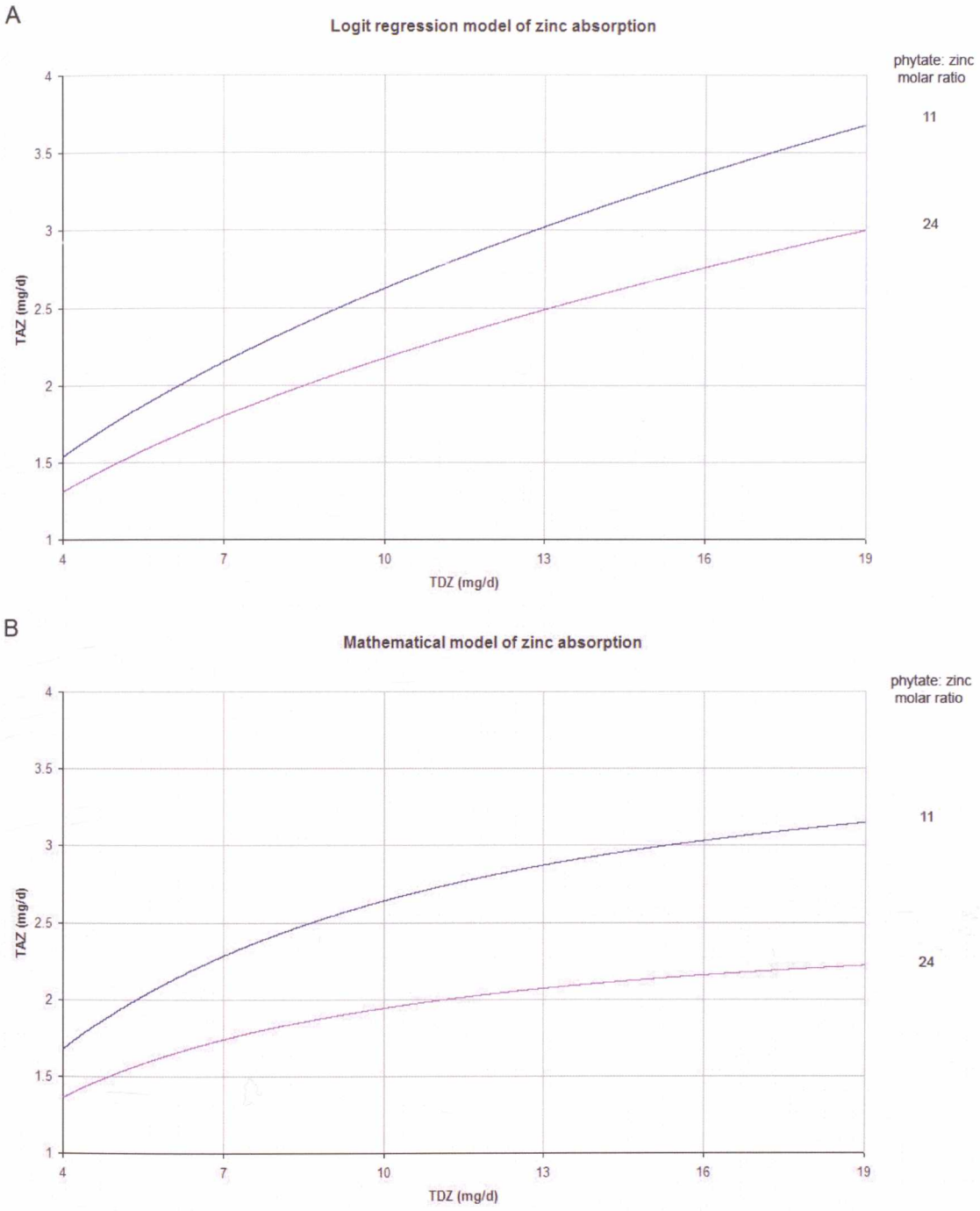


Figure 7.6 Comparison of the mathematical model predictions of TAZ with the predictions of the IZiNCG model.

Prediction of the TAZ versus TDZ by the logit regression model of IZiNCG [16] (A) and the mathematical model of zinc absorption [394] (B) across the range of the data used in the current investigation for two levels of phytate: zinc molar ratios (Rpz=11 or Rpz=24). The models diverge at higher TDZ values.

Overall, predictions of the logit regression model of IZiNCG [16] appear to be more constant with our current understanding of the estimated physiologic zinc requirements and the zinc and phytate contents of normal diets. For example, the model of IZiNCG shows that the dietary zinc required to reach the absorption level of 3.29 mg/day (estimated physiologic zinc requirement for UK adult men) for the

hypothetical phytate: zinc molar ratio of 11 is 14.84 mg/day (Figure 7.6A). This indicates a 22% zinc absorption, which is expected from this dietary pattern.

7.3 Estimated average absorption of zinc for the UK population

The logit regression model suggested by the IZiNCG expert committee (Equation 7.1) [16] was used in this study. The median phytate: zinc molar ratios of the UK adult men and women (Chapter 5) was applied to the IZiNCG model to predict total absorbed zinc, for several hundred hypothetical values of dietary zinc intake. This gave rise to a curve, illustrating the relationship between zinc intake and the absorbed zinc for men and women. The curve was then used with the estimated physiologic requirement of adult men (3.29 mg/day) and women (2.61 mg/day), (from chapter 4) to predict the amount of dietary zinc needed (Figure 7.7).

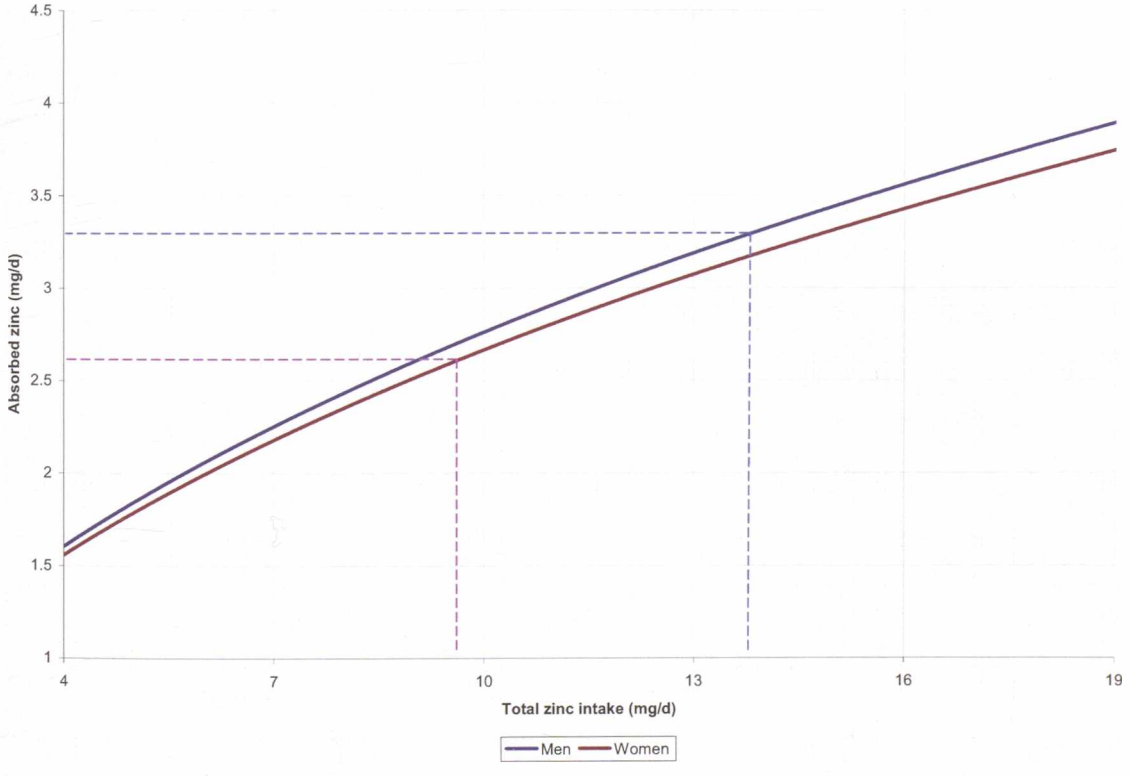


Figure 7.7 Derivation of the estimated average zinc absorption for UK adult men and women.

The curves are drawn for the median phytate: zinc molar ratio of UK adult men (8.82) and women (10.28).

The percentage of zinc absorption at this level of intake is considered the 'estimated average absorption of zinc' (also called critical level of zinc absorption [16] or estimated proportion of zinc absorption). The estimated average absorption of zinc for UK adult men was calculated as 3.29 mg zinc/day (physiologic zinc requirement) ÷ 13.80 mg/day (dietary zinc requirement) × 100 ≈ 24%. Following

this example, the estimated average absorption of zinc for UK adult women was approximately 27% (2.61 mg zinc/day ÷ 9.63 mg zinc/day × 100 ≈ 27%).

Estimated average zinc absorption for several gender and age groups of the UK population were calculated and presented in Table 7.2. The average level of zinc absorption was estimated for all age/gender groups who had significantly different dietary phytate: zinc molar ratios compared with the other groups of the population (chapter 5). The model was not used for children aged less than 10 years, as the usual dietary zinc intake of these children is much lower than for adults, and the estimates could be inaccurate [16].

For boys and girls aged 11 to 14 years, the estimated average zinc absorption was calculated to be 32% (Table 7.2). This level of zinc absorption is considered valid until further data, particularly an algorithm exclusively derived for children, become available. Nevertheless, this level of zinc absorption was close to the value suggested by IZiNCG (31%) and the value applied by the FNB/IOM committee (30%).

Because young people aged 15-18 years, had lower phytate: zinc molar ratios compared with the other young people age groups (Chapter 5), the estimated average zinc absorption was calculated for them separately. Among adults, the youngest age group had phytate: zinc molar ratios significantly lower than the other adult age groups; although, the calculated proportion of zinc absorption was only slightly different to the values calculated for older adults (Table 7.2).

Age	gender	Estimated physiologic requirement of zinc (mg/day)	Phytate: zinc molar ratio	Estimated dietary requirement of zinc (mg/day)	Estimated average absorption of zinc (%)
11-14 yrs	Males	2.10	10.48	6.62	32
	Females				
15-18 yrs	Males	2.87	9.34	10.99	26
	Females	2.33	10.19	7.84	30
19-24 yrs	Males	3.29	8.21	13.36	25
	Females	2.61	9.28	9.23	28
25-64 yrs	Males	3.29	8.82	13.80	24
	Females	2.61	10.28	9.63	27
65 yrs and over	Males	2.85	8.70	10.53	27
	Females	2.39		7.71	31

Table 7.2 Estimated average absorption of zinc for the UK population

None of the expert committees has proposed a different level of zinc absorption for the elderly population. Some expert committees assumed that there is no evidence suggesting that aging affects zinc absorption negatively [16, 237].

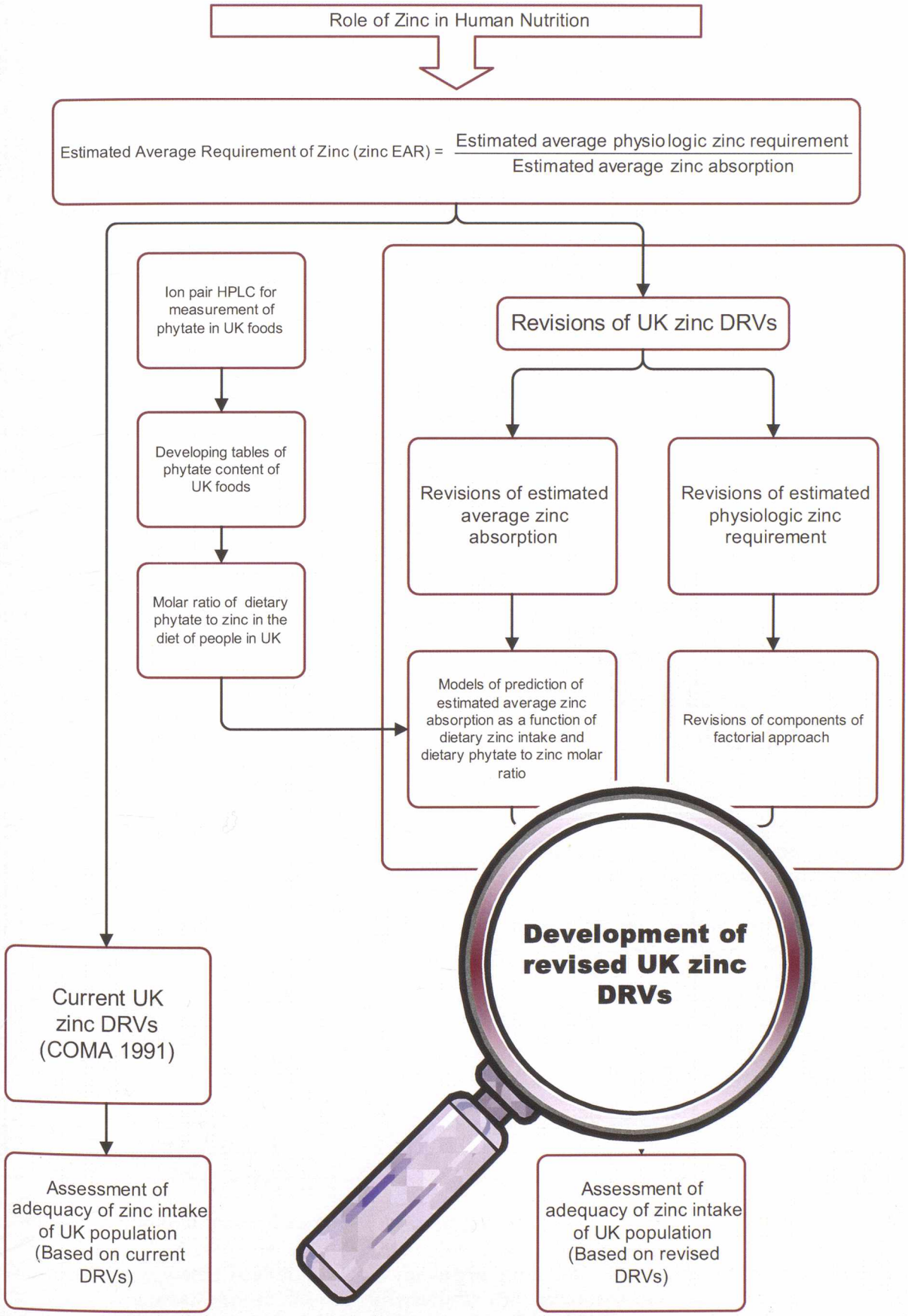
Nevertheless, because the UK elderly population have lower phytate intake (Chapter 5), the level of zinc absorption for the elderly population is calculated and presented separately.

7.4 Conclusion

In conclusion, although the study provided more evidence to support the relative validity of the mathematical model, in practice, the model is unable to predict a critical level of zinc absorption for the population. An assessment of the various criteria of the validity including the goodness of fit, generalisation and diagnostics examinations of the model's accuracy, evaluations of the parameters estimates, and comparison of the predicted values of TAZ to another model confirmed that the model is useful (but not yet applicable) for its intended predictive purposes. At the same time, further investigations are required to provide better estimates of the parameters by fitting the model to the new sets of data.

This chapter used the logit regression model, suggested by the IZiNCG, together with estimates of phytate: zinc molar ratio of the UK population and provided and estimate of the average absorption of zinc for subgroups of UK population.

Chapter 8: Development of the Revised Zinc DRVs and Assessment of the Zinc Adequacy in the UK Using the Revised Zinc DRVs



The magnifier symbol shows where you are in the conceptual framework of the thesis.

The aim of this chapter is to develop the revised zinc DRVs and then use them for the assessment of zinc adequacy in the UK population. This chapter consists of two sections: The first section discusses the development of the revised UK zinc DRVs including derivation of the revised zinc EAR, RNI and LRNI for the UK population and comparing them with the other available DRVs. The second section uses the revised zinc DRVs for the assessment of zinc adequacy in the UK population.

8.1 Development of the revised zinc DRVs

8.1.1 Derivation of the estimated average requirement of zinc for the UK population

The necessary compartments of zinc EAR have been discussed in the previous chapters: Chapter 4 provided an estimate of the average physiologic zinc requirement and chapter 7 provided an estimate of the average proportion of zinc absorption.

The revised EARs were derived by dividing the estimated average physiologic zinc requirement by the estimated average absorption of zinc (Equation 4.1). For instance, the revised EAR for UK adult women consuming normal mixed diets would be calculated as 2.61 mg absorbed zinc/day ÷ 0.28 = 9.32 mg zinc/day. The revised EAR, for all gender and age groups of the UK population is given in Table 8.1.

Age and gender	Estimated physiologic zinc requirement (mg/day)	Estimated average zinc absorption (%)	Estimated average zinc requirement (mg/day)
1-3 years (M+F)	0.64	32	2
4-6 years (M+F)	0.90	32	2.81
7-10 years (M+F)	1.38	32	4.31
11-14 years (M+F)	2.10	32	6.56
15-18 years (M)	2.87	26	11.03
15-18 years (F)	2.33	30	7.76
19-64 years (M)	3.29	25	13.16
19-64 years (F)	2.61	28	9.32
65 years & over (M)	2.85	27	10.63
65 years & over (F)	2.39	31	7.71

Table 8.1 Revision of EAR of zinc for the UK population

The values suggested in this table are for mixed diets as major dietary type consumed in the UK. The current report did not differentiate the estimated average zinc absorption of adults aged 19 to 24 years because the calculated percentages (25% and 28% for men and women respectively) were close to the percentages calculated for other adults aged 25 to 64 years (24% and 27% for men and women respectively). Thus, the estimated average zinc absorption of adults were assumed at 25% for men and 28% for women overall.

8.1.2 This investigation's zinc EAR compared with other expert committees

Table 8.2 compares the estimation of the average requirement of zinc as developed by expert committees of the COMA, WHO, US FNB/IOM and IZiNCG with the current investigation.

The current investigation proposes a more robust estimate of the zinc requirement for the UK population compared with other expert committees because it:

1. Reviewed a larger number and more recent studies to estimate a physiologic zinc requirement.
2. Used a model based on the presence of both zinc and phytate.
3. Is based on the most recent estimates of the factorial model of estimating the physiologic requirement and logit regression model of zinc absorption.
4. Included the current estimates of dietary zinc and phytate intakes of different age/gender groups of the UK population.

For children aged less than 14 years, these revised UK EARs are lower than the current UK EARs [217], mostly because of lower estimates of the physiologic zinc requirement. For example, COMA suggested faecal zinc excretion of endogenous zinc for children to be 0.077 mg/kg/day, while more recent data indicated this to be much lower - in the region of 0.020-0.034 mg/kg/day [16, 237]. COMA also overestimated the non-intestinal zinc excretion and levels of zinc requirement for growth of children, and therefore, the 1991 EARs are markedly higher than values suggested by other committees. For children aged 1 to 14 years, the revised UK EARs are similar to the values suggested by the IZiNCG and FNB/IOM committees.

The revised EARs for adults are higher than the values suggested by COMA and higher than the other expert committees. While COMA assumed a 30% absorption efficacy for all age groups, the current investigation indicated that different UK age/gender groups have a differing level of dietary zinc and phytate intakes, and therefore, different levels of zinc absorption. The current investigation indicated that the level of absorption for adults aged 19 to 64 years is less than 30%, and this gives a higher EAR value.

COMA assumed total endogenous zinc losses were in the order of 2.2 and 1.6 mg/day for UK men and women respectively. However, more recent studies indicate that the total endogenous zinc excretion to be much higher (i.e. 3.29 and

2.61 mg/day for UK men and women respectively). Thus, a lower zinc absorption together with high zinc losses gives a higher EAR for this age group.

These revised UK EARs, are the only currently available estimates of the average zinc requirements that have used an algorithm of zinc absorption as a function of dietary zinc and phytate intake together with a real set of data on dietary zinc and phytate intake of the population to predict the level of zinc absorption.

The FNB/IOM acknowledged that there is a need for an algorithm to predict zinc absorption [237] but at that time (2002), the model suggested by the IZiNCG was not available, so the FNB/IOM used an asymptotic regression of absorbed zinc on zinc intake to predict the proportion of zinc absorption. This asymptotic regression model was derived from seven studies [263, 272, 274, 279-281, 287]. Because the inhibiting role of phytate on zinc absorption was not included in this model, it overestimated the proportion of zinc absorption. Reviews of the phytate adjusted dietary reference intakes are scheduled to be discussed in the 2008 spring and summer meetings of the FNB/IOM expert committee [406].

The estimated physiologic zinc requirement of adults suggested in the current investigation is lower than the values suggested by the FNB/IOM; however, as the estimate of percentage of zinc absorption used in the present report (25-28%) is lower than the estimate made by the FNB/IOM committee (41-48%), the revised UK EARs are higher than the values suggested by the FNB/IOM committee.

The expert committees of the WHO and IZiNCG did include phytate in their estimate of the level of zinc absorption; however, both these committees aimed to provide general advice for global use. Therefore, the suggested EARs did not take anthropometric and nutritional status of the different populations into account.

The WHO committee divided the range of phytate: zinc molar ratios of global diets into three categories, considered to represent diets of relatively high (R_{pz}<5), moderate (R_{pz}=5-15) and low (R_{pz}>15) absorption levels [236]. The IZiNCG committee divided the available data into two categories, based on phytate: zinc molar ratios, where values of 4 to 18 represented mixed or refined vegetarian diets and values of 18 to 30 represented unrefined cereal based diets. The IZiNCG then used the midpoint of these ranges (i.e. phytate: zinc molar ratios of 11 and 24) to calculate the associated amounts of absorbed zinc [16].

The actual dietary phytate: zinc molar ratio of the UK population was estimated from the nationally representative surveys of the NDNS; therefore, there was no need for categories representing different diets.

These revised UK EARs suggested in the present report are more accurate and representative of the UK population. These values are generally higher than the EAR suggested by the IZiNCG because:

1. The estimated physiologic zinc requirement of the UK population is higher than the values suggested by IZiNCG.
2. There is a statistical error in the calculations of the estimated physiologic requirement conducted by the IZiNCG [407, 408].
3. The estimated average zinc absorption for UK men and women is lower than the values suggested by the IZiNCG.
4. The level of absorbed zinc for the UK population was estimated based on different levels of phytate: zinc molar ratios for several gender/age groups, while the IZiNCG estimated the level of zinc absorption based on a single value, thought to be representative of all diet types.

Age and gender	WHO 1996 EAR (mg/day)	Age and gender	FNB/IOM 2002 EAR (mg/day)	IZiNCG 2004 EAR (mg/day)	Age and gender	COMA 1991 EAR (mg/day)	Current revisions EAR (mg/day)
1-3 yrs	3.4	**1-3 yrs**	2.5	2	**1-3 yrs**	3.8	2
3-6 yrs	3.9	**4-8 yrs**	4	3	**4-6 yrs**	5	2.81
6-10 yrs	4.6				**7-10 yrs**	5.4	4.31
10-12 yrs, M	5.7	**9-13 yrs**	7	5	**11-14 yrs**	7.0	6.56
10-12 yrs, F	5.1	**14-18 yrs, M**	8.5	8	**15-18 yrs, M**	7.3	11.03
12-15 yrs, M	7.4	**14-18 yrs, F**	7.3	7	**15-18 yrs, F**	5.5	7.76
12-15 yrs, F	6.3	**19^{+} yrs, M**	9.4	10	**19-64 yrs, M**	7.3	13.16
15-18 yrs, M	8.1	**19^{+} yrs, F**	6.8	6	**19-64 yrs, F**	5.5	9.32
15-18 yrs, F	6.3				**65^{+} yrs, M**	7.3	10.63
18-60^{+}yrs, M	5.7				**65^{+} yrs, F**	5.5	7.71
18-60^{+}yrs, F	4.0						

Table 8.2 EAR of zinc, as developed by expert committees of the COMA [217], WHO [236], FNB/IOM [237], IZiNCG [16] and as reviewed by the current investigation for the UK population.

8.1.3 Derivation of zinc Dietary Reference Values for the UK population

The calculation of the true zinc requirement of a particular individual is not possible. There is also insufficient information to accurately calculate the distribution of zinc requirement in a group of individuals. However, if we assume that the zinc requirement is normally distributed, the suggested level of zinc intake can be set at two notional standard deviations (2SD) above the zinc EAR. Theoretically, based on this approach, almost all individuals whose zinc intake meet or exceed this amount will meet their physiologic requirements and indeed contribute to optimum zinc status.

In COMA 1991, this guidline level of zinc intake was defined as the zinc Reference Nutrient Intake (RNI) [217] while the Canadian/American RDA (Recommended Daily Allowance) is conceptually equivalent [409].

The notion of multiple requirement levels was also introduced by COMA. The panel of COMA defined the Lower Reference Nutrient Intake (LRNI) as a point in the distribution that is two standard deviations below the EAR. The LRNI represents the lowest intakes which will meet the requirement of bottom 2.5% of the population and intake below this point is almost certainly inadequate for most individuals [217].

The FNB/IOM committee adopted a different approach and decided that for all nutrients, for which additional information on the distribution and standard deviation of the requirement was not available, the requirements were thought to be different by ± 10% (CV = 10%) in the population. The RDA was therefore set as 120% of the EAR.

The WHO and COMA did not report on how the variability in the zinc requirement was estimated. The IZiNCG estimated that the coefficient of variation of the zinc requirement is 12.5% and therefore the RDA for dietary zinc was calculated as EAR+(12.5%×2) resulting in the RDA being set at 125% of the EAR. The IZiNCG noted that there would be a negligible difference in the resulting RDAs, when the coefficient of variation of the physiologic requirement is set at 10% versus 12.5% [16].

The current investigation concluded that an estimate of ± 10% for the variability in the zinc requirement is more appropriate and this figure is adopted for the present report (Table 8.3). Nevertheless, the actual value of the coefficient of variation of

zinc requirement is unknown and a selection of the number remains an unsolved problem in development of the RDA or RNI [219].

Age	Gender	LRNI (mg/day)	EAR (mg/day)	RNI (mg/day)
1-3 years	M+F	1.6	2.0	2.4
4-6 years	M+F	2.2	2.8	3.4
7-10 years	M+F	3.4	4.3	5.2
11-14 years	M+F	5.2	6.6	7.9
15-18 years	M	8.8	11.0	13.2
15-18 years	F	6.2	7.8	9.3
19-64 years	M	10.5	13.2	15.8
19-64 years	F	7.5	9.3	11.2
65^{+} years	M	8.5	10.6	12.8
65^{+} years	F	6.2	7.7	9.3

Table 8.3 Revised Dietary Reference Values (DRVs) for zinc, by age and gender, as suggested by the current investigation.

Table 8.4 shows the dietary reference values of zinc, as suggested by the expert committees of the COMA, WHO, FNB/IOM and IZiNCG, compared with the values suggested by the current investigation. The differences are mostly due to different calculated estimated average requirements (discussed in section 8.1.2) and/or the different estimates of the coefficient of variation of zinc requirement.

There are also different approaches in rounding and presenting decimal numbers. In the approach used by the IZiNCG, the EAR and RDA were calculated and rounded to the nearest 1 mg. In the present report, the DRVs are presented with one decimal point accuracy, as in the report of the WHO and COMA [217, 236].

For example, in the IZiNCG report [16], the published EAR for girls aged 14-18 years, consuming mixed or refined vegetarian diets is 7 mg/day. However, based on the calculations by the IZiNCG, this EAR should be calculated as 1.98 mg/day (physiologic zinc requirement) ÷ 0.31 (proportion of zinc absorption) ≈ 6.39 mg/day or 6.4 mg/day to 1 decimal point.

The corresponding RDA was calculated as 7 × 125% = 8.75, rounded to 9 mg/day as opposed to being calculated as 6.39 × 125% ≈ 7.99 mg/day, rounded to 8 mg/day.

8.2 Assessment of zinc adequacy in the UK using the revised zinc DRVs

8.2.1 Introduction

Zinc deficiency can occur due to at least five general causes occurring either in isolation or in combination. These include inadequate intake, increased requirements, malabsorption, increased losses and impaired utilization [23].

Risk of zinc deficiency can be assessed using the following techniques:

1. Assessment of the adequacy of dietary zinc intake in comparison to requirements for absorbed zinc.
2. Evaluation of biochemical measures of zinc status including zinc concentration and/or activity of zinc dependent-enzymes in relation to reference values or established cut-off points.
3. Measurement of the prevalence or presence of the clinical outcomes of zinc deficiency (e.g. stunting, diarrhoea).
4. Assessment of the functional responses following zinc supplementation [16].

This section examines if the Dietary Reference Values of zinc suggested in the previous chapter, can be used as a benchmark for the assessment of dietary adequacy of zinc in the UK.

8.2.1.1 Remit of the investigation

The panel on Dietary Reference Values of the Committee On Medical Aspects of Food Policy (COMA) reviewed the previous Recommended Daily Intakes (RDI, published in 1969) [410] and Recommended Daily Amounts (RDA, published in 1979) [411]. In 1991, the panel produced its guidelines as Dietary Reference Value (DRVs) [217].

Since 1991, two major government reports about zinc requirements have been published. The first provided a review on the role of zinc in nutrition. This report included two tables demonstrating UK zinc intake and recommended daily zinc requirements in the UK, US and Norway [266]. The second review provided a revision on safe upper levels for vitamin and minerals (including zinc) and focused on toxicity of nutrients [412]. None of these reports revised the UK zinc DRVs.

One of the uses of the DRVs is in the evaluation of the risk of inadequate intake by determining the proportion of the population whose intake falls below the DRVs (particularly below the LRNI). Using the revised zinc DRVs, we can examine how the picture of the UK zinc status would differ from the current understanding (discussed in Chapter 3).

8.2.1.2 Primary aspects about the objectives and limitations of the study

There are several points that must be taken into account prior to the assessment of the UK zinc status using the revised UK zinc DRVs.

1. The National Diet and Nutrition Surveys are cross-sectional studies. The primary purpose of surveys of this kind are to establish baseline nutritional intake data, assess the overall nutritional status of the population and to identify and describe those population subgroups 'at risk' to chronic malnutrition. These surveys are unable to detect an acute malnutrition or provide information on possible causes of malnutrition, because they measure both exposure and outcome at the same point in time [206, 413].

Therefore, information about the extent of inadequate dietary zinc intake can be used to allocate resources to vulnerable population subgroups, to suggest the direction for further research and to formulate policies to improve nutritional zinc status; but this information by its nature does not identify zinc deficiency and/or discover possible causes of zinc deficiency.

2. Dietary nutrient intake is highly correlated and the attribution of causation to one nutrient considered to be acting on its own is misleading [413]. This is another methodological limitation that avoids drawing any conclusion to a zinc deficiency endpoint or its causes and consequences.

Isolated zinc deficiency is rare and zinc deficiency often occurs in association with a variety of other health conditions. For example, zinc deficiency can be associated with iron deficiency [414, 415]. It is known that both the distribution of iron and zinc in the food supply and the dietary factors that modify their absorption, are similar; thus, there might be a comparable risk for their deficiencies [16]; therefore, the intervention strategy must include other nutrients and correct factors predisposing to zinc deficiency and its consequences.

3. Groups at risk of zinc deficiency can be categorised based on physiologic status (e.g. age, gender, reproductive cycle, presence of illness), political or geographical status (e.g. region, district, urban versus rural) and socioeconomic status (e.g. income, employment status of head of household, level of education) [16]. In this study, risk groups were defined by gender and age alone. Further research is required to identify vulnerable groups in terms of their geopolitical and socioeconomic status.

4. In this study, the prevalence of 'inadequate zinc intake' is estimated by comparison with the suggested theoretical values of zinc requirement. However, zinc intake data alone cannot provide a proof for the existence of clinical zinc deficiency. The suggestion of a high risk of zinc deficiency must be based on a combination of dietary, biochemical, anthropometric and clinical findings [416].

5. Assessment of the nutritional status of the population is different from that of individuals. The nutritional evaluations of individuals leads to treatment of the case, though nutritional assessment of the population aims to plan and evaluate population based interventions. Therefore, this is not critical for the population assessment methods to provide certainty regarding any particular individual's true status. This is a central aspect in the epidemiological assessment of zinc adequacy because although current method may misclassify some individuals, it can be useful for detecting a population at risk of zinc deficiency [16].

8.2.2 Methodology

8.2.2.1 Methodology of National Diet and Nutrition Surveys

The details of the dietary, anthropometry and blood sampling methodologies and procedures used in the National Diet and Nutrition Surveys are described in the survey reports [213, 222-228].

8.2.2.2 Statistical analysis

The results of the descriptive statistical analysis are presented in Chapter 3. The analysis excluded all participants who failed to complete their four- or seven-day period of the weighed dietary record. Where necessary, variables were weighted to equalise the sample sizes or new variables were computed or recoded. For the

NDNS young people aged 4 to 18 years, the results are unweighted, because there was no weighting variable available at the time of the analysis.

Subjects with low zinc intake (i.e. below the revised LRNI) were flagged. Then a series of independent sample t-tests were performed to explore if nutrition and health indices of this group is different from the other participants.

8.2.3 Results

8.2.3.1 NDNS: children aged 1½ to 4½ years

The mean daily zinc intake from food sources was more than the revised UK RNI for all age groups of both boys and girls. The average daily dietary intake of zinc was 179.9% of the revised RNI for boys and 171.9% of the revised RNI for girls.

Overall, 0.5% of boys and 1% of girls had an intake below the revised UK LRNIs. The highest percentage of children with intake below the revised LRNI was among girls aged 1½ to 2½ years (Table 8.5).

Age group	1½ - 2½ years	2½ - 3½ years	3½ - 4½ years	All
Gender	Boys			
Average zinc intake (mg/day) from food sources	4.40	4.43	4.66	4.49
Base (N)	298	300	250	848
Revised Zinc RNI (mg/day)	2.4	2.4	2.4 | 3.4	NA
Zinc Intake (as % revised RNI)	183.5	184.7	170.0	179.9
Revised Zinc LRNI (mg/day)	1.6	1.6	1.6 | 2.2	NA
Percentage of people with intake below revised LRNI	0.0	0.3	1.2	0.5
Base (N)	0	1	3	4
Gender	Girls			
Average zinc intake (mg/day) from food sources	4.18	4.32	4.45	4.31
Base (N)	278	306	243	827
Revised Zinc RNI (mg/day)	2.4	2.4	2.4 | 3.4	NA
Zinc Intake (as % revised RNI)	174.0	180.0	159.2	171.9
Revised Zinc LRNI (mg/day)	1.6	1.6	1.6 | 2.2	NA
Percentage of people with intake below revised LRNI	1.4	0.7	0.8	1.0
Base (N)	4	2	2	8

Table 8.5 Average daily zinc intake of children aged 1½ to 4½ years compared with the revised RNI and LRNI.

The table demonstrates two indices of zinc adequacy: 1) average daily zinc intake as a percentage of the revised RNI, and 2) percentage of the population with a dietary zinc intake below the revised LRNI.

8.2.3.2 NDNS: young people aged 4 to 18 years

The average daily intake of zinc from food sources was less than the revised RNI for boys and girls aged over 10 years. The mean daily dietary zinc intake was 109.9% of the revised RNI for boys and 96.4% of the revised RNI for girls.

When the intake of zinc from food sources was compared with the revised LRNI overall, 16.2% of boys and 23.3% of girls had an intake below this cut-off point. Among subgroups, young people aged 15 to 18 years were the highest

percentage of the population with an intake below the revised LRNI including 56.4% of boys and 51.9% of girls with zinc intake below the revised LRNI (Table 8.6).

Age group	4 - 6 years	7-10 years	11-14 years	15-18 years	All
Gender	Males				
Average zinc intake (mg/day) from food sources	5.59	6.13	7.22	8.80	6.88
Base (N)	184	256	237	179	856
Zinc RNI (mg/day)	3.4	5.2	7.9	13.2	NA
Zinc Intake (as % revised RNI)	164.5	117.9	91.4	66.7	109.9
Zinc LRNI (mg/day)	2.2	3.4	5.2	8.8	NA
Percentage of people with intake below revised LRNI	1.1	2.3	12.7	56.4	16.2
Base (N)	2	6	30	101	139
Gender	Females				
Average zinc intake (mg/day) from food sources	4.89	5.73	6.01	6.14	5.74
Base (N)	172	225	238	210	845
Zinc RNI (mg/day)	3.4	5.2	7.9	9.3	NA
Zinc Intake (as % revised RNI)	143.7	110.1	76.1	66.0	96.4
Zinc LRNI (mg/day)	2.2	3.4	5.2	6.2	NA
Percentage of people with intake below revised LRNI	1.2	2.7	33.6	51.9	23.3
Base (N)	2	6	80	109	197

Table 8.6 Average daily zinc intake of young people aged 4 to 18 years compared with the revised RNI and LRNI.

8.2.3.3 NDNS: adults aged 19 to 64 years

For adults, the mean daily zinc intake from food sources was less than the revised RNI. The mean daily dietary zinc was 64.6% of the revised RNI for men and 65.8% of the revised RNI for women (Table 8.7).

58.9% of the male population and 54.7% of the female population had dietary zinc intake below the revised UK LRNI. Among different age groups, the highest percentage below the LRNI was among the youngest age group for both men and women (71.1% and 71.0% respectively).

Age group	19-24 years	25-34 years	35-49 years	50-64 years	All
Gender	Males				
Average zinc intake (mg/day) from food sources	8.99	10.25	10.56	10.35	10.21
Base (N)	108	219	253	253	833
Zinc RNI (mg/day)	15.8				
Zinc Intake (as % revised RNI)	56.9	64.9	66.9	65.5	64.6
Zinc LRNI (mg/day)	10.5				
Percentage of people with intake below revised LRNI	71.1	62.9	53.0	56.6	58.9
Base (N)	77	138	134	143	491
Gender	Females				
Average zinc intake (mg/day) from food sources	6.85	6.73	7.59	7.84	7.37
Base (N)	104	210	318	259	891
Zinc RNI (mg/day)	11.2				
Zinc Intake (as % revised RNI)	61.1	60.1	67.8	70.0	65.8
Zinc LRNI (mg/day)	7.5				
Percentage of people with intake below revised LRNI	71.0	68.4	46.3	47.2	54.7
Base (N)	74	144	147	122	487

Table 8.7 Average daily zinc intake of adults aged 19 to 64 years compared with the revised RNI and LRNI.

8.2.3.4 NDNS: People aged 65 years and over

The average dietary intakes of zinc were 68.2% of the revised RNI for men and 74.4% of the revised RNI for women. The mean daily intake of zinc from food sources was less than the revised RNI for all age groups of both men and women.

Overall, 50.2% of men and 40.7% of women had zinc intake below the revised LRNI. Elderly individuals and particularly men aged 85 years and over, seemed to be at increased risk of inadequate zinc intake, as 57.7% of men and 46.2% of women aged 85 years and over had a dietary zinc intake below the revised LRNI (Table 8.8).

Age group	65-74 years	75-84 years	85 years and over	All
Gender	Males			
Average zinc intake (mg/day) from food sources	9.02	8.36	8.20	8.73
Base (N)	371	200	62	633
Revised Zinc RNI (mg/day)	12.8			
Zinc Intake (as % revised RNI)	70.5	65.3	64.1	68.2
Revised Zinc LRNI (mg/day)	8.5			
Percentage of people with intake below revised LRNI	46.1	55.5	57.7	50.2
Base (N)	171	111	36	318
Gender	Females			
Average zinc intake (mg/day) from food sources	7.09	6.92	6.62	6.92
Base (N)	434	368	251	1054
Revised Zinc RNI (mg/day)	9.3			
Zinc Intake (as % revised RNI)	76.2	74.4	71.2	74.4
Revised Zinc LRNI (mg/day)	6.2			
Percentage of people with intake below revised LRNI	35.9	42.6	46.2	40.7
Base (N)	156	157	116	429

Table 8.8 Average daily zinc intake of adults aged 65 years and over compared with the revised RNI and LRNI.

8.2.4 Discussion

8.2.4.1 Limitation of use of revised DRVs for children aged 1 to 4 years

For children, the revised zinc DRVs are considerably lower than the current UK DRVs. Therefore, when the zinc intake of children aged 1½ to 4½ years was compared to the revised DRVs, a smaller proportion of the population was thought to be at risk. For children, the revised zinc DRVs and the detected vulnerable groups based on them are subject to limited validity for the reasons set below:

1. In the absence of better information in children, estimates of physiological zinc requirements are based on the extrapolation from the adults per kg body weight. However, estimates of the physiological zinc requirements in adults are derived from several studies conducted on subjects with different weights. The model that estimated endogenous and total zinc excretion in relation to zinc absorption (Chapter 4) was not adjusted for weight.
2. A recent investigation (2007) indicated that the estimates of total zinc requirement for absorbed zinc of children 1 to 4 years of age is higher than the estimates based on the extrapolation from the adults per kg body weight used by the expert committees (i.e. the FNB/IOM and IZiNCG) and this report [240].
3. The estimated fractional zinc absorption from the adults diet is dependent on total zinc intake [16]. In children, it is unclear whether such a relationship exists as the current information is not conclusive. For instance, de Ramona *et al* (2005) showed that in a study of stunted 3-4 year old Peruvian children, the fractional zinc absorption was negatively related to zinc intake [417]. Alternatively, Griffin *et al* (2007) studied US children aged 1 to 4 years consuming a similar range of zinc intake as the study of de Ramona *et al* and found no significant relationship between zinc intake and fractional absorption [240]. These contrasting results have also been found in previous studies [306, 418].

Bioavailability from the diets, length of the adaptation period and age and health status of subjects in those studies is different. Because of this, deriving a conclusion is difficult.

4. If the distribution of zinc intake of children is identical to that of their requirements, in theory there is still 2.5% of the population with zinc intake

below the revised LRNI demonstrating them at risk of inadequate zinc intake. However, comparing the intake to the revised LRNI, a very small proportion of the population (0.5-1%) had zinc intake below the revised LRNI. This small proportion of the population with an inadequate zinc intake was much lower than that seen among other age groups. One possible explanation is that the revised zinc DRVs of children underestimates their zinc requirements.

8.2.4.2 Adequacy of zinc intake

The average daily zinc intake was compared with the revised RNI, which provided an initial index of zinc adequacy. For boys and girls aged 4 to 6 years, the average intake was above the revised RNI; therefore, the risk of zinc inadequacy in these groups was considered small. Alternatively, for boys and girls aged over 10 years, the average daily zinc intake was less than the revised RNI; thus, a higher percentage of these children were categorised in the group with a zinc intake below their requirements.

The LRNI is a theoretical measure whereby an intake below this level is unlikely to be adequate for 97.5% of the population. The population who had zinc intake below the revised LRNI in this report are regarded as 'at risk' or 'vulnerable' to inadequate zinc intake.

For children aged 4 to 10 years, the revised LRNI is lower than the current UK LRNI; therefore, a smaller proportion of the population had zinc intake below this cut-off points. For children aged 11 to 14 years, the revised LRNI was very close to the current LRNI, so the population with an inadequate zinc intake did not differ considerably from what was detected by comparing to the current LRNI. Only for young people aged 15 to 18 years was the revised LRNI notably higher than the current values. Only a small percentage of the population could meet this measure (Table 8.6 and Figure 8.1).

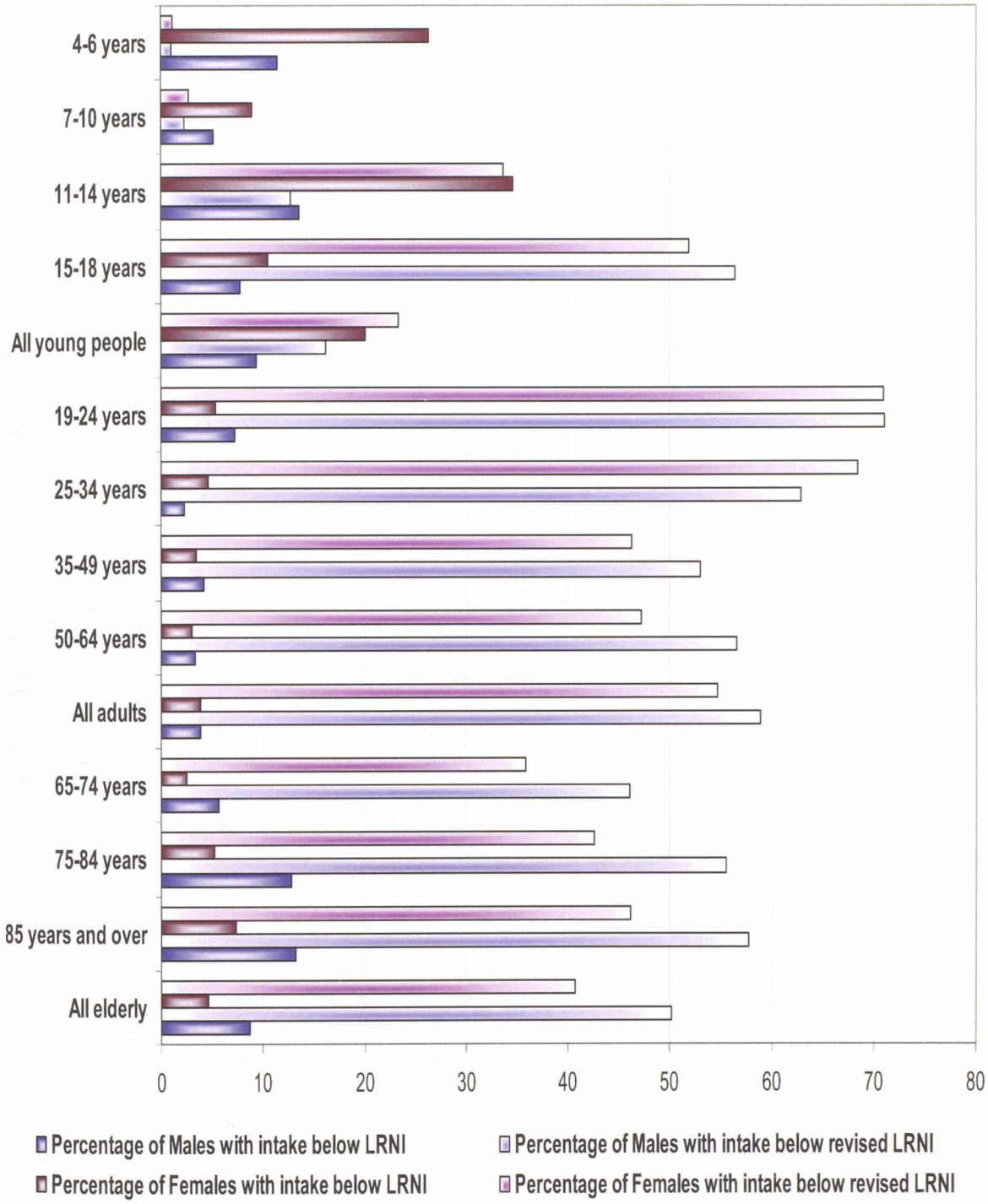


Figure 8.1 Percentage of young, adult and elderly males and females who had zinc intake below the revised LRNI compared with those who had zinc intake below the current LRNI.

Factors that limit the validity of the revised zinc DRVs in children had a lower impact on the revised zinc DRVs of young people. For young people, normal intake, body weight and dietary requirements are closer to adults. Unlike children, there is no direct investigation to question the estimate of zinc requirements for absorbed zinc. For older age groups, the model used for adults was successfully used and that confirmed the assumption that the level of zinc absorption in young people is close to that of adults (Chapter 7).

For all adults, the average zinc intake was below the revised RNI. As a result, the risk of zinc inadequacy for adults was substantial. The revised LRNI of adults is notably higher than the current UK LRNI; therefore, a smaller percentage of the population could meet this criterion (Table 8.7 and Figure 8.1).

For all age/gender groups of the elderly population, the average zinc intake was below the revised RNI; therefore, the risk of zinc inadequacy in these groups was large. The revised LRNI is also higher than the current UK LRNI; therefore, a larger proportion of population had zinc intake below this cut-off point (Table 8.8 and Figure 8.1).

8.2.4.3 Health and nutritional status of vulnerable groups

For groups at risk of inadequate zinc intake, certain health or nutritional consequences may be associated with zinc inadequacy. However, mild zinc inadequacy, of the type identified here, may not have obvious clinical symptoms. Potential asssociated consequences are considered in the following section.

8.2.4.3.1 Anthropometry

Marginal zinc status can affect pattern of growth and energy cost of tissue deposition. Lean body tissue is rich in zinc and proteins whereas, adipose tissue has a relatively less amount of zinc and a high amount of stored energy. In zinc deficiency, there is a tendency to partition dietary energy into fat stores instead of skeletal muscle, maybe because of the critical role of zinc in protein synthesis [419-424] and the higher energy cost of muscle tissue deposition compared with fat tissue [11, 425, 426]. A similar trend was found in growing animals with experimental inadequate dietary zinc intake when a reduction in the synthesis of lean body mass compared with adipose tissue occurred [427, 428].

The relationship between indices of zinc status and anthropometry has been the subject of several investigations:

Gibson *et al* (2000) reported that in young people aged 11 years in New Zealand, those with a low hair zinc concentration had a higher body fat percentage and BMI compared to peers with higher hair zinc groups [429]. In that study, the dietary data was not available. In another study, Cavan *et al* (1993) observed that Guatemalan children aged 6 to 7 years with low hair zinc levels were heavier and fatter than their peers with superior zinc status [426].

Several zinc supplementation studies have confirmed that in growing young people, body composition, indirectly assessed by anthropometry, can be influenced by zinc deficiency. For instance, in malnourished children in the Gambia [430] and in underprivileged boys in Chile [431], zinc supplementation resulted in an increase to the mid upper arm circumference or arm muscle area. In an earlier study, in severely malnourished Jamaican children, zinc supplementation generated a higher rate of lean tissue gain [425].

At the same time, in the study of Guatemalan children, zinc supplementation resulted in an increase in body fat rather than muscle tissue, possibly because of the stimulating effect of zinc supplementation on appetite resulting in an increase in energy intake [426].

In the current study, it was postulated that young people, with marginal zinc intake would have higher indices of body fat and anthropometry. Young people who had zinc intake below the revised LRNI, had a significantly higher weight, height, BMI and mid upper arm circumference (Appendix C).

The availability of serum ALP, plasma zinc concentration along with dietary data was a particular advantage of the NDNS data compared to the data analysed in previous studies. It was found that young people with inadequate zinc intake also had higher intake of rice, potato chips, spirits, beer (oldest age group), sugar, tea and coffee and a lower consumption of zinc food sources such as beef and veal dishes, high fibre and whole grain breakfast cereals and wholemeal breads (Appendix C).

It was postulated that there would be an inverse relationship between the indices of zinc status and lean body mass. Cavan *et al* (1993) have shown that in young people with suboptimal zinc status, as indicated by low plasma ALP activity, there is a shift toward fat tissue deposition in place of muscle [426]. In the current study, plasma ALP showed a significant inverse relationship with mid upper arm circumference ($r=-0.371$, $p<0.001$), weight ($r=-0.439$, $p<0.001$) and BMI ($r=-0.373$, $p<0.001$). Plasma zinc showed a much weaker, but still significant inverse relationship with weight ($r=-0.079$, $p<0.05$) and height ($r=-0.082$, $p<0.05$).

Overall, there was no evidence of impaired linear growth, which is a classical feature of frank zinc deficiency. Young people with inadequate zinc intake were taller than those with an adequate zinc intake. This agrees with the finding of Gibson *et al* (2000), who noted that young people with suboptimal zinc status (low

hair zinc concentration) were taller than their counterparts with normal zinc status [429].

This finding is the opposite of the general hypothesis expecting a high prevalence of stunting as a clinical manifestation of zinc deficiency in children who had inadequate zinc intake. The author could not think of any possible explanation for this finding; however, because of the cross-sectional nature of the NDND that measures both exposure and outcome at the same point in time, establishing a cause and effect relationship was not possible. Furthermore, it is not clear if this finding was not confounded by the socio-economic condition and/or background nutritional status of the young people with inadequate zinc intake since these factors were not controlled between the groups.

The association of higher indices of body fat with poorer zinc status was not found for adult and elderly populations. Adults who had a zinc intake below the revised LRNI, had significantly lower weight, height, BMI, waist and hip circumferences and waist-to-hip ratio. In the elderly population, none of the indices of anthropometry (except for hip circumference and mid upper arm circumference) was significantly different for subjects who had zinc intake below or above the revised LRNI (Appendix C).

Other human studies in adults have reported finding no relationship between zinc status and anthropometric indices of body fatness. Hashim *et al* (1996) reported that among healthy adult men and women, there was no relationship between plasma zinc levels and height or BMI [432]. Bales *et al* (2000) also reported that zinc status (as measured by salivary zinc concentration) was not significantly related to body weight [433].

There are three possible explanations as to why adults and elderly populations with inadequate zinc intake did not demonstrate higher indices of body fat as observed in younger people:

1. The mechanisms affected by inadequate zinc intake are mostly growth related. For example, alteration in muscle tissue deposition is expected to be more evident in growing young people compared with the adult and/or elderly populations as youngsters have a higher rate of protein synthesis and muscle tissue deposition.

Most evidence that supported the inverse relationship between zinc status and body fat are based on studies conducted in growing children or in growing experimental animals.

2. In young people, inadequate zinc intake was accompanied with a higher consumption of 'unhealthy' food and drinks such as potato chips, sugar and alcoholic drinks. In adults, this trend was weaker (Appendix C). This high consumption of high energy and low zinc foods may enforce the impact of marginal zinc status on body fat observed among young people.
3. The impact of marginal zinc status on body composition is not clear. Adults might have been more exposed to prolonged inadequate zinc intake, and show the classical manifestations of marginal zinc deficiency (i.e. compromised height, weight and anthropometric indices).

8.2.4.3.2 Blood pressure

There is increasing interest in a possible role of zinc in pathogenesis of cardiovascular diseases [434, 435] because many enzymes that are involved in the regulation of blood pressure (e.g. nitric oxide synthase (NOS), angiotensin-converting enzyme (ACE) and neutral endopeptidase) are zinc dependent [436]. It was postulated that people with inadequate zinc intake may have a higher blood pressure.

Mean systolic blood pressure and mean arterial pressure among young people with inadequate zinc intake (112.2 and 75.0 mmHg respectively) were higher than those who had adequate zinc intake (107.3 and 72.8 mmHg, $p<0.001$). Plasma ALP showed a weak, but significant inverse relationship with systolic ($r=-0.256$, $p<0.001$), diastolic ($r=-0.146$, $p<0.001$) and arterial blood pressure ($r=-0.226$, $p<0.001$). Zinc intake showed a weak positive relationship with systolic ($r=0.297$, $p<0.001$) and arterial blood pressure ($r=0.154$, $p<0.001$).

In adults, indices of blood pressure were not significantly different between those who had inadequate zinc intake compared with those with adequate zinc intake. However, mean systolic blood pressure, mean diastolic blood pressure and mean arterial pressure (152.8, 78.3 and 103.1 mmHg respectively) among elderly people who had inadequate zinc intake were higher than those who had adequate zinc intake (148.6, 76.8 and 100.7 mmHg respectively, $p<0.01$, $p<0.05$ and $p<0.01$ respectively).

In the elderly population, systolic blood pressure was inversely correlated with zinc intake ($r=-0.100$, $p<0.001$) and serum zinc concentration ($r=-0.118$, $p<0.001$). In this age group, diastolic blood pressure showed a weak but significant positive relationship with serum zinc concentration ($r=0.143$, $p<0.001$).

The kind of weak relationship as the ones reported here was expected. Correlation coefficients (and consequently coefficient of determination) give an account of the variation predicted by the relationship and zinc status only weakly (but with statistical reliability) accounts for the variation in blood pressure. This is expected considering that many more important factors (e.g. sodium intake) with higher correlations predict the variation in blood pressure.

The inverse relationship between indices of zinc status and blood pressure has been reported in some other studies:

Vivoli *et al* (1987) reported that both systolic and diastolic blood pressure were inversely correlated with the zinc dependent enzymes, alcohol dehydrogenase and ALP [437]. Bergomi *et al* (1997) also reported a negative relationship between blood pressure and serum zinc levels in hypertensive subjects [438].

Tomat *et al* (2005) demonstrated that in growing rats, moderate zinc deficiency resulted in increased blood pressure and oxidative stress and decreased NOS activity [436]. More recently, Tomat *et al* (2008) indicated that moderate zinc deficiency during foetal, lactation and post-weaning growth of rats, induced functional and morphological alterations such as a decrease in the glomerular filtration rate and a reduction in the number and size of nephrons, manifested by high blood pressure and renal dysfunction in adulthood [439].

There is no evidence to suggest that zinc deficiency alone can cause high blood pressure. Sato *et al* (2003) indicated that zinc deficiency was inadequate to alter blood pressure in normotensive rats. In that study, dietary zinc deficiency in the short term did not influence blood pressure and/or NOS activity. Long term zinc deficiency influenced blood pressure by affecting neuronal function, immunity, formation of blood cellular components and disturbance of systemic and/or local blood circulation [440].

Chiplonkar *et al* (2004) reported that inadequate dietary intake of vitamin C, folic acid and zinc were the risk factors of hypertension among lacto-vegetarians in India [326]. This finding agrees with the current results as in the current investigation, subjects who had inadequate zinc intake usually had higher indices

of blood pressure and always had lower indices of vitamin C and folic acid status (Appendix C).

A recent review conducted by Tubek (2007) has suggested that impaired zinc homeostasis is both a cause and effect of hypertension. Tubek explained that in hypertension, the reduced serum lymphocytes and bone zinc concentration and the increased heart, liver, kidney and erythrocytes zinc levels are evidence of impaired zinc homeostasis. Thus, altered urinary zinc excretion and intestinal zinc absorption observed in hypertension is a physiological response to maintain zinc homeostasis [441].

8.2.4.3.3 Blood analytes

The dietary intakes of nutrients are highly correlated [413] and it is very likely that inadequate zinc intake is associated with a coexisting inadequate intake of other micronutrients. In this section, differences between the blood analyte status of groups with inadequate and adequate zinc status are discussed. Although there are possible mechanisms to show that dietary zinc deficiency can influence the metabolism of these blood analytes, we cannot conclude that the altered status of a blood analyte is a cause and/or effect of dietary zinc deficiency.

Plasma zinc and plasma ALP

Plasma zinc concentration is considered to be a poor, insensitive and non-specific measure of zinc status [171, 442]. The level of zinc in plasma is homeostatically controlled, and particularly in marginal zinc status, may remain within normal range [206]. However, in the absence of a reliable and globally accepted biomarker of zinc status, plasma zinc concentration is still the most widely used index of zinc status [153, 443]. For example, several recent investigations in developed [444, 445] and developing [446, 447] countries have used the plasma level of zinc as the indicator of zinc deficiency.

The plasma zinc level of young people who had zinc intake lower than the revised LRNI (14.23 μmol/l) was significantly lower than the ones who had adequate zinc intake (14.78 μmol/l , $p<0.01$). The plasma zinc level of elderly population who had zinc intake lower than the revised LRNI (13.89 μmol/l) was lower (but not significantly lower) than the ones who had adequate zinc intake (14.10 μmol/l , $p>0.01$). This provided some evidence that subjects who had zinc intake below this revised cut-off point had poorer zinc status (Appendix C).

ALP is a zinc-dependent metalloenzyme [57] and one of the biomarkers of zinc status [53, 206, 448]. In both humans [100, 449] and experimental animals [52, 450], serum ALP activity declines in response to inadequate zinc intake. Young people who had zinc intake below the revised LRNI, had a significantly lower serum ALP (147.9 IU/l) compared with those who had zinc intake above the revised LRNI (234.4 IU/l, $p<0.001$).

In the NDNS of adults aged 19 to 64 years, serum ALP was not measured. For the elderly population, serum ALP of people who had inadequate zinc intake was not significantly different from those who had adequate zinc intake. Previous studies have indicated that serum ALP is not a sensitive indicator of marginal zinc status in the elderly. Paterson *et al* (1985) reported that in the hospitalised elderly population, serum ALP of zinc deficient patients was not significantly different from normal patients [451]. In another study, Bales *et al* (1994) indicated that serum ALP among elderly subjects did not change in response to an alteration in zinc intake [433].

Blood lipids and Homocysteine

The protective effect of zinc on lipid peroxidation and the antioxidant role of zinc has been the subject of many investigations [452-459]. Some of the suggested mechanisms include: the effect of zinc on the activity of antioxidant enzymes such as SOD [454, 460, 461], replacing iron and other metals with peroxidant activity [462] and the structural and functional role in Peroxisome Proliferator-Activated Receptors (PPARs) [463]. PPARs are a family of nuclear receptor proteins acting as transcription factors in the regulation of many biochemical processes such as lipid metabolism [464].

Because of this antioxidant role, it was suggested that zinc might have a preventive role against CHD. In animals, several investigations showed that there is a relationship between zinc and lipid status.

Koo *et al* (1981 and 1983) have demonstrated that in adult rats, serum HDL was positively correlated with serum zinc concentration ($r=+0.81$, $p<0.01$) [465, 466]. In 1989, Koo and Lee showed that in rats with a marginal experimental zinc deficiency, the plasma HDL was markedly decreased [467]. Yousef *et al* (2002) reported that zinc deficient rats had a significantly higher liver concentration of total lipids, cholesterol, triglycerides and LDL and lower HDL [450].

Increased serum cholesterol, triglycerides and LDL in response to zinc deficiency was reported by El Hendy *et al* (2001) [468]. These findings were confirmed when Reiterer *et al* (2005) demonstrated that inadequate zinc intake in mice, resulted in a significantly increased level of serum cholesterol and triglycerides; however, in that study, the increase came mostly from the VLDL and HDL fractions [469].

In contrast, Khoja *et al* (2002) reported that in three groups of rats treated with a normal diet, a marginally zinc deficient diet and a severely zinc deficient diet for 8 weeks, the blood lipids were significantly altered only in response to severe zinc deficiency [470].

In human studies, results are contradictory:

He *et al* (1992) reported that in adult men in south China, no significant relationship was found between the serum zinc concentration and the serum lipid variables [471].

Gatto and Samman (1995) showed that zinc supplementation in ten healthy adult men, resulted in no significant alteration in the concentration of plasma lipids [472]. (however the sample size and the fact that these men were not in a marginal zinc status has limited the validity of those results). Neggers *et al* (2001) also reported that there was no significant association between serum zinc and serum cholesterol, HDL, LDL and triglyceride levels in African Americans [473].

In Contrast, Fortes *et al* (1997) showed that in the elderly population, zinc supplementation decreased plasma lipid peroxide levels [474]. In a cross-sectional survey in rural and urban populations of North India (N=3575), among subjects who had a lower dietary zinc intake, there was a significantly higher prevalence of hypertension, hypertriglyceridemia and lower plasma HDL concentrations. The study concluded that inadequate zinc intake is one of the risk factors for CHD [475].

In this investigation, young people who had a zinc intake below the revised LRNI, had higher plasma triglycerides and lower total cholesterol (however mostly due to the significantly lower HDL) compared with those who had an adequate zinc intake. The plasma LDL of young people who had an inadequate zinc intake was not significantly different from those who had an adequate zinc intake (Appendix C). A weak, but significant inverse relationship was found between dietary zinc intake and serum total cholesterol ($r=-0.081$, $p<0.05$), dietary zinc intake and

serum HDL (r=-0.115, p<0.001) as well as serum ALP and serum triglyceride (r=-0.128, p<0.001).

In adults, the plasma lipid profile of subjects with an adequate and inadequate zinc intake was not significantly different. In the elderly population, the plasma total cholesterol and plasma LDL (expressed as plasma non-HDL cholesterol) of people who had inadequate zinc intakes was higher than people who had adequate intake. The plasma HDL for the elderly population was not significantly different from those who had adequate zinc intake (Appendix C). Dietary zinc intake of the elderly population was inversely correlated with their serum levels of total cholesterol (r=-0.110, p<0.001), LDL (r=-0.087, p<0.001) and HDL (r=-0.077, p<0.05). Alternatively, the serum zinc concentration was positively correlated to the serum concentrations of total cholesterol (r=0.210, p<0.001), triglyceride (r=0.162, p<0.001) and LDL (r=0.216, p<0.001).

In recent years, a number of investigations have reported an association between increased plasma homocysteine concentration and the risk of CHD [476-481]. Homocysteine metabolism (methylation of homocysteine to methionine) is catalyzed by methionine synthase, which is thought to be a zinc-dependent enzyme [482]. Data on the plasma homocysteine concentration was available only in the NDNS adults aged 19-64 years. Adults who had zinc intake below the revised LRNI, had significantly higher plasma homocysteine levels compared with adults who had a zinc intake above the revised LRNI (11.5 versus 10.1 µmol/l, p<0.001). A very weak, but significant inverse relationship was found between dietary zinc intake and plasma homocysteine concentration (r=-0.087, p<0.01) supporting the assumption that there is a relationship between zinc and homocysteine status.

Water-soluble vitamins

In 1978, Tamura *et al* demonstrated that in experimental zinc deficiency, intestinal hydrolysis of folate decreased. They suggested that because folate conjugase is a zinc dependent enzyme, its activity and therefore the hydrolysis and absorption of folate, decreased in response to zinc deficiency [483]. Chandler *et al* (1986) reported that the hydrolysis of dietary folate to monoglutamate in the brush border membrane of the jejunum, requires pteroylpolyglutamate hydrolase, which is another zinc-dependent enzyme [484]. Further studies of Tamura *et al* (1987) and Hong *et al* (2000) showed that dietary zinc deficiency induced an increase in

hepatic methionine synthase activity and a decrease in plasma folate concentration [482, 485].

In this investigation, for all age groups (i.e. young people, adults and the elderly), subjects who had a zinc intake below the revised LRNI, had a significantly lower serum folate concentration compared with those who had a zinc intake above the revised LRNI (Appendix C).

Fat-soluble vitamins

Zinc has a role in the absorption, transport and metabolism of vitamin A. Zinc has a regulatory role in the synthesis of retinol binding protein (RBP) in the liver. In rats, zinc deficiency decreased the synthesis of hepatic RBP and resulted in declined serum RBP levels [486]. Zinc also has an important role in oxidative conversion of retinol to retinaldehyde (retinal) via its action on retinol dehydrogenase enzymes [51, 487].

Apart from retinol dehydrogenase, other enzymes that regulate vitamin A absorption and function are thought to be zinc-dependent. For example, decreased activity of alcohol dehydrogenase and increased activity of retinol oxidase in the liver of zinc deficient rats were associated with increased total content and concentration of hepatic retinol, suggesting another factor for the altered metabolism of vitamin A in zinc deficiency [51, 488].

The other potential mechanism that explains the dependence of vitamin A metabolism on zinc is the general hypothesis that zinc has a role in the intestinal absorption of fat and fat-soluble vitamins (including vitamin A and its precursor β-carotene). Studies in experimental animals revealed that an inadequate zinc intake significantly lowered the absorption of retinol [489, 490] and β-carotene [491].

In this study, young people who had zinc intake below the revised LRNI, had significantly lower plasma α-, β-carotene, and plasma α-Cryptoxanthin but higher plasma retinol. Adults who had a zinc intake below the revised LRNI had significantly lower plasma retinol, plasma α-, β-carotene, plasma α-, and β-Cryptoxanthin. Among the elderly population, subjects who had an inadequate zinc intake had statistically lower plasma α- and β-carotene and plasma β-Cryptoxanthin (Table A1-3, Appendix C).

Kozlowski *et al* (1987) showed that among American children (with delayed cognitive development), subjects who had a compromised zinc status (based on plasma zinc concentration) had significantly lower vitamin A concentration

compared with children with normal zinc status [492]. In contrast, Hunt *et al* (1985) reported that between pregnant teenagers with low and normal zinc status, serum vitamin A levels were not significantly different [493].

Many studies in experimental animals have demonstrated that zinc status affects the indices of vitamin E status. For example, Bunk *et al* (1989) reported that in zinc deficient rats, the plasma concentration of vitamin E was lower than the pair-fed controls. Furthermore, in zinc deficient rats, in response to increased dietary intake of vitamin E, plasma concentration of vitamin E did not improve to the pair-fed control level [494]. Hatfield *et al* (2002) confirmed this finding, when they indicated that in sheep, zinc supplementation improved serum α-tocopherol levels [495]. Further studies of Noh and Koo (2001) demonstrated that an inadequate dietary zinc intake significantly lowers the lymphatic absorption and tissue (i.e. liver, heart, kidney, testis and brain) concentrations of α-tocopherol [496].

The mechanism underlying this adversity remains to be elucidated. One possible explanation is that during zinc deficiency, because of a lack of phosphatidylcholine (PC), the enterocyte fails to form chylomicrons at a normal rate and chylomicrons are the principle carriers of fat and fat-soluble vitamins [497].

For adults, subjects who had a zinc intake below the revised LRNI, had statistically lower plasma α-tocopherol (20.6 versus 21.9, $p<0.01$) and plasma γ-tocopherol (1.20 versus 1.27, $p<0.01$) compared with subjects who had an inadequate zinc intake. It should be noted that for many blood analytes such as plasma vitamin E, although a statistically significant difference between the vulnerable group and normal group was reported, this difference biologically was not important; therefore, other blood analytes are not discussed here.

8.2.4.3.4 Dietary factors

Although subjects who had a zinc intake below the revised LRNI had different nutritional and health indices and a lower zinc intake, it was not clear whether they had a different dietary pattern leading to low zinc intake. In other words, one can argue that a lower dietary zinc intake of this highlighted population is simply because of their lower general food consumption. Thus, the data were examined to determine whether it was a significantly lower intake of zinc (and phytate) after allowing for the difference in energy intake.

For all age groups (i.e. young, adult and elderly populations), subjects who had a zinc intake below the revised LRNI, had also a significantly lower zinc and phytate density of the diet (per 1000 kcal) and a higher phytate: zinc molar ratio compared with subjects who had a zinc intake above the revised LRNI (Appendix C).

The lower zinc intake of these subjects was related to their lower consumption of food sources of zinc. Overall, subjects who had a zinc intake below the revised LRNI, had a lower consumption of food sources of zinc (i.e. meat and meat products as well as cereal and cereal products) and a higher consumption of high energy and/or low zinc food and drinks such as potato chips, sugar and sweets, coffee, tea and alcoholic beverages (Appendix C).

8.2.5 Conclusion

The revised DRVs were developed following the conceptual approach used by other national and international expert committees using the most recent studies and the limited empirical information available. Like all DRVs, the revised DRVs are dependent on the quality of information available at the time. For example, in children aged 1 to 4 years, the revised DRVs may have limited validity as the most recent direct investigation has demonstrated that the current estimates of the physiological zinc requirement underestimates the requirement of these children [240]. For all other age groups, the revised DRVs are more useful and valid.

Using the revised LRNI, the risk of an inadequate zinc intake for all age groups (i.e. young people, adults and the elderly population) is higher than has been previously reported. Overall, an inadequate intake of zinc was associated with the quality of the diet and the selection of food sources rich in zinc rather than a generally inadequate consumption of food. Inadequate zinc intake was also associated with some health and nutritional disorders of vulnerable groups. For example, in young people and the elderly population who had an inadequate zinc

intake, several risk factors relating to coronary heart disease (CHD) were significantly different compared with those who had an adequate zinc intake (Figure 8.2).

Significantly different health and/or nutritional status of the people vulnerable to an inadequate zinc intake must be treated with caution as this only demonstrated a coexisting (and perhaps correlated) condition and by no means a cause and effect relationship.

Further research is required to investigate whether a marginally inadequate zinc intake (in the absence of other predisposing factors) can lead to a marginal clinical zinc deficiency. Furthermore, there is a need for intervention studies to investigate whether an inadequate zinc intake can be a cause (and/or effect) of altered health and nutritional status of the population. For example, intervention studies are required to investigate whether an inadequate zinc intake has a role in the alteration of risk factors of CHD in the UK population.

Finally, because this study did not take into account ethnicity, geographic and socioeconomic factors and physiological conditions such as pregnancy and lactation, further research is required to characterise potentially vulnerable groups to an inadequate zinc intake in relation to these factors.

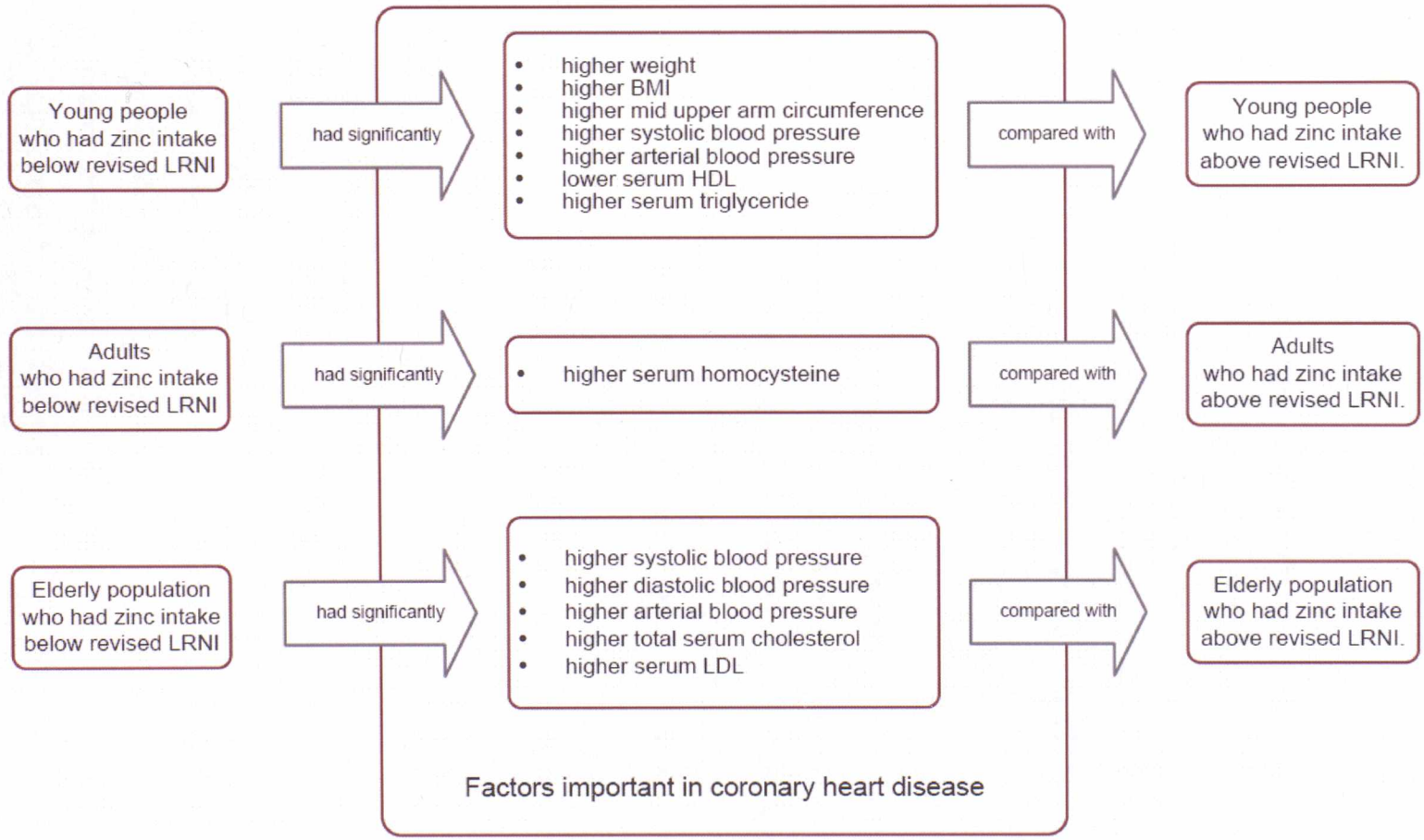


Figure 8.2 Health indices related to coronary heart disease in subjects who had zinc intake below and above the revised LRNI.

Chapter 9: Conclusions

9.1 Hypotheses and research questions revisited

9.1.1 Hypotheses revisited

1. Zinc intake of large proportion of the UK population is less than LRNI.

Confirmed

Using the current and the revised zinc DRVs, this study confirmed that in many subgroups of UK population (e.g. boys and girls aged 15 to 18 years, adult men and women aged 19 to 24 years and elderly men and women aged 75 years and over) there is a large proportion of population with zinc intake below LRNI.

2. The Ion Pair HPLC is a valid method for the assessment of phytate content of foods.

Not confirmed

Although Ion Pair HPLC successfully identified phytate among other food components, the primary measures of method validity (e.g. repeatability, intermediate precision, linearity of response versus concentration and absolute error in accuracy) were unacceptable. The present study concluded that Ion Pair HPLC is not a valid method for the assessment of the phytate content of foods.

3. The mathematical model of zinc absorption as a function of dietary zinc and phytate is a valid model for estimation of the proportion of zinc absorption of the UK population.

Not confirmed

Although the assessment of the various criteria of the model validity (e.g. the goodness of fit, generalisation and diagnostic examination of model accuracy, and evaluations of parameters' estimates) indicated that the model is useful, but in practice, the model is unable to predict the proportion of zinc absorption of the UK population. Further research is required to provide better estimates of the parameters by fitting the model to the new set of data and then the model may become applicable to estimate the proportion of zinc absorption of the UK population.

9.1.2 Research questions revisited

1. Are the current UK zinc DRVs based on the latest knowledge of physiologic requirement?

The present study indicated that for many subgroups of the UK population (i.e. young boys and girls aged 11 years and over and for all adult and elderly men and women), the current estimates of the physiologic requirement need to be updated. Increasing average body weight of the UK population and availability of more detailed information about the estimates of non-intestinal and intestinal zinc excretion are not taken into account in the current UK zinc DRVs. For children aged 10 years and younger, both the current and revised estimates are based on the extrapolation from the adults per kilogram body weight, which is thought to be subject to limited validity.

2. Are the current UK DRVs based on up-to-date estimate of the proportion of zinc absorption, allowing for gender and age differences?

For all age groups, the 1991 UK estimates of zinc absorption were based on 30% absorption efficacy. It is now well documented that the bioavailability of zinc greatly varies depending on dietary zinc and phytate. This report demonstrated that the zinc and phytate intake and proportion of zinc absorption of the UK population varies by age and gender and an estimate of the proportion of zinc absorption should take account of these factors.

3. Using revised zinc DRVs, will the picture of the UK zinc adequacy and vulnerable groups to inadequate zinc intake be different from the current picture?

Zinc intake of UK adults was thought to be generally adequate; however, comparison to the revised zinc DRVs raised some concerns about some subgroups (e.g. men and women aged 19 to 24 years) as a high percentage of these subgroups had a zinc intake below the revised LRNI. For some age groups (e.g. young people aged 11 to 14 years) the revised zinc DRVs are close to the current values, and therefore, using the revised DRVs has confirmed the previous findings.

9.2 Research agenda

In several areas, this research identified gaps in the knowledge and further research is required to fill these gaps:

9.2.1 Zinc requirements and adequacy

For zinc, there is a gap in the knowledge about the definition of zinc inadequacy. This gap has been expanded by lack of reliable biochemical indicators and cut-off points that reflect abnormal function due to poor zinc status. A research priority is the determination of an indicator of zinc status for which a cut-off point has also been defined, below which zinc functions are documented to be impaired.

One approach is to investigate whether there is a relationship between the current indicators of zinc status and the clinical consequences of zinc deficiency in the population. The difficulty is the fact that a marginal zinc status may have no recognised clinical manifestations; therefore, the clinical endpoint or intermediate endpoints of the impaired function associated with zinc needs be characterised and related to the indicators of zinc status.

Depletion-repletion investigations and balance studies have provided useful information for determining zinc requirements. However, the main drawbacks of these studies include the lack of an indicator with a reliable cut-off point, the long duration that is required to reach a new steady state and the need for a sufficient number of subjects to take the variance in requirements into account [237].

An alternative approach to determining the zinc requirements is kinetic modelling of body zinc pools in order to generate a compartmental model that explains the sites of homeostasis regulation of zinc metabolism. Although this approach provides a valuable research technique, the method is complicated, expensive, labour intensive and not practical for assessment of the population [498].

Even if these techniques provide an accurate assessment of zinc body pools, this information will be useful for estimating the zinc requirement only if the zinc body pool size at which functional zinc deficiency occurs, could be established. At the same time, the amount required for restoring the biochemical indicators of zinc status to the baseline level is not necessarily equivalent to the amount of the zinc requirement [237].

For zinc, there are also major gaps in knowledge for setting the zinc requirements of children, young people and the elderly population. Data is lacking regarding both estimates of the physiologic requirements of these groups and their estimates of the proportion of zinc absorption[16].

9.2.2 Methodology

A number of analytical techniques are available for the laboratory measurement of the zinc concentration in serum and food samples. Flame Atomic Absorption Spectrometry (FAAS), Graphite Furnace Atomic Absorption Spectrometry (GFAAS), Inductively Coupled Plasma Mass Spectrometry (ICP-MS), Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES), Instrumental Neutron Activation Analysis (NAA), X-ray spectrometry and Anodic Stripping Voltammetry (ASV) are examples of these techniques [406].

With respect to the methods available for measurement of phytate content of foods, the options are much more limited. In this report, Ion Pair HPLC is shown to have limited precision, accuracy and linearity for measurement of the phytate content of foods. The other methods include Ion Exchange HPLC, HPIC, GCMS and ICP-AES.

Labour intensity, expense and complexity of these analytical techniques are major drawbacks for their usefulness as a routine technique (especially for the assessment of phytate content of foods). Because of the difficulty in the laboratory measurement of phytate, data on the phytate content of foods in food composition tables, and consequently, data on dietary intake of phytate are limited. There is a need for further investigation to develop a simple and feasible method for assessing the phytate content of foods. Further studies are also needed to validate that the technique for the assessment of phytate content of foods consumed in the UK ultimately add to the data of phytate to the UK food composition tables.

In the NDNS, underreporting dietary intake (because of difficulties associated with the assessment of dietary intake using weighed dietary records) and the general decline in response to surveys were potential sources of error. Further research is required to develop and validate either an alternative method for a weighed dietary record or to suggest a method for adjusting the results of a weighed dietary record. Further work is required to investigate the approach that can improve the response rate in order to address the decline in participation. These approaches can include reviewing the incomplete cases and reasons for refusal to participate, improving the training of the interviewers and their managers and enhancing the support offered to them.

9.2.3 Relationship between zinc intake and health and nutrition-related disorders

There are major gaps in knowledge linking zinc intake and health and nutrition-related disorders. For example, despite several studies conducted in the field, the role of dietary zinc in the maintenance of coronary heart health and the effect of inadequate zinc intake on the risk factors of CHD are still not well established.

9.2.4 Adverse effect of high zinc intake

There is a gap in the knowledge about the tolerable upper intake level of zinc and the adverse effects of a chronic high zinc intake. Further research should focus on investigating the highest level of zinc intake that is unlikely to deliver any risk of adverse health effects in the UK population and identify groups at risk of the adverse effects of the chronic high intake of zinc.

9.3 Contribution to the body of knowledge

9.3.1 Derivation of the revised zinc DRVs

To the author's knowledge, the revised UK zinc DRVs suggested in this study is the only set of national zinc dietary reference values that have been developed by using an algorithm for establishing dietary zinc requirements based on the presence of zinc and the food component that affects zinc bioavailability the most - phytate. The implementation of this approach and review of the adjustment for phytate and the dietary reference intake of zinc (zinc DRI) was scheduled to be discussed in the 2008 spring and summer meetings of the US FNB/IOM expert committee [406].

To the author's knowledge, the revised zinc DRVs are the only revisions made to the current UK DRVs and has made a contribution to an area of nutrition neglected in the UK since 1991. This study revised the current UK DRVs by 1) using the standard approach used by other international (e.g. WHO and IZiNCG) expert committees, 2) estimating physiologic zinc requirement of the UK population based on a large number of more recent studies, 3) by taking into account the increasing weight of the UK population and 4) taking into account the current estimates of the dietary zinc and phytate intakes of the UK population in estimating the average zinc absorption, and 5) identifying and correcting the discrepancies in previous expert committee reports (Appendix A).

9.3.2 Providing an overall picture of dietary zinc adequacy for the UK population

Although several studies have commented on the zinc intake of particular groups in the UK, an overall and conclusive picture of zinc status for the entire UK population had been missing. The study provided a clear and coherent picture of the overall zinc adequacy of all gender/age groups of the UK population.

However, These findings must be considered with caution, as higher zinc DRVs would lead to a larger proportion of the UK population being considered zinc inadequate and there is little independent data to suggest that this is a problem.

To achieve a clear and conclusive picture of dietary zinc adequacy, this study reanalysed several NDNSs. The extensive range of the zinc data including common dietary and biochemical indices were available in the NDNS and subsequent surveys of the NDNS followed a relatively similar methodology that allowed drawing a broad and nationally representative picture of zinc adequacy.

The cost effectiveness was one key advantage of this research as the study effectively conducted a review of zinc adequacy at the national level by using minimum resources and keeping to the lowest cost possible.

9.3.3 Developing tables of phytate content of foods consumed in the UK

Very few national food composition tables provided values for the phytate content of local foods. To the author's knowledge, the database of the phytate content of foods consumed in the US (University of Minnesota Nutrition Coordinating Centre Nutrient database) is the only national data validated and updated regularly. In the UK, despite previous efforts by the Institute of Food Research (IFR) to include phytate data on the software of the MBIAT, the data on the phytate content of foods is not available in the UK food composition tables.

This study provided an extensive database on phytate content of the foods consumed in the UK with two key benefits:

First, the tables of phytate content was generated based on food coding of the Food Standard Agency (FSA) which was also used in the nutrient database of the NDNS. Hence, this provided an opportunity to estimate the dietary phytate intake of the UK population. Second, the tables provided a reference list and range of values in different references, and hence could be easily updated when further information becomes available for the phytate value of a particular food.

9.3.4 Providing an overall estimate of dietary phytate intake in the UK population

There is very limited data on phytate intake of the UK population and this research has provided a conclusive and clear estimate of the dietary phytate intake of all age/gender groups of the UK population.

9.3.5 Providing an extensive review on the role of zinc in human nutrition

All expert committees that developed or revised the zinc requirements (i.e. COMA, WHO, FNB/IOM and IZiNCG) have recommended that there is a need for further research into the role of zinc in human nutrition and health.

This study contributed to the body of knowledge by (1) providing an extensive review about the role of zinc in human nutrition citing the most recent studies available in the field, and (2) providing additional information on the nutrition and health status of the UK population who had an inadequate zinc intake and on the possible relationship between dietary zinc intake and health consequences.

9.3.6 Correction of the errors and/or erroneous approaches

In the process of research into the UK zinc requirements and/or in the validation of assessment of phytate content of foods via Ion Pair HPLC, this study noted a number of methodological weaknesses and/or discrepancies in the published data. This study corrected those weaknesses and/or suggested an alternative approach for their corrections (e.g. Appendices 1).

9.4 Limitations of the study

1. The basic assumptions of the development of DRVs are questionable. In the absence of adequate information regarding the distribution of the zinc requirement in the population, it has been assumed that this distribution is normal and this was the basis for allocating a notional mean requirement (or EAR). In practice, however, there is not sufficient information to confirm this assumption.

In this investigation, the LRNI was used as a fixed cut-off point to estimate the prevalence of inadequate zinc intake, whereas the variability in zinc requirements among individuals was not taken into account. This conceptual approach could overestimate or underestimate the proportion of the population vulnerable to

inadequate zinc intake and depends on the position of the zinc requirement in relation to the distribution of zinc intake in the population [217, 416].

2. This study did not take into account the possibility that humans can adapt to prolonged periods of inadequate zinc intake and achieve zinc homeostasis by increasing zinc absorption and/or decreasing zinc excretion [416]. Additionally, the study did not take account of the role of other factors in the absorption process, the validity of phytate data for the UK and the robustness of the data used in the theoretical model of zinc requirement.

3. In a conceptual approach of this thesis, when the data of the NDNS or other investigations were reanalysed, the results inherited the systematic errors of those main stage investigations. For example, for some subgroups of the UK population, the prevalence of inadequate zinc intake was overestimated because the data of those groups in the NDNS were based on intakes that are systematically underreported.

In the NDNS, the error of underreporting occurred because of using a weighed dietary record, which was a time consuming and somewhat invasive method for the assessment of dietary intake and therefore increased the likelihood that the participants changed their normal dietary pattern during the recording period, underreported their intake and/or did not completed the dietary assessment. The current thesis had a very limited option to reduce these inherent errors and therefore these errors have affected the reported results [416, 499].

4. The results presented in this study are hindered by two major factors:

    - The lack of a reliable biomarker and method for the assessment of zinc status.
    - The lack of a reliable method for the assessment of phytate content of foods.

In general, estimates of nutrient requirements are made from the association between dietary nutrient intake and the appropriate biochemical marker of nutrient adequacy [16]. However, because there is no globally agreed reliable biomarker and method for assessment of zinc status, this part of the information is missing. This gap in the current knowledge affected the current results, as this study (like previous studies) did not have a definition of the criteria for adequacy of zinc in the population.

The estimates of dietary phytate intake of the UK population are prone to errors because the tables of phytate content of foods (which are used to calculate these estimates) are derived from published and unpublished data that is not validated for foods consumed in the UK. This study's effort to develop a valid method for the assessment of phytate content of foods consumed in the UK was not successful.

5. Technical, logistic and financial limitations affected this study.

For example, an alternative method such as Ion Exchange HPLC would have been ideal and could have been developed and validated for the assessment of foods consumed in the UK. Equipment such as a vacuum manifold and/or autosampler could have been used to improve the precision of the analytical technique and a software package such as GraphPad Prism (GraphPad Software, Inc. California, USA) could have been used to improve curve fitting and data analysis. However, using these solutions was not feasible for this study because of technical, logistical and financial limitations.

6. This study provided limited information about dietary zinc intake of the UK population based on only age and gender.

Groups at risk of inadequate zinc intake could be categorised based on the ethnic physiologic, demographic and socioeconomic status. This study did not take these factors into account. Furthermore, the kinds of relationship reported in this study were association between variables. The study, by its nature, cannot take cause and effect relationships into account.

In the assessment of UK zinc status, this study focused on the adequacy and inadequacy of zinc intake and did not assess the adverse effects of chronically high dietary intake of zinc.

9.5 Policy recommendations

This study has provided evidence for existing inadequate zinc intake and associated health and nutritional implications for some subgroups of UK populations. This evidence should be used to motivate the public or private sectors to plan further investigation and intervention programmes and promote the public acceptance of these programmes.

A high prevalence of inadequate zinc intake, particularly among the elderly population and young people, has determined that there is a need to target these age groups for intervention programmes. For UK adults, despite a high percentage

of the population who had inadequate zinc intake below the revised LRNI, fewer health and nutritional implications were found. This questions the urgency with which the situation needs to be addressed, and hence, highlights the need for further investigation rather than planning intervention policies.

Three major intervention strategies can be suggested to target the elderly and young people who had inadequate zinc intake:

9.5.1 Supplementation strategies

Zinc supplementation can be an effective strategy in developing intervention policies that target the UK young and elderly populations who had inadequate zinc intake. A successful zinc supplementation programme must be supported by a financial and political commitment at the national and community levels as well as having a well-designed social marketing effort to raise awareness and generate a demand particularly among young people.

There are several implications that preclude the success of zinc supplementation strategies:

Firstly, delivering zinc supplementation in a large-scale intervention programme is expensive and difficult. Secondly, most knowledge about zinc supplementation comes from research trials and not from the experience of previous large-scale or national supplementation programmes. Thirdly, many issues such as chemical form, dosage and frequency of supplementation, administration of zinc supplements with or without foods and mechanisms to reach the vulnerable groups, raise acceptance of zinc supplements, and finally, possible adverse effects of zinc supplementation are yet to be elucidated [16].

9.5.2 Fortification strategies

In developed countries, fortification has played an important role in enhancing the dietary intake of micronutrients for which inadequate intakes are of concern . For example, in the US food fortification with folic acid has resulted in a significant increase in the average folate intake [500]. Experiences of this kind can be learnt to be used for fortification of zinc for food consumed in the UK.

The selection of foods to be fortified, selection of the form of zinc fortificant, determining the level of zinc fortification, consumer acceptability of zinc-fortified foods and regular monitoring of the effectiveness and issues of the programme are

technical concerns that must be considered in the development of a zinc fortification programme [16].

9.5.3 Dietary diversification and modification strategies

Apart from zinc supplementation and fortification, there is also a need for strategies to modify the diet of UK vulnerable groups, in order to improve the intake of absorbable zinc either by increasing total zinc intake or by increasing zinc absorption via altering the food components that affect zinc absorption. These strategies can be implemented at two levels of community and household strategies and agricultural strategies.

At the household and community levels, programmes aim to reduce the phytate content of foods (e.g. through germination and/or soaking grains and cereals) and nutritional education programmes (e.g. through community target groups, schools, religious organisations and community health centres) are examples of available strategies.

Agricultural strategies to increase the zinc content of foods can include the use of zinc fertilisers to enrich soil and plant breeding to produce varieties of plants with higher grain zinc levels and better tolerance of zinc-deficient soils. Genetic modification to increase the micronutrient content of cereals and legumes and to increase the level of proteins that enhance micronutrient absorption recently has successfully produced cereals with a more absorbable level of trace elements [216] and this approach can be used as a new agricultural strategy to increase total absorbable zinc content of food consumed in the UK.

The final consideration that must be taken into account in developing nutritional zinc policies for the UK is the issue of linking a zinc intervention programme with other nutrition and health programmes. This study demonstrated that in all age groups of UK populations, subjects who had zinc intake below the revised zinc LRNI, had also lower nutritional indices of other nutrients. Thus, programmes to address inadequate zinc intake, must be incorporated into wider general ongoing or new nutritional programmes.

The ideal policy to improve zinc intake of the UK population is a combination of short-term (e.g. zinc fortification and supplementation) and long-term (e.g. agricultural and educations programmes) strategies that can be added to the current UK nutritional policies.

References

1. King, J.C. and C.L. Keen, *Zinc*, in *Modern nutrition in health and disease*, O.J. Shile ME, Shike M, Editor. 1994, Lea and Febiger: Philadelphia. p. 214-230.
2. Habashi, F., *Zinc - The metal from the East.* Cim Bulletin, 2001. **94**(1049): p. 71-76.
3. Todd, W.R., C.A. Elvehjem, and E.B. Hart, *Zinc in the nutrition of the rat.* Am J Physiology, 1934. **107**: p. 146.
4. Prasad, A.S., *Historical Aspects of Zinc*, in *Biochemistry of Zinc.* 1993, Plenum Press: New York.
5. Prasad, A., *Zinc deficiency.* BMJ, 2003. **326**: p. 409-10.
6. Ronaghy, H.A. and J.A. Halsted, *Zinc deficiency occurring in females. Report of two cases.* Am J Clin Nutr, 1975. **28**(8): p. 831 - 6.
7. Prasad, A.S., J.A. Halsted, and M. Nadimi, *Syndrome of iron deficiency anemia, hepatosplenomegaly, hypogonadism, dwarfism, and geophagia.* Am J Med, 1961. **31**: p. 532.
8. Czajka-Narins, D.M., *Zinc*, in *Krause's Food, Nutrition and Diet Therapy*, L.K. Mahan and S. Escott-stump, Editors. 1996, W.B Saunders Company: Philadelphia. p. 143-166.
9. Hambidge, M., *Human zinc deficiency.* J Nutr, 2000. **130**(5S Suppl): p. 1344S - 9S.
10. Hambidge, K.M., et al., *Low levels of zinc in hair, anorexia, poor growth, and hypogeusia in children.* Pediatr Res, 1972. **6**(12): p. 868 - 74.
11. King, J.C. and C.L. Keen, *Zinc*, in *Modern Nutrition in Health and Disease*, Maurice E. Shils, et al., Editors. 1999, Lippincott Williams & Wilkins: Baltimore. p. 223-239.
12. Berdanier, C.D., *Advanced Nutrition Micronutrient*, in *Advanced Nutrition.* 1998, CRC Press LLC: Boca Raton. p. 184-215.
13. Groff, J.L. and S. S.Gropper, *Microminerals*, in *Advanced Nutrition and Human Metabolism.* 2000, Wadsworth, a division of Thomson Learning: Belmont. p. 401-430.
14. Whitney, E.N., C.B. Cataldo, and S.R. Rolfes, *Zinc*, in *Understanding Normal and Clinical Nutrition.* 1998, Wadsworth Publishing Company: Belmont. p. 463-479.
15. Anderson, J.J.B., *Minerals*, in *Krause's Food, Nutrition and Diet Therapy*, L.K. Mahan and S. Escott-Stump, Editors. 2004, Elsevier: Philadelphia. p. 120-163.
16. Brown, K.H., et al., *International Zinc Nutrition Consultative Group (IZiNCG) technical document #1. Assessment of the risk of zinc deficiency in populations and options for its control.* Food Nutr Bull, 2004. **25**(1 Suppl 2): p. S99 - 203.
17. Williams, S.R., *Zinc*, in *Nutrition and diet therapy.* 1993, Mosby-Year Book, Inc.: St. Louis. p. 252-266.
18. Hambidge, K.M., C.E. Casey, and N.F. Krebs, *Zinc*, in *Trace Element in Human and Animal Nutrition*, W. Mertz, Editor. 1986, Academic Press Inc. (London) Ltd. p. 1-137.
19. Iyengar GV, *Reevaluation of the trace element content in reference man.* Radiat Phys Chem, 1998. **51**: p. 545-60.
20. Groff, J.L., S.S. Gropper, and S.M. Hunt, *Microminerals*, in *Advanced Nutrition and Human Metablism.* 1995, West Publishing Company: Minneaplis/St.Paul. p. 366-375.
21. Lönnerdal, B., *Dietary factors influencing zinc absorption.* J Nutr, 2000. **130**(5S Suppl): p. 1378S - 83S.
22. Krebs, N.F., *Overview of zinc absorption and excretion in the human gastrointestinal tract.* J Nutr, 2000. **130**(5S Suppl): p. 1374S - 7S.
23. King, J.C. and R.J. Cousins, *Zinc*, in *Modern nutrition in health and disease*, M.E. Shils, et al., Editors. 2006, Lippincott Williams & Wilkins: Philadelphia. p. 271-285.
24. Favier, M. and I. Hininger-Favier, *[Zinc and pregnancy].* Gynecol Obstet Fertil, 2005. **33**(4): p. 253 - 8.
25. Sian, L., et al., *Zinc homeostasis during lactation in a population with a low zinc intake.* Am J Clin Nutr, 2002. **75**(1): p. 99 - 103.

26. Abrams, S.A., I.J. Griffin, and P.M. Davila, *Calcium and zinc absorption from lactose-containing and lactose-free infant formulas.* Am J Clin Nutr, 2002. **76**(2): p. 442 - 6.

27. Bender, D.A. and A.E. Bender, *Zinc*, in *Nutrition: a reference handbook.* 1997, Oxford University Press: New York. p. 424-427.

28. Aggett, P.J., P.K. Fenwick, and H. Kirk, *An in vitro study of the effect of picolinic acid on metal translocation across lipid bilayers.* J Nutr, 1989. **119**(10): p. 1432 - 7.

29. Kohlmeier, M., *Zinc*, in *Nutrient Metabolism.* 2003, Academic Press: San Diego. p. 685-693.

30. Coudray, C., et al., *Long-term consumption of red wine does not modify intestinal absorption or status of zinc and copper in rats.* J Nutr, 2000. **130**(5): p. 1309 - 13.

31. Johnson, P.E., *Effect of food processing and preparation on mineral utilization.* Adv Exp Med Biol, 1991. **289**: p. 483 - 98.

32. Fairweather-Tait, S.J., S. Southon, and Z. Piper, *The effect of alcoholic beverages on iron and zinc metabolism in the rat.* Br J Nutr, 1988. **60**(2): p. 209 - 15.

33. Brzozowska, A., *[Interaction of iron, zinc and copper in the body of animals and humans].* Rocz Panstw Zakl Hig, 1989. **40**(4-6): p. 302 - 12.

34. Welch, R.M., W.A. House, and D. Van Campen, *Effects of oxalic acid on availability of zinc from spinach leaves and zinc sulfate to rats.* J Nutr, 1977. **107**(6): p. 929 - 33.

35. Drews, L.M., C. Kies, and H.M. Fox, *Effect of dietary fiber on copper, zinc, and magnesium utilization by adolescent boys.* Am J Clin Nutr, 1979. **32**(9): p. 1893 - 7.

36. Sandström, B., et al., *A high oat-bran intake does not impair zinc absorption in humans when added to a low-fiber animal protein-based diet.* J Nutr, 2000. **130**(3): p. 594 - 9.

37. Wisker, E., et al., *Calcium, magnesium, zinc, and iron balances in young women: effects of a low-phytate barley-fiber concentrate.* Am J Clin Nutr, 1991. **54**(3): p. 553 - 9.

38. Semrad, C.E., *Zinc and intestinal function.* Curr Gastroenterol Rep, 1999. **1**(5): p. 398 - 403.

39. Matsui, T., *Zinc deficiency in Crohn's disease.* J Gastroenterol, 1998. **33**(6): p. 924 - 5.

40. Naber, T.H., et al., *The value of methods to determine zinc deficiency in patients with Crohn's disease.* Scand J Gastroenterol, 1998. **33**(5): p. 514 - 23.

41. Green, T.J., et al., *Effect of folic acid supplementation on plasma zinc concentrations of young women.* Nutrition, 2003. **19**(6): p. 522 - 3.

42. King, J.C., D.M. Shames, and L.R. Woodhouse, *Zinc homeostasis in humans.* J Nutr, 2000. **130**(5S Suppl): p. 1360S - 6S.

43. Maret, W., *Zinc coordination environments in proteins determine zinc functions.* J Trace Elem Med Biol, 2005. **19**(1): p. 7 - 12.

44. Stefanidou, M., et al., *Zinc: a multipurpose trace element.* Arch Toxicol, 2006. **80**(1): p. 1 - 9.

45. Frassinetti, S., et al., *The role of zinc in life: a review.* J Environ Pathol Toxicol Oncol, 2006. **25**(3): p. 597 - 610.

46. McCall, K.A., C. Huang, and C.A. Fierke, *Function and mechanism of zinc metalloenzymes.* J Nutr, 2000. **130**(5S Suppl): p. 1437S - 46S.

47. Brandão-Neto, J., et al., *The essential role of zinc in growth.* Nutrition Research, 1995. **15**(3): p. 335-358.

48. Prasad, A.S., *Clinical, biochemical and nutritional spectrum of zinc deficiency in human subjects: an update.* Nutr Rev, 1983. **41**(7): p. 197 - 208.

49. Prasad, A.S., *Zinc deficiency in human subjects.* Prog Clin Biol Res, 1983. **129**: p. 1 - 33.

50. Kang, Y.J. and Z. Zhou, *Zinc prevention and treatment of alcoholic liver disease.* Mol Aspects Med, 2005. **26**(4-5): p. 391 - 404.

51. Christian, P. and K.P. West, *Interactions between zinc and vitamin A: an update.* Am J Clin Nutr, 1998. **68**(2 Suppl): p. 435S - 441S.

52. Okegbile, E.O., O. Odunuga, and A. Oyewo, *Effect of dietary zinc deficiency on alkaline phosphatase and nucleic acids in rats.* Afr J Med Med Sci, 1998. **27**(3-4): p. 189 - 92.

53. Roth, H.P. and M. Kirchgessner, *[Diagnosis of zinc deficiency].* Z Gerontol Geriatr, 1999. **32 Suppl 1**: p. I55 - 63.

54. Landis, G.N. and J. Tower, *Superoxide dismutase evolution and life span regulation.* Mechanisms Of Ageing And Development, 2005. **126**(3): p. 365-379.

55. Faraci, F.M. and S.P. Didion, *Vascular protection - Superoxide dismutase isoforms in the vessel wall.* Arteriosclerosis Thrombosis And Vascular Biology, 2004. **24**(8): p. 1367-1373.

56. Zalewski, P.D., *Zinc metabolism in the airway: basic mechanisms and drug targets.* Curr Opin Pharmacol, 2006. **6**(3): p. 237 - 43.

57. Coleman, J.E., *Structure And Mechanism Of Alkaline-Phosphatase.* Annual Review Of Biophysics And Biomolecular Structure, 1992. **21**: p. 441-483.

58. Tapiero, H. and K.D. Tew, *Trace elements in human physiology and pathology: zinc and metallothioneins.* Biomed Pharmacother, 2003. **57**(9): p. 399 - 411.

59. Dreosti, I.E., *Zinc and the gene.* Mutat Res, 2001. **475**(1-2): p. 161 - 7.

60. Favier, A.E., *The role of zinc in reproduction. Hormonal mechanisms.* Biol Trace Elem Res, 1992. **32**: p. 363 - 82.

61. Jantz, D., et al., *The design of functional DNA-binding proteins based on zinc finger domains.* Chem Rev, 2004. **104**(2): p. 789 - 99.

62. Matthews, J.M. and M. Sunde, *Zinc fingers--folds for many occasions.* IUBMB Life, 2002. **54**(6): p. 351 - 5.

63. Hartwig, A., *Zinc finger proteins as potential targets for toxic metal ions: differential effects on structure and function.* Antioxid Redox Signal, 2001. **3**(4): p. 625 - 34.

64. King, J.C., *Determinants of maternal zinc status during pregnancy.* Am J Clin Nutr, 2000. **71**(5 Suppl): p. 1334S - 43S.

65. Castillo-Durán, C. and G. Weisstaub, *Zinc supplementation and growth of the fetus and low birth weight infant.* J Nutr, 2003. **133**(5 Suppl 1): p. 1494S - 7S.

66. Seyoum, G., *The embryopathic effects of zinc deficiency and the influnce of zinc supplementation on growth and organogenesis in zinc deficient rat embroyos.* Ethiop Med J, 2006. **44**(4): p. 353 - 62.

67. Zhang, Y., et al., *[Zinc deficiency on pathological changes of femur epiphyseal growth plate in rats].* Wei Sheng Yan Jiu, 2003. **32**(1): p. 16 - 9.

68. Browning, J.D., et al., *Reduced food intake in zinc deficient rats is normalized by megestrol acetate but not by insulin-like growth factor-I.* J Nutr, 1998. **128**(1): p. 136 - 42.

69. Cossack, Z.T., *Decline in somatomedin-C (insulin-like growth factor-1) with experimentally induced zinc deficiency in human subjects.* Clin Nutr, 1991. **10**(5): p. 284 - 91.

70. McNall, A.D., T.D. Etherton, and G.J. Fosmire, *The impaired growth induced by zinc deficiency in rats is associated with decreased expression of the hepatic insulin-like growth factor I and growth hormone receptor genes.* J Nutr, 1995. **125**(4): p. 874 - 9.

71. Ripa, S. and R. Ripa, *[Zinc and the growth hormone system].* Minerva Med, 1996. **87**(1-2): p. 25 - 31.

72. Salgueiro, M.J., et al., *The role of zinc in the growth and development of children.* Nutrition, 2002. **18**(6): p. 510 - 9.

73. Siklar, Z., et al., *Zinc deficiency: a contributing factor of short stature in growth hormone deficient children.* J Trop Pediatr, 2003. **49**(3): p. 187 - 8.

74. O'Dell, B.L. and P.G. Reeves, *Zinc Status and Food Intake*, in *Zinc in Human Biology*, C.F. Mills, Editor. 1989, Springer-Verlag: Berlin Heidelberg. p. 173-183.

75. Su, J.C. and C.L. Birmingham, *Zinc supplementation in the treatment of anorexia nervosa.* Eat Weight Disord, 2002. **7**(1): p. 20 - 2.

76. Essatara, M.B., et al., *Zinc deficiency and anorexia in rats: normal feeding patterns and stress induced feeding.* Physiol Behav, 1984. **32**(3): p. 469 - 74.

77. Rains, T.M., et al., *Food intake patterns are altered during long-term zinc deficiency in rats.* Physiol Behav, 1998. **65**(3): p. 473 - 8.

78. Wallwork, J.C., G.J. Fosmire, and H.H. Sandstead, *Effect of zinc deficiency on appetite and plasma amino acid concentrations in the rat.* Br J Nutr, 1981. **45**(1): p. 127 - 36.

79. Wallwork, J.C. and H.H. Sandstead, *Effect of zinc deficiency on appetite and free amino acid concentrations in rat brain.* J Nutr, 1983. **113**(1): p. 47 - 54.

80. Selvais, P.L., et al., *Cyclic feeding behaviour and changes in hypothalamic galanin and neuropeptide Y gene expression induced by zinc deficiency in the rat.* J Neuroendocrinol, 1997. **9**(1): p. 55 - 62.

81. Lee, R.G., et al., *Zinc deficiency increases hypothalamic neuropeptide Y and neuropeptide Y mRNA levels and does not block neuropeptide Y-induced feeding in rats.* J Nutr, 1998. **128**(7): p. 1218 - 23.

82. Levenson, C.W., *Zinc regulation of food intake: new insights on the role of neuropeptide Y.* Nutr Rev, 2003. **61**(7): p. 247 - 9.

83. Shay, N.F. and H.F. Mangian, *Neurobiology of zinc-influenced eating behavior.* J Nutr, 2000. **130**(5S Suppl): p. 1493S - 9S.

84. Lee, S.L., et al., *Leptin gene expression and serum leptin levels in zinc deficiency: implications for appetite regulation in rats.* J Med Food, 2003. **6**(4): p. 281 - 9.

85. Baltaci, A.K., R. Mogulkoc, and A. Ozturk, *Testosterone and zinc supplementation in castrated rats: Effects on plasma leptin levels and relation with LH, FSH and testosterone.* Life Sci, 2006. **78**(7): p. 746 - 52.

86. Ott, E.S. and N.F. Shay, *Zinc deficiency reduces leptin gene expression and leptin secretion in rat adipocytes.* Exp Biol Med (Maywood), 2001. **226**(9): p. 841 - 6.

87. Konukoglu, D., et al., *Relationship between plasma leptin and zinc levels and the effect of insulin and oxidative stress on leptin levels in obese diabetic patients.* J Nutr Biochem, 2004. **15**(12): p. 757 - 60.

88. Chen, M.D., Y.M. Song, and P.Y. Lin, *Zinc may be a mediator of leptin production in humans.* Life Sci, 2000. **66**(22): p. 2143 - 9.

89. Tomita, H., *[Zinc-deficient disorders of sense organs--dark adaptation, taste and smell disorders].* Nippon Rinsho, 1996. **54**(1): p. 141 - 7.

90. McDaid, O., et al., *Dietary zinc intake and sex differences in taste acuity in healthy young adults.* J Hum Nutr Diet, 2007. **20**(2): p. 103 - 10.

91. Shatzman, A.R. and R.I. Henkin, *Gustin concentration changes relative to salivary zinc and taste in humans.* Proc Natl Acad Sci U S A, 1981. **78**(6): p. 3867 - 71.

92. Bicknell, J.M. and R.V. Wiggins, *Taste disorder from zinc deficiency after tonsillectomy.* West J Med, 1988. **149**(4): p. 457 - 60.

93. MacDonald, R.S., *The role of zinc in growth and cell proliferation.* J Nutr, 2000. **130**(5S Suppl): p. 1500S - 8S.

94. Wang, X., et al., *Short-term zinc deficiency inhibits chondrocyte proliferation and induces cell apoptosis in the epiphyseal growth plate of young chickens.* J Nutr, 2002. **132**(4): p. 665 - 73.

95. Cen, X., R. Wang, and Z. Wu, *[Zinc promotes proliferation and differentiation of osteoblast in rats in vitro].* Zhonghua Yu Fang Yi Xue Za Zhi, 1999. **33**(4): p. 221 - 3.

96. Cen, X., et al., *[Role of metallothionein in the resistance of rat osteoblasts to zinc excess and deficiency].* Wei Sheng Yan Jiu, 2001. **30**(2): p. 67 - 9.

97. Cen, X., et al., *[Zinc deficiency inhibited the proliferation and differentiation of rat osteoblast in vitro].* Wei Sheng Yan Jiu, 1999. **28**(1): p. 41 - 4.

98. Coleman, J.E., *Zinc Proteins - Enzymes, Storage Proteins, Transcription Factors, And Replication Proteins.* Annual Review Of Biochemistry, 1992. **61**: p. 897-946.

99. Wu, F.Y. and C.W. Wu, *Zinc in DNA replication and transcription.* Annu Rev Nutr, 1987. **7**: p. 251 - 72.

100. Nakamura, T., et al., *Mild to moderate zinc deficiency in short children: effect of zinc supplementation on linear growth velocity.* J Pediatr, 1993. **123**(1): p. 65 - 9.

101. Zhang, Y.H., et al., *[Effects of zinc deficiency on bone mineralization and its mechanism in rats].* Zhonghua Yu Fang Yi Xue Za Zhi, 2003. **37**(2): p. 121 - 4.

102. Fernandez-Madrid, F., A.S. Prasad, and D. Oberleas, *Effect of zinc deficiency on nucleic acids, collagen, and noncollagenous protein of the connective tissue.* J Lab Clin Med, 1973. **82**(6): p. 951 - 61.

103. Agren, M.S., *Collagen synthesis in connective tissue of wounded rat mesentery: effect of dietary zinc deficiency.* Eur J Surg, 1991. **157**(8): p. 453 - 5.

104. Ninh, N.X., et al., *Continuous administration of growth hormone does not prevent the decrease of IGF-I gene expression in zinc-deprived rats despite normalization of liver GH binding.* Growth Horm IGF Res, 1998. **8**(6): p. 465 - 72.

105. Lefebvre, D., et al., *Zinc regulation of insulin-like growth factor-I (IGF-I), growth hormone receptor (GHR) and binding protein (GHBP) gene expression in rat cultured hepatocytes.* Mol Cell Endocrinol, 1998. **138**(1-2): p. 127 - 36.

106. Cunnane, S.C., *Role of zinc in lipid and fatty acid metabolism and in membranes.* Prog Food Nutr Sci, 1988. **12**(2): p. 151 - 88.

107. Eder, K. and M. Kirchgessner, *Dietary fat influences the effect of zinc deficiency on liver lipids and fatty acids in rats force-fed equal quantities of diet.* J Nutr, 1994. **124**(10): p. 1917 - 26.

108. Zajac, J. and A. Boznanski, *[Role of zinc in carbohydrate metabolism].* Pol Tyg Lek, 1991. **46**(24-26): p. 474 - 5.

109. Berger, J. and B.O. Schneeman, *Stimulation of bile-pancreatic zinc, protein and carboxypeptidase secretion in response to various proteins in the rat.* J Nutr, 1986. **116**(2): p. 265 - 72.

110. Berger, J. and B.O. Schneeman, *Intestinal zinc and carboxypeptidase A and B activity in response to consumption of test meals containing various proteins by rats.* J Nutr, 1988. **118**(6): p. 723 - 8.

111. Li, X. and B. Solomon, *Zinc-mediated thermal stabilization of carboxypeptidase A.* Biomol Eng, 2001. **18**(4): p. 179 - 83.

112. Mills, C.F., et al., *The effects of zinc deficiency on pancreatic carboxypeptidase activity and protein digestion and absorption in the rat.* Biochem J, 1967. **102**(3): p. 712 - 718.

113. Mock, W.L. and L. Wang, *Synergistic inhibition of carboxypeptidase A by zinc ion and imidazole.* Biochem Biophys Res Commun, 1999. **257**(1): p. 239 - 43.

114. Kettler, S.I., et al., *Zinc deficiency and the activities of lipoprotein lipase in plasma and tissues of rats force-fed diets with coconut oil or fish oil.* J Nutr Biochem, 2000. **11**(3): p. 132 - 8.

115. Cowen, L.A., et al., *Influence of dietary zinc deficiency and parenteral zinc on rat liver fructose 1,6-bisphosphatase activity.* Biochem Biophys Res Commun, 1986. **134**(2): p. 944 - 50.

116. Ruz, M., *Zinc supplementation and growth.* Curr Opin Clin Nutr Metab Care, 2006. **9**(6): p. 757 - 62.

117. Cunningham, B.C., M.G. Mulkerrin, and J.A. Wells, *Dimerization of human growth hormone by zinc.* Science, 1991. **253**(5019): p. 545 - 8.

118. Root, A.W., et al., *Effects of zinc deficiency upon pituitary function in sexually mature and immature male rats.* J Nutr, 1979. **109**(6): p. 958 - 64.

119. Roth, H.P. and M. Kirchgessner, *Course of concentration changes of growth hormone, IGF-1, insulin and C-peptide in serum, pituitary and liver of zinc-deficient rats.* Journal Of Animal Physiology And Animal Nutrition-Zeitschrift Fur Tierphysiologie Tierernahrung Und Futtermittelkunde, 1997. **77**(2): p. 91-101.

120. Roth, H.P. and M. Kirchgessner, *Influence of alimentary zinc deficiency on the concentration of growth hormone (GH), insulin-like growth factor I (IGF-I) and insulin in the serum of force-fed rats.* Horm Metab Res, 1994. **26**(9): p. 404 - 8.

121. Cha, M.C. and A. Rojhani, *Zinc deficiency inhibits the direct growth effect of growth hormone on the tibia of hypophysectomized rats.* Biol Trace Elem Res, 1997. **59**(1-3): p. 99 - 111.

122. Yamaguchi, M., *Role of zinc as an activator of bone formation.* J Nutr Sci Vitaminol (Tokyo), 1992. **Spec No**: p. 522 - 5.

123. Dicks, D., A. Rojhani, and Z.T. Cossack, *The Effect Of Growth-Hormone Treatment On Growth In Zinc-Deficient Rats.* Nutrition Research, 1993. **13**(6): p. 701-713.

124. Oner, G., B. Bhaumick, and R.M. Bala, *Effect Of Zinc-Deficiency On Serum Somatomedin Levels And Skeletal Growth In Young-Rats.* Endocrinology, 1984. **114**(5): p. 1860-1863.

125. Matsui, T. and M. Yamaguchi, *Zinc modulation of insulin-like growth factor's effect in osteoblastic MC3T3-E1 cells.* Peptides, 1995. **16**(6): p. 1063 - 8.

126. Cossack, Z.T., *Somatomedin-C and zinc status in rats as affected by Zn, protein and food intake.* Br J Nutr, 1986. **56**(1): p. 163 - 9.

127. Favier, A.E., *Hormonal effects of zinc on growth in children.* Biol Trace Elem Res, 1992. **32**: p. 383 - 98.

128. Freake, H.C., et al., *Actions and interactions of thyroid hormone and zinc status in growing rats.* J Nutr, 2001. **131**(4): p. 1135 - 41.

129. Chausmer, A.B., *Zinc, insulin and diabetes.* J Am Coll Nutr, 1998. **17**(2): p. 109 - 15.

130. Bhatnagar, S. and S. Taneja, *Zinc and cognitive development.* Br J Nutr, 2001. **85 Suppl 2**: p. S139 - 45.

131. Dreosti, I.E., *Zinc in brain development and function.* Prog Clin Biol Res, 1993. **380**: p. 81 - 90.

132. Black, M.M., *Zinc deficiency and child development.* Am J Clin Nutr, 1998. **68**(2 Suppl): p. 464S - 469S.

133. Koh, J.Y., *Zinc and disease of the brain.* Mol Neurobiol, 2001. **24**(1-3): p. 99 - 106.

134. Salgueiro, M.J., et al., *Zinc deficiency and growth: current concepts in relationship to two important points: intellectual and sexual development.* Biol Trace Elem Res, 2004. **99**(1-3): p. 49 - 69.

135. Black, M.M., *The evidence linking zinc deficiency with children's cognitive and motor functioning.* J Nutr, 2003. **133**(5 Suppl 1): p. 1473S - 6S.

136. Hamadani, J.D., et al., *Randomized controlled trial of the effect of zinc supplementation on the mental development of Bangladeshi infants.* Am J Clin Nutr, 2001. **74**(3): p. 381 - 6.

137. Ali, H., et al., *Relationship of serum and seminal plasma zinc levels and serum testosterone in oligospermic and azoospermic infertile men.* J Coll Physicians Surg Pak, 2005. **15**(11): p. 671 - 3.

138. Kumar, N., et al., *Effect of different levels and sources of zinc supplementation on quantitative and qualitative semen attributes and serum testosterone level in crossbred cattle (Bos indicus x Bos taurus) bulls.* Reprod Nutr Dev, 2006. **46**(6): p. 663 - 75.

139. Vivoli, G., et al., *Relationship between zinc in serum and hair and some hormones during sexual maturation in humans.* Sci Total Environ, 1990. **95**: p. 29 - 40.

140. Hamdi, S.A., O.I. Nassif, and M.S. Ardawi, *Effect of marginal or severe dietary zinc deficiency on testicular development and functions of the rat.* Arch Androl, 1997. **38**(3): p. 243 - 53.

141. Ebisch, I.M., et al., *The importance of folate, zinc and antioxidants in the pathogenesis and prevention of subfertility.* Hum Reprod Update, 2007. **13**(2): p. 163 - 74.

142. Li, S., et al., *GLP-1: a novel zinc finger protein required in somatic cells of the gonad for germ cell development.* Dev Biol, 2007. **301**(1): p. 106 - 16.

143. Hammond, G.L., G.V. Avvakumov, and Y.A. Muller, *Structure/function analyses of human sex hormone-binding globulin: effects of zinc on steroid-binding specificity.* J Steroid Biochem Mol Biol, 2003. **85**(2-5): p. 195 - 200.

144. Bedwal, R.S. and A. Bahuguna, *Zinc, copper and selenium in reproduction.* Experientia, 1994. **50**(7): p. 626 - 40.

145. Antoniou, L.D., et al., *Reversal of uraemic impotence by zinc.* Lancet, 1977. **2**(8044): p. 895 - 8.

146. Solomons, N.W., M. Ruz, and C. Castillo-Durán, *Putative Therapeutic Roles for Zinc*, in *Zinc in Human Biology*, C.F. Mills, Editor. 1989, Springer-Verlag: Berlin Heidelberg. p. 297-321.

147. El-Tawil, A.M., *Zinc deficiency in men with Crohn's disease may contribute to poor sperm function and male infertility.* Andrologia, 2003. **35**(6): p. 337 - 41.

148. Tikkiwal, M., R.L. Ajmera, and N.K. Mathur, *Effect of zinc administration on seminal zinc and fertility of oligospermic males.* Indian J Physiol Pharmacol, 1987. **31**(1): p. 30 - 4.

149. Karaca, Z., et al., *Pubertal arrest due to Zn deficiency The effect of zinc supplementation.* Hormones (Athens), 2007. **6**(1): p. 71 - 4.

150. Salem, S.I., et al., *Response of the reproductive system of male rats to protein and zinc deficiency during puberty.* Ann Nutr Metab, 1984. **28**(1): p. 44 - 51.

151. Hidiroglou, M. and J.E. Knipfel, *Zinc in mammalian sperm: a review.* J Dairy Sci, 1984. **67**(6): p. 1147 - 56.

152. Tuormaa, T.E., *The adverse effects of zinc deficiency: A reveiw from the literature.* Journal of Orthomolecular Medicine, 1995. **10**(3 & 4): p. 149-164.

153. Prasad, A.S., *Zinc in growth and development and spectrum of human zinc deficiency.* J Am Coll Nutr, 1988. **7**(5): p. 377 - 84.

154. Thompson, P., et al., *Zinc status and sexual development in adolescent girls.* J Am Diet Assoc, 1986. **86**(7): p. 892 - 7.

155. Cavdar, A.O., et al., *Geophagia in Turkey: iron and zinc deficiency, iron and zinc absorption studies and response to treatment with zinc in geophagia cases.* Prog Clin Biol Res, 1983. **129**: p. 71 - 97.

156. Eby, G.A., *Zinc treatment prevents dysmenorrhea.* Med Hypotheses, 2007.

157. Golub, M.S., et al., *Adolescent growth and maturation in zinc-deprived rhesus monkeys [see comment].* Am J Clin Nutr, 1996. **64**(3): p. 274 - 82.

158. Gibson, R.S., A.L. Heath, and E.L. Ferguson, *Risk of suboptimal iron and zinc nutriture among adolescent girls in Australia and New Zealand: causes, consequences, and solutions.* Asia Pac J Clin Nutr, 2002. **11 Suppl 3**: p. S543 - 52.

159. Bouglé, D.L., et al., *Zinc status and bone mineralisation in adolescent girls.* J Trace Elem Med Biol, 2004. **18**(1): p. 17 - 21.

160. Shah, D. and H.P. Sachdev, *Zinc deficiency in pregnancy and fetal outcome.* Nutr Rev, 2006. **64**(1): p. 15 - 30.

161. Scheplyagina, L.A., *Impact of the mother's zinc deficiency on the woman's and newborn's health status.* J Trace Elem Med Biol, 2005. **19**(1): p. 29 - 35.

162. Pathak, P. and U. Kapil, *Role of trace elements zinc, copper and magnesium during pregnancy and its outcome.* Indian J Pediatr, 2004. **71**(11): p. 1003 - 5.

163. Seshadri, S., *Prevalence of micronutrient deficiency particularly of iron, zinc and folic acid in pregnant women in South East Asia.* Br J Nutr, 2001. **85 Suppl 2**: p. S87 - 92.

164. Hambidge, K.M. and N.F. Krebs, *Zinc, low birth weight, and breastfeeding.* Pediatrics, 2003. **112**(6 Pt 1): p. 1419 - 20.

165. Bhatnagar, S. and U.C. Natchu, *Zinc in child health and disease.* Indian J Pediatr, 2004. **71**(11): p. 991 - 5.

166. Bozalioglu, S., et al., *Prevalence of zinc deficiency and immune response in short-term hemodialysis.* J Trace Elem Med Biol, 2005. **18**(3): p. 243 - 9.

167. Baum, M.K., G. Shor-Posner, and A. Campa, *Zinc status in human immunodeficiency virus infection.* J Nutr, 2000. **130**(5S Suppl): p. 1421S - 3S.

168. Ibs, K.H. and L. Rink, *Zinc-altered immune function.* J Nutr, 2003. **133**(5 Suppl 1): p. 1452S - 6S.

169. Wintergerst, E.S., S. Maggini, and D.H. Hornig, *Immune-enhancing role of vitamin C and zinc and effect on clinical conditions.* Ann Nutr Metab, 2006. **50**(2): p. 85 - 94.

170. Brown, K.H., *Effect of infections on plasma zinc concentration and implications for zinc status assessment in low-income countries.* Am J Clin Nutr, 1998. **68**(2 Suppl): p. 425S - 429S.

171. King, J.C., *Assessment of zinc status.* J Nutr, 1990. **120 Suppl 11**: p. 1474 - 9.

172. Sohnle, P.G., C. Collins-Lech, and J.H. Wiessner, *The zinc-reversible antimicrobial activity of neutrophil lysates and abscess fluid supernatants.* J Infect Dis, 1991. **164**(1): p. 137 - 42.

173. Shankar, A.H. and A.S. Prasad, *Zinc and immune function: the biological basis of altered resistance to infection.* Am J Clin Nutr, 1998. **68**(2 Suppl): p. 447S - 463S.

174. Prasad, A.S., *Zinc and immunity.* Mol Cell Biochem, 1998. **188**(1-2): p. 63 - 9.

175. Fraker, P.J., *Roles for cell death in zinc deficiency.* J Nutr, 2005. **135**(3): p. 359 - 62.

176. Taylor, C.G. and J.A. Giesbrecht, *Dietary zinc deficiency and expression of T lymphocyte signal transduction proteins.* Can J Physiol Pharmacol, 2000. **78**(10): p. 823 - 8.

177. Prasad, A.S., et al., *Nutritional and zinc status of head and neck cancer patients: an interpretive review.* J Am Coll Nutr, 1998. **17**(5): p. 409 - 18.

178. Prasad, A.S. and O. Kucuk, *Zinc in cancer prevention.* Cancer Metastasis Rev, 2002. **21**(3-4): p. 291 - 5.

179. Kupka, R. and W. Fawzi, *Zinc nutrition and HIV infection.* Nutr Rev, 2002. **60**(3): p. 69 - 79.

180. Scott, M.E. and K.G. Koski, *Zinc deficiency impairs immune responses against parasitic nematode infections at intestinal and systemic sites.* Arch Dis Child, 2000. **83**(3): p. 275.

181. Bhandari, N., et al., *Increased diarrhoeal and respiratory morbidity in association with zinc deficiency--a preliminary report.* Acta Paediatr, 1996. **85**(2): p. 148 - 50.

182. Cuevas, L.E. and A. Koyanagi, *Zinc and infection: a review.* Ann Trop Paediatr, 2005. **25**(3): p. 149 - 60.

183. Truong-Tran, A.Q., et al., *New insights into the role of zinc in the respiratory epithelium.* Immunol Cell Biol, 2001. **79**(2): p. 170 - 7.

184. Bogden, J.D., *Influence of zinc on immunity in the elderly.* J Nutr Health Aging, 2004. **8**(1): p. 48 - 54.

185. Fischer Walker, C. and R.E. Black, *Zinc and the risk for infectious disease.* Annu Rev Nutr, 2004. **24**: p. 255 - 75.

186. McClung, J.P. and A.G. Scrimgeour, *Zinc: an essential trace element with potential benefits to soldiers.* Mil Med, 2005. **170**(12): p. 1048 - 52.

187. Dardenne, M., *Zinc and immune function.* Eur J Clin Nutr, 2002. **56 Suppl 3**: p. S20 - 3.

188. Moyad, M.A., *Zinc for prostate disease and other conditions: a little evidence, a lot of hype, and a significant potential problem.* Urol Nurs, 2004. **24**(1): p. 49 - 52.

189. Grahn, B.H., et al., *Zinc and the eye.* J Am Coll Nutr, 2001. **20**(2 Suppl): p. 106 - 18.

190. Kim, H.J., Y.Y. Kim, and S.Y. Kim, *Effect of zinc on the visual sensitivity of the bullfrog's eye.* Korean J Ophthalmol, 2000. **14**(2): p. 53 - 9.

191. Morris, B., et al., *Age-related macular degeneration and recent developments: new hope for old eyes?* Postgrad Med J, 2007. **83**(979): p. 301 - 7.

192. Age-Related Eye Disease Study Research Group, *The effect of five-year zinc supplementation on serum zinc, serum cholesterol and hematocrit in persons randomly assigned to treatment group in the age-related eye disease study: AREDS Report No. 7.* J Nutr, 2002. **132**(4): p. 697 - 702.

193. Stur, M., et al., *Oral zinc and the second eye in age-related macular degeneration.* Invest Ophthalmol Vis Sci, 1996. **37**(7): p. 1225 - 35.

194. Kuzniarz, M., et al., *Use of vitamin and zinc supplements and age-related maculopathy: the Blue Mountains Eye Study.* Ophthalmic Epidemiol, 2002. **9**(4): p. 283 - 95.

195. Frederickson, C.J., J.Y. Koh, and A.I. Bush, *The neurobiology of zinc in health and disease.* Nat Rev Neurosci, 2005. **6**(6): p. 449 - 62.

196. Lansdown, A.B., et al., *Zinc in wound healing: Theoretical, experimental, and clinical aspects.* Wound Repair Regen, 2007. **15**(1): p. 2 - 16.

197. Rostan, E.F., et al., *Evidence supporting zinc as an important antioxidant for skin.* Int J Dermatol, 2002. **41**(9): p. 606 - 11.

198. Fivenson, D.P., *The mechanisms of action of nicotinamide and zinc in inflammatory skin disease.* Cutis, 2006. **77**(1 Suppl): p. 5 - 10.

199. Jones, P.W. and D.R. Williams, *The use and role of zinc and its compounds in wound healing.* Met Ions Biol Syst, 2004. **41**: p. 139 - 83.

200. Gray, M., *Does oral zinc supplementation promote healing of chronic wounds?* J Wound Ostomy Continence Nurs, 2003. **30**(6): p. 295 - 9.

201. Patel, G.K. and K.G. Harding, *Wound problems due to zinc deficiency.* Int Wound J, 2004. **1**(2): p. 150 - 1.

202. Lim, Y., M. Levy, and T.M. Bray, *Dietary zinc alters early inflammatory responses during cutaneous wound healing in weanling CD-1 mice.* J Nutr, 2004. **134**(4): p. 811 - 6.

203. Agren, M.S., *Zinc in wound repair.* Arch Dermatol, 1999. **135**(10): p. 1273 - 4.

204. Kienast, A., et al., *Zinc-deficiency dermatitis in breast-fed infants.* Eur J Pediatr, 2007. **166**(3): p. 189 - 194.

205. Richelle, M., et al., *Skin bioavailability of dietary vitamin E, carotenoids, polyphenols, vitamin C, zinc and selenium.* Br J Nutr, 2006. **96**(2): p. 227 - 38.

206. Gibson, R.S., *Principle of Nutritional Assessment.* 1990, Oxford: Oxford university Press, Inc.

207. Prasad, A.S., *Zinc deficiency in women, infants and children.* J Am Coll Nutr, 1996. **15**(2): p. 113 - 20.

208. Prasad, A.S., *Clinical manifestations of zinc deficiency.* Annu Rev Nutr, 1985. **5**: p. 341 - 63.

209. Sandstead, H.H., et al., *Zinc nutriture in the elderly in relation to taste acuity, immune response, and wound healing.* Am J Clin Nutr, 1982. **36**(5 Suppl): p. 1046 - 59.

210. Ueda, C., et al., *Zinc nutrition in healthy subjects and patients with taste impairment from the view point of zinc ingestion, serum zinc concentration and angiotensin converting enzyme activity.* Auris Nasus Larynx, 2006. **33**(3): p. 283 - 8.

211. Takeda, N., et al., *Zinc deficiency in patients with idiopathic taste impairment with regard to angiotensin converting enzyme activity.* Auris Nasus Larynx, 2004. **31**(4): p. 425 - 8.

212. Stewart-Knox, B.J., et al., *Zinc status and taste acuity in older Europeans: the ZENITH study.* Eur J Clin Nutr, 2005. **59 Suppl 2**: p. S31 - 6.

213. Henderson, L., et al., *The national diet & nutrition survey: adults aged 19 to 64 years, Volume 3: Vitamin and mineral intake and urinary analytes.* Vol. 3. 2003, London: TSO. 5 v.

214. Hunt, J.R., *Bioavailability of iron, zinc, and other trace minerals from vegetarian diets.* Am J Clin Nutr, 2003. **78**(3 Suppl): p. 633S - 639S.

215. Hunt, J.R., *Moving toward a plant-based diet: are iron and zinc at risk?* Nutr Rev, 2002. **60**(5 Pt 1): p. 127 - 34.

216. Lönnerdal, B., *Genetically modified plants for improved trace element nutrition.* J Nutr, 2003. **133**(5 Suppl 1): p. 1490S - 3S.

217. Great Britain. Dept. of, H., *Report of the Panel on Dietary Reference Values of the Committee on Medical Aspects of Food Policy: Dietary Reference Values for Food Energy and Nutrients for the United Kingdom.* Report on Health and Social Subjects. 1991, London: The Stationary Office.

218. Fosmire, G.J., *Zinc toxicity.* Am J Clin Nutr, 1990. **51**(2): p. 225 - 7.

219. Maret, W. and H.H. Sandstead, *Zinc requirements and the risks and benefits of zinc supplementation.* J Trace Elem Med Biol, 2006. **20**(1): p. 3 - 18.

220. Ashwell, M., et al., *National diet and nutrition surveys: the British experience.* Public Health Nutr, 2006. **9**(4): p. 523 - 30.

221. Swan, G., *Findings from the latest National Diet and Nutrition Survey.* Proc Nutr Soc, 2004. **63**(4): p. 505 - 12.

222. Gregory, J.R., et al., *National Diet and Nutrition Survey: children aged 1 1/2 to 4 1/2 years; volume 1 - report of the diet and nutrition survey.* 1995: HMSO.

223. Finch, S., et al., *National diet and nutrition survey: people aged 65 years and over; volume 1 report of the diet and nutrition survey.* 1998: The Stationary Office.

224. Gregory, J., et al., *National Diet and Nutrition Survey: young people aged 4 to 18 years; volume 1: report of the diet and nutrition survey.* 2000: The Stationery Office.

225. Henderson, L., J. Gregory, and G. Swan, *The national diet & nutrition survey: adults aged 19 to 64 years, Volume 1: Types and quantities of foods consumed.* Vol. 1. 2002, London: TSO. 5 v.

226. Henderson, L., et al., *The national diet & nutrition survey: adults aged 19 to 64 years, Volume 2: Energy,Protein, carbohydrate, fat and alcohol intake.* Vol. 2. 2003, London: TSO. 5 v.

227. Ruston, D., et al., *The national diet & nutrition survey: adults aged 19 to 64 years, Volume 4: Nutritional status (anthropometry and blood analytes), blood pressure and physical activity.* Vol. 4. 2004, London: TSO. 5 v.

228. Hoare, J., et al., *The national diet & nutrition survey: adults aged 19 to 64 years, Volume 5: Summary Report.* Vol. 5. 2004, London: TSO. 5 v.

229. Gibson, S., *Micronutrient intakes, micronutrient status and lipid profiles among young people consuming different amounts of breakfast cereals: further analysis of data from the National Diet and Nutrition Survey of Young People aged 4 to 18 years.* Public Health Nutr, 2003. **6**(8): p. 815 - 20.

230. Amirabdollahian, F., *Personal Communication (Email); Re: Methodology of NDNS*, M. Stather, Editor. 05/02/2009, (unpublished): London.

231. Stather, M., *Personal Communication (Email); Re: Methodology of NDNS*, F. Amirabdollahian, Editor. 06/02/2009, (unpublished): London.

232. Cook, A., J. Pryer, and P. Shetty, *The problem of accuracy in dietary surveys. Analysis of the over 65 UK National Diet and Nutrition Survey.* J Epidemiol Community Health, 2000. **54**(8): p. 611 - 6.

233. Bates, C.J., et al., *Micronutrients: highlights and research challenges from the 1994-5 National Diet and Nutrition Survey of people aged 65 years and over.* Br J Nutr, 1999. **82**(1): p. 7 - 15.

234. Tsung-Shan Tsou, *Comparing two population means and variances: A parametric robust way.* Communications in Statistics - Theory and Methods, 2003. **32**(10): p. 2013-2029.

235. Briefel, R.R., et al., *Total energy intake of the US population: the third National Health and Nutrition Examination Survey, 1988-1991.* Am J Clin Nutr, 1995. **62**(5 Suppl): p. 1072S - 1080S.

236. World Health Organization (WHO). Food and Agriculture Organization (FAO). and International Atomic Energy Agency (IAEA). *Trace elements in human nutrition and health.* 1996, Geneva: World Health Organization.

237. Institute of Medicine, *DRI, Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc: a Report of the Panel on Micronutrients, Subcommittees on Upper Reference Levels of Nutrients and Interpretation and Uses of Dietary Reference Intakes, and the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine.* 2002, Washington, D.C.: National Academy Press. xxii, 773p.

238. Alaimo, K., et al., *Dietary intake of vitamins, minerals, and fiber of persons ages 2 months and over in the United States: Third National Health and Nutrition Examination Survey, Phase 1, 1988-91.* Adv Data, 1994(258): p. 1 - 28.

239. Briefel, R.R., et al., *Zinc intake of the U.S. population: findings from the third National Health and Nutrition Examination Survey, 1988-1994.* J Nutr, 2000. **130**(5S Suppl): p. 1367S - 73S.

240. Griffin, I.J., et al., *Zinc homeostasis in 1-4 year olds consuming diets typical of US children.* Br J Nutr, 2007. **98**(2): p. 358 - 63.

241. Briefel, R., et al., *Feeding Infants and Toddlers Study: do vitamin and mineral supplements contribute to nutrient adequacy or excess among US infants and toddlers?* J Am Diet Assoc, 2006. **106**(1 Suppl 1): p. S52 - 65.

242. Nelson, M. and S.A. Bingham, *Assessment of food consumption and nutrient inatake*, in *Design concepts in nutritional epidemiology*, B.M. Margetts and M. Nelson, Editors. 1997, Oxford University Press: Oxford. p. xv,451p.

243. Piwoz, E.G., et al., *Potential for misclassification of infants' usual feeding practices using 24-hour dietary assessment methods.* J Nutr, 1995. **125**(1): p. 57 - 65.

244. Cowin, I., P. Emmett, and A.S. Team, *Diet in a group of 18-month-old children in South West England, and comparison with the results of a national survey.* J Hum Nutr Diet, 2007. **20**(3): p. 254 - 67.

245. Emmett, P., et al., *Food and nutrient intakes of a population sample of 3-year-old children in the south west of England in 1996.* Public Health Nutr, 2002. **5**(1): p. 55 - 64.

246. Thane, C.W., C.J. Bates, and A. Prentice, *Zinc and vitamin A intake and status in a national sample of British young people aged 4-18 y.* Eur J Clin Nutr, 2004. **58**(2): p. 363 - 75.

247. Rennie, K.L., et al., *Secular trends in under-reporting in young people.* Br J Nutr, 2005. **93**(2): p. 241 - 7.

248. Wood, R.J., *Assessment of marginal zinc status in humans.* J Nutr, 2000. **130**(5S Suppl): p. 1350S - 4S.

249. Droke, E.A., T.S. Kennedy, and L. Hubbs-Tait, *Potential for misclassification of micronutrient status in children participating in a Head Start program.* J Am Diet Assoc, 2006. **106**(3): p. 376 - 82.

250. Brown, K.H., et al., *Potential magnitude of the misclassification of a population's trace element status due to infection: example from a survey of young Peruvian children.* Am J Clin Nutr, 1993. **58**(4): p. 549 - 54.

251. Pilch, S.M.E. and F.R.E. Senti, *Assessment of the iron nutritional status of the U.S. population based on data collected in the second national health and nutrition examination survey, 1976-1980.* 1984: National Technical Information Service.

252. Hotz, C., J.M. Peerson, and K.H. Brown, *Suggested lower cutoffs of serum zinc concentrations for assessing zinc status: reanalysis of the second National Health and Nutrition Examination Survey data (1976-1980).* Am J Clin Nutr, 2003. **78**(4): p. 756 - 64.

253. Amirabdollahian, F. and R. Ash, *Is the zinc intake of young people in the UK adequate?* Eur J Clin Nutr, 2008 March 5th. [Epublication ahead of print].

254. Skinner, C. and D. Holmes, *The 2000-1 National Diet and Nutrition Survey of adults aged 19-64 years: the impact of non-response.* 2001, University of Southampton: (The report forms Appendix.E of the 'The National Diet & Nutrition Survey: adults aged 19 to 64 years, User Guide').

255. Amirabdollahian, F. and R. Ash, *The effect of supplemental zinc on the zinc intake of British adults.* Public Health Nutrition, 2008. **11**(6): p. 650.

256. Wright, J.D., et al., *Dietary intake of ten key nutrients for public health, United States: 1999-2000.* Adv Data, 2003(334): p. 1 - 4.

257. Mensink, G.B. and R. Beitz, *Food and nutrient intake in East and West Germany, 8 years after the reunification--The German Nutrition Survey 1998.* Eur J Clin Nutr, 2004. **58**(7): p. 1000 - 10.

258. Schulze, M.B., et al., *Macronutrient, vitamin, and mineral intakes in the EPIC-Germany cohorts.* Ann Nutr Metab, 2001. **45**(5): p. 181 - 9.

259. Schulze, A., G. Karg, and M. Steinel, *[Comparison of dietary surveys in Germany and Great Britain regarding objectives, methodology and results].* Z Ernahrungswiss, 1995. **34**(3): p. 190 - 7.

260. Bailey, A.L., et al., *Relationships between micronutrient intake and biochemical indicators of nutrient adequacy in a "free-living' elderly UK population.* Br J Nutr, 1997. **77**(2): p. 225 - 42.

261. Ma, J. and N.M. Betts, *Zinc and copper intakes and their major food sources for older adults in the 1994-96 continuing survey of food intakes by individuals (CSFII).* J Nutr, 2000. **130**(11): p. 2838 - 43.

262. Volkert, D., et al., *Energy and nutrient intake of young-old, old-old and very-old elderly in Germany.* Eur J Clin Nutr, 2004. **58**(8): p. 1190 - 200.

263. Taylor, C.M., et al., *Homeostatic regulation of zinc absorption and endogenous losses in zinc-deprived men.* Am J Clin Nutr, 1991. **53**(3): p. 755 - 63.

264. King, J.C. and J.R. Turnlund, *Human zinc requirements*, in *Zinc in human biology*, C.F. Mills, Editor. 1989, Springer: Berlin Heidelberg. p. xix,388p.

265. Ezzati, M. and World Health Organization. *Comparative quantification of health risks global and regional burden of disease attributable to selected major risk factors.* 2004 [cited; 3 v. in 1 CD-ROM].

266. Export Group on Vitamins and Minerals, *Review of Zinc (report number:EVM/99/18)*, Archive of Food Standard Agency, Editor. 1999, MAFF and the Department of Health.

267. Baer, M.T. and J.C. King, *Tissue zinc levels and zinc excretion during experimental zinc depletion in young men.* Am J Clin Nutr, 1984. **39**(4): p. 556 - 70.

268. Behall, K.M., et al., *Mineral balance in adult men: effect of four refined fibers.* Am J Clin Nutr, 1987. **46**(2): p. 307 - 14.

269. Coudray, C., et al., *Effect of soluble or partly soluble dietary fibres supplementation on absorption and balance of calcium, magnesium, iron and zinc in healthy young men.* Eur J Clin Nutr, 1997. **51**(6): p. 375 - 80.

270. Hallfrisch, J., et al., *Mineral balances of men and women consuming high fiber diets with complex or simple carbohydrate.* J Nutr, 1987. **117**(1): p. 48 - 55.

271. Hunt, J.R., L.K. Mullen, and G.I. Lykken, *Zinc Retention From An Experimental Diet Based On The United-States Fda Total Diet Study.* Nutrition Research, 1992. **12**(11): p. 1335-1344.

272. Jackson, M.J., et al., *Zinc homeostasis in man: studies using a new stable isotope-dilution technique.* Br J Nutr, 1984. **51**(2): p. 199 - 208.

273. Johnson, P.E., et al., *Homeostatic control of zinc metabolism in men: zinc excretion and balance in men fed diets low in zinc.* Am J Clin Nutr, 1993. **57**(4): p. 557 - 65.

274. Lee, D.Y., et al., *Homeostasis of zinc in marginal human zinc deficiency: role of absorption and endogenous excretion of zinc.* J Lab Clin Med, 1993. **122**(5): p. 549 - 56.

275. Mahalko, J.R., et al., *Effect of a moderate increase in dietary protein on the retention and excretion of Ca, Cu, Fe, Mg, P, and Zn by adult males.* Am J Clin Nutr, 1983. **37**(1): p. 8 - 14.

276. Milne, D.B., et al., *Effect of dietary zinc on whole body surface loss of zinc: impact on estimation of zinc retention by balance method.* Am J Clin Nutr, 1983. **38**(2): p. 181 - 6.

277. Snedeker, S.M., S.A. Smith, and J.L. Greger, *Effect of dietary calcium and phosphorus levels on the utilization of iron, copper, and zinc by adult males.* J Nutr, 1982. **112**(1): p. 136 - 43.

278. Spencer, H., et al., *Metabolic balances of cadmium, copper, manganese, and zinc in man.* Am J Clin Nutr, 1979. **32**(9): p. 1867 - 75.

279. Turnlund, J.R., et al., *Stable isotope studies of zinc absorption and retention in young and elderly men.* J Nutr, 1986. **116**(7): p. 1239 - 47.

280. Turnlund, J.R., et al., *A stable isotope study of zinc absorption in young men: effects of phytate and alpha-cellulose.* Am J Clin Nutr, 1984. **40**(5): p. 1071 - 7.

281. Wada, L., J.R. Turnlund, and J.C. King, *Zinc utilization in young men fed adequate and low zinc intakes.* J Nutr, 1985. **115**(10): p. 1345 - 54.

282. Holbrook, J.T., J.C. Smith, and S. Reiser, *Dietary fructose or starch: effects on copper, zinc, iron, manganese, calcium, and magnesium balances in humans.* Am J Clin Nutr, 1989. **49**(6): p. 1290 - 4.

283. Johnson, M.A., M.J. Baier, and J.L. Greger, *Effects of dietary tin on zinc, copper, iron, manganese, and magnesium metabolism of adult males.* Am J Clin Nutr, 1982. **35**(6): p. 1332 - 8.

284. King, J.C., et al., *Effect of acute zinc depletion on zinc homeostasis and plasma zinc kinetics in men.* Am J Clin Nutr, 2001. **74**(1): p. 116 - 24.

285. Spencer, H., et al., *Effect of oat bran muffins on calcium absorption and calcium, phosphorus, magnesium and zinc balance in men.* J Nutr, 1991. **121**(12): p. 1976 - 83.

286. Jacob, R.A., et al., *Whole body surface loss of trace metals in normal males.* Am J Clin Nutr, 1981. **34**(7): p. 1379 - 83.

287. Hunt, C.D., et al., *Effects of dietary zinc depletion on seminal volume and zinc loss, serum testosterone concentrations, and sperm morphology in young men.* Am J Clin Nutr, 1992. **56**(1): p. 148 - 57.

288. Pasqualotto, F.F., et al., *Cigarette smoking is related to a decrease in semen volume in a population of fertile men.* BJU Int, 2006. **97**(2): p. 324 - 6.

289. Leitzmann, M.F., et al., *Ejaculation frequency and subsequent risk of prostate cancer.* JAMA, 2004. **291**(13): p. 1578 - 86.

290. Milne, D.B., et al., *Ethanol metabolism in postmenopausal women fed a diet marginal in zinc.* Am J Clin Nutr, 1987. **46**(4): p. 688 - 93.

291. Miller, L.V., N.F. Krebs, and K.M. Hambidge, *Human zinc metabolism: advances in the modeling of stable isotope data.* Adv Exp Med Biol, 1998. **445**: p. 253 - 69.

292. Swanson, C.A. and J.C. King, *Zinc utilization in pregnant and nonpregnant women fed controlled diets providing the zinc RDA.* J Nutr, 1982. **112**(4): p. 697 - 707.

293. Taper, L.J., M.L. Hinners, and S.J. Ritchey, *Effects of zinc intake on copper balance in adult females.* Am J Clin Nutr, 1980. **33**(5): p. 1077 - 82.

294. Turnlund, J.R., et al., *A stable-isotope study of zinc, copper, and iron absorption and retention by young women fed vitamin B-6-deficient diets.* Am J Clin Nutr, 1991. **54**(6): p. 1059 - 64.

295. Colin, M.A., L.J. Taper, and S.J. Ritchey, *Effect of dietary zinc and protein levels on the utilization of zinc and copper by adult females.* J Nutr, 1983. **113**(8): p. 1480 - 8.

296. Hunt, J.R., L.A. Matthys, and L.K. Johnson, *Zinc absorption, mineral balance, and blood lipids in women consuming controlled lactoovovegetarian and omnivorous diets for 8 wk.* Am J Clin Nutr, 1998. **67**(3): p. 421 - 30.

297. Greger, J.L., et al., *Calcium, magnesium, phosphorus, copper, and manganese balance in adolescent females.* Am J Clin Nutr, 1978. **31**(1): p. 117 - 21.

298. Kim, J., et al., *Zinc supplementation reduces fractional zinc absorption in young and elderly korean women.* J Am Coll Nutr, 2004. **23**(4): p. 309 - 15.

299. Kim, J., et al., *Effect of dietary phytate on zinc homeostasis in young and elderly Korean women.* J Am Coll Nutr, 2007. **26**(1): p. 1 - 9.

300. Hess, F.M., J.C. King, and S. Margen, *Zinc excretion in young women on low zinc intakes and oral contraceptive agents.* J Nutr, 1977. **107**(9): p. 1610 - 20.

301. Knudsen, E., B. Sandström, and P. Solgaard, *Zinc, copper and magnesium absorption from a fibre-rich diet.* J Trace Elem Med Biol, 1996. **10**(2): p. 68 - 76.

302. Hunt, J.R., et al., *High- versus low-meat diets: effects on zinc absorption, iron status, and calcium, copper, iron, magnesium, manganese, nitrogen, phosphorus, and zinc balance in postmenopausal women.* Am J Clin Nutr, 1995. **62**(3): p. 621 - 32.

303. Sian, L., et al., *Zinc absorption and intestinal losses of endogenous zinc in young Chinese women with marginal zinc intakes.* Am J Clin Nutr, 1996. **63**(3): p. 348 - 53.

304. Lowe, N.M., et al., *A compartmental model of zinc metabolism in healthy women using oral and intravenous stable isotope tracers.* Am J Clin Nutr, 1997. **65**(6): p. 1810 - 9.

305. Lowe, N.M., et al., *Comparison of estimates of zinc absorption in humans by using 4 stable isotopic tracer methods and compartmental analysis.* Am J Clin Nutr, 2000. **71**(2): p. 523 - 9.

306. Griffin, I.J., et al., *Metabolic adaptations to low zinc intakes in premenarcheal girls.* Am J Clin Nutr, 2004. **80**(2): p. 385 - 90.

307. Griffin, I.J., J.C. King, and S.A. Abrams, *Body weight-specific zinc compartmental masses in girls significantly exceed those reported in adults: a stable isotope study using a kinetic model.* J Nutr, 2000. **130**(10): p. 2607 - 12.

308. McKenna, A.A., et al., *Zinc balance in adolescent females consuming a low- or high-calcium diet.* Am J Clin Nutr, 1997. **65**(5): p. 1460 - 4.

309. Manary, M.J., et al., *Dietary phytate reduction improves zinc absorption in Malawian children recovering from tuberculosis but not in well children.* J Nutr, 2000. **130**(12): p. 2959 - 64.

310. Manary, M.J., et al., *Zinc homeostasis in Malawian children consuming a high-phytate, maize-based diet.* Am J Clin Nutr, 2002. **75**(6): p. 1057 - 61.

311. Sheng, X.Y., et al., *Major variables of zinc homeostasis in Chinese toddlers.* Am J Clin Nutr, 2006. **84**(2): p. 389 - 94.

312. Chujian, C., et al., *Zinc metabolism and requirement in Chinese preschool children consuming different diets.* J Nutr, 1998. **128**(12): p. 2369 - 73.

313. Price, N.O., G.E. Bunce, and R.W. Engel, *Copper, manganese, and zinc balance in preadolescent girls.* Am J Clin Nutr, 1970. **23**(3): p. 258 - 60.

314. Meiners, C.R., et al., *The relationship of zinc to protein utilization in the preadolescent child.* Am J Clin Nutr, 1977. **30**(6): p. 879 - 82.

315. Greger, J.L., R.P. Abernathy, and O.A. Bennett, *Zinc and nitrogen balance in adolescent females fed varying levels of zinc and soy protein.* Am J Clin Nutr, 1978. **31**(1): p. 112 - 6.

316. Greger, J.L., et al., *Zinc, nitrogen, copper, iron, and manganese balance in adolescent females fed two levels of zinc.* J Nutr, 1978. **108**(9): p. 1449 - 56.

317. Ritchey, S.J., et al., *Zinc retention and losses of zinc in sweat by preadolescent girls.* Am J Clin Nutr, 1979. **32**(4): p. 799 - 803.

318. Reilly, J.J., A.R. Dorosty, and P.M. Emmett, *Prevalence of overweight and obesity in British children: cohort study.* BMJ, 1999. **319**(7216): p. 1039.

319. Reilly, J.J. and A.R. Dorosty, *Epidemic of obesity in UK children.* Lancet, 1999. **354**(9193): p. 1874 - 5.

320. Stamatakis, E., et al., *Overweight and obesity trends from 1974 to 2003 in English children: what is the role of socioeconomic factors?* Arch Dis Child, 2005. **90**(10): p. 999 - 1004.

321. Wood, R.J. and J.J. Zheng, *High dietary calcium intakes reduce zinc absorption and balance in humans.* Am J Clin Nutr, 1997. **65**(6): p. 1803 - 9.

322. Brooks, S.P.J. and B.J. Lampi, *Problems associated with measuring phytate in infant cereals.* Journal Of Agricultural And Food Chemistry, 2001. **49**(2): p. 564-569.

323. Dost, K. and O. Tokul, *Determination of phytic acid in wheat and wheat products by reverse phase high performance liquid chromatography.* Analytica Chimica Acta, 2006. **558**(1-2): p. 22-27.

324. Lehrfeld, J., *High-Performance Liquid Chromatography Analysis of Phytic Acid on a pH-Stable, Macroporous Polymer Column.* Cereal Chemistry, 1989. **66**(6): p. 510-515.

325. Brian, K. and P. Lawrance, *Nutrient analysis of bread and morning goods; Analytical report, MAFF/DH JFSSG Project number: AN 1062 (Ref CSA 4768).* 1999, unpublished government report; Report no. LGC/FA50/99/51.

326. Holland, B., I.D. Unwin, and D.H. Buss, *Cereals and cereal products: The third supplement to McCance and Widdowson's The composition of foods.* 4th ed. 1988, Nottingham: Royal Society of Chemistry.

327. Holland, B., I.D. Unwin, and D.H. Buss, *Vegetables, herbs and spices: The fifth supplement to McCance and Widdowson's The composition of foods.* 4th ed. 1991, Cambridge: Royal Society of Chemistry.

328. Holland, B., I.D. Unwin, and D.H. Buss, *Fruit and nuts: The first supplement to the fifth edition of McCance and Widdowson's The composition of foods.* 5th ed. 1992, Cambridge: Royal Society of Chemistry.

329. Holland, B., et al., *Vegetable dishes: The second supplement to the fifth edition of McCance and Widdowson's The composition of foods.* 5th ed. 1992, Cambridge: Royal Society of Chemistry and Ministry of Agriculture, Fisheries and Food.

330. Davies, N.T. and S. Warrington, *The phytic acid mineral, trace element, protein and moisture content of UK Asian immigrant foods.* Hum Nutr Appl Nutr, 1986. **40**(1): p. 49 - 59.

331. Wise, A., G.M. Lockie, and J. Liddell, *Dietary intakes of phytate and its meal distribution pattern amongst staff and students in an institution of higher education.* Br J Nutr, 1987. **58**(3): p. 337 - 46.

332. Davies NT and, *Effects of phytic acid on mineral availability*, in *Dietary Fiber in Health and Disease*, Vahoung GV and Kritchevsky K, Editors. 1982, Plenum Press: New York. p. 105-116.

333. McCance, R.A. and E.M. Widdowson, *McCance and Widdowson's The composition of foods.* 6th summary ed / compiled by Food Standards Agency and Institute of Food Research. ed. 2002, Cambridge: Royal Society of Chemistry. xv, 537 p.

334. Joung, H., et al., *Bioavailable zinc intake of Korean adults in relation to the phytate content of Korean foods.* Journal Of Food Composition And Analysis, 2004. **17**(6): p. 713-724.

335. Harland, B.F., *Table A.7*, in *CRC Handbook of dietary fiber in human nutrition, 3rd Ed.*, G.A. Spiller, Editor. 2001, CRC Press: Boca Raton, Fla, London. p. 659-666.

336. Harland, B.F. and D. Oberleas, *Phytate in foods.* World Rev Nutr Diet, 1987. **52**: p. 235 - 59.

337. Oberleas, D. and B.F. Harland, *Phytate content of foods: effect on dietary zinc bioavailability.* J Am Diet Assoc, 1981. **79**(4): p. 433 - 6.

338. Vinh, L.T. and E. Dworschák, *Phytate content of some foods from plant origin from Vietnam and Hungary.* Nahrung, 1985. **29**(2): p. 161 - 6.

339. Harland, B.F., S. Smikle-Williams, and D. Oberleas, *High performance liquid chromatography analysis of phytate (IP6) in selected foods.* Journal Of Food Composition And Analysis, 2004. **17**(2): p. 227-233.

340. Lehrfeld, J., *Hplc Separation and Quantitation of Phytic Acid and Some Inositol Phosphates in Foods: Problems and Solutions.* Journal Of Agricultural And Food Chemistry, 1994. **42**(12): p. 2726-2731.

341. Talamond, P., et al., *Comparison of high-performance ion chromatography and absorptiometric methods for the determination of phytic acid in food samples.* Analusis, 1998. **26**(10): p. 396-400.

342. Davies, N.T. and H. Reid, *Evaluation Of The Phytate, Zinc, Copper, Iron And Manganese Contents Of, And Zn Availability From, Soya-Based Textured Vegetable-Protein Meat-Substitutes Or Meat-Extenders.* British Journal Of Nutrition, 1979. **41**(3): p. 579-589.

343. Nitithan, S., S. Komindr, and A. Nichachotsalid, *Phytate and fiber content in Thai fruits commonly consumed by diabetic patients.* J Med Assoc Thai, 2004. **87**(12): p. 1444 - 6.

344. Chen, Q.C., *Determination of phytic acid and inositol pentakisphosphates in foods by high-performance ion chromatography.* Journal Of Agricultural And Food Chemistry, 2004. **52**(15): p. 4604-4613.

345. Lind, T., et al., *Effects of weaning cereals with different phytate contents on hemoglobin, iron stores, and serum zinc: a randomized intervention in infants from 6 to 12 mo of age.* Am J Clin Nutr, 2003. **78**(1): p. 168 - 75.

346. Bunch, S. and S. Murphy, *World Food Dietary Assessment System. Version 2.0.* 1996, Food and Agriculture Organization of the United Nations (FAO).www.fao.org/infoods/software_worldfood_en.stm (accessed 5 January 2008).

347. Roe, M., *Personal Communication, email; Re: Phytate content of foods in MBIAT*, F. Amirabdollahian, Editor. 14/01/2008, (Unpublished): Norwich.

348. Lind, T., *Personal Communication, email; Re: Phytate content of milk based cereal drinks*, F. Amirabdollahian, Editor. 17/01/2008, (Unpublished): Umea.

349. Heath, A.L.M., et al., *Meal-based intake assessment tool: relative validity when determining dietary intake of Fe and Zn and selected absorption modifiers in UK men.* British Journal Of Nutrition, 2005. **93**(3): p. 403-416.

350. Oberleas, D. and B.F. Harland, *Diagnosis of zinc deficiency in population studies.* Trace Elements And Electrolytes, 2005. **22**(4): p. 282-287.

351. Dai, F., et al., *Genotypic and environmental variation in phytic acid content and its relation to protein content and malt quality in barley.* Food Chemistry, 2007. **105**(2): p. 606-611.

352. Kumar, V., et al., *Phytic acid in Indian soybean: genotypic variability and influence of growing location.* Journal Of The Science Of Food And Agriculture, 2005. **85**(9): p. 1523-1526.

353. Chitra, U., et al., *Variability In Phytic Acid Content And Protein Digestibility Of Grain Legumes.* Plant Foods For Human Nutrition, 1995. **47**(2): p. 163-172.

354. Liu, Z.H., et al., *Genotypic and spike positional difference in grain phytase activity, phytate, inorganic phosphorus, iron, and zinc contents in wheat (Triticum aestivum L.).* Journal Of Cereal Science, 2006. **44**(2): p. 212-219.

355. Merchant, A.T. and M. Dehghan, *Food composition database development for between country comparisons.* Nutr J, 2006. **5**: p. 2.

356. West CE and WA van Staveren, *Food consumption, nutrient intake, and the use of food composition tables*, in *Design Concepts in Nutritional Epidemiology*, BM. Margetts and M. Nelson, Editors. 1997, Oxford University Press Inc: New York. p. 107-122.

357. Heath, A.L.M., *Personal Communication, Email; Re: Phytate content of foods in MBIAT*, F. Amirabdollahian, Editor. 14/01/2008, (Unpublished): Dunedin.

358. Roe, M., *Personal Communication, Email; Re: Phytate content of foods in MBIAT*, F. Amirabdollahian, Editor. 01/02/2008, (Unpublished): Norwich.

359. Campbell, M.J., *Teaching non-parametric statistics to students in health sciences*, in *7th International Conference on Teaching Statistics (ICOTS-7)*. 2006: Salvador, Bahia, Brazil.

360. Ma, G., et al., *Phytate intake and molar ratios of phytate to zinc, iron and calcium in the diets of people in China.* Eur J Clin Nutr, 2007. **61**(3): p. 368 - 74.

361. Harland, B.F. and M. Peterson, *Nutritional-Status Of Lacto-Ovo Vegetarian Trappist Monks.* Journal Of The American Dietetic Association, 1978. **72**(3): p. 259-264.

362. Plaami, S. and J. Kumpulainen, *Inositol phosphate content of some cereal-based foods.* J Food Comp Anal, 1995. **8**: p. 324-335.

363. Khokhar, S., Pushpanjali, and G.R. Fenwick, *Phytate Content of Indian Foods and Intakes by Vegetarian Indians of Hisar Region, Haryana State.* J Agric Food Chem, 1994. **42**: p. 2440-2444.

364. Kwun, I.S. and C.S. Kwon, *Dietary molar ratios of phytate:zinc and millimolar ratios of phytate x calcium:zinc in South Koreans.* Biol Trace Elem Res, 2000. **75**(1-3): p. 29 - 41.

365. Harland, B.F., O.L. Oke, and R. Felix-Phipps, *Preliminary studies on the phytate content of Nigerian foods.* J Food Comp Anal, 1988. **1**: p. 202-205.

366. Carnovale, E., G. Lombardiboccia, and E. Lugaro, *Phytate And Zinc Content Of Italian Diets.* Human Nutrition-Applied Nutrition, 1987. **41A**(3): p. 180-186.

367. Chen, Q.C. and B.W. Li, *Separation of phytic acid and other related inositol phosphates by high-performance ion chromatography and its applications.* Journal Of Chromatography A, 2003. **1018**(1): p. 41-52.

368. Harland, B.F. and G. Narula, *Food phytate and its hydrolysis products.* Nutrition Research, 1999. **19**(6): p. 947-961.

369. Lehrfeld, J. and E.R. Morris, *Overestimation Of Phytic Acid In Foods By The Aoac Anion-Exchange Method.* Journal Of Agricultural And Food Chemistry, 1992. **40**(11): p. 2208-2210.

370. Park, H.R., et al., *Determination of the phytic acid levels in infant foods using different analytical methods.* Food Control, 2006. **17**(9): p. 727-732.

371. AOAC, *AOAC official method 986.11, Phytate in foods, Anion Exchange Method, First action 1986, Final Action 1988*, in *Official methods of analysis of AOAC International. Vol.*

2, Food composition; additives; natural contaminants, 17th, Editor. 2000, AOAC International: Maryland.

372. Harland, B.F. and D. Oberleas, *Anion-exchange method for determination of phytate in foods: collaborative study.* J Assoc Off Anal Chem, 1986. **69**(4): p. 667 - 70.

373. Sandberg, A.S., *The effect of food processing on phytate hydrolysis and availability of iron and zinc.* Adv Exp Med Biol, 1991. **289**: p. 499 - 508.

374. Phillippy, B.Q., M. Lin, and B. Rasco, *Analysis of phytate in raw and cooked potatoes.* Journal of Food Composition and Analysis, 2004. **17**(2): p. 217.

375. Wise, A., *Phytate and zinc bioavailability.* Int J Food Sci Nutr, 1995. **46**(1): p. 53 - 63.

376. Burbano, C., et al., *Determination Of Phytate And Lower Inositol Phosphates In Spanish Legumes By Hplc Methodology.* Food Chemistry, 1995. **52**(3): p. 321-325.

377. Rounds, M.A. and S.S. Nielsen, *Anion-Exchange High-Performance Liquid-Chromatography With Postcolumn Detection For The Analysis Of Phytic Acid And Other Inositol Phosphates.* Journal Of Chromatography A, 1993. **653**(1): p. 148-152.

378. Snyder, L.R., J. J.Kirkland, and J. L.Glajch, *Practical HPLC Method Development.* 2nd ed. 1997, New York: John Wiley & Sons,Inc.

379. Amari, J.V., *Characterization of Linearity for an HPLC-UV Detector Using a Recombinant Protein.* American Laboratory, 2005. **37**(14): p. 19-24.

380. Dorschel, C.A., et al., *Evaluation and practical implications of linearity. Anal Chem, 1989.* **61**(17): p. 951A.

381. Brooks, S.P.J., et al., *Proposed phytic acid standard including a method for its analysis.* Journal Of Aoac International, 2001. **84**(4): p. 1125-1129.

382. Hatzack, F., et al., *Inositol phosphates from barley low-phytate grain mutants analysed by metal-dye detection HPLC and NMR.* Biochemical Journal, 2001. **354**: p. 473-480.

383. Oberleas, D., et al., *HPLC method for phytate, its validation and application to zinc defeciency diagnosis.* 2005, Texas Tech University (Emeritus), Lubbock, Texas USA and Howard University, Washington DC, USA.

384. Gibson, R.S., E.L. Ferguson, and J. Lehrfeld, *Complementary foods for infant feeding in developing countries: their nutrient adequacy and improvement.* European Journal Of Clinical Nutrition, 1998. **52**(10): p. 764-770.

385. Meyer, V.R., *Practical high-performance liquid chromotography.* 3rd ed. 1998, Chichester: John Wiley & Sons Ltd.

386. Hanai, T., *HPLC: a practical guide.* RSC Chromatography Monographs, ed. Roger.M.Smith. 1999, Cambridge: The Royal Society of Chemistry.

387. W.J.Lough and I.W.Wainer, *High Performance Liquid Chromatography (fundamental principles and practice).* 2nd ed. 1996, London: Blackie Academic & Professional (an Imprint of Chapman and Hall).

388. Dean, J.R., et al., *Practical Skills in Chemistry.* First ed. 2002, Harlow: Prentice Hall, Pearson Education Limited.

389. Dong, M.W., *Precision in HPLC.* Today's Chemist At Work, 2000. **9**(8): p. 28-33.

390. Paré, J.R.J. and J.M.R. Bélanger, *Instrumental methods in food analysis.* Techniques and instrumentation in analytical chemistry; v. 18. 1997, Amsterdam; Oxford: Elsevier.

391. Oberleas, D., *Personal communication (Email)*, F. Amirabdollahian, Editor. 07/06/2006, (Unpublished): Washington DC.

392. Harland, B., *Personal Communication (Letter)*, F. Amirabdollahian, Editor. 07/03/2006, (Unpublished): Washington DC.

393. Amirabdollahian, F. and R. Ash, *Precision, Linearity, and Accuracy of the Assessment of Phytic Acid via Ion-Pair HPLC.* American Laboratory, On-Line Edition, 2008. **1**(1): p. 35-42.

394. Miller, L.V., N.F. Krebs, and K.M. Hambidge, *A mathematical model of zinc absorption in humans as a function of dietary zinc and phytate.* J Nutr, 2007. **137**(1): p. 135 - 41.

395. Sandström, B., *Bioavailability of zinc.* Eur J Clin Nutr, 1997. **51 Suppl 1**: p. S17 - 9.

396. Liuzzi, J.P. and R.J. Cousins, *Mammalian zinc transporters.* Annu Rev Nutr, 2004. **24**: p. 151 - 72.

397. Pinna, K., *Effect of a low zinc intake on zinc homeostasis and immune function in adult men.* 1999, University of California: Berkeley (CA).

398. Adams, C.L., et al., *Zinc absorption from a low-phytic acid maize.* Am J Clin Nutr, 2002. **76**(3): p. 556 - 9.

399. Hambidge, K.M., et al., *Zinc absorption from low-phytate hybrids of maize and their wild-type isohybrids.* Am J Clin Nutr, 2004. **79**(6): p. 1053 - 9.

400. Hunt, J.R., J.M. Beiseigel, and L.K. Johnson, *Adaptation in human zinc absorption as influenced by dietary zinc and bioavailability.* Am J Clin Nutr, 2008. **87**(5): p. 1336 - 45.

401. Ellis, R., et al., *Phytate:zinc and phytate X calcium:zinc millimolar ratios in self-selected diets of Americans, Asian Indians, and Nepalese.* J Am Diet Assoc, 1987. **87**(8): p. 1043 - 7.

402. Amirabdollahian, F., *Personal Communication (Email); Estimate of the proportion of absorption of Zinc*, L. Miller, Editor. 20/02/2008, (Unpublished): London.

403. Miller, L., *Personal Communication (Email); Re:Estimate of the proportion of absorption of Zinc*, F. Amirabdollahian, Editor. 24/02/2008, (unpublished): Denver.

404. Miller, L., *Personal Communication (Email); Re:Estimate of the proportion of absorption of Zinc*, F. Amirabdollahian, Editor. 26/02/2008, (unpublished): Denver.

405. Amirabdollahian, F., *Personal Communication (Email); Re: Estimate of the proportion of absorption of Zinc*, L. Miller, Editor. 25/02/2008, (Unpublished): London.

406. Hambidge, K.M., *Personal communication, Email; Re:Mathematical model of zinc absorption*, F. Amirabdollahian, Editor. 23/02/2008, (Unpublished): Denver.

407. Amirabdollahian, F., *Personal communication, Email; Estimates of physiologic zinc requirement*, K.H. Brown, Editor. 20/02/2008, (unpublished): London.

408. Amirabdollahian, F. and R. Ash. *Errors in Estimating Zinc Requirements in Populations.* in *TEMA 13 (13th International Symposium on Trace Elements in Man And Animals).* 2008. Pucon, Chile.

409. Mackerras, D. and I. Rutishauser, *24-hour national dietary survey data: how do we interpret them most effectively?* Public Health Nutr, 2005. **8**(6): p. 657 - 65.

410. Department of Health and Social Security, *Reports on public health and medical subjects No.120. Recommended Intakes of Nutrients for the United Kingdom.* 1969, HMSO, London.

411. Department of Health and Social Security, *Reports on health and social subjects No 15. Recommended Daily Amounts of Food Energy and Nutrients for Groups of People in the United Kingdom.* 1979, HMSO, London.

412. Export Group on Vitamins and Minerals, *Safe Upper Levels for Vitamins and Minerals*, F.S. Agency, Editor. 2003, Food Standard Agency.

413. Margetts, B.M. and M. Nelson, *Overview of the principles of nutritional epidemiology*, in *Design concepts in nutritional epidemiology*, B.M. Margetts and M. Nelson, Editors. 1997, Oxford University Press: Oxford. p. 3-39.

414. Gürgöze, M.K., et al., *Serum and hair levels of zinc, selenium, iron, and copper in children with iron-deficiency anemia.* Biol Trace Elem Res, 2006. **111**(1-3): p. 23 - 9.

415. Bhutta, Z.A., *Iron and zinc deficiency in children in developing countries.* BMJ, 2007. **334**(7585): p. 104 - 5.

416. Gibson, R.S. and E.L. Ferguson, *Assessment of dietary zinc in a population.* Am J Clin Nutr, 1998. **68**(2 Suppl): p. 430S - 434S.

417. de Romaña, D.L., et al., *Longitudinal measurements of zinc absorption in Peruvian children consuming wheat products fortified with iron only or iron and 1 of 2 amounts of zinc.* Am J Clin Nutr, 2005. **81**(3): p. 637 - 47.

418. Etcheverry, P., et al., *Effect of beef and soy proteins on the absorption of non-heme iron and inorganic zinc in children.* J Am Coll Nutr, 2006. **25**(1): p. 34 - 40.

419. Ma, Z.J. and M. Yamaguchi, *Role of endogenous zinc in the enhancement of bone protein synthesis associated with bone growth of newborn rats.* J Bone Miner Metab, 2001. **19**(1): p. 38 - 44.

420. Walther, U.I., J. Schulze, and W. Forth, *Inhibition of protein synthesis by zinc: comparison between protein synthesis and RNA synthesis.* Hum Exp Toxicol, 1998. **17**(12): p. 661 - 7.

421. Ehara, Y. and M. Yamaguchi, *Zinc stimulates protein synthesis in the femoral-metaphyseal tissues of normal and skeletally unloaded rats.* Res Exp Med (Berl), 1997. **196**(6): p. 363 - 72.

422. Kimball, S.R., et al., *Effects of zinc deficiency on protein synthesis and expression of specific mRNAs in rat liver.* Metabolism, 1995. **44**(1): p. 126 - 33.

423. Hicks, S.E. and J.C. Wallwork, *Effect of dietary zinc deficiency on protein synthesis in cell-free systems isolated from rat liver.* J Nutr, 1987. **117**(7): p. 1234 - 40.

424. Golden, B.E. and M.H. Golden, *Plasma zinc, rate of weight gain, and the energy cost of tissue deposition in children recovering from severe malnutrition on a cow's milk or soya protein based diet.* Am J Clin Nutr, 1981. **34**(5): p. 892 - 9.

425. Golden, M.H. and B.E. Golden, *Effect of zinc supplementation on the dietary intake, rate of weight gain, and energy cost of tissue deposition in children recovering from severe malnutrition.* Am J Clin Nutr, 1981. **34**(5): p. 900 - 8.

426. Cavan, K.R., et al., *Growth and body composition of periurban Guatemalan children in relation to zinc status: a cross-sectional study.* Am J Clin Nutr, 1993. **57**(3): p. 334 - 43.

427. Guigliano R and Millward DJ, *The effects of severe zinc deficiency on protein turnover in muscle and thymus.* Br J Nutr, 1987. **57**: p. 139-55.

428. Guigliano R and Millward DJ, *Growth and zinc homeostasis in the severely Zn-deficient rat.* Br J Nutr, 1984. **52**: p. 545-60.

429. Gibson, R.S., M. Skeaff, and S. Williams, *Interrelationship of indices of body composition and zinc status in 11-yr-old New Zealand children.* Biol Trace Elem Res, 2000. **75**(1-3): p. 65 - 77.

430. Bates, C.J., et al., *A trial of zinc supplementation in young rural Gambian children.* Br J Nutr, 1993. **69**(1): p. 243 - 55.

431. Ruz, M., et al., *A 14-mo zinc-supplementation trial in apparently healthy Chilean preschool children.* Am J Clin Nutr, 1997. **66**(6): p. 1406 - 13.

432. Hashim, Z., L. Woodhouse, and J.C. King, *Interindividual variation in circulating zinc concentrations among healthy adult men and women.* Int J Food Sci Nutr, 1996. **47**(5): p. 383 - 90.

433. Bales, C.W., et al., *Marginal zinc deficiency in older adults: responsiveness of zinc status indicators.* J Am Coll Nutr, 1994. **13**(5): p. 455 - 62.

434. Soinio, M., et al., *Serum zinc level and coronary heart disease events in patients with type 2 diabetes.* Diabetes Care, 2007. **30**(3): p. 523 - 8.

435. Vlad, M., et al., *Concentration of copper, zinc, chromium, iron and nickel in the abdominal aorta of patients deceased with coronary heart disease.* J Trace Elem Electrolytes Health Dis, 1994. **8**(2): p. 111 - 4.

436. Tomat, A.L., et al., *Moderate zinc deficiency influences arterial blood pressure and vascular nitric oxide pathway in growing rats.* Pediatr Res, 2005. **58**(4): p. 672 - 6.

437. Vivoli, G., et al., *Zinc and copper levels in serum, urine, and hair of humans in relation to blood pressure.* Sci Total Environ, 1987. **66**: p. 55 - 64.

438. Bergomi, M., et al., *Zinc and copper status and blood pressure.* J Trace Elem Med Biol, 1997. **11**(3): p. 166 - 9.

439. Tomat, A.L., et al., *MODERATE ZINC RESTRICTION DURING FETAL AND POSTNATAL GROWTH OF RATS: EFFECTS ON ADULT ARTERIAL BLOOD PRESSURE AND KIDNEY.* Am J Physiol Regul Integr Comp Physiol, 2008.

440. Sato, M., et al., *Dietary Zn deficiency does not influence systemic blood pressure and vascular nitric oxide signaling in normotensive rats.* Biol Trace Elem Res, 2003. **91**(2): p. 157 - 72.

441. Tubek, S., *Role of zinc in regulation of arterial blood pressure and in the etiopathogenesis of arterial hypertension.* Biol Trace Elem Res, 2007. **117**(1-3): p. 39 - 51.

442. Fung, E.B., et al., *Plasma zinc is an insensitive predictor of zinc status: use of plasma zinc in children with sickle cell disease.* Nutr Clin Pract, 2002. **17**(6): p. 365 - 72.

443. Angelova, M., et al., *Serum zinc in children with enterocolitis, chronic diarrhoea with malabsorption syndrom and type 1 diabetes.* Trakia Journal of Sciences, 2006. **4**(2): p. 11-17.

444. Sánchez, C., et al., *Assessment of iron and zinc intake and related biochemical parameters in an adult Mediterranean population from southern Spain: influence of lifestyle factors.* J Nutr Biochem, 2008.

445. Meydani, S.N., et al., *Serum zinc and pneumonia in nursing home elderly.* Am J Clin Nutr, 2007. **86**(4): p. 1167 - 73.

446. Obeid, O., et al., *Plasma copper, zinc, and selenium levels and correlates with metabolic syndrome components of lebanese adults.* Biol Trace Elem Res, 2008. **123**(1-3): p. 58 - 65.

447. Weisstaub, G., et al., *Plasma zinc concentration, body composition and physical activity in obese preschool children.* Biol Trace Elem Res, 2007. **118**(2): p. 167 - 74.

448. Roth, H.P. and M. Kirchgessner, *[Diagnosis of zinc deficiency using alkaline phosphatase activity in serum before and after zinc injection].* Zentralbl Veterinarmed A, 1980. **27**(4): p. 290 - 7.

449. Kasarskis, E.J. and A. Schuna, *Serum alkaline phosphatase after treatment of zinc deficiency in humans.* Am J Clin Nutr, 1980. **33**(12): p. 2609 - 12.

450. Yousef, M.I., et al., *Dietary zinc deficiency induced-changes in the activity of enzymes and the levels of free radicals, lipids and protein electrophoretic behavior in growing rats.* Toxicology, 2002. **175**(1-3): p. 223 - 34.

451. Paterson, P.G., et al., *Zinc levels of hospitalized elderly.* J Am Diet Assoc, 1985. **85**(2): p. 186 - 91.

452. Duzguner, V. and S. Kaya, *Effect of zinc on the lipid peroxidation and the antioxidant defense systems of the alloxan-induced diabetic rabbits.* Free Radic Biol Med, 2007. **42**(10): p. 1481 - 6.

453. Jenner, A., et al., *Zinc supplementation inhibits lipid peroxidation and the development of atherosclerosis in rabbits fed a high cholesterol diet.* Free Radic Biol Med, 2007. **42**(4): p. 559 - 66.

454. Goel, A., V. Dani, and D.K. Dhawan, *Protective effects of zinc on lipid peroxidation, antioxidant enzymes and hepatic histoarchitecture in chlorpyrifos-induced toxicity.* Chem Biol Interact, 2005. **156**(2-3): p. 131 - 40.

455. Baltaci, A.K., et al., *Effect of zinc deficiency and supplementation on lipid peroxidation of renal tissue in ovariectomized rats.* Biol Trace Elem Res, 2004. **101**(3): p. 231 - 9.

456. Baltaci, A.K., et al., *The effects of zinc deficiency and supplementation on lipid peroxidation in bone tissue of ovariectomized rats.* Toxicology, 2004. **203**(1-3): p. 77 - 82.

457. Padmaja, K. and R. Ramamurthi, *Effect of zinc on lipid peroxidation and antioxidant enzymes in hepatic and brain tissues of chick embryos.* J Enzyme Inhib, 1997. **12**(4): p. 281 - 90.

458. Chen, S.M. and T.K. Young, *Effects of zinc deficiency on endogenous antioxidant enzymes and lipid peroxidation in glomerular cells of normal and five-sixths nephrectomized rats.* J Formos Med Assoc, 1998. **97**(11): p. 750 - 6.

459. Zhang, S.S., et al., *The effect of zinc supplementation on antioxidant and lipid peroxidation status during Brachiaria decumbens intoxication in sheep.* Vet Hum Toxicol, 2001. **43**(2): p. 83 - 7.

460. Dimitrova, A.A., et al., *Effect of zinc on the activity of Cu/Zn superoxide dismutase and lipid profile in Wistar rats.* Folia Med (Plovdiv), 2005. **47**(1): p. 42 - 6.

461. Dimitrova, A.A., et al., *Changes in the activity of Cu/Zn superoxide dismutase, lipid profile and aorta morphology of spontaneously hypertensive rats on zinc diet.* Folia Med (Plovdiv), 2007. **49**(3-4): p. 52 - 7.

462. Zago, M.P., S.V. Verstraeten, and P.I. Oteiza, *Zinc in the prevention of Fe2+-initiated lipid and protein oxidation.* Biol Res, 2000. **33**(2): p. 143 - 50.

463. Shen, H., et al., *Zinc deficiency alters lipid metabolism in LDL receptor deficient mice treated with rosiglitazone.* J Nutr, 2007. **137**(11): p. 2339 - 45.

464. Berger, J. and D.E. Moller, *The mechanisms of action of PPARs.* Annu Rev Med, 2002. **53**: p. 409 - 35.

465. Koo, S.I. and J.S. Ramlet, *Dietary cholesterol decreases the serum level of zinc: further evidence for the positive relationship between serum zinc and high-density lipoproteins.* Am J Clin Nutr, 1983. **37**(6): p. 918 - 23.

466. Koo, S.I. and D.A. Williams, *Relationship between the nutritional status of zinc and cholesterol concentration of serum lipoproteins in adult male rats.* Am J Clin Nutr, 1981. **34**(11): p. 2376 - 81.

467. Koo, S.I. and C.C. Lee, *Cholesterol and apolipoprotein distribution in plasma high-density-lipoprotein subclasses from zinc-deficient rats.* Am J Clin Nutr, 1989. **50**(1): p. 73 - 9.

468. El Hendy, H.A., M.I. Yousef, and N.I. Abo El-Naga, *Effect of dietary zinc deficiency on hematological and biochemical parameters and concentrations of zinc, copper, and iron in growing rats.* Toxicology, 2001. **167**(2): p. 163 - 70.

469. Reiterer, G., et al., *Zinc deficiency increases plasma lipids and atherosclerotic markers in LDL-receptor-deficient mice.* J Nutr, 2005. **135**(9): p. 2114 - 8.

470. Khoja, S.M., et al., *Effect of dietary zinc deficiency on rat lipid concentrations.* Saudi Med J, 2002. **23**(1): p. 82 - 6.

471. He, J.A., et al., *Relation of serum zinc and copper to lipids and lipoproteins: the Yi People Study.* J Am Coll Nutr, 1992. **11**(1): p. 74 - 8.

472. Gatto, L.M. and S. Samman, *The effect of zinc supplementation on plasma lipids and low-density lipoprotein oxidation in males.* Free Radic Biol Med, 1995. **19**(4): p. 517 - 21.

473. Neggers, Y.H., J.R. Bindon, and W.W. Dressler, *The relationship between zinc and copper status and lipid levels in African-Americans.* Biol Trace Elem Res, 2001. **79**(1): p. 1 - 13.

474. Fortes, C., et al., *Zinc supplementation and plasma lipid peroxides in an elderly population.* Eur J Clin Nutr, 1997. **51**(2): p. 97 - 101.

475. Singh, R.B., et al., *Current zinc intake and risk of diabetes and coronary artery disease and factors associated with insulin resistance in rural and urban populations of North India.* J Am Coll Nutr, 1998. **17**(6): p. 564 - 70.

476. B. Vitamin Treatment Trialists' Collaboration, *Homocysteine-lowering trials for prevention of cardiovascular events: a review of the design and power of the large randomized trials.* Am Heart J, 2006. **151**(2): p. 282 - 7.

477. Clarke, R., *Homocysteine-lowering trials for prevention of heart disease and stroke.* Semin Vasc Med, 2005. **5**(2): p. 215 - 22.

478. Clarke, R. and S. Lewington, *Homocysteine and coronary heart disease.* Semin Vasc Med, 2002. **2**(4): p. 391 - 9.

479. Abby, S.L., I.M. Harris, and K.M. Harris, *Homocysteine and cardiovascular disease.* J Am Board Fam Pract, 1998. **11**(5): p. 391 - 8.

480. Doncheva, N., et al., *Study of homocysteine concentration in coronary heart disease patients and comparison of two determination methods.* Ann Nutr Metab, 2007. **51**(1): p. 82 - 7.

481. Genser, D., et al., *Homocysteine, folate and vitamin b12 in patients with coronary heart disease.* Ann Nutr Metab, 2006. **50**(5): p. 413 - 9.

482. Hong, K.H., et al., *Effects of dietary zinc deficiency on homocysteine and folate metabolism in rats.* J Nutr Biochem, 2000. **11**(3): p. 165 - 9.

483. Tamura, T., et al., *Absorption of mono- and polyglutamyl folates in zinc-depleted man.* Am J Clin Nutr, 1978. **31**(11): p. 1984 - 7.

484. Chandler, C.J., T.T. Wang, and C.H. Halsted, *Pteroylpolyglutamate hydrolase from human jejunal brush borders. Purification and characterization.* J Biol Chem, 1986. **261**(2): p. 928 - 33.

485. Tamura, T., et al., *Increased methionine synthetase activity in zinc-deficient rat liver.* Arch Biochem Biophys, 1987. **256**(1): p. 311 - 6.

486. Mobarhan, S., et al., *Zinc deficiency reduces hepatic cellular retinol-binding protein in rats.* Int J Vitam Nutr Res, 1992. **62**(2): p. 148 - 54.

487. Huber, A.M. and S.N. Gershoff, *Effects of zinc deficiency on the oxidation of retinol and ethanol in rats.* J Nutr, 1975. **105**(11): p. 1486 - 90.

488. Boron, B., et al., *Effect of zinc deficiency on hepatic enzymes regulating vitamin A status.* J Nutr, 1988. **118**(8): p. 995 - 1001.

489. Ahn J and Koo SI, *Effects of zinc and essential fatty acid deficiencies on the lymphatic absorption of vitamin A and secretion of phospholipids.* J Nutr Biochem, 1995. **6**(11): p. 595-603.

490. Noh, S.K. and S.I. Koo, *Intraduodenal infusion of lysophosphatidylcholine restores the intestinal absorption of vitamins A and E in rats fed a low-zinc diet.* Exp Biol Med (Maywood), 2001. **226**(4): p. 342 - 8.

491. Noh, S.K. and S.I. Koo, *Low zinc intake decreases the lymphatic output of retinol in rats infused intraduodenally with beta-carotene.* J Nutr Biochem, 2003. **14**(3): p. 147 - 53.

492. Kozlowski, B.W., et al., *Anticonvulsant medication use and circulating levels of total thyroxine, retinol binding protein, and vitamin A in children with delayed cognitive development.* Am J Clin Nutr, 1987. **46**(2): p. 360 - 8.

493. Hunt, I.F., et al., *Zinc supplementation during pregnancy in low-income teenagers of Mexican descent: effects on selected blood constituents and on progress and outcome of pregnancy.* Am J Clin Nutr, 1985. **42**(5): p. 815 - 28.

494. Bunk, M.J., et al., *Dietary zinc deficiency decreases plasma concentrations of vitamin E.* Proc Soc Exp Biol Med, 1989. **190**(4): p. 379 - 84.

495. Hatfield, P.G., et al., *Serum alpha-tocopherol and immune function in yearling ewes supplemented with zinc and vitamin E.* J Anim Sci, 2002. **80**(5): p. 1329 - 34.

496. Noh, S.K. and S.I. Koo, *Feeding of a low-zinc diet lowers the tissue concentrations of alpha-tocopherol in adult rats.* Biol Trace Elem Res, 2001. **81**(2): p. 153 - 68.

497. Noh, S.K. and S.I. Koo, *Enteral infusion of phosphatidylcholine increases the lymphatic absorption of fat, but lowers alpha-tocopherol absorption in rats fed a low zinc diet*.* J Nutr Biochem, 2001. **12**(6): p. 330 - 337.

498. Lowe, N.M., *In search of a reliable marker of zinc status-are we nearly there yet?* Nutrition, 2005. **21**(7-8): p. 883 - 4.

499. Briefel, R.R., et al., *Dietary methods research in the third National Health and Nutrition Examination Survey: underreporting of energy intake.* Am J Clin Nutr, 1997. **65**(4 Suppl): p. 1203S - 1209S.

500. Ganji, V. and M.R. Kafai, *Population reference values for plasma total homocysteine concentrations in US adults after the fortification of cereals with folic acid.* Am J Clin Nutr, 2006. **84**(5): p. 989 - 94.

501. Nititham, S., et al., *Phytate and fiber content in Thai fruits commonly consumed by diabetic patients.* J Med Assoc Thai, 2004. **87**(12): p. 1444 - 6.

502. Lind, T., *Personal Communication, email; Re:Phytate content of milk based cereal drinks*, F. Amirabdollahian, Editor. 17/01/2008, (Unpublished): Umea.

List of Abbreviations

ACE	Angiotensin converting enzyme
ALP	Alkaline phosphatase
ALSPAC	Avon longitudinal study of pregnancy and children
A_{max}	Maximum absorbed zinc
AOAC	Association of analytical communities
ARMD	Age related macular degeneration
ASV	Anodic stripping voltammetry
CAPI	Computer assisted personal interviewing
CDC	Centre for disease control and prevention
CHD	Coronary heart disease
CIF	Children in focus
CNS	Central nervous system
COMA	Committee of medical aspects of food policy
CRIP	Cysteine-rich intestinal protein
CRP	C- reactive protein
CSFII	Continuing survey of food intake by individuals
CV	Coefficient of variation
DH	Department of health
DNA	Deoxyribonucleic acid
DRI	Dietary reference intake
DRV	Dietary reference value
EAR	Estimated average requirement
EPIC	European prospective investigation into cancer and nutrition
Exp	Exponential function
FAAS	Flame atomic absorption spectrometry
FAO	Food and agriculture organisation
FDA	Food and drug administration
FNB/IOM	Food and nutrition board/ institute of medicine
FSA	Food standard agency
FSH	Follicle stimulating hormone
G	Gram
GABA	Gama (γ-) amino butyric acid
GCMS	Gas chromatography mass spectrometry
GeNuS	German nutrition survey
GFAAS	Graphite furnace atomic absorption spectrometry
GH	Growth hormone
GNHIES	German national health interview and examination survey
GRAS	Generally recognised as safe
HCl	Hydrochloric acid

HDL	High density lipoprotein
HIV	Human immunodeficiency virus
HPIC	High performance ion chromatography
HPLC	High performance liquid chromatography
IAEA	International atomic energy agency
ICP-AES	Inductively coupled plasma atomic emission spectroscopy
ICP-MS	Inductively coupled plasma mass spectrometry
ID	Identification
IFN	Interferon
IFR	Institute of food research
IGF-1	Insulin-like growth factor-1
IgG	Immunoglobulin G
IL-1	Interleukin-1
IL-2	Interleukin-2
IL-6	Interleukin-6
IOM	Institute of medicine
IP1	Inositol monophosphate
IP2	Inositol diphosphate
IP3	Inositol triphosphate
IP4	Inositol tetraphosphate
IP5	Inositol pentaphosphate
IP6	Inositol hexaphosphate (also called IP-6, phytic acid, phytate, myo-inositol hexaphosphate and myo-inositol hexakisphosphate)
IU	International unit
IUB	International union of biochemistry
IZINCG	International zinc nutrition consultative group
K_p	Equilibrium dissociation constant of a zinc-phytate constant
K_R	Equilibrium dissociation constant of a zinc-receptor binding reaction
LCMS	Liquid chromatography mass spectrometry
LDL	Low density lipoprotein
LH	Luteinizing hormone
LLE	liquid-liquid extraction
Ln	Natural logarithm
Log	Logarithm
Logit	Logit function in mathematics and statistics
LRNI	Lower reference nutrient intake
MAFF	Ministry of agriculture, fisheries and food
MBIAT	Meal based intake assessment tool
MDI	Mental development index
mRNA	Messenger RNA
MT	Metallothionein

N	Number
NA	Not available
NAA	(Instrumental) neutron activation analysis
NCHS	National centre for health statistics
NDNS	National diet and nutrition survey
NHANES	National health and nutrition examination survey
NK Cells	Natural killer cells
NMR	Nuclear magnetic resonance
NOS	nitric oxide synthase
PCD	programmed cells death
PPARs	Peroxisome proliferator-activated receptors
PRP-1	Polymeric reversed phase-1 (HPLC column)
R or r	Response or Pearson correlation coefficient
R^2	Coefficient of determination
R_a^2	Adjusted coefficient of determination
RBP	Retinol binding protein
RDA	Recommended daily allowance and/or Recommended daily amounts
RDI	Recommended daily intakes
RF	Response factor also called sensitivity
RI	Refractive index
RNA	Ribonucleic acid
RNI	Reference nutrient intake
RPE	Retinal pigment epithelium
R_{PZ}	Phytate: zinc molar ratio
RSD	Relative standard deviation
SAX	Silica based quaternary amine column
SD	Standard deviation
SE	Standard error of the mean
SHBG	Sex hormone-binding globulin
SOD	Superoxide dismutase
SPE	Solid phase extraction
SPSS	Statistical package for the social sciences
SS_R	Sum of the squared residuals
TAZ	Total daily absorbed zinc
TBNOH	Tetra butyl ammonium hydroxide
TDP	Total daily dietary phytate
TDZ	Total daily dietary Zinc
TH	T-helper
UV	Ultra violet
VLDL	Very low density lipoprotein

WHO	World health organisation
$\bar{X}$	Mean
ZIP	Zinc and iron regulated proteins
Zn	Zinc
ZnT	Zinc transporter

Appendix A: Publications and presentations from this thesis

- Amirabdollahian, F. and R. Ash, *Precision, Linearity, and Accuracy of the Assessment of Phytic Acid via Ion-Pair HPLC.* American Laboratory, On-Line Edition, 2008. **1**(1): p. 35-42.
- Amirabdollahian, F. and R. Ash, *The effect of supplemental zinc on the zinc intake of British adults.* Public Health Nutr, 2008. **11**(6): p. 650.
- Amirabdollahian, F. and R. Ash, *Is the zinc intake of young people in the UK adequate?* Eur J Clin Nutr, 2008 March 5th. [E-publication ahead of print].
- Amirabdollahian, F. and R. Ash. *An assessment of zinc adequacy in the UK population using measured zinc intake and adequacy thresholds derived from theoretical model of requirement.* In *TEMA 12*. 2005. University of Ulster, Coleraine, Northern Ireland. (Book of Abstracts, p. 49).
- Amirabdollahian F and R. Ash. *Zinc intake and status in the UK population.* In *IZiNCG Symposium; Moving Zinc into the Micronutrient Program Agenda*. 2004. Lima, Peru (Book of Abstracts, p. 13-14).
- Amirabdollahian, F. and R. Ash. *Mild zinc deficiency and quality of growth in UK children and adolescents*, In *PhD and Post-Doctoral Symposium; London Metabolism Iron Research Group Scientific Meeting*. 2004: King's College London, London, UK.
- Amirabdollahian, F. and R. Ash. *Phytate Intake and Molar Ratio of Phytate: Zinc in the Diet of the UK Population.* In *TEMA 13*. November 9-13, 2008. Pucón, Chile.
- Amirabdollahian, F. and R. Ash. *Errors in Estimating Zinc Requirements in Populations.* In *TEMA 13*. November 9-13, 2008. Pucón, Chile.
- Amirabdollahian, F. and Ash, R. (awaiting decision) Physiologic zinc requirement estimated by IZiNCG appears to be too low. Food Nutr Bull. 2008

Appendix B: Phytate content of foods

No	Code	Food	Phytate mg/100g	Reference
1	Pasta, Rice and other miscellaneous cereals			
	1A	**Pasta**	**100**	
All types-dried, fresh and canned; including egg noodles, macaroni cheese, ravioli, canned spaghetti bolognaise				
1	1A1	Noodles, dry form	92-409	[334]
2	1A2	Noodles, boiled	23-69	[335-337]
3	1A3	Macaroni, boiled	81-260	[347]
4	1A4	Pasta (white or whole meal)	76-292	[323]
	1B	**Rice**	**150**	
Fried and boiled, savoury rice, egg fried rice, rice flakes, rice flour. (Not rice pudding)				
5	1B1	Rice	1020	[326, 346]
6	1B2	White rice raw	350-420	[326, 346]
7	1B3	White rice fried	10-94	[346]
8	1B4	Egg fried rice	0-66	[326, 335, 336]
9	1B5	White rice polished raw	255-420	[346]
10	1B6	Rice flakes	785	[323, 338, 346]
11	1B7	Rice flour	162-560	[334-336, 339]
12	1B8	Brown Rice, dry	518-955	[335, 336, 340]
13	1B9	Wild rice	420-1935	[326]
	1C	**Pizza**	**70**	
14	1C1	Pizza (Unspecified)	40	[329, 347]
15	1C2	Cheese & tomato	50	[329]
16	1C3	Cheese & tomato, whole meal	160	[329]
17	1C4	Cheese & tomato, frozen	50	[329]
18	1C5	Tomato	50	[329]
19	1C6	Tomato, whole meal	140	[335, 336, 346]
	1R	**Other cereals**	**400**	
Includes flour, bran, oats, dry semolina, papadums, dumplings, Yorkshire pudding				
20	1R1	Wheat flour, all purpose, 70% extraction	282	[346]
21	1R2	Wheat flour, 85% extraction	564	[326]
22	1R3	White flour (Bread making or self-raising)	180	[326]
23	1R4	White flour, plain	130	[323]
24	1R5	Hard wheat flour	1190	[326, 335, 336]
25	1R6	Soy flour	1398-1500	[323, 326, 335, 336]
26	1R7	Rye flour	919-1400	[326, 335, 336, 339]
27	1R8	Wheat flour (Brown/whole)	500-854	[323, 324, 346]
28	1R9	Oats	687-1250	[346]
29	1R10	Rice Bran	3011	[346]
30	1R11	Sorghum, millet or maize bran	263	[326]
31	1R12	Dumpling	40	[326]
32	1R13	Yorkshire pudding	40	[325, 326, 339, 346, 347]
2	White bread			
	2R	**White bread**	**70**	
Sliced, unsliced, toast, fried; includes French stick, milk loaf, slimmers, pitta bread, rolls, chappatis, soda bread				
33		White bread (average, fried or toasted)	30-277	[326, 335, 336]
34	2R1	White roll (crusty or soft)	50-64	[335-337]
35	2R2	White bread (enriched)	69	[326]
36	2R3	Pitta Bread	123	[326, 346]
37	2R4	Soda Bread	90-169	[346]

No	Code	Food	Phytate mg/100g	Reference
38	2R5	White Bread, Arabic, 30% extraction	30	[334-336]
39	2R6	French bread	17-20	[326, 330]
40	2R7	Chappatis, made without fat	254-370	[325, 326, 347]
3	Wholemeal bread			
	3R	**Wholemeal bread**	**350**	
Sliced, unsliced, toast, fried; includes chappatis, pitta bread, rolls, hi-bran bread, wholemeal breads				
41	3R1	Whole meal bread (average, fried or toasted)	358-600	[335, 336]
42	3R2	High fibre bread	232	[325, 335, 336]
43	3R3	Whole meal rolls	60-285	[330]
44	3R4	Dark flour Chappatis	341	[335, 336]
45	3R5	All bran loaf	520	[335-337, 346]
46	3R6	Whole wheat bread	334-845	[325, 326]
4	Other breads			
	4A	**Soft grain bread**	**70**	
Sliced, unsliced, toast, fried, rolls, fortified and not fortified				
	4R	**Other breads**	**400**	
Sliced, unsliced, toast, fried; includes brown, granary, high fibre white, rye bread, gluten free, garlic bread, continental breads e.g. ciabatta, oatmeal bread, Vitbe, Hovis, crumpets, English muffins (white & wholemeal), pikelets, brown and granary rolls, bagels, brioche, naan, paratha				
47	4R1	Brown bread (average or toasted)	169-450	[347]
48	4R2	Granary	845	[335, 336]
49	4R3	High fibre white	79	[326, 335-337]
50	4R4	Rye bread	155-942	[326]
51	4R5	Vitbe	440	[325]
52	4R6	Soda bread, brown	458	[326]
53	4R7	Hovis (average or toasted)	250-320	[326, 335, 336]
54	4R8	English Muffins (white)	73-110	[326, 335, 336]
55	4R9	English Muffins (whole)	498-680	[326]
56	4R10	Brown rolls (soft or crusty)	350	[338]
57	4R11	Brioche	350	[326]
58	4R12	Naan	110	[326]
59	4R13	Paratha	320	[326, 335, 336]
5	Whole grain and high fibre breakfast cereals			
	5R	**Wholegrain and high fibre breakfast cereals**	**750**	
All with non-starch polysaccharide (Englyst fibre) of 4g/100g or more, e.g. All Bran, muesli, Shredded Wheat. Includes porridge and Ready Brek				
60	5R1	All bran cereal	3168-3500	[326]
61	5R2	Muesli	680	[324, 326, 335, 339]
62	5R3	Shredded wheat	940-1530	[346]
63	5R4	Porridge	666	[326]
64	5R5	Ready Brek	840	[326, 334-336, 346]
6	Other breakfast cereals			
	6R	**Other breakfast cereals**	**200**	
All with non-starch polysaccharide (Englyst fibre) of less than 4g/100g, e.g. cornflakes, Coco Pops, Sugar Puffs. Includes Pop Tarts				
65	6R1	Cornflakes	40-800	[335, 336, 347]
66	6R2	Corn Pops	94-214	[346]
7	Biscuits			
	7R	**Biscuits**	**180**	
All types, sweet and savoury; includes cream crackers, flapjacks, breadsticks, crispbread, cereal crunchy bars, ice cream cornet				
67	7R1	Biscuits	180	[346]

No	Code	Food	Phytate mg/100g	Reference
68	7R2	Salty biscuits	180	[326]
69	7R3	Flapjack	370	[326]
70	7R4	Crispbread, rye	40	[335-337, 346]
71	7R5	Cracker	84-317	[347]
72	7R6	Cereal crunchy bar	788	[326, 347]
8	**Buns, cakes, pastries and fruit pies**			
	8A	**Fruit pies**	**20**	
All types, one and two crusts; includes apple strudel, individual fruit pies from takeaways				
73	8A1	Fruit pie (one crust)	20	[326, 347]
74	8A2	Fruit pie (pastry top and bottom)	40	[335, 336]
75	8A3	Peach pie	3	[347]
	8R	**Buns, cakes and pastries**	**70**	
Includes Danish pastries, currant bun, doughnuts, Eccles cakes, Bakewell tarts, jam tarts, scones (sweet and savoury), sponge cakes, fruit cakes, éclairs, currant bread, malt loaf, gateaux, pastry, mince pies, sponge fingers, scotch pancakes, croissants, custard tart, lemon meringue pie				
76	8R1	Danish Pasties	104	[326, 335, 336]
77	8R2	Buns (including plain burger buns, Chelsea buns, hot cross buns)	30-82	[335, 336]
78	8R3	Doughnut, cake, sugar coated	366	[347]
79	8R4	Doughnut (jam or custard)	44	[326, 347]
80	8R5	Scones (sweet or savoury)	70	[347]
81	8R6	Scones, wholemeal	291	[326]
82	8R7	Éclairs	20	[326]
83	8R8	Eccles cake	40	[347]
84	8R9	Fruit mince pie	50	[326]
85	8R10	Gateaux	20	[326]
86	8R11	Scotch Pancakes	60	[347]
87	8R12	Lemon Meringue pie	30	[347]
9	**Puddings**			
	9A	**Cereal-based milk puddings**	**10**	
Rice pudding (including canned), custard (not egg custard), Angel Delight, blancmange, confectioners custard, semolina, sweet white sauce				
88	9A1	Rice pudding	38	[347]
89	9A2	Custard	0	[347]
90	9A3	Custard tarts	40	[326]
91	9A4	Confectioners custard	10	[326]
	9B	**Sponge puddings**	**40**	
Steamed, canned, suet pudding, jam roly poly, sponge flan, upside down pudding				
92	9B1	Sponge pudding (including sponge pudding with dried fruit or with jam or treacle)	40	[326]
93	9B2	Suet pudding	30	[326, 347]
	9R	**Other cereal-based puddings**	**30**	
Includes trifle, fruit fritters, pancakes, crumble, bread pudding, cheesecakes, tiramisu, rum baba, Christmas pudding				
94	9R1	Pancakes (sweet or savoury)	30	[326]
95	9R2	Cheesecake	20	[326]
96	9R3	Rum baba	20	[326]
97	9R4	Christmas pudding	20	[347]
98	9R5	Crumble, fruit	20	[347]
99	9R6	Crumble, wholemeal	75	[347]
10	**Whole milk**			
	10R	**Whole milk**	**0**	[347]
All types of cow's milk including pasteurised, UHT, sterilised, Channel Island				
11	**Semi-skimmed milk**			

No	Code	Food	Phytate mg/100g	Reference
	11R	**Semi-skimmed milk**	**0**	[346, 347]
All types of cow's milk including pasteurised, UHT, sterilised, canned, milk with added vitamins				
12	**Skimmed milk**			
	12R	**Skimmed milk**	**0**	[346]
All types of cow's milk including pasteurised, UHT, sterilised, canned, milk with added vitamins, Vital, Calcia				
13	**Other milk and cream**			
	13A	**Infant formula**	**5**	
100	13A1	Commercial infant formula e.g., Lactona, Neslac, SGM and Lactogen)	0	[335, 336]
101	13A2	Soy based infant formula	8	[346, 347]
	13B	**Cream**	**0**	[346, 347]
All types, including imitation cream, aerosol, dream topping, Tip Top, crème fraiche				
	13R	**Other milk**	**0**	[347]
Includes soya alternative to milk, goats, sheep, evaporated, condensed, dried milk, milk shake, coffee whitener, buttermilk, flavoured milk drink				
14	**cheese**			
	14A	**Cottage cheese**	**0**	[346, 347]
Includes diet and flavoured				
	14R	**Other cheese**	**0**	[347]
All types, including hard, soft, cream cheese				
15	**Yogurt, fromage frais and other dairy desserts**			
	15A	**Fromage frais**	**0**	[346, 347]
Includes fromage frais mousse, Quark				
	15B	**Yogurt**	**0**	[347]
All types including soya, goats, sheep, yogurt mousse, yogurt drink, frozen yogurt, custard style yogurt, Greek yogurt				
	15R	**Other dairy desserts**	**5**	
Includes chocolate and fruit cream desserts, mousse, milk jelly, junket, egg custard, buttermilk desserts, fruit fools, crème caramel				
102	15R1	Chocolate mousse	38	[346]
53	**Ice cream**			
	53R	**Ice cream**	**0**	[347]
All types, including non dairy, choc ices, ice cream desserts, ice cream containing lollies, milk ice lollies, low fat/low calorie ice cream				
16	**Eggs and egg dishes**			
	16A	**Eggs**	**0**	[347]
Includes boiled, fried, scrambled, poached, dried, omelettes (sweet and savoury)				
	16B	**Egg dishes**	**50**	
Includes quiches, flans, souffles, scotch eggs, eggy bread, apple snow, meringue, pavlova, curried eggs				
103	16B1	Quiches, cheese and egg	25	[347]
104	16B2	Quiches, cheese and egg, wholemeal	96	[347]
105	16B3	Creme eggs	52	[329]
106	16B4	Flan (including broccoli/ cauliflower cheese/ lentil and tomato/ cheese, onion and potato/ cheese and mushroom/ spinach/ vegetable)	20-90	[329]
107	16B5	Wholemeal flan (including broccoli/ cauliflower cheese/ lentil and tomato/ cheese, onion and potato/cheese and mushroom/ Spinach/ vegetable)	80-140	[347]
108	16B6	Scotch eggs and Cheese Soufflés	0	[346]
17	**Butter**			

No	Code	Food	Phytate mg/100g	Reference
	17R	**Butter**	**0**	[346]
Salted and unsalted, butter ghee, spreadable butter				
18	**Polyunsaturated margarine and oils**			
	18A	**Polyunsaturated margarine**	**0**	[346]
Margarine claiming to be high in polyunsaturated fatty acids				
	18B	**Polyunsaturated oils**	**0**	[346, 347]
Includes corn oil, sunflower oil, solid sunflower oil				
19	**Low fat spread**			
	19A	**Low fat spread polyunsaturated**	**0**	NA
Spreads containing 40% or less fat, claiming to be high in polyunsaturated fatty acids				
	19R	**Other low fat spread polyunsaturated**	**0**	NA
Spreads containing 40% or less fat, not claiming to be high in polyunsaturated fatty acids				
20	**Margarine and other cooking fats and oils not polyunsaturated**			
	20A	**Block margarine**	**0**	NA
All hard margarine				
	20B	**Soft margarine, not polyunsaturated**	**0**	NA
Tub margarine not claiming to be high in polyunsaturated fatty acids				
	20C	**Other cooking fats and oils, not polyunsaturated**	**0**	NA
Includes blended vegetable oil, suet, lard, compound cooking fat, dripping, olive oil, rapeseed oil				
21	**Reduced fat spread**			
	21A	**Reduced fat spread, polyunsaturated**	**0**	NA
Spreads containing more than 40% and less than 80% fat, claiming to be high in polyunsaturated fatty acids				
	21B	**Other reduced fat spread**	**0**	NA
Spreads containing more than 40% and less than 80% fat, not claiming to be high in polyunsaturated fatty acids; includes spreads made with olive oil, rapeseed oil or fish oil				
22	**Bacon and ham**			
	22R	**Bacon and ham**	**0**	[346, 347]
Including bacon and gammon joints, steaks, chops and rashers; all types of ham, pork shoulder, bacon and cheese grills				
23	**Beef, veal and dishes**			
	23R	**Beef, veal and dishes**	**2**	[346, 347]
Includes beef and veal joints, steaks, minced beef, stewing steak, beef stews, casseroles, meat balls, lasagne, chilli con carne, beef curry, bolognaise sauce, shepherds pie, canned beef				
109	23R1	Beef, veal, minced beef	0	[347]
110	23R2	Beef curry	21	[346, 347]
24	**Lamb and dishes**			
	24R	**Lamb and dishes**	**0**	[346, 347]
Includes lamb joints, chops, cutlets, fillets, lamb curries, Irish stew, lamb casseroles and stews				
25	**Pork and dishes**			
	25R	**Pork and dishes**	**2**	[347]
Includes joints, chops, steaks, belly rashers, pork stews and casseroles, sweet and sour pork, spare ribs, roast roll				
111	25R1	Pork (steak, scratching, minced, diced, casseroled, stewed, roasted)	0	[347]
112	25R2	Sweat and sour pork	10	[346, 347]
26	**Coated chicken and turkey**			
	26R	**Coated chicken and turkey**	**14**	[347]
Chicken and turkey pieces coated in egg and crumb; drumsticks, nuggets, fingers, burgers etc. Includes Kentucky Fried Chicken, chicken Kiev				
113	26R1	Chicken pie	47	[347]

No	Code	Food	Phytate mg/100g	Reference
114	26R2	Chicken Kiev	14	[347]
115	26R3	Chicken nuggets	22	[347]
116	26R4	Chicken fingers	23	[346, 347]
27	**Chicken and turkey dishes**			
	27R	**Chicken and turkey dishes**	**0**	[346, 347]
Includes roast chicken and turkey, barbecued, fried (no coating), curries, stews, casseroles, chow mien, tandoori, in sauce, spread, chicken/turkey roll				
117	27R1	Chicken (including breast, drumstick, wing, thigh)	0	[346, 347]
28	**Liver, liver products and dishes**			
	28R	**Liver, liver products and dishes**	**0**	[347]
Includes all types of liver - fried, stewed, grilled, braised; liver casserole, liver sausage, liver pate				
29	**Burgers and kebabs**			
	29R	**Burgers and kebabs**	**24**	
Includes beefburgers, hamburgers, cheeseburgers, (with or without roll) doner/shish/kofte kebabs (with or without pitta bread and salad), grillsteaks, steaklets				
118	29R1	Beefburgers	10	[347]
119	29R2	Burger, Big Mac	28	[347]
120	29R3	Burger, Cheeseburger	21	[347]
121	29R4	Burger, Hamburger	24	[347]
122	29R5	Burger, Quarterpounder	36	[347]
123	29R6	Burger, Whopper	35	[347]
30	**Sausages**			
	30R	**Sausages**	**20**	[347]
Includes beef, pork, turkey sausages, polony, sausage in batter, saveloy, frankfurters, sausage dishes				
124	30R1	Pork sausage	20	[347]
31	**Meat pies and pastries**			
	31R	**Meat pies and pastries**	**33**	
Any type of meat; includes chicken/turkey pies, vol-au-vents, beef pies, steak and kidney pudding, pork pies, veal and ham pie, pasties, sausage roll, meat samosas, pancake rolls				
125	31R1	Meat samosas (e.g., lamb samosas)	33	[347]
126	31R2	Pork pies	44	[347]
127	32R3	Chicken pie	47	[347]
128	33R4	Steak and kidney pie	21	[347]
129	33R5	Beef pie	36	[346, 347]
32	**Other meat and meat products**			
	32R	**Other meat and meat products**	**0**	[347]
Includes game (e.g. venison, grouse, rabbit, pheasant), duck, goose, all offal (except liver), faggots, black pudding, haggis, haslet, meat paste, tongue, luncheon meats, corned beef, salami, pepperami, meat loaf				
33	**White fish coated and/or fried including fish fingers**			
	33R	**White fish coated and/or fried including fish fingers**	**13**	
Cod, haddock, plaice, etc. fried without coating, or coated in egg and crumb, batter or flour and fried, grilled or baked. Includes fish fingers and fish cakes - fried and grilled, fried cartilaginous fish, scampi, filet-o-fish, cod roe fried, prawn balls, fish feasts, fish pancakes				
130	33R1	Fish fingers	10	[347]
131	33R2	Fish cakes	13	[347]
132	33R3	Fisherman's pie	23	[347]
133	33R4	Scampi, in crumbs	27	[347]
134	33R5	Cod, battered	13	[347]
135	33R6	Plaice, battered	23	[347]
136	33R7	Plaice, in crumbs	10	[347]
34	**Other white.fish, shellfish and fishes**			

No	Code	Food	Phytate mg/100g	Reference
	34A	**Other white fish and fish dishes**	**2**	
Cod, haddock, plaice etc. poached, steamed, baked, grilled, smoked, dried; includes curried fish, fish in sauce, fish pie, kedgeree				
137	34A1	Fish pie	10	[346, 347]
138	34A2	Cod, haddock, plaices, etc (not coated or fried)	0	[347]
	34B	**Shellfish**	**0**	[346, 347]
All types including mussels, prawns, crabs, shellfish dishes				
	35R	**Oily fish**	**2**	
Includes herrings, kippers, mackerel, sprats, eels, herrings roe (baked, fried, grilled), salmon, tuna, sardines, trout, taramasalata, mackerel pate, fish paste				
139	35R1	Rock Salmon/Dogfish, battered	23	[346, 347]
140	35R2	Herring. tuna, trout (steamed, grilled, canned)	0	[323, 334-336, 346, 347]
36	**Salad and other raw vegetables**			
	36A	**Carrots raw**	**9**	
141	36A1	Carrot raw	5-76	[329]
	36B	**Salad and other vegetables (raw)**	**30**	
All types of raw vegetables, including coleslaw, fresh herbs. Not salads made with cooked vegetables or potato salad				
142	36B1	Salad, carrot and nut with French dressing	110	[329]
143	36B2	Salad, carrot and nut with mayonnaise	80	[329]
144	36B3	Salad, Florida	Trace	[329]
145	36B4	Salad, Greek	20	[329]
146	36B5	Salad, green	30	[329]
147	36B7	Salad, tomato and Onion	20	[347]
148	36B8	Salad, vegetable	10	[347]
	36C	**Tomatoes (raw)**	**52**	[334, 346, 347]
149	36C1	Tomatoes (including green, ripe and cherry tomatoes)	4-52	[335, 336, 347]
37	**Vegetables (not raw)**			
	37A	**Peas (not raw)**	**86**	
Includes canned, dried, mushy, frozen, mange tout, pease pudding canned				
150	37A1	Peas, boiled	20-28	[347]
151	37A2	Mushy peas	86	[347]
152	37A3	Mange-tout peas, stir-fried	10	[347]
153	37A4	Split peas	250	[327]
154	37A5	Petit pois, frozen and boiled	20	[341, 346]
155	37A6	Cow peas	358-970	[327, 346]
156	37A7	Pigeon peas, dried and boiled	290-1154	[327, 334-336]
	37B	**Green beans (not raw)**	**50**	
Includes French, runner, green beans; fresh, canned, frozen				
157	37B1	Green beans/French beans, boiled	10-91	[327]
	37C	**Baked beans**	**160**	
Canned baked beans in sauce. Includes baked beans with additions e.g. sausages, burgers, pasta				
158	37C1	Baked beans	160	[327]
159	37C2	Baked beans (reduced sugar, reduced sugar and reduced salts or re-heated)	170	[327]
160	37C3	Baked beans, with burgers	130	[327]
161	37C4	Baked beans, with sausages	120	[347]
	37D	**Leafy green vegetables (not raw)**	**10**	
Includes broccoli, spinach, cabbage (all types), Brussels sprouts; fresh and frozen				
162	37D1	Broccoli, boiled	18	[334, 347]
163	37D2	Spinach, cabbage or Brussels sprouts,	0-7	[346]

No	Code	Food	Phytate mg/100g	Reference
		boiled		
	37 E	**Carrots (not raw)**	**9**	[334, 346]
Includes fresh, frozen, canned				
164	37E1	Carrots, boiled	9-16	[335-337]
	37F	**Tomatoes (not raw)**	**60**	
Includes fried, grilled, canned, sundried tomatoes				
165	37F1	Tomatoes, canned	6	[327, 347]
166	37F2	Tomatoes, grilled	60	[347]
167	37F3	Tomatoes, fried	57	[347]
168	37F4	Tomatoes, sundried	320	[329]
	37G	**Vegetable dishes (not raw)**	**100**	
Includes curries, pulse dishes, casseroles and stews, pies, vegetable lasagne, cauliflower cheese, veggieburgers, bubble and squeak, vegetable samosas, pancake rolls, ratatouille, vegetable fingers etc.				
169	37G1	Curry (various dishes)	10-300	[329]
170	37G2	Casseroles (various dishes)	10-70	[329]
171	37G3	Cauliflower cheese	40	[329]
172	37G4	Vegetable lasagne	10-20	[329]
173	37G5	Pies (various dishes)	10-220	[329]
174	37G6	Ratatouille	20	[347]
175	37G7	Vegetable samosas	37	[347]
176	37G8	Veggieburger, retail	233	[327, 330, 338]
	37R	**Other vegetables (not raw)**	**80**	
Includes lentils, dried beans and pulses, mushrooms, onion, aubergine, parsnips, sweetcorn, peppers, leeks, courgettes, cauliflower, mixed vegetables, TVP/soya mince, quorn, tofu				
177	37R1	Lentils, dried, boiled	80-340	[327, 330, 334, 338]
178	37R2	Dried beans	80-1300	[327, 334]
179	37R3	Onions, baked or boiled	8-30	[327]
180	37R4	Mushrooms, boiled	80	[327]
181	37R5	Peppers, boiled	20	[334]
182	37R6	Sweetcorn, steamed	111	[327]
183	37R7	Sweetcorn, boiled	20	[347]
184	37R8	Leeks, boiled	5	[347]
185	37R9	Parsnip, boiled	50	[347]
186	37R10	Cauliflower, courgettes, aubergine boiled	0	[335, 336, 342]
187	37R11	Soy-based TVP, unflavoured or flavoured	884-1720	[327]
188	37R12	Tofu, steamed	290	[327]
189	37R13	Tofu, fried	840	[335, 336, 346, 347]
38	**Chips, fried and roast potatoes and potato products**			
	38A	**Chips**	**147**	
Fresh and frozen, including oven and microwave, French fries				
190	38A1	Potato Chips and potato French fries	100-196	[347]
	38B	**Fried or roast potatoes and fried potato products**	**61**	
Roast potato, fried sliced potato with or without batter, fried waffles, croquettes, crunchies, alphabites, fritters, hash browns				
191	38B1	Old potatoes, roasted	61	[347]
192	38B2	Potato waffles	39	[347]
193	38B3	Potato croquettes	34	[347]
	38R	**Potato products not fried**	**50**	
Croquettes, waffles, fritters, hash browns, alphabites, Ketchips, grilled or oven baked				
194	38R1	Old potatoes, baked	50	[329, 335, 336]
39	**Other potatoes, potato salads and dishes**			
	39R	**Other potatoes, potato salads and**	**60**	

No	Code	Food	Phytate mg/100g	Reference
		dishes		
Includes boiled, mashed, baked (with or without fat), canned, potato salad, instant potato, potato based curries, cheese and potato pie				
195	39R1	Potato salad	Trace-84	[329]
196	39R2	Potato curries, various	10-50	[347]
197	39R3	Old potatoes, mashed	20-30	[327, 335-337]
198	39R4	New potatoes, boiled	80-81	[334, 347]
199	39R5	Old potatoes, boiled	41-49	[347]
42	**Crisps and savoury snacks**			
	42R	**Crisps and savoury snacks**	**196**	
Includes all potato and cereal based savoury snacks, popcorn (not sweet), twiglets				
200	42R1	Potato crisps	196	[347]
201	42R2	Twiglets	105	[347]
202	42R3	Popcorn	630	[334, 346, 347]
43	**Fruit**			
	40A	**Apples and pears not canned**	**63**	
Includes raw, baked, stewed (with or without sugar), dried, apple sauce				
203	40A1	Apple and pear, raw	1-63	[347]
204	40A2	Apple, stewed	52	[334]
	40B	**Citrus fruit not canned**	**5**	
Includes oranges, grapefruit, limes, tangerines, etc.				
205	40B1	Citrus fruit, mandarin	50	[346, 347]
206	40B2	Oranges	0	[334, 346, 347]
	40C	**Bananas**	**3**	
Includes baked bananas, banana chips				
207	40C1	Banana, fresh	0-3	[346, 347]
	40D	**Canned fruit in juice**	**10**	
Includes canned in water				
208	40D1	Canned (apricot, cherries, grapefruit, guava, mandarin oranges, peaches, pineapple, rhubarb,	0	[346, 347]
209	40D2	Canned pear	84	[347]
	40D3	Fruit cocktail canned	35	[346, 347]
210	**40E**	**Canned fruit in syrup**	**10**	
211	40E1	Canned (apricot, cherries, grapefruit, guava, mandarin oranges, peaches, pineapple, rhubarb)	0	[346, 347]
212	40E2	Canned pear	84	[347]
213	40E3	Fruit cocktail canned	35	[347]
	40R	**Other fruit, not canned**	**60**	
Includes plums, grapes, apricots (raw and stewed) etc. fruit pie fillings, dried fruit, fruit salad				
214	40R1	Plum, raw	63	[347]
215	40R2	Apricot (raw or dried), passion fruit, cherry-like fruits,	0	[328, 501]
216	40R3	Mango	30-86	[501]
217	40R4	Guava	80	[501]
218	40R5	Pineapple	90	[328]
219	40R6	Date, raw	40	[328]
220	40R7	Date, dried	90	[328, 335, 336, 347]
221	40R8	Figs, dried	30-385	[328]
222	40R9	Raisin	10	[328, 334, 339, 344]
56	**Nuts and seeds**			
	56R	**Nuts and seeds**	**850**	
Includes fruit and nut mixes, salted peanuts, peanut butter, tahini, Bombay mix				
223	56R1	Peanuts (roasted)	680-2008	[328]
224	56R2	Peanuts and raisins	440	[337, 339, 347]

No	Code	Food	Phytate mg/100g	Reference
225	56R3	Peanut butter	443-1252	[328, 335, 336, 339, 344]
226	56R4	Almonds	970-2111	[328, 335, 336, 339, 344]
227	56R5	Cashews (roasted)	937-1229	[328, 344]
228	56R6	Macadamia nuts	290-340	[328, 335, 336, 344]
229	56R7	Walnuts	580-1977	[328, 335, 336]
230	56R8	Brazil nuts	1320-1799	[328, 335, 336]
231	56R9	Chestnuts	10-47	[328]
232	56R10	Pistachio nuts	340	[347]
233	56R11	Bombay mix	577	[328]
234	56R12	Sunflower seeds	3000	[328]
235	56R13	Sesame seeds	1380	[346]
41	**Sugars, preserves and sweet spreads**			
	41A	**Sugar**	**0**	[346, 347]
All types, including golden syrup, fructose				
	41B	**Preserves**	**0**	[347]
Includes jam, fruit spreads, marmalade, honey, lemon curd				
	41R	**Sweet spreads, fillings icings**	**25**	
Includes ice cream topping sauce, chocolate spread, mincemeat, glace cherries, mixed peel, icing, brandy/rum butter, marzipan				
236	41R1	Chocolate nut spread	308	[347]
237	41R2	Marzipan	482	[347]
238	41R3	Chocolate covered ice cream bar	33	[335-337, 346, 347]
	43R	**Sugar confectionery**	**40**	
Includes boiled sweets, gums, pastilles, fudge, chews, mints, rock, liquorice, toffees, chewing gum, sweet popcorn, ice lollies (without ice cream)				
239	43R1	Popcorn (plain)	614-800	[346, 347]
240	43R2	Toffees, fudge and gums	0	[346, 347]
44	**Chocolate confectionery**			
	44R	**Chocolate confectionery**	**125**	
Includes chocolate bars, filled bars, assortments				
241	44R1	Chocolate (plain)	84-127	[335, 336, 347]
242	44R2	Chocolate (milk)	127	[346]
243	44R3	Chocolate tablet with sugar	301	[346]
244	44R4	Chocolate tablet without sugar	1109	[347]
45	**Fruit juice**			
	45R	**Fruit juice**	**10**	
Includes 100% single or mixed fruit juices, vegetable juices, canned, bottled, cartons; carbonated, still, freshly squeezed				
245	45R1	Blackcurrant juice drink, made up	61	[346]
246	45R2	Carrot juice	9	[346]
247	45R3	Mango Juice	8	[347]
248	45R4	Tomato juice	6	[334, 346, 347]
249	45R5	Orange juice	0-8	[346, 347]
250	45R6	Fruit juice (including apple, grape, grapefruit, orange, apricot, lime, pineapple, guava)	0	[346, 347]
57	**Soft drinks, not low calorie**			
	57A	**Concentrated soft drinks, not low calorie**	**0**	[346, 347]
All types including squashes and cordials				
	57B	**Carbonated soft drinks, not low calorie**	**0**	[346, 347]
All types, including tonic water. Not carbonated mineral water; not alco-pops				

No	Code	Food	Phytate mg/100g	Reference
	57C	**Ready to drink soft drinks, not low calorie**	**0**	[346, 347]
All types of still soft drinks, not carbonated				
58	**Soft drinks, low calorie**			
	58A	**Concentrated soft drinks, low calorie**	**0**	[346, 347]
All low calorie, no added sugar, sugar free types				
	58B	**Carbonated soft drinks, low calorie**	**0**	[346]
All low calorie, no added sugar, sugar free types, including slimline tonic water. Not carbonated mineral water				
	58C	**Ready to drink soft drinks low calorie**	**0**	[347]
All low calorie, no added sugar, sugar free types. Not carbonated.				
47	**Spirits and liqueurs**			
	47A	**Liqueurs**	**0**	[347]
Includes cream liqueurs, Pernod, Southern Comfort, Tia Maria, Cherry Brandy				
	47B	**Spirits**	**0**	[346, 347]
70 % proof spirits - brandy, gin, rum, vodka, whisky				
48	**Wine**			
	48A	**Wine**	**0**	[347]
White, red, sparkling, rose				
	48B	**Fortified wine**	**0**	[347]
Port, sherry, champagne, vermouth				
	48C	**Low alcohol and alcohol free wine**	**0**	NA
Includes fruit juice and wine drinks				
49	**Beer, lager, cider and perry**			
	49A	**Beers and lagers**	**0**	[347]
Premium and non premium, stout, strong ale (bottled, draft and canned)				
	49B	**Low alcohol and alcohol free lager and beer**	**0**	NA
Includes shandy				
	49C	**Cider and perry**	**0**	[347]
Includes Babycham				
	49D	**Low alcohol and alcohol free cider and perry**	**0**	[335, 336, 346, 347]
	49E	**Alco-pops**	**0**	NA
Includes alcoholic lemonade				
51	**Tea, coffee and water**			
	51A	**Coffee (made up)**	**10**	
Includes instant and leaf bean, decaffeinated, vending machine with whitener, coffee essence				
251	51A1	Coffee (instant)	1-387	[339, 346, 347]
252	51A2	Infusion (infusion)	6-7	[346, 347]
	51B	**Tea (made up)**	**2**	
Infusion, instant, decaffeinated, vending machine with whitener				
253	51B1	Tea (black)	0-8	[346]
	51C	**Herbal tea (made up)**	**2**	
Includes fruit teas				
254	51C1	Tea (herbal)	0-2	[347]
255	51C2	Tea (camomile, lemon, Spearmint,, orange, green Chinese, cinnamon, fenugreek, tamarind and anis)	0	[347]
	51D	**Bottled water**	**0**	NA
Includes carbonated and still, herbal tonics, (not sweetened drinks or tonic water)				
	51R	**Tap water**	**0**	[347]
Includes tap water as a drink or used as a diluent for powdered beverages only. Includes filtered tap water. Not water as a diluent for concentrated soft drinks, instant coffee or instant				

No	Code	Food	Phytate mg/100g	Reference
tea				
50	**Miscellaneous**			
	50A	**Beverages (dry weight)**	**500**	
Includes drinking chocolate, cocoa, ovaltine, horlicks, malted drinks etc.				
256	50A1	Drinking chocolate powder	508	[347]
257	50A2	Horlicks powder	285	[335, 336, 347]
258	50A3	Ovaltine powder	111	[335, 336]
259	50A4	Cocoa powder	1880	[335, 336]
260	50A5	Hot chocolate, instant	1	[346]
	50B	**Soups**	**30**	
Includes homemade, dried, condensed, cartons, canned				
261	50B1	Soup (tomato)	6	[347]
262	50B2	Soup (pasta)	22	[347]
263	50B3	Soup (lentil)	35	[347]
264	50B4	Soup (minestrone)	20	[347]
265	50B5	Soup (pea and ham)	161	[347]
266	50B6	Soup (scotch broth)	25	[347]
267	50B7	Soup (potato and leek)	16	[347]
268	50B8	Soup (including bouillabaisse, cream of chicken soup, French onion, cream of mushroom soup and oxtail)	0	[346, 347]
	50R	**Savoury sauces, pickles, gravies, condiments**	**40**	
Includes white sauces, cook in sauces, sauce mixes, tomato ketchup, pickles, chutney, stuffing, gravy, mayonnaise, salad cream, dried herbs, spices				
269	50R1	Mango Chutney	12	[346, 347]
270	50R2	Tomato Chutney	6	[346]
271	50R3	Pickle (Herring, sweet, beetroot, eggplant, garlic, lettuce, onion, radish, spinach, turnip, cabbage, cauliflower, cucumber)	0	[347]
272	50R4	Pickle (parsley)	29	[346]
273	50R5	Pickle (pepper)	30	[346]
274	50R6	Pickle (white potato)	72	[346]
275	50R7	Pickle (chilli including Jalapeno, Serrano and Pilipili)	35	[346]
276	50R8	Pickle (carrot)	8	[329]
277	50R9	Pickle (chicory)	34	[329]
278	50R10	Sauce (curry including onion with butter or vegetable oil	20	[346]
279	50R11	Sauce (sweet curry)	10	[346]
280	50R12	Sauce (green)	42	[329, 346]
281	50R13	Sauce (spicy mole)	48	[346]
282	50R14	Sauce (tomato and mushroom or tomato base)	9-10	[335, 336, 346, 347]
283	50R15	Sauce (hot chilli)	129	[347]
284	50R16	Tomato Ketchup	7	[346]
285	50R17	Mayonnaise	0	[326, 334]
286	50R18	Dried pepper, red or hot	195-403	[346]
287	50R19	Stuffing (sage and onion)	30	[322, 335, 336]
288	50R20	Spices (including mixed spices, noodle spices and spice's leaf)	0-281	[345, 502]
52	**Commercial toddlers food and drinks**			
	52A	**Commercial toddlers drinks**	**20**	
Includes powdered, concentrated and ready to drink beverages specifically manufactured for young children				
289	52A1	Commercial milk based cereal drink	20	[322, 335, 336]

No	Code	Food	Phytate mg/100g	Reference
	52R	**Commercial toddlers foods**	**750**	
Includes instant and ready to eat foods specifically manufactured for young children				
290	52R1	Barley infant cereal	858-1000	[322, 335, 336]
291	52R2	Oatmeal infant cereal	1000-1071	[322, 335, 336]
292	52R3	Rice infant cereal	366-980	[322]
293	52R4	Mixed grain infant cereal	698-809	[322]
294	52R5	Mixed grain infant cereal with fruit	860	[346]
295	52R6	Soya based infant cereal	1138	[346]
296	52R7	Infant cereal (Cerelac, Milna, Promina, Sun)	290	[346]
297	52R8	Infant food (chicken with cereal and vegetable)	29	[346]
298	52R9	Infant food (vegetable with liver)	16	[347]
54	**Dietary supplements**			
	54A	**Tablets and capsules**	**0**	
Vitamin and mineral tablets and capsules; includes cod liver oil and other oil based capsules				
	54B	**Oils and syrups**	**0**	
Includes cod liver oil etc (not capsules), malt extract, multivitamin syrups, iron syrups and tonics				
299	54B1	Cod liver oil	0	[347]
	54C	**Drops and powders**	**0**	
Includes cold relief powders with vitamin C, multivitamin drops				
	54R	**Nutritionally complete supplements**	**0**	
Liquid or powdered supplement drinks containing protein/fat/carbohydrate plus vitamins/minerals. Includes Complan, Build Up, Fortisip, Ensure, Provide, Fresubin				
300	54R1	Complan powder	0	
	55R	**Artificial sweeteners**	**0**	
Includes granulated table top sweeteners, tablet, liquid or mini cube sweeteners				

Table B1 Phytate content of foods

Appendix C: Health and Nutritional indices of population who had zinc intake below revised LRNI compared with the ones who had zinc intake above revised LRNI

Index of health and nutrition	Unit	Mean value for the group at risk of inadequate zinc intake	Mean value for group with normal zinc intake	2-tailed probability
Blood Analytes				
Water-soluble vitamins				
Plasma Vitamin C	µmol/l	52.7	59.9	0.000
Serum Folate	nmol/l	17.3	22.1	0.000
Red cell Folate	nmol/l	518.6	614.2	0.000
Plasma Vitamin B_{12}	pmol/l	287.2	421.9	0.000
ETKAC	µmol/g Hb/min	1.13	1.12	0.002
ETK-B	µmol/g Hb/min	0.816	0.866	0.000
EGRAC	µmol/g Hb/min	1.54	1.43	0.000
EAATAC	µmol/g Hb/min	1.82	1.79	0.034
Fat-soluble vitamins				
Plasma Retinol	µmol/l	1.41	1.29	0.000
Plasma Retinyl palmitate	µmol/l	0.107	0.117	0.046
Plasma α-Carotene	µmol/l	0.040	0.050	0.001
Plasma β-Carotene	µmol/l	0.282	0.325	0.015
Plasma α-Cryptoxanthin	µmol/l	0.055	0.061	0.015
Plasma 25-OHD	nmol/l	48.41	52.49	0.009
Blood Lipids				
Total Cholesterol	Mmol/l	3.97	4.16	0.004
HDL Cholesterol	Mmol/l	1.22	1.29	0.009
Plasma Triglycerides	Mmol/l	1.05	0.91	0.000
Other analytes				
Plasma Zinc	µmol/l	14.23	14.78	0.001
Plasma ALP	IU/l	147.9	234.4	0.000
Plasma Creatinine	µmol/l	58.11	53.53	0.000
Plasma urea	Mmol/l	4.1	4.6	0.000
Eosinophils count	$\times 10^9$/l	0.20	0.30	0.000
Neutrophils count	$\times 10^9$/l	3.0	2.8	0.003
Monocytes count	$\times 10^9$/l	0.44	0.41	0.016
Hb	g/dl	13.6	13.2	0.000
MCV	fl	91.0	88.1	0.000
TIBC	µmol/l	62.0	60.7	0.038
Anthropometric Indices				
Mean Weight	kg	55.1	37.8	0.000
Mean Height	cm	161.0	139.1	0.000
Body Mass Index	Kg/m²	21.0	18.5	0.000
MUAC	cm	25.3	22.0	0.000
Indices of Blood Pressure				
Mean Systolic BP	mmHg	112.2	107.3	0.000
Mean Arterial Pressure	mmHg	75.0	72.8	0.000
Urinary Analytes				
Urinary Na/Cr ratio	Mol/mol	12.0	14.8	0.000
Urinary K/Cr ratio	Mol/mol	3.7	5.1	0.000
Urine Creatinine	Mmol/l	14.64	11.48	0.000
Zinc food sources				
Beef, veal & dishes	gr7 days	93.3	140.2	0.000
High fibre & whole grain breakfast cereals	g/7 days	41.4	104.7	0.000
Buns, cakes & pastries	g/7 days	107.3	162.7	0.000
Biscuits	g/7 days	89.5	132.8	0.000
Wholemeal breads	g/7 days	29.3	49.9	0.000

Index of health and nutrition	Unit	Mean value for the group at risk of inadequate zinc intake	Mean value for group with normal zinc intake	2-tailed probability
Whole milk	g/7 days	259.5	648.2	0.000
Semi-skimmed milk	g/7 days	468.7	709.2	0.000
Other foods				
Rice	g/7 days	167.8	116.7	0.007
Soft margarine, not polyunsaturated	g/7 days	10.4	7.2	0.014
Potato chips	g/7 days	408.1	311.5	0.000
Sugar	g/7 days	42.6	33.8	0.021
Spirits	g/7 days	7.8	1.5	0.045
Beers	g/7 days	531.1	89.6	0.000
Coffee	g/7 days	510.1	230.4	0.000
Tea	g/7 days	747.9	480.9	0.002
Nutrients				
Phytate intake	mg/day	591.8	723.2	0.000
Phytate density	mg/1000 kcal	386.3	409.9	0.011
Zinc density	mg/1000 kcal	3.28	3.79	0.000
Phytate: zinc molar ratio		11.85	11.07	0.005

Table C1 The average values of indices of blood, anthropometry, blood pressure, urine and dietary intake for young people aged 4-18 years who had zinc intake below the revised LRNI compared with the population who had zinc intake above the revised LRNI.

Index of health and nutrition	Unit	Mean value for the group at risk of inadequate zinc intake	Mean value for group with normal zinc intake	2-tailed probability
Blood Analytes				
Water-soluble vitamins				
Plasma Vitamin C	µmol/l	54.7	58.8	0.009
Serum Folate	nmol/l	21.0	23.4	0.000
Red cell Folate	nmol/l	664.4	739.6	0.000
Serum Vitamin B12	Pmol/l	279.4	315.8	0.000
ETKAC	µmol/g Hb/min	1.15	1.14	0.032
EGRAC	µmol/g Hb/min	1.41	1.35	0.000
Homocysteine	µmol/l	11.5	10.1	0.000
Fat-soluble vitamins				
Plasma retinol	µmol/l	1.90	1.96	0.032
Plasma Retinyl palmitate	µmol/l	0.024	0.033	0.000
Plasma α-Carotene	µmol/l	0.068	0.082	0.001
Plasma β-Carotene	µmol/l	0.242	0.306	0.000
Plasma β-Cryptoxanthin	µmol/l	0.122	0.150	0.000
Plasma Lycopene	µmol/l	0.436	0.505	0.000
Plasma Lutein	µmol/l	0.264	0.306	0.000
Plasma 25-OHD	nmol/l	47.13	51.90	0.000
Plasma α-Tocopherol	µmol/l	20.59	21.93	0.001
Plasma γ-Tocopherol	µmol/l	1.202	1.267	0.005
Other analytes				
Plasma Selenium	µmol/l	1.086	1.136	0.000
Red cell selenium	µmol/l	1.649	1.728	0.007
Plasma α-ACT	g/l	0.310	0.301	0.021
Anthropometric Indices				
Mean weight	kg	74.8	78.3	0.000
Mean height	cm	168.1	169.3	0.007
Body Mass Index	Kg/m²	23.7	25.8	0.000
Waist circumference	cm	87.6	90.0	0.000

Index of health and nutrition	Unit	Mean value for the group at risk of inadequate zinc intake	Mean value for group with normal zinc intake	2-tailed probability
Hip circumference	cm	95.2	101.1	0.000
Waist/Hip Ratio		0.839	0.848	0.042
Urinary Analytes				
Urinary Sodium	Mmol/l	102.5	92.5	0.000
Urinary Sodium	Mmol/24hr	149.1	169.7	0.000
Mean urine weight	kg	1.67	2.03	0.000
Urinary Creatinine	Mmol/l	9.67	8.63	0.000
Urinary urea	Mmol/24hr	275.7	360.5	0.000
Urinary potassium	Mmol/24hr	65.6	79.2	0.000
Dietary Indices				
Zinc food sources				
Beef, veal & dishes	g/7 days	210.0	348.6	0.001
Chicken, turkey & dishes	g/7 days	305.4	353.0	0.007
Burger & kebabs	g/7 days	51.3	74.7	0.005
Bacon & ham	g/7 days	91.1	123.9	0.000
White bread	g/7 days	436.2	496.9	0.002
Wholemeal bread	g/7 days	82.9	143.2	0.000
Other breads	g/7 days	102.6	158.9	0.000
Breakfast cereals	g/7 days	93.1	225.8	0.000
Cheese	g/7 days	81.3	119.1	0.000
Semi-skimmed milk	g/7 days	708.6	1097.4	0.000
Other foods				
Sugar	g/7 days	87.6	70.5	0.029
Savoury snacks	g/7 days	54.6	47.4	0.048
Carbonated soft drinks	g/7 days	614.0	506.4	0.047
Nutrients				
Phytate intake	mg/day	766.3	1118.6	0.000
Phytate density	mg/1000 kcal	488.7	545.0	0.000
Zinc density	mg/1000 kcal	4.16	4.99	0.000
Phytate: zinc molar ratio		10.92	10.33	0.016

Table C2 The average values of indices of blood, anthropometry, blood pressure, urine and dietary intake for adults aged 19-64 years who had zinc intake below the revised LRNI compared with the population who had zinc intake above the revised LRNI.

Index of health and nutrition	Unit	Mean value for the group at risk of inadequate zinc intake	Mean value for group with normal zinc intake	2-tailed probability
Blood Analytes				
Water-soluble vitamins				
Plasma Vitamin C	µmol/l	35.9	43.8	0.000
Serum Folate	nmol/l	14.1	16.5	0.000
ETKAC	µmol/g Hb/min	1.17	1.16	0.041
EGRAC	µmol/g Hb/min	1.32	1.29	0.001
Fat-soluble vitamins				
Plasma α-Carotene	µmol/l	0.062	0.080	0.000
Plasma β-Carotene	µmol/l	0.333	0.379	0.002
Plasma β-Cryptoxanthin	µmol/l	0.126	0.159	0.000
Plasma Lycopene	µmol/l	0.244	0.280	0.004
Plasma Lutein	µmol/l	0.340	0.391	0.000
Plasma 25-OHD	nmol/l	48.41	52.49	0.009
Plasma γ-Tocopherol	µmol/l	2.443	2.280	0.021
Blood Lipids				
Total cholesterol	Mmol/l	5.93	5.76	0.044

Index of health and nutrition	Unit	Mean value for the group at risk of inadequate zinc intake	Mean value for group with normal zinc intake	2-tailed probability
Non-HDL cholesterol	Mmol/l	4.67	4.50	0.039
Other analytes				
Plasma zinc	µmol/l	13.89	14.10	0.158
Plasma creatinine	µmol/l	90.6	84.0	0.004
Plasma α-ACT	g/l	0.405	0.391	0.018
WBC	$\times 10^9$/l	6.9	6.5	0.000
Neutrophils	$\times 10^9$/l	4.0	3.7	0.003
Monocytes	$\times 10^9$/l	0.52	0.48	0.006
Basophils	$\times 10^9$/l	0.08	0.07	0.047
Anthropometric Indices				
Hip circumference	cm	81.6	87.8	0.002
MUAC	cm	28.9	29.6	0.000
Indices of Blood Pressure				
Mean systolic BP	mmHg	152.8	148.6	0.002
Mean Diastolic BP	mmHg	78.3	76.8	0.049
Mean arterial pressure	mmHg	103.1	100.7	0.006
Urinary Analytes				
Urinary Sodium	Mmol/l	98.6	93.8	0.049
Urinary K/Cr ratio	Mol/mol	4.9	5.2	0.029
Dietary Indices				
Zinc food sources				
Beef, veal & dishes	g/7 days	106.4	257.6	0.000
Bacon & ham	g/7 days	84.3	95.5	0.000
Lamb & dishes	g/7 days	37.9	58.7	0.000
High fibre and whole grain breakfast cereals	g/7 days	135.6	336.3	0.000
Wholemeal bread	g/7 days	86.3	219.6	0.000
White bread	g/7 days	429.3	338.6	0.000
Whole milk	g/7 days	752.7	921.0	0.003
Semi-skimmed milk	g/7 days	587.8	973.0	0.000
Cheese	g/7 days	53.6	76.4	0.000
Other foods				
Sugar	g/7 days	142.2	111.7	0.000
Beers	g/7 days	613.9	388.1	0.015
Nutrients				
Phytate intake	mg/day	563.3	948.0	0.000
Phytate density	mg/1000 kcal	397.4	536.8	0.000
Zinc density	mg/1000 kcal	4.06	5.22	0.000
Phytate: zinc molar ratio		9.76	10.36	0.036

Table C3 The average values of indices of blood, anthropometry, blood pressure, urine and dietary intake for adults aged 65 years and over who had zinc intake below the revised LRNI compared with the population who had zinc intake above the revised LRNI.