Human Vitamin and Mineral Requirements

Report of a joint FAO/WHO expert consultation Bangkok, Thailand

Food and Agriculture Organization of the United Nations

World Health Organization

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Foreword

The report of this *joint FAO/WHO expert consultation on human vitamin and mineral requirements* has been long in coming. The consultation was held in Bangkok in September 1998, and much of the delay in the publication of the report has been due to controversy related to final agreement about the recommendations for some of the micronutrients. *A priori* one would not anticipate that an evidence based process and a topic such as this is likely to be controversial. But the reality is that there is surprisingly little data on specific health status indicators on which to draw conclusions, whereas there is more information in relation to overt deficiency disease conditions. The recommended intakes are therefore largely based on the interpretation of the best available scientific information, and this leaves the door open for differences in interpretation.

A fundamental point is that when we look at recommended nutrient intakes (RNIs) in industrialized countries over the last 25 years, the levels for some of the micronutrients have been gradually increasing. The question is whether this comes from better scientific knowledge and understanding of the biochemical role of the nutrients, or whether the criteria for setting the levels of the requirements have changed. Even if the scientific knowledge base has expanded, it appears that the basic criteria for deciding on levels to recommend may bear more of the responsibility. Whereas RNIs for vitamins and minerals were initially established on the understanding that they are meant to meet the basic nutritional needs of over 97 percent of the population, a fundamental criterion in industrialized countries has become one of presumptive role these nutrients may play in the "prevention" against an increasing range of disease conditions that characterise these populations. The latter approach implies the notion of "optimal nutrition", and this may be insidiously pushing requirements to higher levels time and again.

This shift in the goal for setting RNIs is not without reason. The populations that are targeted for prevention through "optimal nutrition" are characterised by sedentary lifestyles and longer life expectations. The populations of developed countries are ageing, and the concern for the health of the ageing has become prominent. By contrast the micronutrient needs of developing countries as a whole have not really changed, and are more appropriately described as those that will satisfy basic needs of younger populations that are physically active. This, nevertheless, is not to deny the double burden of malnutrition, which is rapidly rising in many developing countries, and one needs to also bear that in mind.

The concern raised about possible differences in micronutrient needs of populations with different lifestyles is for a very practical reason. The logic behind the establishment of micronutrient needs of industrialized nations has come about at the same time that there is a large and growing demand for a variety of supplements of all kinds, and manufacturers have responded quickly to meet this market. This phenomenon could be skewing our strategy for nutritional development, with a tendency to want to resolve micronutrient deficiency problems of developing countries through the use of supplements and fortification strategies, rather than through increasing the consumption of an adequate and varied diet. Higher levels of RNIs in developed countries can easily be supported because they can be met with supplementation in addition to food. But when it becomes difficult to meet some of the micronutrient needs of developing countries by consuming locally available food, there is a problem.

The nutrients of concern currently are, first, calcium, for which the RNI may be difficult to meet without dairy products. The recently revised US/Canada Dietary Reference Intakes (DRIs) only propose an Acceptable Intake (AI) for calcium, instead of a Recommended Daily Allowance (RDA), in recognition of the fact that intake data is out of step with the relatively high intake requirements observed with experimentally derived values. Another is iron, particularly during pregnancy, where supplementation appears to be essential during the second half of pregnancy. Folic acid requirements are doubled for women of child-bearing age to prevent the incidence of neural tube defects in the foetus. Conversion factors for carotenoids are under review, with the looming conclusion that servings of green leafy vegetables needed to meet vitamin A requirements would probably need to be at least doubled. In view of this uncertainty, we have only provided for "recommended safe intakes" rather than RNIs. Selenium is undergoing growing interest because of its properties as an antioxidant. The RNIs recommended from the FAO/WHO process for this micronutrient are in a lower range than that provided by the US/Canada process because the latter is calculated on a cellular basis, whereas the former are based on the more traditional whole body estimates.

Are these "developments" or "new understandings" appropriate and applicable for developing countries? There is no clear answer based on our current knowledge, but the time may be coming when RNIs will need to be seen differently for developing countries, and based on *developing* country data. There may be a need to identify some biomarkers that are specific to developing country conditions. There is therefore an urgent need for research to be carried out in developing countries about *their* nutrient needs. The current situation also implies that the RNIs for the micronutrients of concern discussed above will need to be re-evaluated as soon as significant additional data becomes available.

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Chapter 1
Introduction

A Joint Food and Agriculture Organization of the United Nations/World Food Organization of the United Nations *(FAO/WHO) expert consultation on human vitamin and mineral requirements* was held in the FAO Regional Office for Asia and the Pacific, Bangkok, Thailand, from 21 to 30 September 1998. The purpose of the consultation was to complement the information, which had been provided through previous consultations for different nutrients, in order to provide current knowledge on all essential nutrients as the first step towards the production of a new edition of the *manual on human nutritional requirements.*

Background

The Secretariat of the FAO and WHO organized the *joint FAO/WHO expert consultation on human vitamin and mineral requirements* to review the FAO/WHO micronutrient requirements and to develop recommended nutrient intakes. The report of the Expert Consultation (this report) is being published as a technical document, and this will later serve as the basis for developing a revised edition of the *FAO/WHO handbook of human nutrition.*

Consultations such as this one are part of a continuing commitment by both FAO and WHO to promote a reliable, nutritious, and safe food supply and to provide scientifically sound nutritional advice to Member Nations. This commitment was recently reaffirmed by the World Food Summit in November 1996 in Rome.

Written documents providing the criteria that were used in the past to develop the FAO/WHO RNIs were made available to the experts. These documents included the 1974 *FAO/WHO handbook on human nutritional requirements*, the 1988 *FAO/WHO expert consultation report on the requirements for vitamin A, iron, folate, and vitamin* B_{12}, and the 1996 *WHO/FAO/IAEA report on trace elements in human nutrition and health*.

For the purpose of preparation of the background papers, the following working definition of a recommended nutrient intake (RNI) was used. The RNI is the intake level sufficient to meet the daily nutrient requirements of most individuals in a specific life-stage and gender group and is based on an estimated average nutrient requirement (EAR) plus two standard deviations above the mean: $RNI = EAR + 2SD_{EAR}$.

Terms of reference and process

The terms of reference for the Expert Panel were the following:

- To review the full scope of vitamin and minerals requirements, including their role in normal human physiology and metabolism and in deficiency disease conditions. To focus on the requirements of the essential vitamins and minerals, including vitamins A, C, D, E, and K; the B vitamins; calcium; iron; magnesium; zinc; selenium; and iodine.
- To draft and adopt a report which would provide recommended nutrient intakes for vitamins A, C, D, E, and K; the B vitamins; calcium; iron; magnesium; zinc; selenium; and iodine. The report would provide practical advice and recommendations which will constitute an authoritative source of information to all those from member countries who work in the area of nutrition, agriculture, food production and distribution, and health

promotion. This report will form, in large part, the basis for a new edition of the FAO/WHO Handbook on Human Nutritional Requirements, which was published in 1974 and last re-issued in 1980.

- To identify key issues for future research and make preliminary recommendations for the handbook.

The presentations addressed changes in the science base for each of the essential vitamin and mineral nutrients from the time of the most recent FAO/WHO review of those nutrients. The Expert Panel made recommendations for the nutrient requirements, identified key issues for future research, and made preliminary recommendations for the handbook.

Definitions of terms used

The following definitions relate to the nutrient intake from food (including water) that is required to prevent deficiency conditions. Upper limits of nutrient intake are defined for specific vitamins and minerals where there is a potential problem with excess.

Requirement

A requirement is an intake level, which will meet specified criteria of adequacy, preventing risk of deficit or excess. These criteria include a gradient of biological effects related to the nutrient intake. This dose response will be assumed to have a Gaussian distribution unless it is known to be otherwise. A risk function (a probability of 0 to 1) of deficiency and excess can be derived (***Figure 1***).

The relevance of the biological effects starts with the most extreme case, that is, the prevention of death. For nutrients where sufficient data on mortality are not available, the nutrient intake that prevents clinical disease or sub-clinical pathological conditions, identified by biochemical or functional assays, is used. The next sets of biomarkers that are used to define requirements include measures of nutrient stores or critical tissue pools. Intakes to assure replete body stores are important when deficiency conditions are highly prevalent. Presently, approaches to define requirements of most nutrients use several criteria examined in combination, functional assays of sub-clinical conditions are considered the most relevant. These biomarkers ideally should be sensitive to changes in nutritional state while at the same time be specific in terms of identifying sub-clinical deficiency conditions. The use of nutrient balance to define requirements has been avoided whenever possible. However, in the absence of other criteria it has been used. In most cases, balance based on input-output measurements are greatly influenced by level of intake, that is, subjects adjust to high intakes by increasing output, conversely they lower output when intake is low. Thus, if sufficient time is provided balance can be achieved at multiple levels of intake. The same can be said of nutrient blood levels, they usually will reflect level of intake and absorption rather than functional state. Unless balance or plasma level is related to abnormal function or disease conditions, they are inadequate for use as a criteria to support the definition of requirements. Where relevant, requirement estimates should include allowance for variations in bio-availability.

Recommended nutrient intake

Recommended nutrient intake (RNI) is the daily intake, which meets the nutrient requirements of almost all (97.5 percent) apparently healthy individuals in an age and sex-specific population group. Daily intake corresponds to the average over a period of time. Criteria to establish requirements used in this report will be nutrient specific. The estimation of RNI starts with the definition of the criteria for requirement and adds corrections for physiologic and dietary factors. The average requirement value obtained from a group of individuals is then adjusted for inter-individual variability. If the distribution of values is not

known, a Gaussian distribution is assumed, that is, a mean plus 2 SD is expected to cover 97.5 percent of the population. If the SD is not known, a value based on each nutrient's physiology is used. In most cases a variation in the range of 10-12.5 percent was assumed; exceptions are noted within chapters. The definition of RNI used in this report is equivalent to that of recommended dietary allowance (RDA) as used by the Food and Nutrition Board of the US National Academy of Sciences (*1*).

Apparently healthy

Apparently healthy refers to the absence of disease based on clinical signs and symptoms and function, normally assessed by routine laboratory methods and physical evaluation.

Upper tolerable nutrient intake level

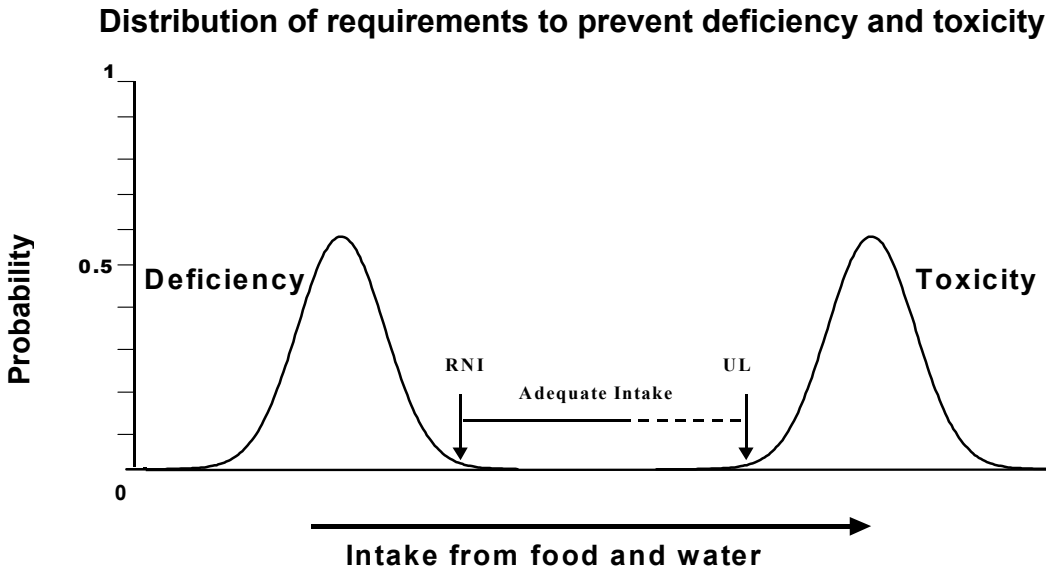
Upper tolerable nutrient intake levels (ULs) have been defined for some nutrients. ULs are the maximum intake from food that is unlikely to pose risk of adverse health effects from excess in almost all (97.5 percent) apparently healthy individuals in an age and sex-specific population group. ULs should be based on long-term exposure from food, including fortified food products. For most nutrients no adverse effects are anticipated when they are consumed as foods, because their absorption and or excretion are regulated. The special situation of consumption of nutritional supplements which when added to the nutrient intake from food may exceed the UL will be addressed in the specific chapters. The ULs as presented here do not meet the strict definition of no observed effect level used in health risk assessment by toxicologists because in most cases a dose-response curve for risk from total exposure to a nutrient will not be available. For more details on how to derive ULs, see the model presented in Nutrition Reviews (2).

The range of intakes encompassed by the RNI and UL should be considered sufficient to prevent deficiency while avoiding toxicity. If no UL can be derived from experimental or observational data in humans, the UL can be defined from available data on upper range of observed dietary intake of apparently healthy populations.

Protective nutrient intake

The concept of protective nutrient intake has been introduced in some cases to refer to an amount greater than the RNI, which may be protective against a specified health or nutritional risk of public health relevance (e.g., vitamin C intake with a meal to promote iron absorption or folic acid to lower the risk of neural tube defects). The text will indicate when existing data provide justifiable differences between RNI values and protective intake levels. These intakes are expressed as a daily value or as an amount to be consumed within a meal.

Figure 1



Report of the consultation

In welcoming the participants, Dr Nath, Assistant Director General, Regional Office for Asia and the Pacific, (FAO), recalled the previous Consultation and publication on this subject from 1974. That and the present Consultation are part of a long series of such expert consultations, which have as a primary objective; a) the review of the state of knowledge on the role of various nutrients in the human diet; and, b) the formulation of practical recommendations where interpretation is needed or controversy exists. The most recent in this series was the *joint FAO/WHO expert consultation on carbohydrates in human nutrition* held in Rome in 1997.

Dr Nath spoke of the increasing evidence for the important role which vitamins and minerals play in preventing disease and promoting overall health. Indeed, the understanding of the role of micronutrients in foods and nutrition has significantly increased over the past 24 years. For many years the basic assumption – which still may be the best assumption – on which nutritionists make their projections has been that all nutrients can be obtained from a diet containing a variety of foods from a variety of sources. Some of the challenges to this assumption rest in the complexities and diversity of worldwide realities, culture, and traditions.

Dr Nath pointed out that for many people with access to an adequate energy intake, an extensive freedom of choice exists in the selection of food. However, the existence of widespread poverty in the majority of the UN member countries precludes the opportunity to consume adequate energy let alone a diet balanced in micronutrients. He observed that the increase in the availability of a wide variety of foods and especially “fast foods” in almost every country in the world, coupled with the increasing pace of urban lifestyles across all cultures and countries, does not necessarily result in adequate vitamin and mineral intake.

The existence of the dichotomies in lifespan was also mentioned by Dr Nath. In different parts of the world and in different segments of society within the same countries, there are broad ranges of life spans in part due to nutritional adequacy. Especially in urban populations, as lifespan increases as a result of nutritional adequacy and despite improved

access to health care, an increase in obesity, diabetes, some forms of cancer, and cardiovascular disease has been recorded in all regions of the globe. Although epidemiological studies do not provide us with cause-and-effect explanations, they do provide impetus for future research into the role of vitamins and minerals in the prevention and management of some non-communicable diseases.

Dr Nath reminded the participants that they had been invited to the Consultation as independent experts and that their participation in the Consultation was to be in their individual capacity and not as a representative of any organization, affiliation, or government. He underscored the importance of drawing conclusions and making recommendations based on science, which is traceable to studies conducted largely in humans. This is necessary for correct food labelling and relevant health claims and for the better use of foods in the dietary management and prevention of non-communicable diseases. These issues have economic implications for agricultural production, the food industry, and public health policy.

Dr Sultana Khanum, Regional Adviser/Nutrition, SEARO (South-East Asia Regional Office), WHO, added her welcome on behalf of the Director-General of WHO, Dr Gro Harlem Brundtland, and the Regional Director for South-East Asia Region, Dr Uton Muchtar Raffle. Dr Khanum noted that the choice of South-East Asia as the site of the Expert Consultation was significant because some of the most tangible successes and achievements have occurred within this geographical region in the realm of identifying, preventing, reducing, and eliminating many forms of malnutrition.

Dr Khanum noted that the FAO and WHO have a long history of collaboration at the country, regional, and global levels towards combating food and nutritional problems. She underlined the importance of using science as the basis of the standard setting process, which took place during the consultation.

Dr Graeme Clugston, Director, WHO Nutrition Programmes, added his welcome to the participants on behalf of the Director-General of WHO. Dr Clugston pointed out that the formulation and implementation of science-based dietary guidelines have become a central issue for the nutritional sciences as well as a major challenge for governments world wide, especially since the *International Conference on Nutrition* held in Rome, December 1992.

Dr Clugston expressed confidence that this Expert Consultation would lead to scientifically sound up-to-date recommendations for vitamin and mineral requirements in human nutrition. FAO and WHO would then ensure that these recommendations would be passed on to all Member States world wide, providing them with the best possible guidance for developing their own appropriate dietary guidelines for health promotion, good nutrition, and disease prevention.

The Consultation elected Dr Donald McCormick as chairperson and Professor Chen Chunming as vice-chair. Dr Glenville Jones and Dr Colin Mills were appointed jointly as rapporteurs. Dr McCormick in his response indicated the importance of this Consultation and outlined the scope of the issues that would be discussed and on which the two agencies, FAO and WHO, were seeking expert guidance from the Consultation.

Recommended nutrient intakes

Appendix 1 at the end of the report provides two composite tables summarising the recommended nutrient intakes (RNIs) for each of the vitamins and minerals. For the purposes of preparing these tables the recommendations made by the experts were adjusted so that the tables could be based on common body weights and age groups. Details are provided in the footnote at the bottom of the tables.

REFERENCES

1. **Food and Nutrition Board, Institute of Medicine**. 1997. *Dietary Reference Intakes*: Washington, DC, National Academy Press.
2. **Anonymous**. 1997. A Model for the Development of Tolerable Upper Intake Levels. *Nutr. Revs.,* 55: 342-351.

Chapter 2

Food-based approaches to meeting vitamin and mineral needs

Dietary patterns have varied over time depending on the agricultural practices and the climatic, ecologic, cultural, and socio-economic factors, which determine available foods. At present, virtually all dietary patterns adequately satisfy or even exceed the nutritional needs of population groups. This is true except where socio-economic conditions limit the capacity to produce and purchase food or aberrant cultural practices restrict the choice of foods. It is thought that if people have access to a sufficient quantity and variety of foods, they will meet their nutritional needs. The current practice of evaluating nutritive value of diets should include not only energy and protein adequacy but also the micronutrient density of the diet.

A healthy diet can be attained in more than one way because of the variety of foods, which can be combined. It is thus difficult to define the ranges of intake for a specific food, which should be included in a given combination to comply with nutritional adequacy. In practice, the set of food combinations which is compatible with nutritional adequacy is restricted by the level of food production sustainable in a given ecologic and population setting. In addition, there are economic constraints, which limit food supply at household level. The development of food-based dietary guidelines (FBDGs) by the FAO and WHO (*1*) recognises this and focuses on the combination of foods that can meet nutrient requirements rather than on how each specific nutrient is provided in adequate amounts.

The first step in the process of setting dietary guidelines is defining the significant diet-related public health problems in a community. Once these are defined, the adequacy of the diet is evaluated by comparing the information available on dietary intake with recommended nutrient intakes (RNIs). Nutrient intake goals under this situation are specific for a given ecologic setting, and their purpose is to promote overall health, control specific nutritional diseases (whether they are induced by an excess or deficiency of nutrient intake), and reduce the risk of diet-related multi-factorial diseases. Dietary guidelines represent the practical way to reach the nutritional goals for a given population. They take into account the customary dietary pattern and indicate what aspects should be modified. They consider the ecologic setting, socio-economic and cultural factors, and biologic and physical environment in which the population lives.

The alternative approach to defining nutritional adequacy of diets is based on the biochemical and physiologic basis of human nutritional requirements in health and disease. The quantitative definition of nutrient needs and its expression as RNIs have been important instruments of food and nutrition policy in many countries and have focused the attention of international bodies. This nutrient-based approach has served many purposes but has not always fostered the establishment of nutritional and dietary priorities consistent with the broad public health priorities at the national and international levels. It has permitted a more precise definition of requirements for essential nutrients when establishing RNIs but unfortunately has often been narrowly focused, concentrating on the precise nutrient requirement amount and not on solving the nutritional problems of the world. In contrast to RNIs, FBDGs are based on the fact that people eat food, not nutrients. As illustrated in this chapter, the notion of nutrient density is

helpful for defining FBDGs and evaluating the adequacy of diets. In addition, they serve to educate the public through the mass media and provide a practical guide to selecting foods by defining dietary adequacy (1).

Advice for a healthy diet should provide both a quantitative and qualitative description of the diet for it to be understood by individuals, who should be given information on both size and number of servings per day. The quantitative aspects include the estimation of the amount of nutrients in foods and their bio-availability in the form they are actually consumed. Unfortunately, available food composition data for most foods currently consumed in the world are incomplete, outdated, or insufficient for evaluating true bio-availability. The qualitative aspects relate to the biologic utilisation of nutrients in the food as consumed by humans and explore the potential for interaction among nutrients. Such an interaction may enhance or inhibit the bio-availability of a nutrient from a given food source.

Including foods in the diet, which have high micronutrient density – such as pulses or legumes, vegetables (including green leafy vegetables), and fruits – is the preferred way of ensuring optimal nutrition including micronutrient adequacy for most population groups. Most population groups afflicted by micronutrient deficiency largely subsist on refined cereal grain or tuber-based diets, which provide energy and protein (with improper amino acid balance) but are insufficient in critical micronutrients. Figures 2-5 and Tables 1-4 included at the end of this chapter illustrate how addition of a variety of foods to the basic four diets (white rice- ***Figure 2***, corn tortilla- ***Figure 3***, refined couscous- ***Figure 4***, and potato- ***Figure 5***) can increase the nutrient density of a cereal or tuber-based diet. There is a need for broadening the food base and diversification of diets. Much can be gained from adding reasonable amounts of these foods, which will add micronutrient density to the staple diet (***Table 1, 2, 3*** *and* ***4***).

The recent interest in the role of phyto-chemicals and antioxidants on health and their presence in plant foods lend further support to the recommendation for increasing vegetables and fruit consumed in the diet. The need for dietary diversification is supported by the knowledge of the interrelationships of food components, which may enhance the nutritional value of foods and prevent undesirable imbalances, which may limit the utilisation of some nutrients. For example, fruits rich in ascorbic acid will enhance the absorption of ionic iron.

If energy intake is low (<8.368 MJ/day), for example, in the case of young children, sedentary women, or the elderly, the diet may not provide vitamin and mineral intakes sufficient to meet the RNIs. This situation may be of special relevance to the elderly, who are inactive, have decreased lean body mass, and typically decrease their energy intake. Young children, pregnant women, and lactating women, who have greater micronutrient needs relative to their energy needs, will also require increased micronutrient density.

The household is the basic unit for food consumption under most settings, and if there is sufficient food, individual members of the household can consume a diet with the recommended nutrient densities and meet their specific RNIs. However, appropriate food distribution within the family must be considered to ensure that children and women receive adequate food with high micronutrient density. Household food distribution must be considered when establishing general dietary guidelines and addressing the needs of vulnerable groups in the community. In addition, education detailing the appropriate storage and processing of foods to prevent micronutrient losses at the household level is important.

Dietary diversification when consuming cereal and tuber-based diets (rice, corn, wheat, potato, and cassava)

Dietary diversification is important to improve the intake of critical nutrients. The micronutrients selected discussed here, although limited in number, are of public health relevance or serve as markers for overall micronutrient intake. The chapters on individual nutrients will provide further details on food-related considerations for micronutrient adequacy. The nutrients selected for discussion below include some of the nutrients, which are most difficult to obtain in cereal and tuber-based diets. Nutrient deficiencies of vitamin A, iron, and zinc are widespread.

Vitamin A

The vitamin A content of most staple diets can be significantly improved with the addition of a relatively small portion of plant foods rich in carotenoids, the precursors of vitamin A. For example, a usual portion of cooked carrots (50 g) added to a daily diet, or 21 g of carrots per 4.184 MJ, provides 500 μg retinol equivalents, which is the recommended nutrient density for this vitamin. The biologic activity of pro-vitamin A varies among different plant sources, and fruits and vegetables such as carrots, mango, papaya, and melon contain large amounts of nutritionally active carotenoids, (*2, 3*). Green leafy vegetables such as ivy gourd have been successfully used in Thailand as a source of vitamin A, and carotenoid-rich red palm oil serves as an easily available and excellent source of vitamin A in other countries. Consequently, a regular portion of these foods included in an individual's diet may provide 100 percent or more of the daily requirement for retinol equivalents. Vitamin A is also present in animal food sources in a highly bio-available form. Therefore it is important to consider the possibility of meeting vitamin A needs by including animal foods in the diet. For example, providing minor amounts of fish or chicken liver (20–25 g) in the diet provides more than the recommended vitamin A nutrient density for virtually all age and sex groups.

Vitamin C

A real gain in vitamin C intake can be achieved by including citrus fruit or other foods rich in ascorbic acid in the diet. For example, an orange or a small amount of other vitamin C-rich fruit (60 g of edible portion) provides the recommended ascorbic acid density. Adding an orange to a potato-based diet increases the level of vitamin C threefold. Other good vitamin C food sources are guava, amla, kiwi, cranberries, strawberries, papaya, mango, melon, cantaloupe, spinach, Swiss chard, tomato, asparagus, and Brussels sprouts. All these foods, when added to a diet or meal in regular portion sizes, will significantly improve the vitamin C density. Because ascorbic acid is heat labile, minimal cooking (steaming or stir-frying) is recommended to maximise the bio-available nutrient. The significance of consuming vitamin C with meals will be discussed relative to iron absorption (see ***Chapter 13***).

Folate

Folate is now considered significant not only for the prevention of macrocytic anaemia, but also for normal foetal development. Recently, this vitamin was implicated in the maintenance of cardiovascular health and cognitive function in the elderly. Staple diets consisting largely of cereal grains and tubers are very low in folate but can be improved by the addition of legumes or green leafy vegetables. For example, a regular portion of cooked lentils (95 g) added to a rice-based diet can provide an amount of folate sufficient to meet the desirable nutrient density for this vitamin. Other legumes such as beans and peas are also good sources of this vitamin, but larger portions are needed for folate sufficiency (100 g beans and 170 g peas). Cluster bean and colacasia leaves are excellent folate sources used in the Indian diet.

Another good source of folate is chicken liver; only one portion (20–25 g) is sufficient to meet the desirable nutrient density for folate and vitamin A simultaneously. The best sources of folate are organ meats, green leafy vegetables, and sprouts. However, 50 percent or more of food folate is destroyed during cooking. Prolonged heating in large volumes of water should be avoided, and it is advisable to consume the water used in the cooking of vegetables.

Iron and zinc

Minerals such as iron and zinc are low in cereal and tuber-based diets, but the addition of legumes can slightly improve the iron content of those diets. However, the bio-availability of this non-heme iron source is low. Therefore, it is not possible to meet the recommended levels of iron and zinc in the staple-based diets through a food-based approach unless some meat, poultry, or fish is included. For example adding a small portion (50 g) of meat, poultry, or fish will increase the total iron content as well as the amount of bio-available iron. For zinc the presence of a small portion (50 g) of meat, poultry, or fish will secure dietary sufficiency of most staple diets.

The consumption of ascorbic acid along with the food rich in iron will enhance absorption. There is a critical balance between enhancers and inhibitors of iron absorption. Nutritional status can be improved significantly by educating households on food preparation practices, which minimise the consumption of inhibitors of iron absorption; for example, the fermentation of phytate-containing grains before the baking of breads to enhance iron absorption.

How to accomplish dietary diversity in practice

It is essential to work on strategies, which promote and facilitate dietary diversification to achieve complementarity of cereal or tuber-based diets with foods rich in micronutrients in populations with limited economics or limited access to food. A recent FAO and International Life Sciences Institute (*4*) publication proposed strategies to promote dietary diversification within the implementation of food-based approaches. These strategies, which follow, have been adapted or modified based on the discussions held in this consultation:

1. Community or home vegetable and fruit gardens. These projects should lead to increased production and consumption of micronutrient-rich foods (legumes, green leafy vegetables, and fruits) at the household level. The success of such projects requires a good knowledge and understanding of local conditions as well as the involvement of women and the community in general. These are key elements for supporting, achieving, and sustaining beneficial nutritional change at the household level. Land availability and water supply may present common constraints, which require local government intervention or support before they are overcome. The educational effort should be directed towards securing appropriate within-family distribution, which considers the needs of the most vulnerable members of the family, especially infants and young children. Separate FBDGs for vulnerable groups, such as pregnant and lactating women, children, and the elderly, should be developed.

2. Production of fish, poultry, and small animals (rabbits, goats, and guinea pigs). These are excellent sources of highly bio-available essential micronutrients such as vitamin A, iron, and zinc. The production of animal foods at the local level may permit communities to access foods which otherwise are not available because of their high costs. These types of projects also need some support from local governments or non-governmental organizations to overcome cost constraints of programme implementation, including the training of producers.

3. Implementation of large-scale commercial vegetable and fruit production. The objective of this initiative is to provide micronutrient-rich foods at reasonable prices through effective

and competitive markets, which lower consumer prices without reducing producer prices. This will serve predominantly the urban and non-food-producing rural areas.

4. Reduction of post-harvest losses of the nutritional value of micronutrient-rich foods, such as fruits and vegetables. Improvement of storage and food-preservation facilities significantly reduces post-harvest losses. At the household level, the promotion of effective cooking methods and practical ways of preserving foods (solar drying of seasonal micronutrient-rich foods such as papaya, grapes, mangoes, peaches, tomatoes, and apricots) may significantly increase the access to bio-available micronutrient-rich foods. At the commercial level, grading, packing, transport, and marketing practices reduce losses, stimulate economic growth, and optimise income generation.

5. Improvement of micronutrient levels in soils and plants, which will improve the composition of plant foods and enhance yields. Current agricultural practices can improve the micronutrient content of foods through correcting soil quality and pH and increasing soil mineral content depleted by erosion and poor soil conservation. Long-term food-based solutions to micronutrient deficiencies will require improvement of agricultural practices, seed quality, and plant breeding (by means of a classical selection process or genetic modification).

The green revolution made important contributions to cereal supplies, and it is time to address the need for improvements in the production of legumes, vegetables, fruits, and other micronutrient-rich foods. FBDGs can serve to reemphasise the need for these crops.

It is well recognised that the strategies proposed to promote dietary diversity need a strong community-level commitment. For example, the increase in price of legumes associated with decreased production and lower demand needs to be corrected. The support of local authorities and government may facilitate the implementation of such projects because these actions require economic resources, which sometimes are beyond the reach of the most needy.

Practices which will enhance the success of food-based approaches

To achieve dietary adequacy of vitamin A, vitamin C, folate, iron, and zinc by using food-based approaches, food preparation and dietary practices must be considered. For example, it is important to recommend that vegetables rich in vitamin C, folate, and other water-soluble or heat-labile vitamins be minimally cooked in small amounts of water. For iron bio-availability it is essential to reduce the intake of inhibitors of iron absorption and to increase the intake of enhancers of absorption in a given meal. Following this strategy, it is recommended to increase the intake of: germinated seeds, fermented cereals, heat-processed cereals, meats, and fruits and vegetables rich in vitamin C and to encourage the consumption of tea, coffee, chocolate, or herbal teas at times other than with meals (see ***Chapter 13*** and ***Chapter 16***). Consumption of flesh foods improves zinc absorption whereas it is inhibited by consumption of diets high in phytate, such as diets based on unrefined cereal. Zinc availability can be estimated according to the phytate-to-zinc (molar) ratio of the meal (*5*).

This advice is particularly important for people who consume cereal and tuber-based diets. These foods constitute the main staples for most populations of the world, populations that are also most at risk for micronutrient deficiencies. Other alternatives – fortification and supplementation – have been proposed as stopgap measures when food-based approaches are not feasible or are still in progress. There is a definite role for fortification in meeting iron, folate, iodine, and zinc needs. Fortification and supplementation should be seen as complementary to food-based strategies and not as a replacement. Combined, all these strategies can go a long way toward stabilising the micronutrient status of populations at risk.

Food-based approaches usually take longer to implement but once established are truly sustainable.

Delineating the role of supplementation and food fortification for nutrients which cannot be supplied by regular foods

Under ideal conditions of food access and availability, food diversity should satisfy micronutrient and energy needs of the general population. Unfortunately, for many people in the world, the access to a variety of micronutrient-rich foods is not possible. As demonstrated in our analysis of cereal and tuber-based diets (***see appendixes***), micronutrient-rich foods including small amount of flesh foods and a variety of plant foods (vegetables and fruits) are needed daily. This may not be realistic at present for many communities living under conditions of poverty. Food fortification and food supplementation are important alternatives that complement food-based approaches to satisfy the nutritional needs of people in developing and developed countries.

Fortification

Fortification refers to the addition of nutrients to a commonly eaten food (the vehicle). It is possible for a single nutrient or group of micronutrients (the fortificant) to be added to the vehicle, which has been identified through a process in which all stakeholders have participated. This strategy is accepted as sustainable under most conditions and often is cost effective on a large scale when successfully implemented. Iron fortification of wheat flour and iodine fortification of salt is examples of fortification strategies with excellent results (*6*).

There are at least three essential conditions that must be met in any fortification programme(*6, 7*): the fortificant should be effective, bio-available, acceptable, and affordable; the selected food vehicle should be easily accessible and a specified amount of it should be regularly consumed in the local diet; and detailed production instructions and monitoring procedures should be in place and enforced by law.

Iron fortification

Food fortification with iron is recommended when dietary iron is insufficient or the dietary iron is of poor bio-availability, which is the reality for most people in the developing world and for vulnerable population groups in the developed world. Moreover, the prevalence of iron deficiency and anaemia in vegetarians and in populations of the developing world which rely on cereal or tuber foods is significantly higher than in omnivore populations.

Iron is present in foods in two forms, as heme iron, which is derived from flesh foods (meats, poultry, and fish), and as non-heme iron, which is the inorganic form present in plant foods such as legumes, grains, nuts, and vegetables (*8, 9*). Heme iron is highly (20–30 percent) absorbed and its bio-availability is relatively unaffected by dietary factors. Non-heme iron has a lower rate of absorption (2–10 percent), depending on the balance between iron absorption inhibitors (phytates, polyphenols, calcium, and phosphate) and iron absorption enhancers (ascorbic and citric acids, cysteine-containing peptides, ethanol, and fermentation products) present in the diet (*8, 9*). Because staple foods around the world provide predominantly non-heme iron sources of low bio-availability, the traditionally eaten staple foods represent an excellent vehicle for iron fortification. Examples of foods, which have been fortified, are wheat flour, corn (maize) flour, rice, salt, sugar, cookies, curry powder, fish sauce, and soy sauce (*8*). Nevertheless the beneficial effects of consumption of iron absorption enhancers have been extensively proven and should always be promoted (i.e., consumption of vitamin C–rich food together with the non-heme iron source).

Iodine fortification

Iodine is sparsely distributed in the Earth's surface and foods grown in soils with little or no iodine lack an adequate amount of this micronutrient. This situation had made iodine deficiency disorders exceedingly common in most of the world and highly prevalent in many countries before the introduction of salt iodisation (*10*). Only foods of marine origin are naturally rich sources of iodine. Salt is a common food used by most people worldwide, and the establishment of an well-implemented permanent salt-iodisation programme has been proven to eradicate iodine deficiency disorders (see ***Chapter 12***). Universal salt iodisation is the best way to virtually eliminate iodine deficiency disorders by the year 2000 (*4*).

However, salt iodisation is not simply a matter of legislating mandatory iodisation of salt. It is important to determine the best fortification technique, co-ordinate the implementation at all salt production sites, establish effective monitoring and quality control programmes, and measure iodine fortification level periodically. The difficulties in implementing salt iodisation programmes arise primarily when the salt industry is widely dispersed among many small producers. The level of iodine fortification usually lies between 25 and 50 mg/kg salt. The actual amount should be specified according to the level of salt intake and magnitude of deficit at the country level, because iodine must be added within safe and effective ranges. Additionally, it is very important to implement a monitoring plan to control the amount of iodine in the salt at the consumer's table, (*10, 11*). United Nations agencies responsible for assisting governments in establishing iodisation programmes should provide technical support for programme implementation, monitoring, and evaluation to ensure sustainability.

Zinc fortification

The body depends on a regular zinc supply provided by the daily diet because stores are quite limited. Food diversity analysis demonstrates that it is virtually impossible to achieve zinc adequacy in the absence of a flesh food source. Among flesh foods, beef is the best source of zinc and is followed by poultry and then fish. Zinc fortification programmes are being studied, especially for populations, which consume predominately plant foods. Fortification of cereal staple foods is a potentially attractive intervention, which could benefit the whole population as well as target the vulnerable population groups of children and pregnant women. Such addition of zinc to the diet would perhaps decrease the prevalence of stunting in many developing countries with low-zinc diets, because linear growth is affected by zinc supply.

Folic acid fortification

The recommended nutrient density by the developers of the FAO/WHO (*1*) FBDGs for folic acid is 200 μg/4.184 MJ. Although this reference value is higher than other standards of reference, the increase in folic acid consumption by women of childbearing age is very important: it may improve birth weight and reduce the prevalence of neural tube defects by 50 percent. Elevated plasma homo-cysteine levels are considered to be an independent risk factor for heart disease; a higher intake of folic acid may also benefit the rest of the population because it may lower homo-cysteine levels in adults (see ***Chapter 4***). In addition, folate may improve the mental condition of the elderly population (*12, 13*).

Although the desirable folic acid density may be achieved through dietary diversity, it requires the daily presence of organ meats, green leafy vegetables, pulses, legumes, or nuts in the diet (*14*). Most population groups may not easily reach the appropriate level of folic acid consumption; therefore, folic acid fortification has been recommended. The United States initiated mandatory folic acid fortification of cereal-grain products in January 1998. The

fortification level approved in the United States is 140 μg/100 g product, which will increase the average woman's intake by only 100 μg/day. This amount is considered safe (a dose, which will not mask pernicious anaemia, which results from vitamin B12 deficiency,) but it may be ineffective in lowering the occurrence of neural tube defects (*15*).

Supplementation

Supplementation refers to periodic administration of pharmacologic preparations of nutrients as capsules or tablets or by injection when substantial or immediate benefits are necessary for the group at risk. As established at the *International Conference on Nutrition* (*16*), nutritional supplementation should be restricted to vulnerable groups, which cannot meet their nutrient needs through food (women of childbearing age, infants and young children, elderly people, low socio-economic groups, displaced people, refugees, and populations experiencing other emergency situations). For example, iron supplementation is recognised as the only option to control or prevent iron deficiency anaemia in pregnant women. Supplementation with folic acid should be considered for women of childbearing age who have had a child with neural tube defect to prevent recurrence.

Food-based dietary guidelines

Food-based dietary guidelines (FBDGs) are an instrument of and expression of food and nutrition policy and should be based directly on diet and disease relationships of particular relevance to the individual country. Their primary purpose is to educate healthcare professionals and consumers about health promotion and disease prevention. In this way priorities in establishing dietary guidelines can address the relevant public health concerns whether they are related to dietary insufficiency or excess. In this context, meeting the nutritional needs of the population takes its place as one of the components of food and nutrition policy goals along with the priorities included in the FBDGs for improved health and nutrition for a given population.

The world nutrition and health situation demonstrates that the major causes of death and disability have been traditionally related to undernutrition in developing countries and to the imbalance between energy intake and expenditure (which lead to obesity and other chronic diseases – diabetes, cardiovascular disease, hypertension, and stroke) in industrialized countries. The tragedy is that many suffer from too little food while others have diseases resulting from too much food, but both would benefit from a more balanced distribution of food and other resources. Although the nature of the health and nutrition problems in these two contrasting groups is very different, the dietary guidelines required to improve both situations are not. Most countries presently have the combined burden of malnutrition from deficit and increasing prevalence of obesity and other chronic diseases from over consumption. The approaches to address the problems, nevertheless, should be country and population specific.

Although two-thirds of the world's population depends on cereal or tuber-based diets, the other one-third consumes significant amounts of animal food products. The latter group places an undue demand on land, water, and other resources required for intensive food production, which makes the typical Western diet not only undesirable from the standpoint of health but also environmentally unsustainable. If we balance energy intake with the expenditure required for basal metabolism, physical activity, growth, and repair, we will find that the dietary quality required for health is essentially the same across population groups.

Efforts in nutrition education and health promotion should include a strong encouragement for active lifestyles. Improving energy balance for rural populations in developing countries may mean increasing energy intake to normalise low body mass index (BMI, weight/height^2, calculated as kg/m^2), ensuring adequate energy stores and energy for

appropriate social interactions. In sedentary urban populations, improving energy balance will mean increasing physical activity to decrease energy stores (body fat mass) and thus normalise BMI. Thus, the apparent conflicting goals – eradicating undernutrition while preventing overnutrition – are resolved by promoting sufficient energy for a normal BMI. Moreover, if we accept that FBDGs should be ecologically sustainable, the types and amounts of foods included in a balanced diet are not very different for promoting adequate nutrition in the undernourished and preventing overnutrition in the affluent.

This is well exemplified by the similarities in the FBDGs across countries, whether represented by pyramids, rainbows, dishes, pots, etc. It is obvious that consumption of excess energy will induce an increase in energy stores, which may lead to obesity and related health complications. Populations should consume nutritionally adequate and varied diets, based primarily on foods of plant origin with small amounts of added flesh foods. Households should select predominantly plant-based diets rich in a variety of vegetables and fruits, pulses or legumes, and minimally processed starchy staple foods. The evidence that such diets will prevent or delay a significant proportion of non-communicable chronic diseases is consistent. A predominantly plant-based diet has a low energy density, which may protect against obesity. This should not exclude small amounts of animal foods, which may make an important nutritional contribution to plant-food-based diets, as illustrated in the examples presented earlier. Inadequate diets occur when food is scarce or when food traditions change rapidly, as is seen in societies undergoing demographic transitions or rapid urbanisation. Traditional diets, when adequate and varied, are likely to be generally healthful and more protective against chronic non-communicable diseases than the typical Western diet, consumed predominantly in industrialized societies (*17)*.

Reorienting food production, agricultural research, and commercialisation policies needs to take into consideration FBDGs, which increase the demand for a variety of micronutrient-rich foods and thus stimulate production to meet the consumption needs. Prevailing agricultural policies encourage research on and production and importation of foods, which do not necessarily meet the requirements of FBDG implementation. For example, great emphasis is placed on cereals, horticultural crops for export, legumes for export, non-food cash crops, and large livestock. Necessary policy reorientation is required to ensure increased availability of micronutrient-rich foods within the local food system. Norway has successfully implemented agricultural and food production policies based on a National Nutrition Plan of Action, providing economic incentives for the producer and consumer in support of healthful diets. The results speak for themselves, as Norway has experienced a sustained improvement in life expectancy and a reduction in deaths from cardiovascular disease and other chronic non-communicable conditions.

Recommendations for the future

The Consultation acknowledged the limitations in our knowledge of these important aspects, which affect nutrient utilisation and recommended that the International Food Data System (INFoods) effort led by FAO/UNU be strengthened. Special emphasis should be placed on the micronutrient composition of local diets as affected by ecologic setting; analysis of food components (nutrients or bio-active components), which may affect the bio-availability and utilisation of critical micronutrients; and the analysis of cooked foods and typical food combinations as actually consumed by population groups. In addition the development of FBDGs at the country level should be supported by UN agencies.

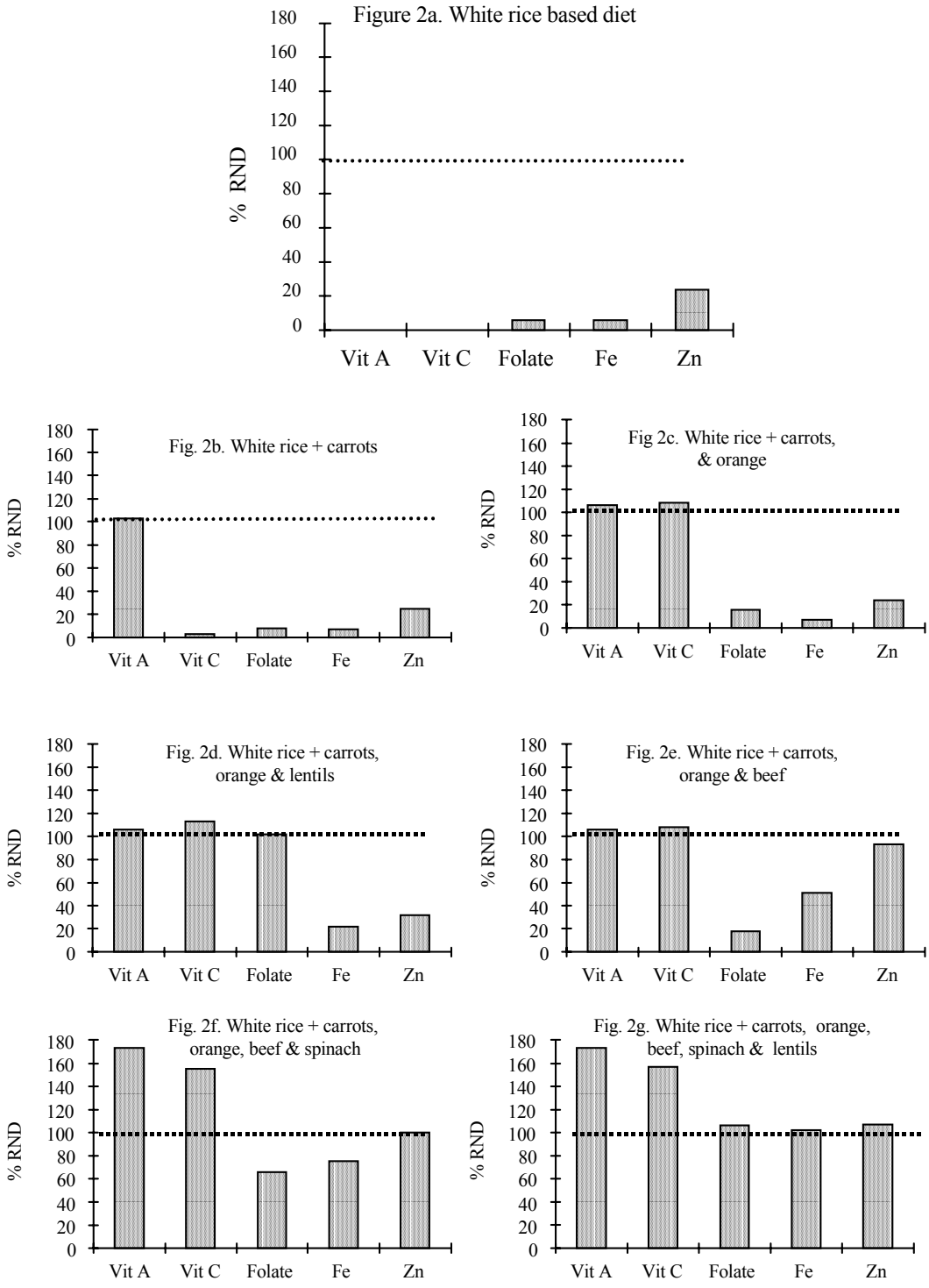
Future research

The following research needs were identified to facilitate the implementation of a food-based approach in the prevention of micronutrient deficiencies:

- food data system development, which includes development of methodology for micronutrient composition of foods, organizing data retrieval, and reporting and dissemination through electronic means; this effort should include phyto-chemicals, antioxidants, and other components which may affect health and nutrition, with special emphasis on local foods which may be important for given food cultures;
- identification and evaluation of optimal methods for cooking foods to preserve the nutrient value and enhance the bio-availability of micronutrients;
- development of better methods to preserve foods, especially micronutrients, at the household and community levels;
- identification and propagation of agricultural methods which will enhance the food yields, content, and biologic value of micronutrient-rich foods;
- identification of optimal food combinations and serving size which will be most effective in preventing micronutrient deficits and methods of promotion for these food combinations at the community level;
- development of agricultural research to support the implementation of FBDGs; and
- evaluation of the nutritional impact and cost benefit of food-based approaches in combating micronutrient deficiencies.

Figure 2

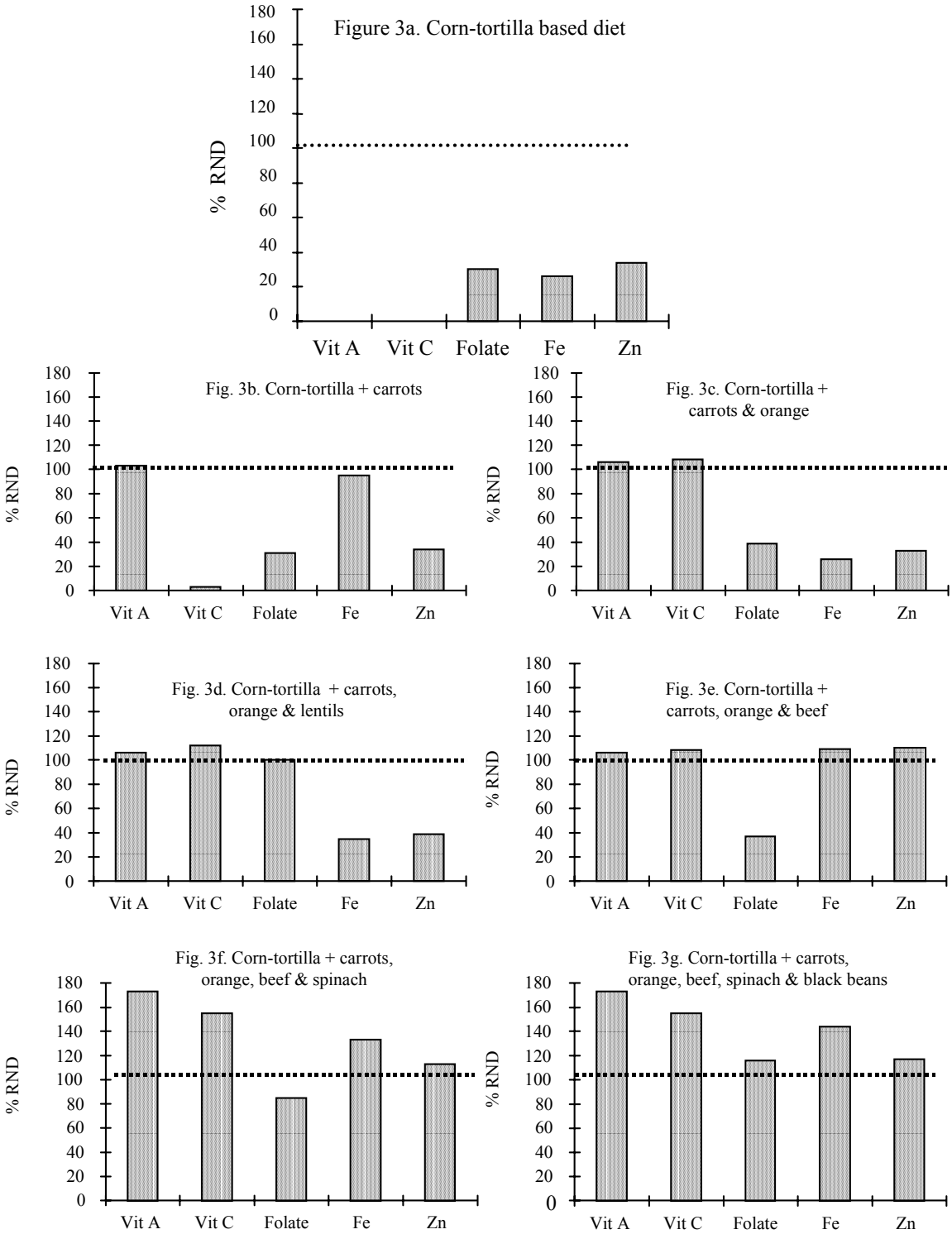
Percentage of recommended nutrient density (RND) for a diet of white rice and the addition of a variety of foods



Note: Data in ***Tables 1*** *and* ***3***

Figure 3

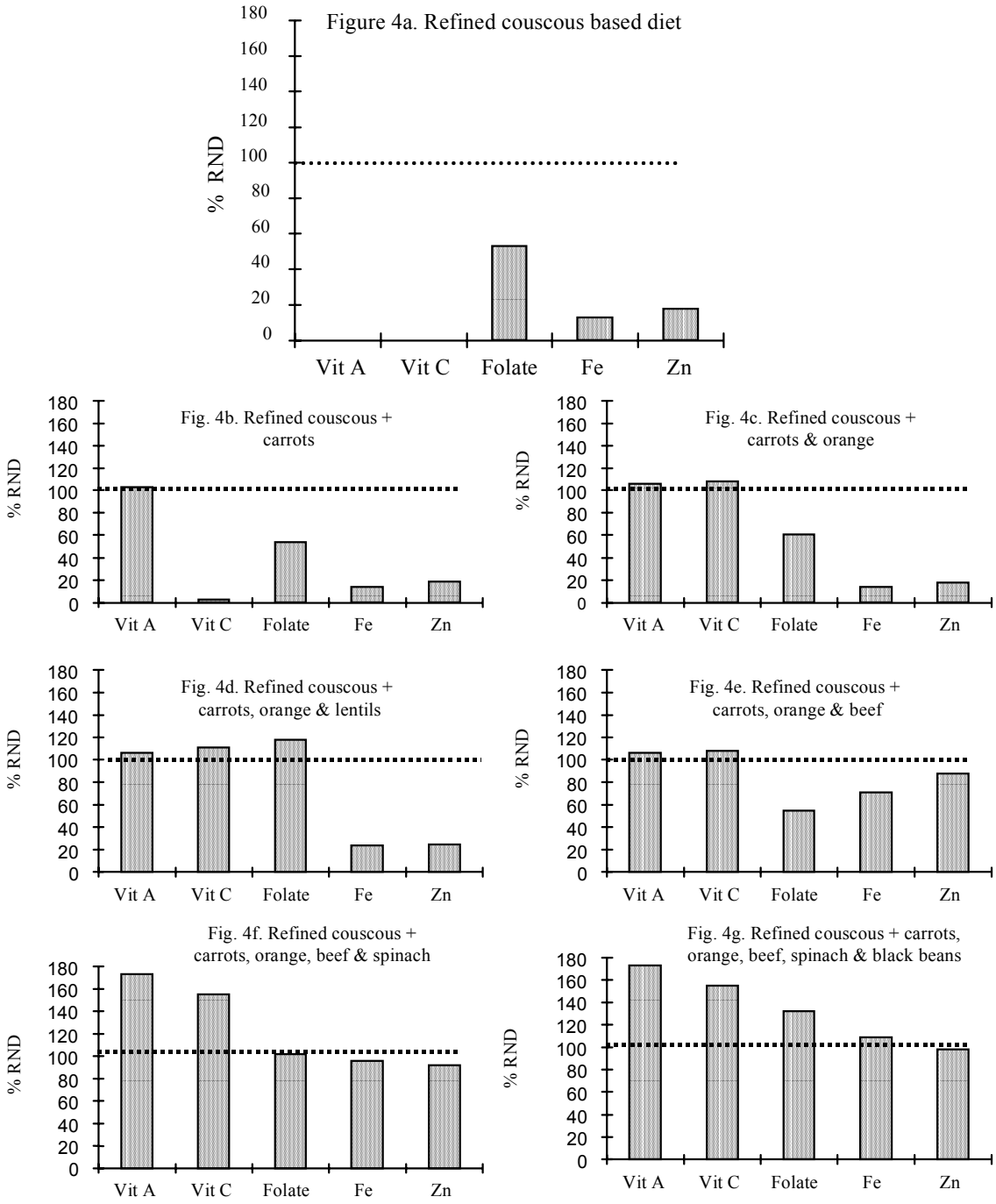
Percentage of recommended nutrient density (RND) for a diet of corn-tortilla and the addition of a variety of foods



Note: Data in ***Tables 1*** *and* ***3***

Figure 4

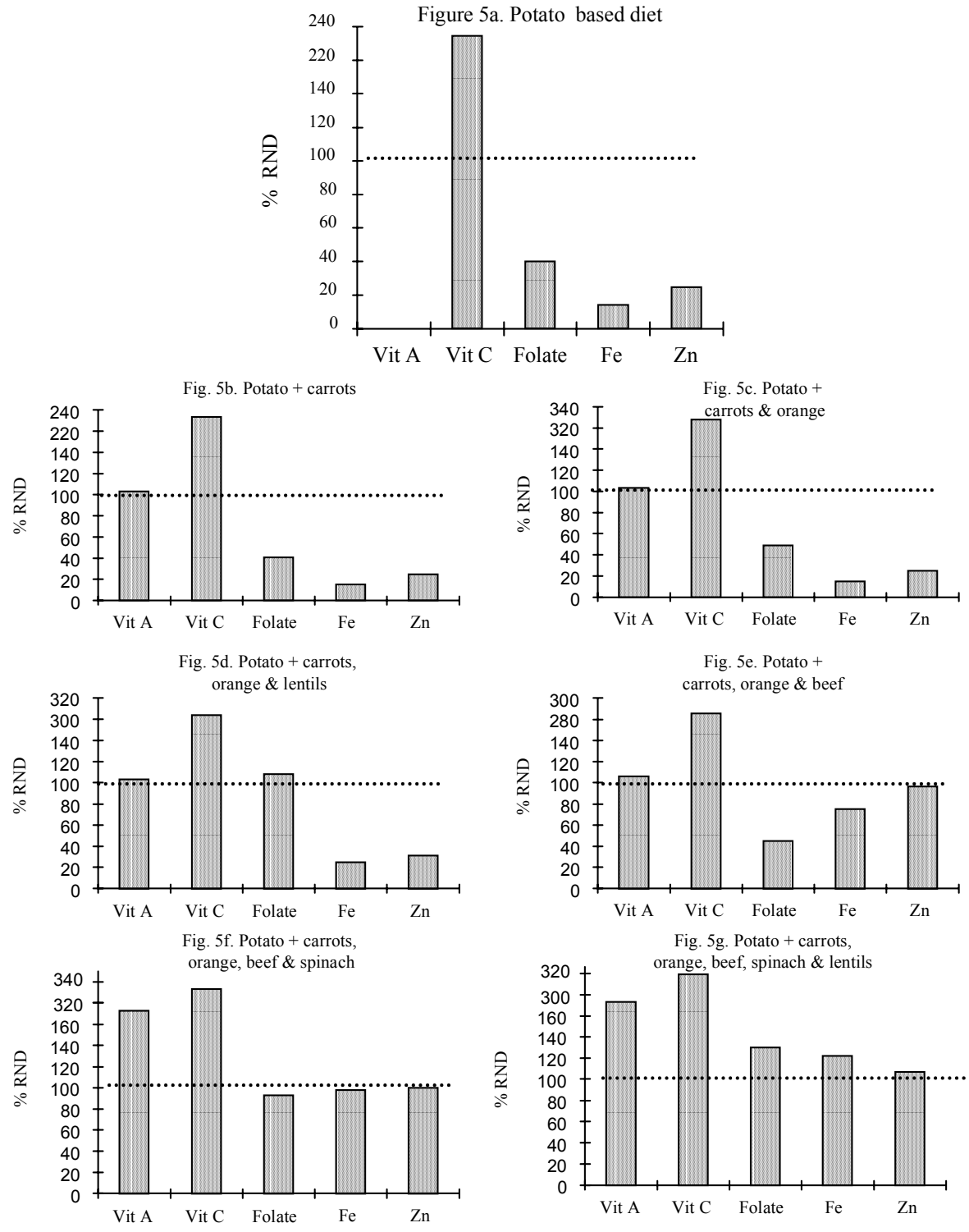
Percentage of recommended nutrient density (RND) for a diet of refined couscous and the addition of a variety of foods



Note: Data in ***Tables 2*** *and* ***4***

Figure 5

Percentage of recommended nutrient density (RND) for a diet of potatoes and the addition of a variety of foods



Note: Data in ***Tables 2*** *and* ***4***

Table 1: White rice and corn-tortilla based diets composition and nutrient density values per 1000 kcals for vitamin A, vitamin C, folate, iron and zinc

	WHITE RICE BASED DIET						
	White rice 598 g Vegetable oil 25 g	White rice 590g Vegetable oil 25 g Carrots 21 g	White rice 570 g Vegetable oil 25 g Carrots 21 g Orange 60 g	White rice 483 g Vegetable oil 25 g Carrots 21 g Orange 60 g Lentils 95g	White rice 477 g Vegetable oil 25 g Carrots 21 g Orange 60 g Beef 55 g	White rice 468 g Vegetable oil 25 g Carrots 21 g Orange 60 g Beef 55 g Spinach raw 50 g	White rice 428 g Vegetable oil 25 g Carrots 21 g Orange 60 g Beef 55 g Spinach raw 50 g Lentils 45 g
% E as Protein	5	6	6	8	11	12	13
% E as CHO	72	72	72	69	61	60	59
% E as Fat	23	22	22	23	28	28	28
Vitamin A (μg)	0	516	528	529	528	864	864
Vitamin C (mg)	0	0.5	32.5	33.9	32.5	46.5	47.0
Folate (μg)	12	15	32	203	35	131	212
Iron (mg)	1.2	1.3	1.3	4.3	2.8	4.1	5.6
Zinc (mg)	2.4	2.5	2.4	3.2	5.8	6.0	6.4
Calcium (mg)	18.0	24.2	47.7	62.8	49.4	98.6	105.8
	CORN-TORTILLA BASED DIET						
	Corn-tortilla 368 g Vegetable oil 20 g	Corn-tortilla 363 g Vegetable oil 20 g Carrots 21 g	Corn-tortilla 351 g Vegetable oil 20 g Carrots 21 g Orange 60 g	Corn-tortilla 314 g Vegetable oil 20 g Carrots 21 g Orange 60 g Lentils 71 g	Corn-tortilla 297 g Vegetable oil 20 g Carrots 21 g Orange 60 g Beef 55 g	Corn-tortilla 292g Vegetable oil 20 g Carrots 21 g Orange 60 g Beef 55 g Spinach raw 50 g	Corn-tortilla 266 g Vegetable oil 20 g Carrots 21 g Orange 60 g Beef 55 g Spinach raw 50 g Black beans 45 g
% E as Protein	8	8	8	10	13	14	15
% E as CHO	67	67	67	66	58	57	57
% E as Fat	25	25	25	24	29	29	28
Vitamin A (μg)	0	516	528	529	528	864	864
Vitamin C (mg)	0	0.5	32.5	33.5	32.5	46.5	46.5
Folate (μg)	59	61	77	200	73	169	232
Iron (mg)	5.2	5.2	5.1	6.9	6.0	7.3	7.9
Zinc (mg)	3.4	3.4	3.3	3.9	6.6	6.8	7.0
Calcium (mg)	647.7	645.4	648.3	596.5	557.8	598.5	565.0

Table 2: Refined Couscous and potato based diets composition and nutrient density values per 1000 kcal for vitamin A, vitamin C, folate, iron and zinc

	REFINED COUSCOUS BASED DIET						
	Ref. Couscous 697 g Vegetable oil 25 g	Ref. Couscous 690 g Vegetable oil 25 g Carrots 21 g	Ref. Couscous 665g Vegetable oil 25 g Carrots 21 g Orange 60 g	Ref. Couscous 590 g Vegetable oil 25 g Carrots 21 g Orange 60 g Lentils70 g	Ref. Couscous 555 g Vegetable oil 25 g Carrots 21 g Orange 60 g Beef 55 g	Ref. Couscous 546g Vegetable oil 25 g Carrots 21 g Orange 60 g Beef 55 g Spinach raw 50 g	Ref. Couscous 493 g Vegetable oil 25 g Carrots 21 g Orange 60 g Beef 55 g Spinach raw 50 g Black beans 45 g
% E as Protein	11	11	10	12	15	16	17
% E as CHO	66	66	66	64	56	55	55
% E as Fat	23	23	24	24	29	29	28
Vitamin A (μg)	0	516	528	529	528	864	864
Vitamin C (mg)	0	0.5	32.5	33.5	32.5	46.5	46.5
Folate (μg)	105	107	121	236	109	204	263
Iron (mg)	2.6	2.8	2.7	4.7	3.9	5.3	6.0
Zinc (mg)	1.8	1.9	1.8	2.5	5.3	5.5	5.9
Calcium (mg)	55.7	61.6	83.7	90.8	79.4	128.2	136.2

	POTATO BASED DIET						
	Potato 907 g Vegetable oil 25 g	Potato 895 g Vegetable oil 25 g Carrots 21 g	Potato 865 g Vegetable oil 25 g Carrots 21 g Orange 60 g	Potato 770 g Vegetable oil 25 g Carrots 21 g Orange 60 g Lentils 70 g	Potato 723 g Vegetable oil 25 g Carrots 21 g Orange 60 g Beef 55 g	Potato 710 g Vegetable oil 25 g Carrots 21 g Orange 60 g Beef 55 g Spinach raw 50 g	Potato 649 g Vegetable oil 25 g Carrots 21 g Orange 60 g Beef 55 g Spinach raw 50 g Lentils 45 g
% E as Protein	6	6	6	8	12	12	13
% E as CHO	71	71	71	69	61	60	59
% E as Fat	23	23	23	23	27	28	28
Vitamin A (μg)	0	516	528	529	528	864	864
Vitamin C (mg)	67.2	67.0	96.5	90.5	86.0	99.1	95.2
Folate (μg)	80	82	97	216	89	185	261
Iron (mg)	2.8	2.9	2.9	4.9	4.1	5.4	6.7
Zinc (mg)	2.5	2.5	2.5	3.1	5.8	6.0	6.4
Calcium (mg)	67.2	72.8	94.6	100.7	88.7	137.1	141.0

Table 3: White rice and corn-tortilla based diets composition and percent of nutrient density values for vitamin A, vitamin C, folate, iron and zinc

	WHITE RICE BASED DIET						
	White rice 598 g Vegetable oil 25 g	White rice 590g Vegetable oil 25 g Carrots 21 g	White rice 570 g Vegetable oil 25 g Carrots 21 g Orange 60 g	White rice 483 g Vegetable oil 25 g Carrots 21 g Orange 60 g Lentils 95g	White rice 477 g Vegetable oil 25 g Carrots 21 g Orange 60 g Beef 55 g	White rice 468 g Vegetable oil 25 g Carrots 21 g Orange 60 g Beef 55 g Spinach raw 50 g	White rice 428 g Vegetable oil 25 g Carrots 21 g Orange 60 g Beef 55 g Spinach raw 50 g Lentils 45 g
% E as Protein	5	6	6	8	11	12	13
% E as CHO	72	72	72	69	61	60	59
% E as Fat	23	22	22	23	28	28	28
Vitamin A (μg)	0	103 - 147	106 - 151	106 - 151	106 - 151	173 - 247	173 - 247
Vitamin C (mg)	0	2	108	113	108	155	157
Folate	6 - 8	8 - 10	16 - 21	102 - 135	18 - 23	66 - 87	106 - 141
Iron	6	7	7	22	51	75	102
Zinc	24	25	24	32	97	100	107
Calcium	5 - 7	6 - 10	12 - 19	16 - 25	12 - 20	25 - 39	26 - 42

	CORN-TORTILLA BASED DIET						
	Corn-tortilla 368 g Vegetable oil 20 g	Corn-tortilla 363 g Vegetable oil 20 g Carrots 21 g	Corn-tortilla 351 g Vegetable oil 20 g Carrots 21 g Orange 60 g	Corn-tortilla 314 g Vegetable oil 20 g Carrots 21 g Orange 60 g Lentils 71 g	Corn-tortilla 297 g Vegetable oil 20 g Carrots 21 g Orange 60 g Beef 55 g	Corn-tortilla 292 g Vegetable oil 20 g Carrots 21 g Orange 60 g Beef 55 g Spinach raw 50 g	Corn-tortilla 266 g Vegetable oil 20 g Carrots 21 g Orange 60 g Beef 55 g Spinach raw 50 g Black beans 45 g
% E as Protein	8	8	8	10	13	14	15
% E as CHO	67	67	67	66	58	57	57
% E as Fat	25	25	25	24	29	29	28
Vitamin A (μg)	0	103 - 147	106 - 151	106 - 151	106 - 151	173 - 247	173 - 247
Vitamin C (mg)	0	2	108	112	108	155	155
Folate	30 - 39	31 - 41	39 - 51	100 - 133	37 - 49	85 - 113	116 - 155
Iron	26	26	26	35	109	133	144
Zinc	34	34	33	39	110	113	117
Calcium	162 - 259	161 - 258	162 - 259	149 - 239	139 - 223	150 - 239	141 - 226

***Table 4**:* Refined couscous and potato based diets composition and percent of nutrient density values for vitamin. A, vitamin C, folate, iron and zinc

	REFINED COUSCOUS BASED DIET						
	Ref. Couscous 697 g Vegetable oil 25 g	Ref. Couscous 690 g Vegetable oil 25 g Carrots 21 g	Ref. Couscous 665g Vegetable oil 25 g Carrots 21 g Orange 60 g	Ref. Couscous 590 g Vegetable oil 25 g Carrots 21 g Orange 60 g Lentils70 g	Ref. Couscous 555 g Vegetable oil 25 g Carrots 21 g Orange 60 g Beef 55 g	Ref. Couscous 546 g Vegetable oil 25 g Carrots 21 g Orange 60 g Beef 55 g Spinach raw 50 g	Ref. Couscous 492g Vegetable oil 25 g Carrots 21 g Orange 60 g Beef 55 g Spinach raw 50 g Black beans 45 g
% E as Protein	11	11	10	12	15	16	17
% E as CHO	66	66	66	64	56	55	55
% E as Fat	23	23	24	24	29	29	28
Vitamin A (μg)	0	103 - 147	106 - 151	106 - 151	106 - 151	173 - 247	173 - 247
Vitamin C (mg)	0	2	108	112	108	155	155
Folate	53 - 70	54 - 71	61 - 81	118 - 157	55 - 73	102 - 136	132 - 175
Iron	13	14	14	24	71	96	109
Zinc	18	19	18	25	88	92	98
Calcium	14 - 22	15 - 25	21 - 33	23 - 36	20 - 32	32 - 51	34 - 54

	POTATO BASED DIET						
	Potato 907 g Vegetable oil 25 g	Potato 895 g Vegetable oil 25 g Carrots 21 g	Potato 865 g Vegetable oil 25 g Carrots 21 g Orange 60 g	Potato 770 g Vegetable oil 25 g Carrots 21 g Orange 60 g Lentils 70 g	Potato 725 g Vegetable oil 25 g Carrots 21 g Orange 60 g Beef 55 g	Potato 710 g Vegetable oil 25 g Carrots 21 g Orange 60 g Beef 55 g Spinach raw 50 g	Potato 649 g Vegetable oil 25 g Carrots 21 g Orange 60 g Beef 55 g Spinach raw 50 g Lentils 45 g
% E as Protein	6	6	6	8	12	12	13
% E as CHO	71	71	71	69	61	60	59
% E as Fat	23	23	23	23	27	28	28
Vitamin A (μg)	0	103 - 147	106 - 151	106 - 151	106 - 151	173 - 247	173 - 247
Vitamin C (mg)	224	223	322	302	287	330	317
Folate	40 - 53	41 - 55	49 - 65	108 - 144	45 - 59	93 - 123	131 - 174
Iron	14	15	15	25	75	98	122
Zinc	25	25	25	31	97	100	107
Calcium	17 - 27	18 - 29	24 - 38	25 - 40	22 - 35	34 - 55	35 - 56

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Chapter 3

Thiamin, riboflavin, niacin, vitamin B_6, pantothenic acid and biotin

The B-complex vitamins covered here are presented in ***Table 5*** along with the biochemical and physiologic roles of the co-enzyme forms and a brief description of clinical deficiency symptoms.

Table 5

Physiologic roles and deficiency signs of B-complex vitamins

Vitamin	Physiologic roles	Deficiency
Thiamin (B_1)	Co-enzyme functions in metabolism of carbohydrates and branched-chain amino acids	Beri-beri, polyneuritis, and Wernicke-Korsakoff syndrome
Riboflavin (B_2)	Co-enzyme functions in numerous oxidation and reduction reactions	Growth, cheilosis, angular stomatitis, and dermatitis
Niacin (nicotinic acid and nicotinamide)	Co-substrate/co-enzyme for hydrogen transfer with numerous dehydrogenases	Pellagra with diarrhoea, dermatitis, and dementia
Vitamin B_6 (pyridoxine, pyridoxamine, and pyridoxal)	Co-enzyme functions in metabolism of amino acids, glycogen, and sphingoid bases	Naso-lateral seborrhoea, glossitis, and peripheral neuropathy (epileptiform convulsions in infants)
Pantothenic acid	Constituent of co-enzyme A and phosphopantetheine involved in fatty acid metabolism	Fatigue, sleep disturbances, impaired coordination, and nausea
Biotin	Co-enzyme functions in bicarbonate-dependent carboxylations	Fatigue, depression, nausea, dermatitis, and muscular pains

Rice and wheat are the staples for many populations of the world. Excessive refining and polishing of cereals removes considerable proportions of B vitamins contained in these cereals. Clinical manifestations of deficiency of some B vitamins – such as beri-beri (cardiac and dry), peripheral neuropathies, pellagra, and oral and genital lesions (related to riboflavin deficiency) – were once major public health problems in parts of the world. These manifestations have now declined, the decline being brought about not through programmes,

which distribute synthetic vitamins but through changes in the patterns of food availability and consequent changes in dietary practices of the populations.

Although these clinical manifestations of B-vitamin deficiencies have decreased, there is evidence of widespread sub-clinical deficiency of these vitamins (especially of riboflavin and pyridoxine). These sub-clinical deficiencies, although less dramatic in their manifestations, exert deleterious metabolic effects. Despite the progress in reduction of large-scale deficiency in the world, there are periodic reports of outbreaks of B-complex deficiencies, which are linked to deficits of B vitamins in populations under various distress conditions.

Refugee and displaced population groups (20 million people by current United Nations estimates) are at risk for B-complex deficiency because most cereal foods used under emergency situations are not fortified with micronutrients (*1*). Recent reports have implicated the low B-complex content of diets as a factor in the outbreak of peripheral neuropathy and visual loss observed the adult population of Cuba (*2-4*). This deficiency in Cuba resulted from the consequences of an economic blockade (*4*).

Because of the extensive literature pertaining to the study of the B-complex vitamins, the references cited here were selected from those published after the FAO/WHO *handbook on human nutritional requirements* was published in 1974 (*5*). Greater weight has been given to studies which used larger numbers of subjects over longer periods, more thoroughly assessed dietary intake, varied the level of the specific vitamin being investigated, and used multiple indicators, including those considered functional in the assessment of status. These indicators have been the main basis for ascertaining requirements. Although extensive, the bibliographic search of recently published reports presented in this chapter most likely underestimates the extent of B-complex deficiency considering that many cases are not reported in the medical literature. Moreover, outbreaks of vitamin deficiencies in populations are usually not publicised because governments may consider the existence of these conditions to be politically sensitive information. Additional references are listed in the publication by the Food and Nutrition Board of the Institute of Medicine of the US National Academy of Sciences (*6*).

Thiamin

Background with requisite function in human metabolic processes

Deficiency

Thiamin (vitamin B_1, aneurin) deficiency results in the disease called beri-beri, which has been classically considered to exist in dry (paralytic) and wet (oedematous) forms (*7, 8*). Beri-beri occurs in human-milk-fed infants whose nursing mothers are deficient. It also occurs in adults with high carbohydrate intakes mainly from milled rice and with intakes of anti-thiamin factors. Beri-beri is still endemic in Asia. In relatively industrialized nations, the neurologic reflections of Wernicke-Korsakoff syndrome are frequently associated with chronic alcoholism with limited food consumption (*9*). Some cases of thiamin deficiency have been observed with patients who are hypermetabolic, are on parenteral nutrition, are undergoing chronic renal dialysis, or have undergone a gastrectomy. Thiamin deficiency has also been observed in Nigerians who ate silk worms, Russian schoolchildren (in Moscow), Thai rural elderly, Cubans, Japanese elderly, Brazilian Xavante Indians, French Guyanense, Southeast Asian schoolchildren who were infected with hookworm, Malaysian detention inmates, and people with chronic alcoholism.

Toxicity

Toxicity is not a problem with thiamin because renal clearance of levels conceivably ingested is rapid.

Functions

Thiamin functions as the co-enzyme thiamin pyrophosphate (TPP) in the metabolism of carbohydrates and branched-chain amino acids. Specifically the Mg^{2+}-coordinated TPP participates in the formation of α-ketols (e.g., among hexose and pentose phosphates) as catalysed by transketolase and in the oxidation of α-keto acids (e.g., pyruvate, α-ketoglutarate, and branched-chain α-keto acids) by dehydrogenase complexes (*10, 11*). Hence, when there is insufficient thiamin, the overall decrease in carbohydrate metabolism and its inter-connection with amino acid metabolism (via α-keto acids) have severe consequences, such as a decrease in the formation of acetylcholine for neural function.

Biochemical indicators

Indicators used to estimate thiamin requirements are urinary excretion, erythrocyte transketolase activity coefficient, erythrocyte thiamin, blood pyruvate and lactate, and neurologic changes. The excretion rate of the vitamin and its metabolites reflects intake, and the validity of the assessment of thiamin nutriture is improved with load test. Erythrocyte transketolase activity co-efficient reflects TPP levels and can indicate rare genetic defects. Erythrocyte thiamin is mainly a direct measure of TPP but also is a measure of thiamin and thiamin monophosphate by high performance liquid chromatography (HPLC) separation.

Thiamin status has been assessed by measuring urinary thiamin excretion under basal conditions or after thiamin loading, transketolase activity, and free and phosphorylated forms in blood or serum (*6, 9*). Although overlap with baseline values for urinary thiamin was found with oral doses below 1 mg, a correlation of 0.86 between oral and excreted amounts was found by Bayliss *et al.* (*12*). The erythrocyte transketolase assay, in which an activity coefficient based on a TPP stimulation of the basal level is given, continues to be a main functional indicator (*9*), but some problems have been encountered. Gans and Harper (*13*) found a wide range of TPP effect when thiamin intakes were adequate above 1.5 mg/day over a 3-day period. In some cases the activity coefficient may appear normal after prolonged deficiency (*14*). This measure seemed poorly correlated with dietary intakes estimated for a group of English adolescents (*15*). Certainly, there are both inter-individual and genetic factors affecting the transketolase (*16*). Baines and Davies (*17*) suggested that it is useful to determine erythrocyte TPP directly because the co-enzyme is less susceptible to factors that influence enzyme activity; however, there are also methods for determining thiamin and its phosphate esters in whole blood (*18*).

Factors affecting requirements

Because thiamin facilitates energy utilisation, its requirements have traditionally been expressed on the basis of energy intake, which can vary depending on activity levels. However, Fogeholm *et al.* (*19*) found no difference in activation coefficients for erythrocyte transketolase from a small group of skiers and from less physically active control subjects. Also, a study with thiamin-restricted Dutch males whose intake averaged 0.43 mg/day for 11 weeks did not reveal an association between short bouts of intense exercise and the decreases in indicators of thiamin status (*20*). Alcohol consumption may interfere with thiamin absorption (*9*).

Findings by age and life stage

Recommendations for infants are based on adequate food intake. Mean thiamin content of human milk is 0.21 mg/l (0.62 μmol/l) (*21*), which corresponds to 0.16 mg (0.49μmol) thiamin per 0.75 l of secreted milk per day. The blood concentration for total thiamin averages 210 ± 53 nmol/l for infants up to 6 months but decreases over the first 12–18 months of life (*22*).

A study of 13–14-year-old children related dietary intake of thiamin to several indicators of thiamin status (*15*). Sauberlich *et al.* (*23*) concluded from a carefully controlled depletion-repletion study of seven healthy young men that 0.3 mg thiamin per 4184 kJ met their requirements. Intakes below this amount lead to irritability and other symptoms and signs of deficiency (*24*). Anderson *et al*. (*25*) reported thiamin intakes of 1.0 and 1.2 mg/day as minimal for women and men, respectively. Hoorn et al. (*26*) reported that 23 percent of 153 patients aged 65–93 years were deemed deficient based on a transketolase activation coefficient greater than 1.27, which was normalised after thiamin administration. Nichols and Basu (*27*) found that only 57 percent of 60 adults aged 65–74 years had TPP effects of less than 14 percent and suggested that ageing may increase thiamin requirements.

An average total energy cost of 230 MJ has been estimated for pregnancy (*28*). With an intake of 0.4 mg thiamin/4184 kJ, this amounts to 22 mg total, or 0.12 mg/day for an additional thiamin need for the second and third trimesters (180 days). Taking into account an increased growth in maternal and foetal compartments, an overall additional requirement of 0.3 mg/day is adequate (6).

Lactating women are estimated to transfer 0.2 mg thiamin in their milk each day, and an additional 0.2 mg is estimated as a need for the increased energy cost of lactation of about 2092 kJ/day.

Recommendations

The recommendations for thiamin are given in ***Table 6***.

Table 6

Recommended nutrient intakes for thiamin

Group	Recommended nutrient intake mg/day
Infants and children	
0–6 months	0.2
7–12 months	0.3
1–3 years	0.5
4–6 years	0.6
7–9 years	0.9
Adolescents, 10–18 years	
Females	1.1
Males	1.2
Adults	
Females, 19+ years	1.1
Males, 19+ years	1.2
Pregnancy	1.4
Lactation	1.5

Riboflavin

Background with requisite function in human metabolic processes

Deficiency

Riboflavin (vitamin B_2) deficiency results in the condition of hypo- or ariboflavinosis, with sore throat; hyperaemia; oedema of the pharyngeal and oral mucous membranes; cheilosis; angular stomatitis; glossitis; seborrheic dermatitis; and normochromic, normocytic bone marrow (*8, 29*). Because the deficiency almost invariably occurs combined with a deficiency of other B-complex vitamins, some of the symptoms (e.g., glossitis and dermatitis) may result from other complicating deficiencies. The major cause of hypo-riboflavinosis is inadequate dietary intake as a result of limited food supply, which is sometimes exacerbated by poor food storage or processing. Children in developing countries will commonly demonstrate clinical signs of riboflavin deficiency during periods of the year when gastrointestinal infections are prevalent. Decreased assimilation of riboflavin also results from abnormal digestion such as that which occurs with lactose intolerance. This condition is highest in African and Asian populations and can lead to a decreased intake of milk as well as an abnormal absorption of the vitamin. Absorption of riboflavin is also affected in some other conditions, for example, tropical sprue, celiac disease, malignancy and resection of the small bowel, and decreased gastrointestinal passage time. In relatively rare cases the causes of deficiency are inborn errors in which the genetic defect is in the formation of a flavoprotein (e.g., acyl-co-enzyme A [co-A] dehydrogenases). Also at risk are those receiving phototherapy for neonatal jaundice and perhaps those with inadequate thyroid hormone. Some cases of riboflavin deficiency were also observed in Russian schoolchildren (Moscow) and Southeast Asian schoolchildren (infected with hookworm).

Toxicity

Riboflavin toxicity is not a problem because of limited intestinal absorption.

Functions

Conversion of riboflavin to flavin mononucleotide (FMN) and further to the predominant flavin adenine dinucleotide (FAD) occurs before these flavins form complexes with numerous flavoprotein dehydrogenases and oxidases. These flavoco-enzymes (FMN and FASD) participate in oxidation-reduction reactions in metabolic pathways and in energy production via the respiratory chain (*10, 11*).

Biochemical indicators

Indicators used to estimate riboflavin requirements are urinary flavin excretion, erythrocyte glutathione reductase activity coefficient, and erythrocyte flavin. The urinary flavin excretion rate of vitamin and metabolites reflects intake; validity of assessment of riboflavin adequacy is improved with load test. Erythrocyte glutathione reductase activity coefficient reflects FAD levels; results are confounded by such genetic defects as glucose-6-phosphate dehydrogenase deficiency and heterozygous β thalassemia.

Erythrocyte flavin is mainly a measure of FMN and riboflavin after hydrolysis of labile FAD and HPLC separation.

Riboflavin status has been assessed by measuring urinary excretion of the vitamin in fasting, random, and 24-hour specimens or by load returns tests (amounts measured after a specific amount of riboflavin is given orally); erythrocyte glutathione reductase; or erythrocyte flavin concentration (*6, 9, 29*). The HPLC method with fluorometry gives lower values for urinary riboflavin than do fluorometric methods, which measure the additive fluorescence of similar flavin metabolites (*30*). The metabolites can comprise as much as one-third of total

urinary flavin (*31, 32*) and in some cases may depress assays dependent on a biologic response because certain catabolites can inhibit cellular uptake (*33*). Under conditions of adequate riboflavin uptake (≈1.5 mg/day) by adults, an estimated 120 μg (320 nmol) total riboflavin or 80 μg/g of creatinine is excreted daily (*32*). The erythrocyte glutathione reductase assay, with an activity coefficient (AC) expressing the ratio of activities in the presence and absence of added FAD, continues to be used as a main functional indicator, but some limits have been noted. The reductase in erythrocytes from individuals with glucose-6-phosphate dehydrogenase deficiency (often present in blacks) has an increased avidity for FAD, which makes this test invalid (*34*). Sadowski (*35*) has set an upper limit of normality for the AC at 1.34 based on the mean value plus 2 standard deviations from several hundreds of apparently healthy individuals aged 60 years and over. Suggested guidelines for the interpretation of such enzyme ACs are as follows: less than 1.2, acceptable; 1.2–1.4, low; greater than 1.4, deficient (*9*). In general agreement with earlier findings on erythrocyte flavin, Ramsay *et al*. (*36*) found a correlation between cord blood and maternal erythrocyte deficiencies and suggested that values greater than 40 nmol/l are considered adequate.

Factors affecting requirements

Several studies reported modest effects of physical activity on the erythrocyte glutathione reductase AC (*37-41*). A slight increase in the AC and decrease in urinary flavin of weight-reducing women (*39*) and older women undergoing exercise training (*41*) were "normalised" with 20 percent additional riboflavin. However, riboflavin supplementation did not lead to an increase in work performance when such subjects were not clinically deficient (*42-45*).

Bio-availability of riboflavin in foods, mostly as digestible flavoco-enzymes, is excellent at nearly 95 percent (*6*), but absorption of the free vitamin is limited to about 27 mg per single meal or dose in an adult (*46*). No more than about 7 percent of food flavin is found as 8α-FAD covalently attached to certain flavoprotein enzymes. Although some portions of the 8α-(amino acid)-riboflavins are released by proteolysis of these flavoproteins, they do not have vitamin activity (*47*). A lower fat-to-carbohydrate ratio may decrease the riboflavin requirements of the elderly (*48*). Riboflavin interrelates with other B vitamins, notably niacin, which requires FAD for its formation from tryptophan, and vitamin B_6, which requires FMN for conversion of the phosphates of pyridoxine and pyridoxamine to the co-enzyme pyridoxal 5'-phosphate (PLP) (*49*). Contrary to earlier reports, no difference was seen in riboflavin status of women taking oral contraceptives when dietary intake was controlled by providing a single basic daily menu and meal pattern after 0.6 mg riboflavin/418 kJ was given in a 2-week acclimation period (*50*).

Findings by age and life stage

As reviewed by Thomas et al. (*51*), early estimates of riboflavin content in human milk showed changes during the post-partum period. More recent investigations of flavin composition of both human (*52*) and cow (*53*) milk have helped clarify the nature of the flavins present and provide better estimates of riboflavin equivalence. For human milk consumed by infants up to age 6 months, the riboflavin equivalence averages 0.35mg (931 nmol) /l (*6*) or 0.26 mg (691nmol) /0.75 l of milk per day. For low-income Indian women with erythrocyte glutathione reductase activity ratios averaging 1.80 and a milk riboflavin content of 0.22 mg/l, breast-fed infants averaged AC ratios near 1.36 (*54*). Hence, a deficiency sufficient to reduce human-milk riboflavin content by one-third can lead to a mild sub-clinical deficiency in infants.

Studies of riboflavin status in adults include those by Belko *et al.* (*38, 39*) in modestly obese young women on low-energy diets, by Bates *et al.* (*55*) on deficient Gambians, and by

Kuizon *et al.* (*56*) on Filipino women. Most of a 1.7-mg dose of riboflavin given to healthy adults consuming at least this amount was largely excreted in the urine (*32*). Such findings corroborate earlier work indicating a relative saturation of tissue with intakes above 1.1 mg/day. Studies by Alexander *et al.* (*57*) on riboflavin status in the elderly show that doubling the estimated riboflavin intakes of 1.7 mg/day for women aged 70 years and over, with a reductase AC of 1.8, led to a doubling of urinary riboflavin from 1.6–3.4 µg (4.2 to 9.0 nmol) /mg creatinine and a decrease in AC to 1.25. Boisvert *et al.* (*48*) obtained normalisation of the glutathione reductase AC in elderly Guatemalans with approximately 1.3 mg/day of riboflavin, with a sharp increase in urinary riboflavin occurring at intakes above 1.0–1.1 mg/day.

Pregnant women have an increased erythrocyte glutathione reductase AC (*58, 59*). Kuizon *et al.* (*56*) found that riboflavin at 0.7 mg /4184 kJ was needed to lower the AC of four of eight pregnant women to 1.3 within 20 days, whereas only 0.41 mg/4184 kJ was needed for five of the seven non-pregnant women. Maternal riboflavin intake was positively associated with foetal growth in a study of 372 pregnant women (*60*). The additional riboflavin requirement of 0.3 mg/day for pregnancy is an estimate based on increased growth in maternal and foetal compartments. For lactating women, an estimated 0.3 mg riboflavin is transferred in milk daily and, because utilisation for milk production is assumed to be 70 percent efficient, the value is adjusted upward to 0.4 mg/day.

Recommendations

The recommendations for riboflavin are given in ***Table 7***.

Table 7

Recommended nutrient intakes for riboflavin

Group	Recommended nutrient intake mg/day
Infants and children	
0–6 months	0.3
7–12 months	0.4
1–3 years	0.5
4–6 years	0.6
7–9 years	0.9
Adolescents, 10–18 years	
Females	1.0
Males	1.3
Adults	
Females, 19+ years	1.1
Males, 19+ years	1.3
Pregnancy	1.4
Lactation	1.6

Niacin

Background with requisite function in human metabolic processes

Deficiency

Niacin (nicotinic acid) deficiency classically results in pellagra, which is a chronic wasting disease associated with a characteristic erythematous dermatitis that is bilateral and symmetrical, a dementia after mental changes including insomnia and apathy preceding an overt encephalopathy, and diarrhoea resulting from inflammation of the intestinal mucous

surfaces (*8, 9, 61*). At present, pellagra occurs endemically in poorer areas of India, China, and Africa. Its cause has been mainly attributed to a deficiency of niacin; however, its biochemical inter-relationship to riboflavin and vitamin B_6, which are needed for the conversion of L-tryptophan to niacin equivalents (NEs), suggests that insufficiencies of these vitamins may also contribute to pellagra (*62*). Pellagra-like syndromes occurring in the absence of a dietary niacin deficiency are also attributable to disturbances in tryptophan metabolism (e.g., Hartnup disease with impaired absorption of the amino acid and carcinoid syndrome where the major catabolic pathway routes to 5-hydroxytryptophan) (*61*). Pellagra also occurs in people with chronic alcoholism (*61*). Cases of niacin deficiency have been found in people suffering from Crohn's disease (*61*).

Toxicity

Although therapeutically useful in lowering serum cholesterol, administration of chronic high oral doses of nicotinic acid can lead to hepatotoxicity as well as dermatologic manifestations. An upper limit (UL) of 35 mg/day as proposed by the US Food and Nutrition Board (*6*) was adopted by this consultation.

Functions

Niacin is chemically synonymous with nicotinic acid although the term is also used for its amide (nicotinamide). Nicotinamide is the other form of the vitamin, which does not have the pharmacologic action of the acid that is administered at high doses to lower blood lipids. It is the amide form that exists within the redox-active co-enzymes, nicotinamide adenine dinucleotide (NAD) and its phosphate (NADP), which function in dehydrogenase-reductase systems requiring transfer of a hydride ion (*10*, *11*). NAD is also required for non-redox adenosine diphosphate–ribose transfer reactions involved in DNA repair (*63*) and calcium mobilisation. NAD functions in intracellular respiration and with enzymes involved in the oxidation of fuel substrates such as glyceraldehyde 3-phosphate, lactate, alcohol, 3-hydroxybutyrate, and pyruvate. NADP functions in reductive biosyntheses such as fatty acid and steroid syntheses and in the oxidation of glucose-6-phosphate to ribose-5-phosphate in the pentose phosphate pathway.

Biochemical indicators

Indicators used to estimate niacin requirements are urinary excretion, plasma concentrations of metabolites, and erythrocyte pyridine nucleotides. The excretion rate of metabolites, mainly N'-methyl-nicotinamide and its 2- and 4-pyridones, reflects intake and is usually expressed as a ratio of the pyridones to N'-methyl-nicotinamide. Concentrations of metabolites, especially 2-pyridone, are measured in plasma after a load test. Erythrocyte pyridine nucleotides measure NAD concentration changes.

Niacin status has been monitored by daily urinary excretion of methylated metabolites, especially the ratio of the 2-pyridone to N'-methyl-nicotinamide; erythrocyte pyridine nucleotides; oral dose uptake tests; erythrocyte NAD; and plasma 2-pyridone (*6, 9*). Shibata and Matsuo (*64*) found that the ratio of urinary 2-pyridone to N'-methyl-nicotinamide was as much a measure of protein adequacy as it was a measure of niacin status. Jacob *et al.* (*65*) found this ratio too insensitive to marginal niacin intake. The ratio of the 2-pyridone to N'-methyl-nicotinamide also appears to be associated with the clinical symptoms of pellagra, principally the dermatitic condition (*66*). In plasma, 2-pyridone levels change in reasonable proportion to niacin intake (*65*). Similarly to the situation for erythrocyte pyridine nucleotide (nicotinamide co-enzymes), NAD concentration decreased 70 percent whereas NADP remained unchanged in adult males fed diets with only 6 or 10 mg NEs/day (*67*). Erythrocyte

NAD provided a marker at least as sensitive as urinary metabolites of niacin in this study (*67*) and in a niacin depletion study of elderly subjects (*68*).

Factors affecting requirements

The biosynthesis of niacin derivatives on the pathway to nicotinamide co-enzymes stems from tryptophan, an essential amino acid found in protein, and as such this source of NEs increases niacin intake. There are several dietary, drug, and disease factors that reduce the conversion of tryptophan to niacin (*61*) (e.g. the use of oral contraceptives [*69*]). Although a 60-to-1 conversion factor represents the average for human utilisation of tryptophan as NEs, there are substantial individual differences (*70, 71*). There is also an interdependence of enzymes within the tryptophan-to-niacin pathway where vitamin B_6 (as pyridoxal phosphate) and riboflavin (as FAD) are functional. Further, riboflavin (as FMN) is required for the oxidase that forms co-enzymic PLP from the alcohol and amine forms of phosphorylated vitamin B_6 (*49*).

Findings by age and life stage

Niacin content of human milk is approximately 1.5 mg (12.3 μmol) /l and the tryptophan content is 210 mg (1.0mmol) /l (*21*). Hence, the total content is approximately 5 mg NEs/l or 4 mg NEs/ 0.75 l secreted daily in human milk. Recent studies (*64, 70*) together with those reported in the 1950s suggest that 12.5 mg NEs, which corresponds to 5.6 mg NEs/4184 kJ, is minimally sufficient for niacin intake in adults.

For pregnant women, where 230 MJ is the estimated energy cost of pregnancy, calculated needs above those of non-pregnant women are 5.6 mg NEs/ 4186 kjoule (1,000 kcal) × 230,000 kjoule (55,000 kcal), or 308 mg NEs for the entire pregnancy or 1.7 mg NEs/day (308 mg NEs/180 days) for the second and third trimester, which is about a 10 percent increase. Also about 2 mg NEs/day is required for growth in maternal and foetal compartments (*6*).

For lactating women, an estimated 1.4 mg preformed niacin is secreted daily, and an additional requirement of less than 1 mg is needed to support the energy expenditure of lactation. Hence, 2.4 mg NEs/day is the added need attributable to lactation.

Recommendations

The recommendations for niacin are given in ***Table 8***.

Table 8

Recommended nutrient intakes for niacin

Group	Recommended nutrient intake, NEs/day[a]
Infants and children	
0–6 months	2[b]
7–12 months	4
1–3 years	6
4–6 years	8
7–9 years	12
Adolescents, 10–18 years	16
Adults	
Females, 19+ years	14
Males, 19+ years	16
Pregnancy	18
Lactation	17

[a] NEs, niacin equivalents. [b] Preformed.

Vitamin B_6

Background with requisite function in human metabolic processes

Deficiency

A deficiency of vitamin B_6 alone is uncommon because it usually occurs in association with a deficit in other B-complex vitamins (*72*). Early biochemical changes include decreased levels of plasma PLP and urinary 4-pyridoxic acid. These are followed by decreases in synthesis of transaminases (aminotransferases) and other enzymes of amino acid metabolism such that there is an increased urinary xanthurenate and a decreased glutamate conversion to the anti-neurotransmitter γ-aminobutyrate. Hypovitaminosis B_6 may often occur with riboflavin deficiency, because riboflavin is needed for the formation of the co-enzyme PLP. Infants are especially susceptible to insufficient intakes, which can lead to epileptiform convulsions. Skin changes include dermatitis with cheilosis and glossitis. There is usually a decrease in circulating lymphocytes and possibly a normocytic, microcytic, or sideroblastic anaemia (*9*). The sensitivity of such systems as sulphur amino acid metabolism to vitamin B_6 availability is reflected in homo-cysteinemia. A decrease in the metabolism of glutamate in the brain, which is found in vitamin B_6 insufficiency, reflects a nervous system dysfunction. As is the case with other micronutrient deficiencies, vitamin B_6 deficiency results in an impairment of the immune system. A current concern is for the rather pandemic occurrence of somewhat low vitamin B_6 intakes in many people who eat poorly (e.g., people with eating disorders). Vitamin B_6 deficiency has also been observed in Russian schoolchildren (Moscow), Southeast Asian schoolchildren (infected with hookworm), elderly Europeans (Dutch), and in some individuals with hyperhomo-cysteinemia or on chronic hemodialysis. Several medical conditions can also affect vitamin B_6 metabolism and lead to deficiency symptoms.

Toxicity

Use of high doses of pyridoxine for dubious treatment of pre-menstrual syndrome, carpal tunnel syndrome, and some neurologic diseases has resulted in neurotoxicity. A UL of 100 mg/day as proposed by the US Food and Nutrition Board (*6*) was adopted by this consultation.

Functions

There are three natural vitamers (different forms of the vitamin) of vitamin B_6, namely pyridoxine, pyridoxamine, and pyridoxal. These must be phosphorylated and the 5'-phosphates of the first two oxidized to the functional PLP, which serves as a carbonyl-reactive co-enzyme to diverse enzymes involved in the metabolism of amino acids. Such enzymes include aminotransferases, decarboxylases, and dehydratases, δ-aminolevulinate synthase in heme biosynthesis, phosphorylase in glycogen breakdown, and sphingoid base biosynthesis, etc. (*10, 11*).

Biochemical indicators

Indicators used to estimate vitamin B_6 requirements are PLP, urinary excretion, erythrocyte aminotransferases activity coefficients, tryptophan catabolites, erythrocyte and whole blood PLP, and plasma homo-cysteine. PLP is the major vitamin B_6 form in tissue and reflects liver PLP; it changes fairly slowly in response to vitamin intake. The excretion rate of vitamin and particularly 4-pyridoxate reflects intake. Erythrocyte aminotransferases for aspartate and alanine reflect PLP levels and show large variations in activity coefficients. The urinary excretion of xanthurenate, a tryptophan catabolite, is used especially after a tryptophan load test.

Vitamin B_6 status is most appropriately evaluated by using a combination of indicators, including those considered direct (e.g., vitamer concentration in cells or fluids) and indirect or functional indicators (e.g., erythrocyte aminotransferase saturation by PLP or tryptophan metabolites) (*9*). Plasma PLP may be the best single indicator because it appears to reflect tissue stores (*73*). Kretsch *et al*. (*74*) found that diets containing less than 0.05 mg vitamin B_6 given to 11 young women led to abnormal electroencephalograph patterns in 2 of the women and a plasma PLP of approximately 9 nmol. Hence, a level of about 10 nmol is considered sub-optimal. A cut-off for plasma PLP of 20 nmol has been proposed as an index of adequacy (*6*) based on recent findings (*73, 75*). Plasma PLP levels have been reported to fall with age (*76*). Urinary 4-pyridoxic acid level changes promptly with changes in vitamin B_6 intake (*73*) and is therefore of questionable value in assessing status. However, a value higher than 3 μmol/day , achieved with an intake of ~1 mg/d, has been suggested to reflect adequate intake (*77*). Erythrocyte aminotransferases for aspartate and alanine are commonly measured before and after addition of PLP to ascertain amounts of apoenzymes, the proportion of which increases with vitamin B_6 depletion. Values of 1.5–1.6 for the aspartate aminotransferase and approximately 1.2 for the alanine aminotransferase have been suggested as being adequate (*9*, *77*). Catabolites from tryptophan and methionine have also been used to assess vitamin B_6 status. In a review of such literature, Leklem (*77*) suggested that a 24-hour urinary excretion less than 65 μmol xanthurenate after a 2-g oral dose of tryptophan indicates normal vitamin B_6 status.

Factors affecting requirements

A recent review by Gregory (*78*) shows that bio-availability of vitamin B_6 in a mixed diet is about 75 percent (*79*), with approximately 8 percent of this total contributed by pyridoxine β-D-glucoside, which is about half as effectively utilised (*78*) as free B vitamers or their phosphates. The amine and aldehyde forms of vitamin B_6 may be about 10 percent less effective than pyridoxine (*80*). Despite the involvement of PLP with many enzymes affecting amino acid metabolism, there seems to be only a slight effect of dietary proteins on vitamin B_6 status (*81*). Studies reported decreases in indicators of vitamin B_6 status in women receiving oral contraceptives (*82, 83*), but this probably reflects hormonal stimulation of tryptophan catabolism rather than any deficiency of vitamin B_6 per se. Subjects with pre-eclampsia or eclampsia have lowered plasma PLP levels than do healthy pregnant women (*84, 85*).

Findings by age and life stage

The average intake for infants, based on human-milk content, is 0.13 mg/l (*86*) or 0.1 mg/0.75 l/day. With an average maternal dietary intake of vitamin B_6 of 1.4 mg/day, human milk was found to contain 0.12 mg/l, and plasma PLP of nursing infants averaged 54 nmol (*87*). Extrapolation on the basis of metabolic body size, weight, and growth suggests 0.3 mg/day as an adequate intake for infants 6–12 months of age (*6*). Information on vitamin B_6 requirements for children is limited, but Heiskanen *et al*. (*88*) found an age-related decrease in erythrocyte PLP and an increase in the aspartate aminotransferase activation.

In a review of earlier studies of men with various protein intakes, Linkswiler (*89*) concluded that normalisation of a tryptophan load test required 1–1.5 mg vitamin B_6. Miller *et al.* (*90*) found that 1.6 mg vitamin B_6 led to plasma PLP levels above 30 nmol/l for young men with various protein intakes. From several investigations of young women (*91-94*), a requirement closer to 1–1.2 mg vitamin B_6 could be estimated.

Limited studies of the elderly indicate that requirements may be somewhat higher, at least to maintain plasma PLP above a 20-nmolar cut-off level (*95, 96*).

For pregnancy it was confirmed that indicators of vitamin B_6 status decrease, especially in the third trimester (*85, 97, 98*). It is not clear, however, whether this is a normal physiologic phenomenon. For a maternal body store of 169 mg and foetal plus placental accumulation of 25 mg vitamin B_6, about 0.1 mg/day is needed on average over gestation (*6*). With additional allowances for the increased metabolic need and weight of the mother and about 75 percent of bio-availability, an additional average requirement in pregnancy of 0.25 mg can be assumed. Because most of this need is in the latter stages of pregnancy and vitamin B_6 is not stored to any significant extent, an extra 0.5 mg/day of vitamin B_6 may be justified to err on the side of safety.

For lactation, it may be prudent to add 0.6 mg vitamin B_6 to the base requirement for women because low maternal intakes could lead to a compromised vitamin B_6 status in the infant (*99*).

Recommendations

The recommendations for vitamin B_6 are given in ***Table 9***.

Table 9

Recommended nutrient intakes for vitamin B_6

Group	Recommended nutrient intake mg/day
Infants and children	
0–6 months	0.1
7–12 months	0.3
1–3 years	0.5
4–6 years	0.6
7–9 years	1.0
Adolescents, 10–18 years	
Females	1.2
Males	1.3
Adults	
Females, 19–50 years	1.3
Males, 19–50 years	1.3
Females, >50 years	1.5
Males, >50 years	1.7
Pregnancy	1.9
Lactation	2.0

Pantothenate

Background with requisite function in human metabolic processes

Deficiency

The widespread occurrence of releasable pantothenic acid in food makes a dietary deficiency unlikely (*8, 9, 100, 101*). If a deficiency occurs, it is usually accompanied by deficits of other nutrients. The use of experimental animals, an antagonistic analogue (ω-methyl-pantothenate) given to humans, and more recently the feeding of semi-synthetic diets virtually free of pantothenate (*102*) have all helped to define signs and symptoms of deficiency. Subjects become irascible; develop postural hypotension, rapid heart rate on exertion, epigastric distress with anorexia and constipation, numbness and tingling of the hands and feet ("burning feet" syndrome); and have hyperactive deep tendon reflexes and weakness of finger extensor

muscles. Some cases of pantothenate deficiency have been observed in patients with acne and other dermatitic conditions.

Toxicity

Toxicity is not a problem with pantothenate.

Functions

Pantothenic acid is a component of CoA, a cofactor that carries acyl groups for many enzymatic processes, and of phosphopantetheine within acyl carrier protein, a component of the fatty acid synthase complex (*10, 11*). Pantothenate is most especially involved in fatty acid metabolism but has a wide-ranging function as a prosthetic group that adds specificity to binding with appropriate enzymes.

Biochemical indicators

Indicators used to estimate pantothenate requirements are urinary excretion and blood levels. Excretion rate reflects intake. Whole blood, which contains vitamin and pantothenate-containing metabolites, has a general correlation with intake; erythrocyte levels seem more meaningful than plasma or serum levels.

Relative correspondence to pantothenate status has been reported for urinary excretion and for blood content of both whole blood and erythrocytes (*6, 9*). Fry *et al.* (*102*) reported a decline in urinary pantothenate levels from approximately 3–0.8 mg/day (13.7–3.6 μmol/day) in young men fed a deficient diet for 84 days. Urinary excretion for a typical American diet was found to be 2.6 mg (12μmol)/d, but it was strongly dependent on diet (*79*). Pantothenate intake estimated for adolescents was significantly correlated with pantothenate in urine (*103*). Whole-blood pantothenate fell from 1.95–1.41 μg/ml (8.8–6.4 μmol/l)when six adult males were fed a pantothenate-free diet (*102*). Whole-blood content corresponded to intake (*103*), and the range in whole blood was reported to be 1.57–2.66 μg/ml (7.2– 12.1μmol/l (*104*). There is an excellent correlation of whole-blood concentrations of pantothenate with the erythrocyte concentration, with an average value being 334 ng/ml (1.5 μmol/l) (*103*). The lack of sufficient population data, however, suggests the current use of an adequate intake rather than a recommended intake as a suitable basis for recommendations.

Factors affecting requirements

A measurement of urinary excretion of pantothenate after feeding a formula diet containing both bound and free vitamin indicates that approximately 50 percent of the pantothenate present in natural foods may be bio-available (*79*).

Findings by age and life stage

Infant requirements are based on an estimation of pantothenic acid content of human milk, which according to reported values is approximately 2 mg/l (*20, 105*). For a reported average human-milk intake of 0.75 l/day (*106-108*) these values suggest that 1.6 mg/day is an adequate intake by the younger (0–6 months) infants. Taking into consideration growth and body size, 1.9 mg/day may be extrapolated for the older (6–12 months) infant (105).

The studies of Eissenstat *et al.* (*103*) of adolescents suggest that intakes of less than 4 mg/day were sufficient to maintain blood and urinary pantothenate. Kathman and Kies (*109*) found a range of pantothenate intake of 4 to approximately 8 mg/day in 12 adolescents who were 11–16 years old. The usual pantothenate intake for American adults has been reported to be 4–7 mg/day (*102, 109-111*). Hence, around 5 mg/day is apparently adequate.

For pregnancy there is only one relatively recent study that found lower blood pantothenate levels but no difference in urinary excretion in pregnant women compared with non-pregnant controls (*112*).

For lactation blood pantothenate concentrations were found significantly lower at 3 months post-partum (*112*). With a loss of 1.7 mg/day (7.8 μmol/d) from a lactating woman and lower maternal blood concentrations found with intakes of about 5–6 mg/d, a recommended intake may be 7 mg/d.

Recommendations

The recommendations for pantothenate are given in ***Table 10***.

Table 10

Recommended nutrient intakes for pantothenate

Group	Recommended nutrient intake, mg/day
Infants and children	
0–6 months	1.7
7–12 months	1.8
1–3 years	2.0
4–6 years	3.0
7–9 years	4.0
Adolescents, 10–18 years	5.0
Adults	
Females, 19+	5.0
Males, 19+	5.0
Pregnancy	6.0
Lactation	7.0

Biotin

Background with requisite function in human metabolic processes

Deficiency

Biotin deficiency in humans has been clearly documented with prolonged consumption of raw egg whites, which contain biotin-binding avidin. Biotin deficiency was also observed in cases of parenteral nutrition with solutions lacking biotin given to patients with short-gut syndrome and other causes of malabsorption (*9, 113, 114*). Some cases of biotin deficiency were noted in infants with intractable diaper dermatitis and in those fed special formulas. Dietary deficiency in otherwise normal people is probably rare. Some patients have multiple carboxylase deficiencies and there are occasional biotinidase deficiencies. Clinical signs of deficiency include dermatitis of an erythematous and seborrheic type; conjunctivitis; alopecia; and central nervous system abnormalities such as hypotonia, lethargy, and developmental delay in infants and depression, hallucinations, and paresthesia of the extremities in adults.

Toxicity

Toxicity is not a problem because of limited intestinal absorption of biotin.

Functions

Biotin functions as a co-enzyme within several carboxylases after the carboxyl function of the vitamin becomes amide linked to the ε-amino of specific lysyl residues of the apoenzymes (*10, 11*). In humans and other mammals, biotin operates within four carboxylases. Three of

the four biotin-dependent carboxylases are mitochondrial (pyruvate carboxylase, methylcrotonyl-CoA carboxylase, and propionyl-CoA carboxylase) whereas the fourth (acetyl-CoA carboxylase) is found both in mitochondria and the cytosol. In all these cases biotin serves as carrier for the transfer of active bicarbonate into a substrate to generate a carboxyl product.

Biochemical indicators

Indicators used to estimate biotin requirements are urinary excretion and 3-hydroxyisovalerate excretion. The excretion rate of vitamin and metabolites in urine is assessed by avidin-based radioimmunoassay with HPLC. 3-Hydroxyisovalerate excretion inversely reflects the activity of β-methyl-crotonyl-CoA carboxylase, which is involved in leucine metabolism.

The present indicators for biotin status are its urinary excretion, as assessed with an avidin-based radioimmunoassay with HPLC, and 3-hydroxyisovalerate excretion (*115*). The isolation and chemical identification of more than a dozen metabolites of biotin established the main features of its use in microbes and mammals (*116, 117*). Quantification of the major biotin metabolites was done by Zempleni *et al*. (*118*). Both biotin and bisnorbiotin excretions decline in parallel in individuals on a diet containing raw egg whites (*115*). In these individuals the levels of urinary 3-hydroxyisovalerate, which increase as a result of decreased activity of β-methylcrotonyl-CoA carboxylase and altered leucine metabolism, rose from a normal mean of 112 to 272 μmol/24 hours. Decreased excretion of biotin, abnormally increased excretion of 3-hydroxyisovalerate, or both have been reported for overt cases of biotin deficiency (*119-124*). The lack of sufficient population data, however, suggests the current use of an adequate intake rather than a recommended intake as a suitable basis for recommendations.

Findings by age and life stage

The biotin content of human milk is estimated to be approximately 6 μg (24nmol)/l based on several studies (*125-127*) that report values ranging from near 4–7 μg (16.4–28.9 nmol) /l. Hence, the estimated intake of biotin for an infant consuming 0.75 l is 5 μg/day during the first half year and for older infants is perhaps 6 μg/day.

Requirements for children and adults have been extrapolated as follows (*6*):

Adequate intake for child or adult = (adequate intake young infant) (weight adult or child/weight infant) $^{0.75}$

For pregnancy there are at present insufficient data to justify an increase in the adequate intake, although Mock *et al.* (*128*) reported decreased urinary biotin and 3-hydroxyisovalerate in a large fraction of seemingly healthy pregnant women.

For lactation the intake may need to be increased by an additional 5 μg/day to cover the losses due to human-milk secretion.

Recommendations

The recommendations for biotin are given in ***Table 11***.

Table 11

Recommended nutrient intakes for biotin

Group	Recommended nutrient intake μg/day
Infants and children	
0–6 months	5
7–12 months	6
1–3 years	8
4–6 years	12
7–9 years	20
Adolescents, 10–18 years	25
Adults	
Females, 19+	30
Males, 19+	30
Pregnancy	30
Lactation	35

General considerations for B-complex vitamins

Notes on suggested recommendations

The recommendations for infants are based largely on the composition and quantity of human milk consumed and are formally considered to be adequate intakes. Younger (0–6 months) infants are considered to derive adequate intake from milk alone; recommendations for older (7–12 months) infants are adjusted by metabolic scaling such that a factor – (weight of 7-12 mo/weight of 0-5 mo) $^{0.75}$ – is multiplied by the recommendation for the younger infant (*6*). Recommendations have been given to use the higher (6–11 months) level for the first year of life.

For most of the B vitamins, there is little or no direct information that can be used to estimate the amounts required by children and adolescents. Hence, an extrapolation from the adult level has been used where a factor – (weight child/weight adult) $^{0.75}$ × (1 + growth factor) – is multiplied by the adult recommendation (*6*).

For most of the B-complex vitamins covered here, data are not sufficient to justify altering recommendations for the elderly.

For pregnancy and lactation, increased maternal needs related to increases in energy and replacement of secretion losses were considered.

Dietary sources of B-complex vitamins

A listing of some better and usual food sources for the vitamins considered is given in ***Table 12.***

Table 12

Dietary sources of water-soluble vitamins

Vitamin	Good-to-moderate dietary sources[a]
Thiamin (B_1)	Pork, organ meats, whole grains, and legumes
Riboflavin (B_2)	Milk and dairy products, meats, and green vegetables
Niacin (nicotinic acid and nicotinamide)	Liver, lean meats, grains, and legumes; can be formed from tryptophan
Vitamin B_6 (pyridoxine, pyridoxamine, and pyridoxal)	Meats, vegetables, and whole-grain cereals
Pantothenic acid	Animal tissues, whole-grain cereals, and legumes; widely distributed
Biotin	Liver, yeast, egg, yolk, soy flour, and cereals

[a]Foods are listed according to the concentrations of vitamin which they contain.

Research suggestions

In view of the issues raised in this section on B-complex vitamins, the following suggestions are noted:

- Actual requirements are least certain for children, adolescents, pregnant and lactating women, and the elderly, and as such they deserve further study.
- Studies need to include graded levels of the vitamin above and below current recommendations and should consider or establish clearly defined cut-off values for clinical adequacy and inadequacy and be conducted for periods of time sufficient for ascertaining equilibrium dynamics.
- For status indicators, additional functional tests would be useful for riboflavin (e.g., the activity of FMN-dependent pyridoxine [pyridoxamine] 5'-phosphate oxidase in erythrocytes), niacin (e.g., sensitive blood measures, especially of NAD), and perhaps pantothenate.
- The food content and bio-availability of pantothenate and biotin need further investigation to establish the available and preferred food sources reasonable for different populations.

Primary efforts should now be in the arena of public health and nutrition education with emphasis on directing people and their governments to available and healthful foods; care necessary for their storage and preparation; and achievable means for adjusting intake with age, sex, and health status.

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Chapter 4
Folate and folic acid

Role of folate and folic acid in human metabolic processes

Folates accept one-carbon units from donor molecules and passes them on via various biosynthetic reactions (*1*). In their reduced form cellular folates function conjugated to a polyglutamate chain. These folates are a mixture of unsubstituted polyglutamyl tetrahydrofolates and various substituted one-carbon forms of tetrahydrofolate (e.g., 10-formyl, 5,10-methylene, and 5-methyl) (***Figure 6***). The reduced forms of the vitamin, particularly the unsubstituted dihydro and tetrahydro forms, are unstable chemically. They are easily split between the C-9 and N-10 bond to yield a substituted pteridine and *p*-aminobenzoylglutamate, which have no biologic activity (*2*). Substituting a carbon group at N-5 or N-10 decreases the tendency of the molecule to split; however, the substituted forms are also susceptible to oxidative chemical rearrangements and, consequently, loss of activity (*2*). The folates found in food consist of a mixture of reduced folate polyglutamates.

Although natural folates rapidly lose activity in foods over periods of days or weeks, folic acid (e.g., in fortified foods) is almost completely stable for months or even years. The chemical lability of all naturally occurring folates results in a significant loss of biochemical activity during harvesting, storage, processing, and preparation. Half or even three-quarters of initial folate activity may be lost during these processes. This is in contrast to the stability of the synthetic form of this vitamin, folic acid (*2*). In this form the pteridine (2-amino-4-hydroxypteridine) ring is not reduced (***Figure 6***), rendering it very resistant to chemical oxidation. However, folic acid is reduced in cells by the enzyme dihydrofolate reductase to the di- and tetrahydro forms (***Figure 7***). This takes place within the intestinal mucosal cells, and 5-methyltetrahydrofolate is released into the plasma.

Natural folates found in foods are all conjugated to a polyglutamyl chain containing different numbers of glutamic acids depending on the type of food. This polyglutamyl chain is removed in the brush border of the mucosal cells by the enzyme folate conjugase, and folate monoglutamate is subsequently absorbed (*1*). The primary form of folate entering human circulation from the intestinal cells is 5-methyltetrahydrofolate monoglutamate. This process is, however, limited in capacity. If enough folic acid is given orally, unaltered folic acid appears in the circulation (*3*), is taken up by cells, and is reduced by dihydrofolate reductase to tetrahydrofolate

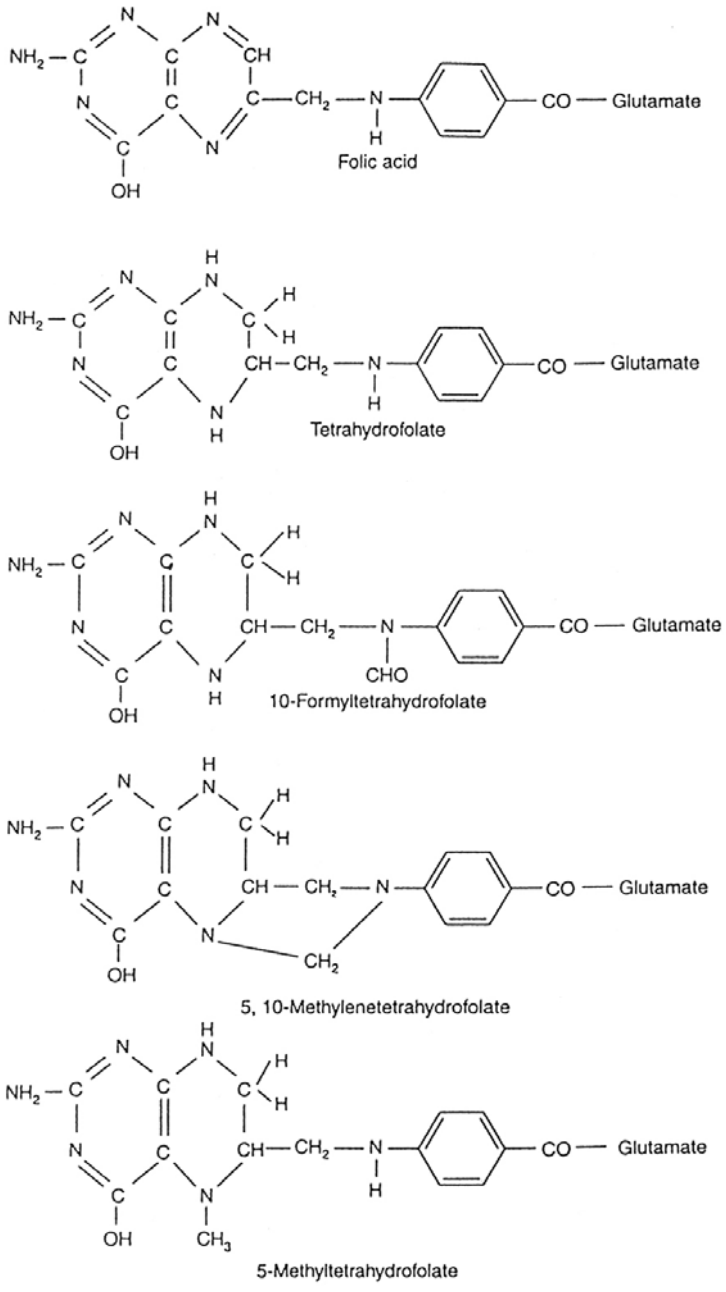
The bio-availability of natural folates is affected by the removal of the polyglutamate chain by the intestinal conjugase. This process is apparently not complete (*4*), thereby reducing the bio-availability of natural folates by as much as 25–50 percent. In contrast, synthetic folic acid appears to have a bio-availability of close to 100 percent (*4, 5*). The low bio-availability and – more importantly – the poor chemical stability of the natural folates has a profound influence on the development of nutrient recommendations. This is particularly true if some of the dietary intake is in the synthetic form, folic acid, which is much more stable and bio-available. Food fortification of breakfast cereals, flour, etc. can add significant amounts of folic acid to the diet.

Functional folates have one-carbon groups derived from several metabolic precursors (e.g., serine, *N*-formino-L-glutamate, folate, etc.). With 10-formyltetrahydrofolate the formyl

group is incorporated sequentially into C-2 and C-8 of the purine ring during its biosynthesis. Likewise the conversion of deoxyuridylate (a precursor to RNA) into thymidylate (a precursor to DNA) is catalysed by thymidylate synthase, which requires 5,10-methylenetetrahydrofofate. Thus, folate in its reduced and polyglutamylated forms is essential for the DNA biosynthesis cycle shown in ***Figure 6***.

Figure 6

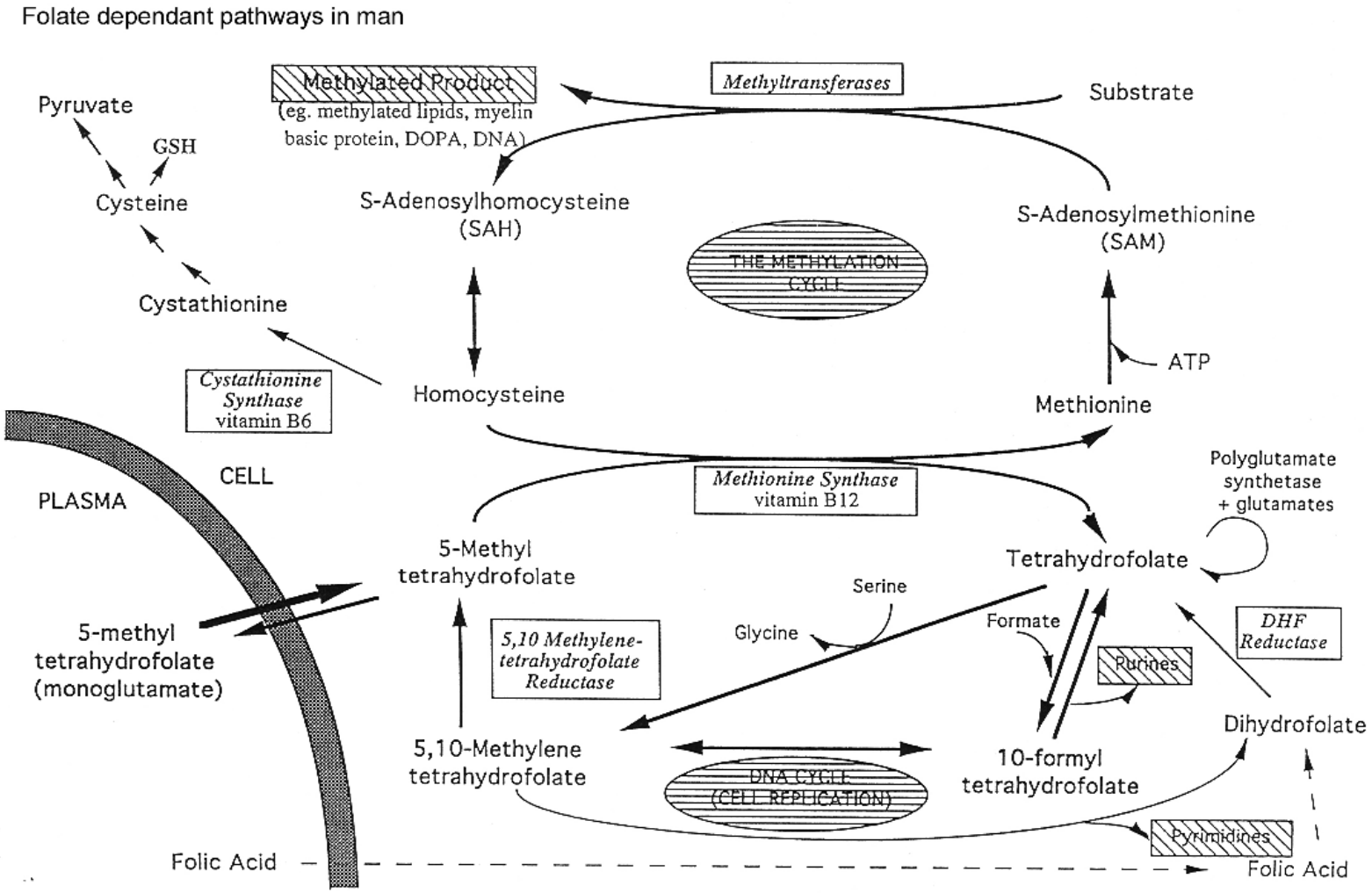
The chemical formula of folic acid (synthetic form) and the most important natural folates.



Note: In cells and thus in food the latter are conjugated to a polyglutamate tail.

Figure 7

The role of the folate co-factors in the DNA cycle and the methylation cycle. The enzyme methionine synthase requires vitamin B_{12} as well as folate for activity.



Alternatively 5,10-methylenetetrahydrofolate can be channelled to the methylation cycle (*1*). This cycle has two functions. It ensures that the cell always has an adequate supply of *S*-adenosylmethionine, an activated form of methionine, which acts as a methyl donor to a wide range of methyltransferases. These enzymes methylate a wide range of substrates including lipids, hormones, DNA, proteins, etc. One such important methylation is that of myelin basic protein, which acts as insulation for nerves cells. When the methylation cycle is interrupted as it is during vitamin B_{12} deficiency, one of the clinical consequences is the demyelination of nerve resulting in a neuropathy which leads to ataxia, paralysis, and, if untreated, ultimately death. Other important methyltransferase enzymes down-regulate DNA and suppress cell division (*1*).

In the liver the methylation cycle also serves to degrade methionine. Methionine is an essential amino acid in Humans and is present in the diet of people in developed countries at about 60 percent over that required for protein synthesis and other uses. The excess methionine is degraded via the methylation cycle to homo-cysteine, which can either be catabolised to sulfate and pyruvate (with the latter being used for energy) or remethylated to methionine. The need to maintain intracellular *S*-adenosylmethionine levels is related to the amount of methionine metabolised via homo-cysteine.

The DNA and methylation cycles both regenerate tetrahydrofolate. However, there is a considerable amount of catabolism of folate (*6*) and a small loss of folate via excretion from the urine, skin, and bile. There is a need to replenish the body's folate content by uptake from the diet. If there is inadequate dietary folate, the activity of both the DNA and the methylation cycles will be reduced. A decrease in the former will reduce DNA biosynthesis and thereby reduce cell division. Although this will be seen in all dividing cells, the deficiency will be most obvious in cells that are rapidly dividing, for example, in a decrease in red cell production, producing anaemia. Other cells derived from bone marrow also decrease, leading to leucopenia and thrombocytopenia. Likewise there is a reduction in cell division in the lining of the gut. Taken together, this reduction in the DNA cycle results in an increased susceptibility to infection, a decrease in blood coagulation, and secondary malabsorption. In folate deficiency, the flux through the methylation cycle is decreased but the DNA cycle may be more sensitive. The most obvious expression of the decrease in the methylation cycle is an elevation in plasma homo-cysteine. This is due to a decreased availability of new methyl groups provided as 5-methyltetrahydrofolate, necessary for the remethylation of plasma homo-cysteine. Previously it was believed that a rise in plasma homo-cysteine was nothing more than a biochemical marker of possible folate deficiency. However, there is increasing evidence that elevations in plasma homo-cysteine are implicated in the aetiology of cardiovascular disease (*7*). This moderate elevation of plasma homo-cysteine occurs in subjects with a folate status previously considered adequate (*8*).

Interruption of the methylation cycle resulting from impaired folate status or deceased vitamin B_{12} or vitamin B_6 status may have serious long-term risks. Such interruption, as seen in vitamin B_{12} deficiency (e.g., pernicious anaemia), causes a very characteristic demyelination and neuropathy known as subacute combined degeneration of the spinal cord and peripheral nerves. If untreated, this leads to ataxia, paralysis, and ultimately death. Such neuropathy is not usually associated with folate deficiency but is seen if folate deficiency is very severe and prolonged (*9*). The explanation may lie in the well-established ability of nerve tissue to concentrate folate to a level of about five times that in the plasma. This may ensure that nerve tissue has an adequate level of folate when folate being provided to the rapidly dividing cells of the marrow has been severely compromised for a prolonged period. The resultant anaemia will thus inevitably present clinically earlier than the neuropathy.

Definition of populations at risk for folate deficiency

Nutritional deficiency of folate is common in people consuming a limited diet (*10*). This can be exacerbated by malabsorption conditions, including coeliac disease and tropical sprue. Pregnant women are at risk of folate deficiency because pregnancy significantly increases the folate requirement, especially during periods of rapid foetal growth (i.e., in the second and third trimester) (*6*). During lactation losses of folate in milk also increase the folate requirement.

During pregnancy there is an increased risk of foetal neural tube defects (NTDs), with risk increasing 10-fold as folate status goes from adequate to poor (*11*). Between days 21 and 27 post-conception, the neural plate closes to form what will eventually be the spinal cord and cranium. Spina bifida, anencephaly, and other similar conditions are collectively called NTDs. They result from improper closure of the spinal cord and cranium, respectively, and are the most common congenital abnormalities (*12*).

Delineation of dietary sources

Although folate is found in a wide variety of foods, it is present in a relatively low density (*10*) except in liver. Diets that contain adequate amounts of fresh green vegetables (i.e., in excess of three servings per day) will be good folate sources. Folate losses during harvesting, storage, distribution, and cooking can be considerable. Likewise, folate derived from animal products is subject to loss during cooking. Some staples, such as white rice and unfortified corn, are low in folate (see ***Chapter 2***).

In view of the increased requirement for folate during pregnancy and lactation and by select population groups and in view of its low bio-availability, it may be necessary to consider fortification of foods or selected supplementation of women of child-bearing years.

Evidence on which to base a recommended intake

The 1988 Food and Agriculture Organization of the United Nations and World Health Organization (FAO/WHO) Expert Consultation report (*13*) indicated that there were three states of folate nutrition: folate adequacy, impending folate deficiency, and overt folate deficiency. This represented the thinking at the time with respect to folate status – that a status necessary to prevent clinical deficiency was clearly present with folate adequacy and that further improvement in folate status would have no benefit. It would thus be appropriate to increase intake in those with impending folate deficiency or more importantly in those with overt folate deficiency but that nothing was to be gained by increasing the status of those who had adequate status. In the 1988 FAO/WHO report (*13)* it was suggested that adequate folate status is reflected in a red cell folate level of greater than 150 μg/L. Of less relevance was a liver folate level of greater than 7.5 μg/g, because such values are only available in rare circumstances. A normal *N*-formino-L-glutamate test was also cited as evidence of sufficiency, but this test has largely been discredited and abandoned as not having any useful function (*10*). In more recent literature red cell folate continues to be used as an important index of folate status (*14*). Plasma folate is also used but is subject to greater fluctuation. Indicators of haematologic status such as raised mean corpuscular volume, hypersegmentation of neutrophils, and, eventually, the first stages of anaemia remain important indicators of reduced folate status (*15*).

The biomarker plasma homo-cysteine is a very sensitive indicator of folate status and must be added to the indicators of folate adequacy. This applies not only to the deficient range of red blood cell folate but includes normal and even above-normal levels of red cell folate (*14*). There is also very strong evidence that plasma homo-cysteine is an independent risk

factor for cardiovascular disease (*8, 16*). Any elevation in homo-cysteine, even at levels where overt folate deficiency is not an issue, may be undesirable because it is a risk factor for chronic disease. Thus, newer thinking would require consideration of a folate intake that would reduce plasma homo-cysteine to a minimum level of less than 7.0 μmol/l. Formerly acceptable levels of red cell folate may therefore be associated with an increased rise of cardiovascular disease and stroke (*17,18*). The possible benefit of lowering plasma homo-cysteine through increased folate intake can be proven only by an intervention trial with folic acid supplementation in large populations. Using plasma homo-cysteine as a biomarker for folate adequacy can only be done on an individual basis after the possibility of a genetic mutation or an inadequate supply of vitamin B_6 or vitamin B_{12} has been eliminated.

There is now conclusive evidence that most NTDs can be prevented by the ingestion of folic acid near the time of conception (*11,12*). Lower red cell folate, including what was previously considered an adequate or normal range, is associated with an increased risk of spina bifida and other NTDs (*19*). Red cell folate levels greater than 150 μg/L, which are completely adequate to prevent anaemia, are associated with increase risk of NTDs (*11*).

Low folate status, including red cell levels in the normal range, increases the risk of colorectal cancer (*20, 21*).

In 1998, the US National Academy of Sciences (*22*) exhaustively reviewed the evidence of folate intake, status, and health for all age groups and also reviewed the literature on the extra requirements during pregnancy and lactation. This review led to calculations of an estimated average requirement (EAR) and a subsequent estimation of the recommended dietary allowances (RDAs) to be the EAR plus 2 standard deviations. This definition of the RDA agrees with the definition of the FAO/WHO recommended nutrient intake (RNI), and members of this FAO/WHO expert group agreed that the values published by the US National Academy of Sciences were the best estimates of folate requirements based on the current literature. Therefore, the FAO/WHO expert group adopted the same approach, and the RNIs suggested in ***Table 13*** were based on the RDAs of the US National Academy of Sciences. These recommendations apply to healthy individuals and populations.

Table 13

Estimated average requirement (EAR) and recommended nutrient intake (RNI) for folic acid expressed as dietary folate equivalents, by age group

Group	EAR (μg/day)	RNI (μg/day)
Infants and children		
0–6 months [a]	65	80
7–12 months	65	80
1–3 years	120	160
4–6 years	160	200
7–9 years	250	300
Adolescents, 10–18 years	300	400
Adults		
19–65 years	320	400
65+ years	320	400
Pregnancy	520	600
Lactation	450	500

Adapted from the US National Academy of Sciences (*22*).

[a] Based on a human milk intake of 0.75 l/day.

Differences in bio-availability of folic acid and food folate

The RNIs suggested for groups in ***Table 13*** used food folate as the source of dietary folate because most societies in developing countries consume folate from naturally occurring sources. As discussed in the introduction, natural folates are found in a conjugated form in food, which reduces its bio-availability by perhaps as much as 50 percent (*4*). In addition, natural folates are much less stable. If chemically pure folic acid (pteroyl monoglutamate) is used to provide part of the RNI, by way of fortification or supplementation, the total dietary folate, which contains conjugated forms (pteroyl polyglutamates), could be reduced by an appropriate amount. On average the conjugated folate in natural foods is considered to be only half as available as synthetic folic acid. For example, the recommendation of usual mixed forms of folate in the diet is 400 μg/day, but 100 μg of this given as pure folic acid would be considered to be equivalent to 200 μg of dietary mixed folate. Hence, only an additional 200 μg of dietary folate would be needed.

The FAO/WHO expert group agreed with the findings of the Food and Nutrition Board of the US National Academy of Sciences (*22*):

> Since folic acid taken with food is 85 percent bio-available but food folate is only about 50 percent bio-available, folic acid taken with food is 85/50 (i.e., 1.7) times more available. Thus, if a mixture of synthetic folic acid plus food folate has been fed, dietary folate equivalents (DFEs) are calculated as follows to determine the EAR:
>
> μg of DFE provided = [μg of food folate + (1.7 x μg of synthetic folic acid)]
>
> To be comparable to food folate, only half as much folic acid is needed if taken on an empty stomach, i.e., 1 μg of DFE = 1 μg of food folate = 0.5 μg of folic acid taken on an empty stomach = 0.6 μg of folic acid with meals (*22*).

The experts from the National Academy of Sciences went on to say that the required estimates for the dietary folate equivalents could be lowered if future research indicates that food folate is more than 50 percent bio-available.

Neural tube defects

It is now agreed that a supplement of 400 μg of folic acid taken near the time of conception will prevent most neural tube defects (NTDs) (*23, 24*). The recommendation to prevent recurrence in women with a previous NTD birth remains 4.0 mg/day because of the high increase in risk in such cases and because that was the amount used in the most definitive trial (*25*). Because of the poorer bio-availability and stability of food folate, a diet based on food folate will not be optimum in prevention. One study determined that risk of NTD is 10-fold higher in people with poor folate status than in those with high normal folate status (*11*). A further study suggests that an extra 200 μg/day or possibly 100 μg/day if taken habitually in fortified food would prevent most if not all of folate-preventable NTDs (*26*). Ideally, an extra 400 μg/day should be provided because this is the amount used in various intervention trials (*12*) and can be achieved by supplementation. This amount could not be introduced by way of fortification, because exposure to high intakes of folic acid by people consuming a large intake of flour would run the risk of preventing the diagnosis of pernicious anaemia in the elderly. It is likely that depending on the staple chosen it would be possible to increase intake in most women by 100 μg/day without causing too high an exposure in other groups. It is suggested that this amount, although not optimal, will prevent most NTDs.

Cardiovascular disease

Plasma homo-cysteine concentration, if only moderately elevated, is an independent risk factor for cardiovascular disease (*7, 8, 16*) and stroke (*18*). Increased risk was associated with values higher than 11 μmol/l (*8*), which is well within what is the normal range (5–15 μmol/l) of plasma homo-cysteine levels (*27*). In addition, even in populations that are apparently normal and consuming diets adequate in folate, there is a range of elevation of plasma homo-cysteine (*14*) that could be lowered by an extra 100 or 200 μg/day of folic acid (*8, 27*). Large-scale intervention trials regarding the significance of interrelationships among folate levels, plasma homo-cysteine levels, and cardiovascular disease have not been completed and therefore it would be premature to introduce public health measures in this area.

Colorectal cancer

Evidence suggests a link between colorectal cancer and dietary folate intake and folate status (20*, 21*). One study reported that women who take multivitamin supplements containing folic acid for prolonged periods have a significantly reduced risk of colorectal cancer (*28*). However, the scientific evidence is not sufficiently clear for recommending increased folate intake in populations at risk for colorectal cancer.

Upper limit

There is no evidence that it is possible to consume sufficient natural folate to pose a risk of toxicity (*22*). However, this clearly does not apply to folic acid given in supplements or fortified foods. The main concern is the masking of the diagnosis of pernicious anaemia, because high levels of folic acid correct the anaemia, allowing the neuropathy to progress undiagnosed to where it may become irreversible even upon treatment with vitamin B_{12} (*1, 29*). Consumption of large amounts of folic acid might also pose other less well-defined risks. Certainly, consumption of milligram amounts of folic acid would be undesirable. Savage and Lindenbaum (*30*) suggest that even at levels of the RNI there is a decreased opportunity to diagnose pernicious anaemia through its presentation via the anaemia.

The US National Academy of Sciences (*22*), after reviewing literature, has suggested an upper level of 1000 μg. Thus, 400 μg/day of folic acid, in addition to dietary folate, would seem safe. There is probably no great risk of toxicity at a range between 400 and 1000 μg of folic acid per day with the exception of some increased difficulty in diagnosing pernicious anaemia resulting from the masking of the anaemia.

Future research

There are many areas for future research:

- Folate status may be related to birth weight. Therefore it is important to study the relationship between folate status and birth weight, especially in populations where low birth weight is prevalent.
- Folate status probably differs widely in different developing countries. Red cell folate levels are an excellent determinant of status. Such estimates in representative populations would determine whether some communities are at risk from poor folate status.
- Some evidence indicates that elevated plasma homo-cysteine is a risk factor for cardiovascular disease and stroke. Elevated plasma homo-cysteine is largely related to poor folate status, with poor vitamin B_6 status, poor vitamin B_{12} status, or both also contributing. There are also genetic differences (*31*). The prevalence of elevated

plasma homo-cysteine and its relationship to cardiovascular disease should be established in different developing countries.

- More data should be generated on the bio-availability of natural folate from diets consumed in developing countries.
- Because the absorption of folate may be more efficient in humans with folate deficiency, folate absorption in these populations requires additional research.
- The relationship between folate deficiency and the incidence of NTDs in developing countries needs further investigation.
- Quantitation of the folate content of foods typically consumed in developing countries should be established for the different regions of the world.

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Chapter 5
Vitamin B_{12}

Role of vitamin B_{12} in human metabolic processes

Although the nutritional literature still uses the term vitamin B_{12}, a more specific name for vitamin B_{12} is cobalamin. Vitamin B_{12} is the largest of the B complex vitamins, with a molecular weight of over 1000. It consists of a corrin ring made up of four pyrroles with cobalt at the center of the ring (*1, 2*).

There are several vitamin B_{12}–dependent enzymes in bacteria and algae, but no species of plants have the enzymes necessary for vitamin B_{12} synthesis. This fact has significant implications for the dietary sources and availability of vitamin B_{12}. In mammalian cells there are only two vitamin B_{12}–dependent enzymes (*3*). One of these enzymes, methionine synthase, uses the chemical form of the vitamin which has a methyl group attached to the cobalt and is called methylcobalamin (see ***Figure 7*** in Chapter 4.). The other enzyme, methylmalonyl CoA mutase, uses vitamin B_{12} with a 5'-adeoxyadenosyl moiety attached to the cobalt and is called 5'-deoxyaldenosylcobalamin, or coenzyme B_{12}. In nature there are two other forms of vitamin B_{12}: hydroxycobalamin and aquacobalamin, where hydroxyl and water groups, respectively, are attached to the cobalt. The synthetic form of vitamin B_{12} found in supplements and fortified foods is cyanocobalamin, which has cyanide attached to the cobalt. These three forms of B_{12} are enzymatically activated to the methyl- or deoxyadenosylcobalamins in all mammalian cells.

Dietary sources and availability

Most microorganisms, including bacteria and algae, synthesise vitamin B_{12}, and they constitute the only source of the vitamin (*4*). The vitamin B_{12} synthesised in microorganisms enters the human food chain through incorporation into food of animal origin. In many animals gastrointestinal fermentation supports the growth of these vitamin B_{12}–synthesising microorganisms, and subsequently the vitamin is absorbed and incorporated into the animal tissues. This is particularly true for the liver, where vitamin B_{12} is stored in large concentrations. Products from these herbivorous animals, such as milk, meat, and eggs, constitute important dietary sources of the vitamin unless the animal is subsisting in one of the many regions known to be geochemically deficient in cobalt (*5*). Milk from cows and humans contains binders with very high affinity for vitamin B_{12}, whether they hinder or promote intestinal absorption is not entirely clear. Omnivores and carnivores, including humans, derive dietary vitamin B_{12} from animal tissues or products (i.e., milk, butter, cheese, eggs, meat, poultry, etc.). It appears that no significant amount of the required vitamin B_{12} by humans is derived from microflora, although vegetable fermentation preparations have also been reported as being possible sources of vitamin B_{12} (6).

Absorption

The absorption of vitamin B_{12} in humans is complex (*1, 2*). Vitamin B_{12} in food is bound to proteins and is released from the proteins by the action of a high concentration of hydrochloric acid present in the stomach. This process results in the free form of the vitamin, which is immediately bound to a mixture of glycoproteins secreted by the stomach and salivary glands. These glycoproteins, called R-binders (or haptocorrins), protect vitamin B_{12}

from chemical denaturation in the stomach. The stomach's parietal cells, which secrete hydrochloric acid, also secrete a glycoprotein called intrinsic factor. Intrinsic factor binds vitamin B_{12} and ultimately enables its active absorption. Although the formation of the vitamin B_{12} – intrinsic factor complex was initially thought to happen in the stomach, it is now clear that this is not the case. At an acidic pH the affinity of the intrinsic factor for vitamin B_{12} is low whereas its affinity for the R-binders is high. When the contents of the stomach enter the duodenum, the R-binders become partly digested by the pancreatic proteases, which causes them to release their vitamin B_{12}. Because the pH in the duodenum is more neutral than that in the stomach, the intrinsic factor has a high binding affinity to vitamin B_{12}, and it quickly binds the vitamin as it is released from the R-binders. The vitamin B_{12}–intrinsic factor complex then proceeds to the lower end of the small intestine, where it is absorbed by phagocytosis by specific ileal receptors (*1, 2*).

Populations at risk for and consequences of vitamin B_{12} deficiency

Vegetarians

Because plants do not synthesise vitamin B_{12}, individuals who consume diets completely free of animal products (vegan diets) are at risk of vitamin B_{12} deficiency. This is not true of lacto-ovo-vegetarians, who consume the vitamin in eggs, milk, and other dairy products.

Pernicious anaemia

Malabsorption of vitamin B_{12} can occur at several points during digestion (*1, 4*). By far the most important condition resulting in vitamin B_{12} malabsorption is the auto-immune disease called pernicious anaemia (PA). In most cases of PA, antibodies are produced against the parietal cells causing them to atrophy, lose their ability to produce intrinsic factor, and secrete hydrochloric acid. In some forms of PA the parietal cells remain intact but auto-antiobodies are produced against the intrinsic factor itself and attach to it, thus preventing it from binding vitamin B_{12}. In another less common form of PA, the antibodies allow vitamin B_{12} to bind to the intrinsic factor but prevent the absorption of the intrinsic factor–vitamin B_{12} complex by the ileal receptors. As is the case with most auto-immune diseases, the incidence of PA increases markedly with age. In most ethnic groups it is virtually unknown to occur before the age of 50, with a progressive rise in incidence thereafter (*4*). However, African American populations are known to have an earlier age of presentation *(4)*. In addition to causing malabsorption of dietary vitamin B_{12}, PA also results in an inability to reabsorb the vitamin B_{12} which is secreted in the bile. Biliary secretion of vitamin B_{12} is estimated to be between 0.3 and 0.5 µg/day. Interruption of this so-called enterohepatic circulation of vitamin B_{12} causes the body to go into a significant negative balance for the vitamin. Although the body typically has sufficient vitamin B_{12} stores to last 3–5 years, once PA has been established the lack of absorption of new vitamin B_{12} is compounded by the loss of the vitamin because of negative balance. When the stores have been depleted, the final stages of deficiency are often quite rapid, resulting in death in a period of months if left untreated.

Atrophic gastritis

Historically, PA was considered to be the major cause of vitamin B_{12} deficiency, but it was a fairly rare condition, perhaps affecting 1 percent to a few percent of elderly populations. More recently it has been suggested that a far more common problem is that of hypochlorhydria associated with atrophic gastritis, where there is a progressive reduction with age of the ability of the parietal cells to secrete hydrochloric acid (*7*). It is claimed that perhaps up to one-quarter of elderly subjects could have various degrees of hypochlorhydria as a result of atrophic gastritis. It has also been suggested that bacterial overgrowth in the stomach and intestine in individuals suffering from atrophic gastritis may also reduce vitamin B_{12}

absorption. This absence of acid is postulated to prevent the release of protein-bound vitamin B_{12} contained in food but not to interfere with the absorption of the free vitamin B_{12} found in fortified foods or supplements. Atrophic gastritis does not prevent the reabsorption of bilary vitamin B_{12} and therefore does not result in the negative balance seen in individuals with PA. However, it is agreed that with time, a reduction in the amount of vitamin B_{12} absorbed from the diet will eventually deplete even the usually adequate vitamin B_{12} stores, resulting in overt deficiency.

When considering recommended nutrient intakes (RNIs) for vitamin B_{12} for the elderly, it is important to take into account the absorption of vitamin B_{12} from sources such as fortified foods or supplements as compared with dietary vitamin B_{12}. In the latter instances, it is clear that absorption of intakes of less than 1.5–2.0 µg/day is complete – that is, for intakes of less than 1.5–2.0 µg of free vitamin B_{12}, the intrinsic factor – mediated system absorbs all of that amount. It is probable that this is also true of vitamin B_{12} in fortified foods, although this has not specifically been examined. However, absorption of food-bound vitamin B_{12} has been reported to vary from 9 percent to 60 percent depending on the study and the source of the vitamin, which is perhaps related to its incomplete release from food (8). This has led many to estimate absorption as being up to 50 percent to correct for bio-availability of absorption from food.

Vitamin B_{12} interaction with folate or folic acid

One of the vitamin B_{12} – dependent enzymes, methionine synthase, functions in one of the two folate cycles (see ***Chapter 4***) – the methylation cycle. This cycle is necessary to maintain availability of the methyl donor *S*-adenosylmethionine; interruption reduces the wide range of methylated products. One such important methylation is that of myelin basic protein. Reductions in the level of *S*-adenosylmethionine seen in PA and other causes of vitamin B_{12} deficiency produce demyelination of the peripheral nerves and the spinal column, called sub-acute combined degeneration (*1, 2*). This neuropathy is one of the main presenting conditions in PA. The other principal presenting condition in PA is a megaloblastic anaemia morphologically identical to that seen in folate deficiency. Disruption of the methylation cycle should cause a lack of DNA biosynthesis and anaemia.

The methyl trap hypothesis is based on the fact that once the cofactor 5,10-methylenetetrahydrofolate is reduced by its reductase to form 5-methyltetrahydrofolate, the reverse reaction cannot occur. This suggests that the only way for the methyltetrahydrofolate to be recycled to tetrahydrofolate, and thus to participate in DNA biosynthesis and cell division, is through the vitamin B_{12} – dependent enzyme methionine synthase. When the activity of this synthase is compromised, as it would be in PA, the cellular folate will become progressively trapped as 5-methyltetrahydrofolate. This will result in a cellular pseudo folate deficiency where despite adequate amounts of folate an anaemia will develop that is identical to that seen in true folate deficiency. Clinical symptoms of PA, therefore, include neuropathy, anaemia, or both. Treatment with vitamin B_{12}, if given intramuscularly, will reactivate methionine synthase, allowing myelination to restart. The trapped folate will be released and DNA synthesis and generation of red cells will cure the anaemia. Treatment with high concentrations of folic acid will treat the anaemia but not the neuropathy of PA. It should be stressed that the so-called masking of the anaemia of PA is generally agreed not to occur at concentrations of folate found in food or at intakes of the synthetic form of folic acid found at usual RNI levels of 200 or 400 µg/day (*1*). However, there is some evidence that amounts less than 400 µg may cause a haematologic response and thus potentially treat the anaemia (*9*). The masking of the anaemia definitely occurs at high concentrations of folic acid (>1000 µg/day). This becomes a concern when considering fortification with synthetic folic acid of a dietary staple such as flour (see ***Chapter 4***).

In humans the vitamin B_{12} – dependent enzyme methylmalonyl coenzyme A (CoA) mutase functions in the metabolism of propionate and certain of the amino acids, converting them into succinyl CoA, and in their subsequent metabolism via the citric acid cycle. It is clear that in vitamin B_{12} deficiency the activity of the mutase is compromised, resulting in high plasma or urine concentrations of methylmalonic acid (MMA), a degradation product of methylmalonyl CoA. In adults this mutase does not appear to have any vital function, but it clearly has an important role during embryonic life and in early development. Children deficient in this enzyme, through rare genetic mutations, suffer from mental retardation and other developmental defects.

Assessment of vitamin B_{12} status

Traditionally it was thought that low vitamin B_{12} status was accompanied by a low serum or plasma vitamin B_{12} level (*4*). Recently this has been challenged by Lindenbaum *et al*.(*10*), who suggested that a proportion of people with normal vitamin B_{12} levels are in fact vitamin B_{12} deficient. They also suggested that elevation of plasma homo-cysteine and plasma MMA are more sensitive indicators of vitamin B_{12} status. Although plasma homo-cysteine may also be elevated because of folate or vitamin B_6 deficiency, elevation of MMA apparently always occurs with poor vitamin B_{12} status. There may be other reasons why MMA is elevated, such as renal insufficiency, so the elevation of itself is not diagnostic. Many would feel that low or decreased plasma vitamin B_{12} levels should be the first indication of poor status and that this could be confirmed by an elevated MMA if this assay was available.

Evidence on which to base a recommended intake

Recommendations for nutrient intake

The Food and Nutrition Board of the National Academy of Sciences (NAS) Institute of Medicine (*8*) has recently exhaustively reviewed the evidence of intake, status, and health for all age groups and during pregnancy and lactation. This review has lead to calculations of what they have called an estimated average requirement (EAR). The EAR is defined by NAS as "the daily intake value that is estimated to meet the requirement, as defined by the specific indicator of adequacy, in half of the individuals in a life-stage or gender group" (*8)*. They have estimated the recommended dietary allowances to be this figure plus 2 standard deviations (SDs). Some members of the Food and Agriculture Organization of the United Nations and World Health Organization (FAO/WHO) Expert Consultation were involved in the preparation and review of the NAS recommendations and judge them to have been the best estimates based on available scientific literature. The FAO/WHO expert group felt it appropriate to adopt the same approach used by the NAS in deriving the RNIs. Therefore the RNIs suggested in ***Table 14*** are based on the NAS EARs plus 2 SDs.

Adults

Several lines of evidence point to an adult average requirement of about 2.0 µg/day. The amount of intramuscular vitamin B_{12} needed to maintain remission in people with PA suggests a requirement of about 1.5 µg/day (*10*), but they would also be losing 0.3–0.5µg/day through interruption of their enterohepatic circulation, which is not typical. This might suggest a requirement of 0.7–1.0 µg/day. Because vitamin B_{12} is not completely absorbed from food, an adjustment of 50 percent has to be added giving a range of 1.4–2.0 µg/day (*4*). Therapeutic response to ingested food vitamin B_{12} suggests a minimum requirement of something less than 1.0 µg/day (*8*). Diets containing 1.8 µg/day seemed to maintain adequate status but lower intakes showed some signs of deficiency. (*8*). Dietary intakes of less than 1.5 µg/day were reported to be inadequate in some subjects (*11*).

In summary, the average requirement could be said to be 2 µg/day (*8*). The variability of the requirements for vitamin B_{12} is accounted for by adding two SDs, that is, 2.4 µg/day as an RNI for adults, including the elderly.

Table 14

Estimated average requirement (EAR) and recommended nutrient intake (RNI) for vitamin B_{12}, by age group

Adapted from the US National Academy of Sciences (8).

Group	EAR µg/day	RNI µg/day
Infants and children		
0–6 months	0.32	0.4
7–12 months	0.32	0.5
1–3 years	0.7	0.9
4–6 years	1.0	1.2
7–9 years	1.5	1.8
Adolescents, 10–18 years	2.0	2.4
Adults		
19–65 years	2.0	2.4
65+ years	2.0	2.4
Pregnancy	2.2	2.6
Lactation	2.4	2.8

Children

The Food and Nutrition Board of the NAS Institute of Medicine (*8*) suggested the same intakes for adolescents with progressive reduction of intake for younger groups.

Pregnancy

The previous FAO/WHO (12) Expert Consultation suggested that 0.1–0.2 µg/day of vitamin B_{12} is transferred to the foetus (*13*) during the last two trimesters of pregnancy. On the basis of on foetal liver content from post-mortem samples (*14*, *15, 16*), there is further evidence that the foetus accumulates on average 0.1–0.2 µg/day during pregnancies of women with diets which have adequate vitamin B_{12}. It has been reported that children born to vegetarians or other women with a low vitamin B_{12} intake subsequently develop signs of clinical vitamin B_{12} deficiency such as neuropathy (*17*). Therefore, when calculating the EAR for pregnant women, 0.2 µg/day of vitamin B_{12} is added to the EAR for adults to result in an EAR of 2.2 µg/day and an RNI of 2.6 µg /day during pregnancy.

Lactation

It is estimated that 0.4 µg/day of vitamin B_{12} is found in the human milk of women with adequate vitamin B_{12} status (*8)*. Therefore an extra 0.4 µg/day of vitamin B_{12} is needed during lactation in addition to the normal adult requirement of 2.0 µg/day, giving a total EAR of 2.4 µg/day during lactation and an RNI of 2.8 µg/day.

Infants

As with other nutrients, the principal way to determine requirements of infants is to examine the levels in milk from mothers on adequate diets. There is a wide difference in the vitamin

B_{12} values reported in human milk because of differences in methodology. The previous FAO/WHO report (12) used milk vitamin B_{12} values of normal women of about 0.4 µg/l. For an average milk production of 0.75 l/day, the vitamin B_{12} intake by infants would be 0.3 µg/day (*18)*. Other studies have reported ranges of vitamin B_{12} in human milk to be 0.4–0.8 µg/L (*17, 19, 20, 21, 22*). Although daily intakes ranging from 0.02 to 0.05 µg/day have been found to prevent deficiency (*23, 24*), these intakes are totally inadequate for long-term health. An EAR of 0.3–0.6 µg/day would result in an RNI of 0.36–0.72 µg/d. It might be prudent to use the lower figure of 0.4 µg/day for the first 6 months of pregnancy and 0.7 µg/day for last trimester.

Upper limits

The absorption of vitamin B_{12} mediated by intrinsic factor is limited to 1.5–2.0 µg per meal because of the limited capacity of the receptors. In addition, between 1 percent and 3 percent of any particular oral administration of vitamin B_{12} is absorbed by passive diffusion. Thus, if 1000 µg vitamin B_{12} (sometimes used to treat those with PA) is taken orally, the amount absorbed would be 2.0 µg by active absorption plus about 30 µg by passive diffusion. This amount has never been reported to have any side effects (*8*). Similar large amounts have been used in some preparations of nutritional supplements without apparent ill effects. However, there are no established benefits for such amounts. Such high intakes thus represent no benefit in those without malabsorption and should probably be avoided.

Future research

Because they do not consume any animal products, vegans are at risk of vitamin B_{12} deficiency. It is generally agreed that in some communities the only source of vitamin B_{12} is from contamination of food by microorganisms. When vegans move to countries where standards of hygiene are more stringent, there is good evidence that risk of vitamin B_{12} deficiency increases in adults and, particularly, in children born to and breast-fed by women who are strict vegans.

- As standards of hygiene improve in developing countries, there is a concern that the prevalence of vitamin B_{12} deficiency might increase. This should be ascertained by estimating plasma vitamin B_{12} levels, preferably in conjunction with plasma MMA levels in representative adult populations and in infants.
- The contribution which fermented vegetable foods make to vitamin B_{12} status of vegan communities should be investigated.
- The prevalence of atrophic gastritis should be investigated in developing countries.

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Chapter 6
Vitamin C

Vitamin C (chemical names: ascorbic acid and ascorbate) is a six-carbon lactone which is synthesised from glucose by many animals. Vitamin C is synthesised in the liver in some mammals and in the kidney in birds and reptiles. However, several species – including humans, non-human primates, guinea pigs, Indian fruit bats, and Nepalese red-vented bulbuls – are unable to synthesise vitamin C. When there is insufficient vitamin C in the diet, humans suffer from the potentially lethal deficiency disease scurvy (*1*). Humans and primates lack the terminal enzyme in the biosynthetic pathway of ascorbic acid, l-gulonolactone oxidase, because the gene encoding for the enzyme has undergone substantial mutation so that no protein is produced (*2*).

Role in human metabolic processes

Background biochemistry

Vitamin C is an electron donor (reducing agent or antioxidant), and probably all of its biochemical and molecular functions can be accounted for by this function. The potentially protective role of vitamin C as an antioxidant is discussed in the antioxidants chapter of this report.

Enzymatic functions

Vitamin C acts as an electron donor for 11 enzymes (*3, 4*). Three of those enzymes are found in fungi but not in humans or other mammals (*5, 6*). They are involved in reutilisation pathways for pyrimidines and the deoxyribose moiety of deoxynucleosides. Of the 8 remaining human enzymes, three participate in collagen hydroxylation (*7-9*) and two in carnitine biosynthesis (*10, 11*); of the three enzymes which participate in collagen hydroxylation, one is necessary for biosynthesis of the catecholamine norepinephrine (*12, 13*), one is necessary for amidation of peptide hormones (*14, 15*), and one is involved in tyrosine metabolism (*4, 16*).

Ascorbate interacts with enzymes having either monooxygenase or dioxygenase activity. The monooxygenases dopamine β-monooxygenase and peptidyl-glycine α-monooxygenase incorporate a single oxygen atom into a substrate, either a dopamine or a glycine-terminating peptide. The remaining enzymes are dioxygenases which incorporate two oxygen atoms in two different ways. The enzyme 4-hydroxyphenylpyruvate dioxygenase incorporates two oxygen atoms into one product. The other dioxygenase incorporates one oxygen atom into succinate and one into the enzyme-specific substrate.

Miscellaneous functions

The concentrations of vitamin C in gastric juice were several fold higher (median, 249 μmol/l; range, 43–909 μmol/l) than those found in the plasma of the same normal subjects (39 μmol/l, 14–101 μmol/l) (*17*). Gastric juice vitamin C may prevent the formation of *N*-nitroso compounds, which are potentially mutagenic (*18*). High intakes of vitamin C correlate with reduced gastric cancer risk (*19*), but a cause-and-effect relationship has not been established.

Vitamin C protects low-density lipoproteins *ex vivo* against oxidation and may function similarly in the blood (*20;* see ***Chapter 17***).

A common feature of vitamin C deficiency is anaemia. The antioxidant properties of vitamin C may stabilise folate in food and in plasma, and increased excretion of oxidized folate derivatives in human scurvy was reported (*21*). Vitamin C promotes absorption of soluble non-haem iron possibly by chelation or simply by maintaining the iron in the reduced (ferrous, Fe^{2+}) form (*22, 23*). The effect can be achieved with the amounts of vitamin C obtained in foods. However, the amount of dietary vitamin C required to increase iron absorption ranges from 25 mg upwards and depends largely on the amount of inhibitors, such as phytates and polyphenols, present in the meal (*24*). (See ***Chapter 13*** on iron for further discussion.)

Overview of significant scientific information

From the 15th century, scurvy was dreaded by seamen and explorers forced to subsist for months on diets of dried beef and biscuits. Scurvy was described by the Crusaders, during the sieges of numerous European cities, and as a result of the famine in 19th century Ireland. Three important manifestations of scurvy – gingival changes, pain in the extremities, and haemorrhagic manifestations – preceded oedema, ulcerations, and ultimately death. Skeletal and vascular lesions in scurvy probably arise from a failure of osteoid formation. In infantile scurvy the changes are mainly at the sites of most active bone growth; characteristic signs are a pseudoparalysis of the limbs caused by extreme pain on movement and caused by haemorrhages under the periosteum, as well as swelling and haemorrhages in areas of the gums surrounding erupting teeth (*25*). In adults one of the early, principle adverse effects of the collagen-related pathology may be impaired wound healing (*26*). Vitamin C deficiency can be detected from early signs of clinical deficiency, such as the follicular hyperkeratosis, petechial haemorrhages, swollen or bleeding gums, and joint pain, or from the very low concentrations of ascorbate in plasma, blood, or leukocytes. The Sheffield studies (*26, 27*) and later studies in Iowa (*28, 29*) were the first major attempts made to quantify vitamin C requirements. The studies indicated that the amount of vitamin C required to prevent or cure early signs of deficiency was between 6.5 and 10 mg/day. This range represents the lowest physiologic requirement. The Iowa studies (*28, 29*) and Kallner et al (*30*) established that at tissue saturation, whole body vitamin C content is approximately 20 mg/kg, or 1500 mg, and that during depletion vitamin C is lost at 3 percent of whole body content per day.

Clinical signs of scurvy appear in men at intakes lower than 10 mg/day (*27*) or when the whole body content falls below 300 mg (*28*). Such intakes are associated with plasma ascorbate concentrations below 11 μmol/l or leukocyte levels less than 2 nmol/10^8 cells. However, the plasma concentrations fall to around 11 μmol/l when dietary vitamin C is between 10 and 20 mg/day. At intakes greater than 25–35 mg/day, plasma concentrations start to rise steeply, indicating a greater availability of vitamin C for metabolic needs. In general, plasma ascorbate closely reflects the dietary intake and ranges between 20 and 80 μmol/l. Note that during infection or physical trauma, an increase in the number of circulating leukocytes occurs and these take up vitamin C from the plasma (*31, 32*). Therefore, both plasma and leukocyte levels may not be very precise indicators of body content or status at such times. However, leukocyte ascorbate remains a better indicator of vitamin C status than plasma ascorbate most of the time and only in the period immediately after the onset of an infection are both values unreliable.

Intestinal absorption of vitamin C is by an active, sodium-dependent, energy-requiring, carrier-mediated transport mechanism (*33*) and as intakes increase, the tissues progressively become more saturated**.** The physiologically efficient, renal-tubular reabsorption mechanism retains vitamin C in the tissues up to a whole body content of

ascorbate of about 20 mg/kg body weight (*30*). However, under steady state conditions, as intakes rise from around 100 mg/day there is an increase in urinary output in so that at 1000 mg/day almost all absorbed vitamin C is excreted (*34, 35*).

Definition of population at risk

The populations at risk of vitamin C deficiency are those for whom the fruit and vegetable supply is minimal. Epidemics of scurvy are associated with famine and war, when people are forced to become refugees and food supply is small and irregular. Persons in whom the total body vitamin C content is saturated can subsist without vitamin C for approximately 2 months before the appearance of clinical signs, and as little as 6.5–10 mg/day vitamin C will prevent the appearance of scurvy. In general, vitamin C status will reflect the regularity of fruit and vegetable consumption but also socio-economic conditions, because intake is determined not just by availability, but by cultural preferences and cost.

In Europe and the United States an adequate intake of vitamin C is indicated by the results of various national surveys (*36-38*). In the United Kingdom and Germany, the mean dietary intakes of vitamin C in adult men and women were 87 and 76 (*37*) and 75 and 72 mg/day (*36*), respectively. In addition, a recent survey of elderly men and women in the United Kingdom reported vitamin C intakes of 72 (SD 61) and 68 (SD 60) mg/day, respectively (*39*). In the United States, in the National Health and Nutrition Examination Survey (*38*), the median consumption of vitamin C from foods during the years 1988–91 was 73 and 84 mg/day in men and women, respectively. In all these studies there was a wide variation in vitamin C intake and 25–30 percent of the US population consumed less than 2.5 servings of fruit and vegetables daily. Likewise a survey of Latin American children in the United States suggested that less than 15 percent consumed the recommended intake of fruits and vegetables (*40*). It is not possible to relate servings of fruits and vegetables to an exact amount of vitamin C, but the World Health Organization (WHO) dietary goal of 400 g (*41*) aimed to provide sufficient vitamin C to meet the 1970 Food and Agriculture Organization of the United Nations (FAO)/WHO guidelines – that is, approximately 20–30 mg/day – and lower the risk of chronic disease. The WHO goal has been roughly translated into the recommendation of five portions per day (*42*).

Reports from India show that the available supply of vitamin C is 43 mg/capita/day, and in the different states of India it ranges from 27 to 66 mg/day. In one study, low-income children consumed as little as 8.2 mg/day of vitamin C in contrast to a well-to-do group of children where the intake was 35.4 mg/day (*43*). Other studies done in developing countries found plasma vitamin C concentrations lower than those reported for developed countries, for example, 20–27 μmol/l for apparently healthy adolescent boys and girls in China and 3–54 μmol/l (median 14 μmol/l) for similarly aged Gambian nurses (*44, 45*), although values obtained in a group of adults from a rural district in Northern Thailand were quite acceptable (17–118 μmol/l, median 44 μmol/l) (*46*). However, it is difficult to assess the extent to which sub-clinical infections are lowering the plasma vitamin C concentrations seen in such countries.

Data describing a positive association between vitamin C consumption and health status are frequently reported, but intervention studies do not support the observations. Low plasma concentrations are reported in patients with diabetes (*47*) and infections (*48*) and in smokers (*49*), but the relative contribution of diet and stress to these situations is uncertain (see ***Chapter 17***). Epidemiologic studies indicate that diets with a high vitamin C content have been associated with lower cancer risk, especially for cancers of the oral cavity, oesophagus, stomach, colon, and lung (*39, 50-52*). However, there appears to be no effect of consumption of vitamin C supplements on the development of colorectal adenoma and

stomach cancer (*52-54*), and data on the effect of vitamin C supplementation on coronary heart disease and cataract development are conflicting (*55-74*). Currently there is no consistent evidence from population studies that heart disease, cancers, or cataract development are specifically associated with vitamin C status. This of course does not preclude the possibility that other components in vitamin C – rich fruits and vegetables provide health benefits, but it is not yet possible to separate such an effect from other factors such as lifestyle patterns of people who have a high vitamin C intake.

Dietary sources of vitamin C and limitations to vitamin C

Ascorbate is found in many fruits and vegetables (*75*). Citrus fruits and juices are particularly rich sources of vitamin C but other fruits including cantaloupe, honeydew melon, cherries, kiwi fruits, mangoes, papaya, strawberries, tangelo, watermelon, and tomatoes also contain variable amounts of vitamin C. Vegetables such as cabbage, broccoli, Brussels sprouts, bean sprouts, cauliflower, kale, mustard greens, red and green peppers, peas, tomatoes, and potatoes may be more important sources of vitamin C than fruits. This is particularly true because the vegetable supply often extends for longer periods during the year than does the fruit supply.

In many developing countries, limitations in the supply of vitamin C are often determined by seasonal factors (i.e., the availability of water, time, and labour for the management of household gardens and the short harvesting season of many fruits). For example, mean monthly ascorbate intakes ranged from 0 to 115 mg/day in one Gambian community in which peak intakes coincided with the seasonal duration of the mango crop and to a lesser extent with orange and grapefruit harvests. These fluctuations in dietary ascorbate intake were closely reflected by corresponding variations in plasma ascorbate (11.4–68.4 μmol/L) and human milk ascorbate (143–342 μmol/L) (*76*).

Vitamin C is also very labile, and the loss of vitamin C on boiling milk provides one dramatic example of a cause of infantile scurvy. The vitamin C content of food is strongly influenced by season, transport to market, shelf life, time of storage, cooking practices, and chlorination of water. Cutting or bruising of produce releases ascorbate oxidase. Blanching techniques inactivate the oxidase enzyme and help to preserve ascorbate as also will low pH, as in the preparation of sauerkraut (pickled cabbage). In contrast, heating and exposure to copper or iron or to mildly alkaline conditions destroys the vitamin, and too much water can leach it from the tissues during cooking.

The use of citrus fruits by the British navy in the 18th century gave rise to the term ‘limey’, a colloquial term for British sailors. However, it is important to realise that the amount of vitamin C in a food is usually not the major determinant of a food’s importance for supply, but rather regularity of intake. For example, in countries where the potato is an important staple food and refrigeration facilities are limited, seasonal variations in plasma ascorbate are due to the considerable deterioration in the potato’s vitamin C content during storage; the content can decrease from 30 to 8 mg/100 g over 8–9 months (*77*). Such data can indicate the important contribution the potato can make to human vitamin C requirements even though the potato vitamin C concentration is low.

An extensive study has been made of losses of vitamin C during the packaging, storage, and cooking of blended foods (maize and soya-based relief foods). Data from a US Agency for Internation Development programme show that vitamin C losses from packaging and storage in polythene bags of such relief foods are much less significant than the 52–82 percent losses attributable to conventional cooking procedures (*78*).

Information used to derive dietary requirement of vitamin C

Calculating the dietary intake from the physiologic requirements

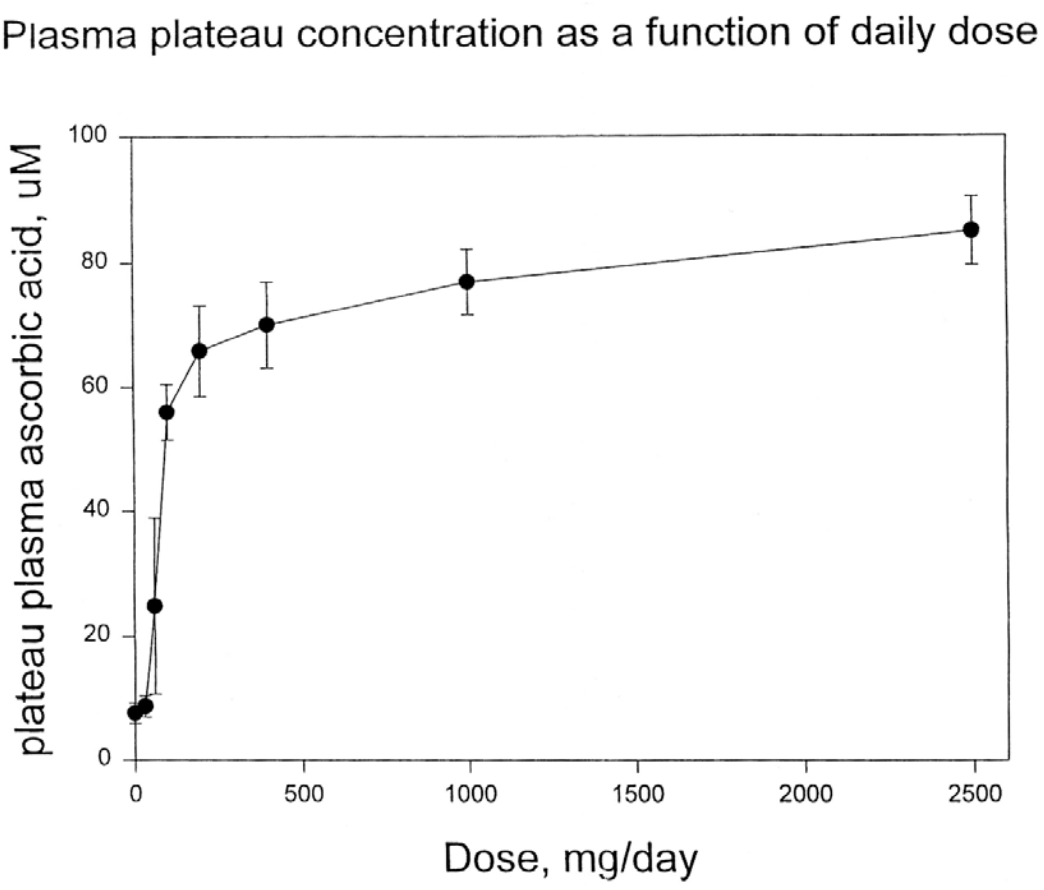
Adults

At saturation the whole body content of ascorbate in adult males is approximately 20 mg/kg, or 1500 mg. Clinical signs of scurvy appear when the whole body content falls below 300–400 mg, and the last signs disappear when the body content reaches about 1000 mg (*28, 30*). In these experiments, ascorbate in the whole body was catabolised at an approximate rate of 3 percent/day (2.9 percent/day, SD 0.6) (*29*).

There is a sigmoidal relationship between intake and plasma concentrations of vitamin C (*79*). Below 30 mg/day, plasma concentrations are around 11 μmol/l. Above this intake, plasma concentrations increase steeply to 60 μmol/l and plateau at around 80 μmol/l, which represents the renal threshold. Under near steady state conditions, plateau concentrations of vitamin C are achieved by intakes in excess of 200 mg/day (***Figure 8***) (*34*). At low doses dietary vitamin C is almost completely absorbed, but over the range of usual dietary intakes (30–180 mg/day), absorption may decrease to 75 percent because of competing factors in the food (35, *80*).

Figure 8

Relationship between intake and plasma concentrations of vitamin C



A body content of 900 mg falls half way between tissue saturation and the point at which clinical signs of scurvy appear. Assuming an absorption efficiency of 85 percent, and a catabolic rate of 2.9, the average intake of vitamin C can be calculated as:

900 x 2.9/100 x 100/85 = 30.7 mg/day, which can be rounded off to 30 mg/day.

The recommended nutrient intake (RNI) would therefore be:

900 x (2.9 + 1.2)/100 x 100/85 = 43.4 mg/day, which can be rounded off to 45 mg/day.

An RNI of 45 mg would achieve 50 percent saturation in the tissues in 97.5 percent of adult males. An intake of 45 mg vitamin C will produce a plasma ascorbate concentrations near the base of the steep slope of the diet-plasma dose response curve (***Figure 8***). No turnover studies have been done in women, but from the smaller body size and whole body content of women, requirements might be expected to be lower. However, in depletion studies plasma concentrations fell more rapidly in women than in men (*81*). It would seem prudent, therefore, to make the same recommendation for non-pregnant, non-lactating women as for men. Thus, an intake of 45 mg/day will ensure that measurable amounts of ascorbate will be present in the plasma of most people and will be available to supply tissue requirements for metabolism or repair at sites of depletion or damage. A whole body content of around 900 mg of vitamin C would provide at least 1 month's safety interval, even for a zero intake, before the body content falls to 300 mg (*82*).

The Sheffield (*27*) and Iowa studies (*28*) indicated that the minimum amount of vitamin C needed to cure scurvy in men was less than 10 mg/day. This level however, is not sufficient to provide measurable amounts of ascorbate in plasma and leukocyte cells, which will remain low. As indicated above, no studies have been done on women and minimum requirements to protect non-pregnant and non-lactating women against scurvy might be slightly lower than in men. Although 10 mg/day will protect against scurvy, this amount provides no safety margin against further losses. The mean requirement is therefore calculated by interpolation between 10 and 45 mg/day, at an intake of 25–30 mg/day.

Pregnancy and lactation

During pregnancy there is a moderate extra drain on vitamin C, particularly during the last trimester, and 8 mg/day of vitamin C is reported to be sufficient to prevent scorbutic signs in infants aged 4–17 months (*83*). Therefore, the additional needs during pregnancy are unlikely to be more, particularly during the last trimester. An extra 10 mg/day throughout pregnancy should enable reserves to accumulate to meet the extra needs of the growing foetus in the last trimester.

During lactation, however, 20 mg/day of vitamin C is secreted in milk. For an assumed absorption efficiency of 85 percent, an extra 25 mg will be needed by the mother. It is therefore recommended that the RNI should be set at 70 mg to fulfil the needs of both the mother and infant during lactation.

Children

As mentioned earlier, 8 mg/day of vitamin C is sufficient to prevent scorbutic signs in infants (*83*). The vitamin C concentration in mature human milk is estimated to be 40 mg/l (mean, SD 10) (*84*), but the amount of vitamin C in human milk appears to reflect maternal dietary intake and not the infants needs (*82, 83*, *85*). RNIs for infants aged 0–6 months are therefore set, somewhat arbitrarily, at 25 mg/day, and the RNI is gradually increased as children got older.

Elderly

Elderly people frequently have low plasma ascorbate values and intakes lower than those in younger people, often because of problems of poor dentition or mobility (*86*). Elderly people are also more likely to have underlying sub-clinical diseases, which can also influence plasma ascorbate concentrations (see ***Chapter 17***). It has been suggested, however, that the requirements of elderly people do not differ substantially from those of younger people in the absence of pathology, which may influence absorption or renal functioning (*82*). The RNIs for the elderly are therefore the same as those for adults (45 mg/day).

Smokers

Kallner *et al.* (*87*) reported that the turnover of vitamin C in smokers was 50 percent greater than that in non-smokers. However, there is no evidence that the health of smokers would be influenced in any way by increasing their RNI. The panel therefore found no justification in making a separate RNI for smokers.

Recommended nutrient intakes for vitamin C

Table 15 presents a summary of the above discussed RNIs for vitamin C by age groups.

Table 15

Recommended nutrient intakes (RNIs) for vitamin C

Group	RNI mg/day [a]
Infants and children	
0–6 months	25
7–12 months	30 [b]
1–3 years	30 [b]
4–6 years	30 [b]
7–9 years	35 [b]
Adolescents, 10–18 years	40 [b]
Adults	
19–65 years	45
65+ years	45
Pregnancy	55
Lactation	70

[a] Amount required to half saturate body tissues with vitamin C in 97.5 percent of the population. Larger amounts may often be required to ensure an adequate absorption of non-haem iron.

[b] Arbitrary values.

Vitamin C toxicity

The potential toxicity of excessive doses of supplemental vitamin C relates to intra-intestinal events and to the effects of metabolites in the urinary system. Intakes of 2–3 g/day of vitamin C produce unpleasant diarrhoea from the osmotic effects of the unabsorbed vitamin in the intestinal lumen in most people (*88*). Gastrointestinal disturbances can occur after ingestion of as little as 1 g because approximately half of the amount would not be absorbed at this dose (*35*).

Oxalate is an end product of ascorbate catabolism and plays an important role in kidney stone formation. Excessive daily amounts of vitamin C produce hyperoxaluria. In four volunteers who received vitamin C in the range of 5–10 g/day, this amounted to

approximately a doubling of urinary oxalate excretion, from 50 to 87 mg/day (range 60–126 mg/day) (*89*). However, the risk of oxalate stones formation may become significant at high intakes of vitamin C (>1 g) (*90*), particularly in subjects with high amounts of urinary calcium (*89*).

Vitamin C may precipitate haemolysis in some people, including those with glucose-6-phosphate dehydrogenase deficiency (*91*), paroxysmal nocturnal haemaglobinuria (*92*), or other conditions where increased risk of red cell haemolysis may occur or where protection against the removal of the products of iron metabolism may be impaired, as in people with the haptoglobin Hp2-2 phenotype (*93*). Of these conditions, only the haptoglobin Hp 2-2 condition was associated with abnormal vitamin C metabolism (lower plasma ascorbate than expected) under conditions where intake of vitamin C was provided mainly from dietary sources. Therefore, 1 g vitamin C appears to be the advisable upper limit of dietary intake.

Future research

Research is needed to gain a better understanding of the following:

- functions of endogenous gastric ascorbate and its effect on iron absorption;
- functional measurements of vitamin C status which reflect the whole body content of vitamin C and are not influenced by infection; and
- reasons for the vitamin C uptake by granulocytes, which is associated with infection.

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Chapter 7
Vitamin A

Role of vitamin A in human metabolic processes

Vitamin A (retinol) is an essential nutrient needed in small amounts by humans for the normal functioning of the visual system; growth and development; and maintenance of epithelial cellular integrity, immune function, and reproduction. These dietary needs for vitamin A are normally provided for as preformed retinol (mainly as retinyl ester) and pro-vitamin A carotenoids.

Overview of vitamin A metabolism

Preformed vitamin A in animal foods occurs as retinyl esters of fatty acids in association with membrane-bound cellular lipid and fat-containing storage cells. Pro-vitamin A carotenoids in foods of vegetable origin are also associated with cellular lipids but are embedded in complex cellular structures such as the cellulose-containing matrix of chloroplasts or the pigment-containing portion of chromoplasts. Normal digestive processes free vitamin A and carotenoids from embedding food matrices, a more efficient process from animal than from vegetable tissues. Retinyl esters are hydrolysed and the retinol and freed carotenoids are incorporated into lipid-containing, water-miscible micellar solutions. Products of fat digestion (e.g., fatty acids, monoglycerides, cholesterol, and phospholipids) and secretions in bile (e.g., bile salts and hydrolytic enzymes) are essential for the efficient solubilisation of retinol and especially for solubilisation of the very lipophilic carotenoids (e.g., α- and β-carotene, β-cryptoxanthin, and lycopene) in the aqueous intestinal milieu. Micellar solubilisation is a prerequisite to their efficient passage into the lipid-rich membrane of intestinal mucosal cells (i.e., enterocytes) (*1-3*). Diets critically low in dietary fat (under about 5–10 g daily) (*4*) or disease conditions that interfere with normal digestion and absorption leading to steatorrhea (e.g., pancreatic and liver diseases and frequent gastroenteritis) can therefore impede the efficient absorption of retinol and carotenoids. Retinol and some carotenoids enter the intestinal mucosal brush border by diffusion in accord with the concentration gradient between the micelle and plasma membrane of enterocytes. Some carotenoids pass into the enterocyte and are solubilized into chylomicrons without further change whereas some of the pro-vitamin A carotenoids are converted to retinol by a cleavage enzyme in the brush border (*3*). Retinol is trapped intracellularly by re-esterification or binding to specific intracellular binding proteins. Retinyl esters and unconverted carotenoids together with other lipids are incorporated into chylomicrons, excreted into intestinal lymphatic channels, and delivered to the blood through the thoracic duct (*2*).

Tissues extract most lipids and some carotenoids from circulating chylomicrons, but most retinyl esters are stripped from the chylomicron remnant, hydrolysed, and taken up primarily by parenchymal liver cells. If not immediately needed, retinol is re-esterified and retained in the fat-storing cells of the liver (variously called adipocytes, stellate cells, or Ito cells). The liver parenchymal cells also take in substantial amounts of carotenoids. Whereas most of the body's vitamin A reserve remains in the liver, carotenoids are also deposited elsewhere in fatty tissues throughout the body (*1*). Usually, turnover of carotenoids in tissues is relatively slow, but in times of low dietary carotenoid intake, stored carotenoids are mobilised. A recent study in one subject using stable isotopes suggests that retinol can be

derived not only from conversion of dietary pro-vitamin carotenoids in enterocytes – the major site of bioconversion – but also from hepatic conversion of circulating pro-vitamin carotenoids (*5*). The quantitative contribution to vitamin A requirements of carotenoid converted to retinoids beyond the enterocyte is unknown.

Following hydrolysis of stored retinyl esters, retinol combines with a plasma-specific transport protein, retinol-binding protein (RBP). This process, including synthesis of the unoccupied RBP (apo-RBP), occurs to the greatest extent within liver cells but it may also occur in some peripheral tissues. The RBP-retinol complex (holo-RBP) is secreted into the blood where it associates with another hepatically synthesised and excreted larger protein, transthyretin. The transthyretin-RBP-retinol complex circulates in the blood, delivering the lipophilic retinol to tissues; its large size prevents its loss through kidney filtration (*1*). Dietary restriction in energy, proteins, and some micronutrients can limit hepatic synthesis of proteins specific to mobilisation and transport of vitamin A. Altered kidney functions or fever associated with infections (e.g., respiratory infections [*6*] or diarrhoea [*7*]) can increase urinary vitamin A loss.

Holo-RBP transiently associates with target-tissue membranes, and specific intracellular binding proteins then extract the retinol. Some of the transiently sequestered retinol is released into the blood unchanged and is recycled (i.e., conserved) (*1,8*). A limited reserve of intracellular retinyl esters is formed, that subsequently can provide functionally active retinol and its oxidation products (i.e., isomers of retinoic acid) as needed intracellularly. These biologically active forms of vitamin A are associated with specific cellular proteins which bind with retinoids within cells during metabolism and with nuclear receptors that mediate retinoid action on the genome (*9*). Retinoids modulate the transcription of several hundreds of genes (*10-12*). In addition to the latter role of retinoic acid, retinol is the form required for functions in the visual (*13*) and reproductive systems (*14*) and during embryonic development (*15*).

Holo-RBP is filtered into the glomerulus but recovered from the kidney tubule and recycled. Normally vitamin A leaves the body in urine only as inactive metabolites which result from tissue utilisation and as potentially recyclable active glucuronide conjugates of retinol in bile secretions (*8*). No single urinary metabolite has been identified which accurately reflects tissue levels of vitamin A or its rate of utilisation. Hence, at this time urine is not a useful biologic fluid for assessment of vitamin A nutriture.

Biochemical mechanisms for vitamin A functions

Vitamin A functions at two levels in the body. The first is in the visual cycle in the retina of the eye; the second is in all body tissues systemically to maintain growth and the soundness of cells. In the visual system, carrier-bound retinol is transported to ocular tissue and to the retina by intracellular binding and transport proteins. Rhodopsin, the visual pigment critical to dim-light vision, is formed in rod cells after conversion of all-*trans* retinol to retinaldehyde, isomerization to the 11-*cis*-form, and binding to opsin. Alteration of rhodopsin through a cascade of photochemical reactions results in ability to see objects in dim light (*13*). The speed at which rhodopsin is regenerated relates to the availability of retinol. Night blindness is usually an indicator of inadequate available retinol, but it can also be due to a deficit of other nutrients, which are critical to the regeneration of rhodopsin, such as protein and zinc, and to some inherited diseases, such as retinitis pigmentosa.

The growth and differentiation of epithelial cells throughout the body are especially affected by vitamin A deficiency (VAD). Goblet cell numbers are reduced in epithelial tissues. The consequence is that mucous secretions with their antimicrobial components diminish. Cells lining protective tissue surfaces fail to regenerate and differentiate, hence

flatten and accumulate keratin. Both factors – the decline in mucous secretions and loss of cellular integrity – diminish resistance to invasion by potentially pathogenic organisms. The immune system is also compromised by direct interference with production of some types of protective secretions and cells (*11*). Classical symptoms of xerosis (drying or nonwetability) and desquamation of dead surface cells as seen in ocular tissue (i.e., xerophthalmia) are the external evidence of the changes also occurring to various degrees in internal epithelial tissues.

Current understanding of the mechanism of vitamin A action within cells outside the visual cycle is that cellular functions are mediated through specific nuclear receptors. These receptors are activated by binding with specific isomers of retinoic acid (i.e., all-*trans* and 9-*cis* retinoic acid). Activated receptors bind to DNA response elements located upstream of specific genes to regulate the level of expression of those genes (*12*). The synthesis of a large number of proteins vital to maintaining normal physiologic functions is regulated by these retinoid-activated genes. There also may be other mechanisms of action that are as yet undiscovered (*10*).

Population at risk and consequences of vitamin A deficiency

Definition of vitamin A deficiency

VAD is not simply defined. WHO defines it as tissue concentrations of vitamin A low enough to have adverse health consequences even if there is no evidence of clinical xerophthalmia (*16*). In addition to the specific signs and symptoms of xerophthalmia and the risk of irreversible blindness, non-specific symptoms include increased morbidity and mortality, poor reproductive health, increased risk of anaemia, and contributions to slowed growth and development. Because these non-specific adverse consequences may occur from other nutrient deficits as well, it is difficult to attribute non-ocular symptoms specifically to VAD in the absence of biochemical measurements reflective of vitamin A status.

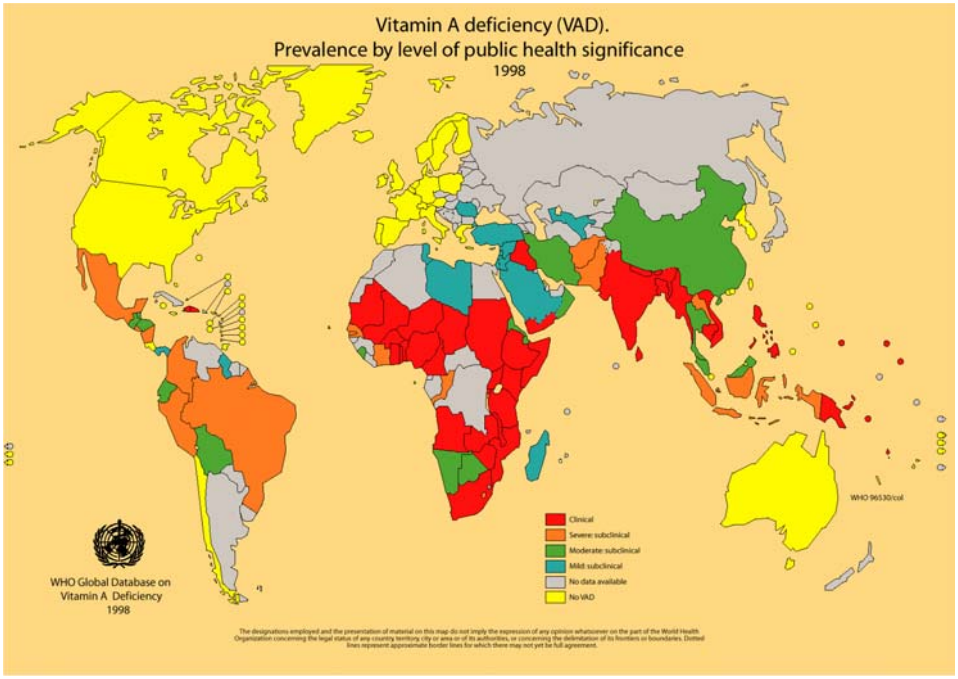
Geographic distribution and magnitude

In 1996 WHO mapped the global distribution of VAD (***Figure 9***) and categorised countries by degree of significance as a public health problem on the basis of both clinical and moderate and severe sub-clinical (prevalence of low blood levels of retinol) indicators of deficiency (*16,18*). In the early 1990s, WHO estimated that about 3 million children had some form of xerophthalmia annually and, on the basis of blood levels, another 250 million were sub-clinically deficient (*18*). The magnitude of the sub-clinical estimate is currently being re-evaluated to quantitatively establish a benchmark for measuring prevalence trends. The actual number of sub-clinical deficiencies based on the prevalence of low serum levels of retinol, however, remains uncertain because of the confounding and poorly quantitative role of infections (see later discussion).

Epidemiologic studies repeatedly report clustering of VAD, presumably resulting from concurrent occurrence of several risk factors. This clustering may occur among both neighbourhoods and households (*19*).

Figure 9

Prevalence of VAD in the world (17)



Age and gender

VAD can occur in individuals of any age. However, it is a disabling and potentially fatal public health problem for children under 6 years of age. VAD-related blindness is most prevalent in children under 3 years of age (*20*). This period is characterised by high requirements for vitamin A to support early rapid growth, the transition from breast-feeding to dependence on other dietary sources of the vitamin, and increased frequency of respiratory and gastrointestinal infections. The increased mortality risk from concurrent infections extends at least to 6 years of age and is associated with both clinical and sub-clinical VAD (*21*). There is little information regarding the health consequences of VAD in school-age children. The prevalence of Bitot's spots (i.e., white foamy patches on the conjunctiva) may be highest in this age group but their occurrence may reflect past more than current history of VAD (*22*). Women of reproductive age are thought also to be vulnerable to VAD during pregnancy and lactation because they often report night blindness (*23,24*) and because their breast-milk is frequently low in vitamin A (*25,26*). Not all night blindness in pregnant women, however, responds to vitamin A (*24*).

There is no consistent, clear indication in humans of a gender differential in vitamin A requirements during childhood. Growth rates and presumably the need for vitamin A from birth to 10 years for boys are consistently higher than those for girls (*27*). In the context of varied cultural and community settings, however, variations in gender-specific child-feeding and care practices are likely to subsume a small gender differential in requirements to account for reported gender differences and prevalence of xerophthalmia. Pregnant and lactating women require additional vitamin A to support maternal and foetal tissue growth and lactation losses, additional vitamin A which is not needed by other post-adolescent adults (*28*).

Risk factors

VAD is most common in populations consuming most of their vitamin A needs from pro-vitamin carotenoid sources and where minimal dietary fat is available (*29*). About 90 percent of ingested preformed vitamin A is absorbed, whereas the absorption efficiency of pro-vitamin A carotenoids varies widely depending on the type of plant source and the fat content of the accompanying meal (*30*). Where it is possible to increase dietary fat, this will likely improve the absorption of vitamin A activity from the diet.

In areas with endemic VAD, fluctuations in the incidence of VAD throughout the year reflect the balance between intake and need. Periods of general food shortage (and specific shortages in vitamin A–rich foods), peak incidence of common childhood infectious diseases (diarrhoea, respiratory infections, and measles), and periodic seasonal growth spurts affect the balance. Seasonal food availability can influence VAD prevalence by directly influencing access to pro-vitamin A sources; for example, the scarcity of mangoes in hot arid months followed by the glutting of the market with mangoes during harvest seasons (*31*). Seasonal growth spurts in children frequently follow seasonal postharvest increases in energy and macronutrient intakes. These increases are usually obtained from staple grains (e.g., rice) and tubers (e.g., light-coloured yams) which are not good sources of some micronutrients (e.g., vitamin A to support the growth spurt) (*32*).

Food habits and taboos often restrict consumption of potentially good food sources of vitamin A (e.g., mangoes and green leafy vegetables). Culture-specific factors for feeding children, adolescents, and pregnant and lactating women are common (*29,33-35*). Illness- and childbirth-related proscription of the use of specific foods pervade in many traditional cultures (*36*). Such influences alter short- and long-term food distribution within families. However, some cultural practices can be protective of vitamin A status and they need to be identified and reinforced.

Morbidity and mortality

The consequences of VAD are manifested differently in different tissues. In the eye, the symptoms and signs, together referred to as xerophthalmia, have a long, well-recognised history and have until recently been the basis for estimating the global burden from the disease (*20*). Although ocular symptoms and signs are the most specific indicators of VAD, they occur only after other tissues have impaired functions that are less specific and less easily assessed.

The prevalence of ocular manifestations (i.e., xerophthalmia or clinical VAD) is now recognised to far underestimate the magnitude of the problem of functionally significant VAD. Many more preschool-age children and perhaps older children and women who are pregnant or lactating have their health compromised when they are sub-clinically deficient. In young children, sub-clinical deficiency, like clinical deficiency, increases the severity of some infections, particularly diarrhoea and measles, and the risk of dying (*21,37*). The incidence (*38*) and prevalence (*39*) of diarrhoea may also increase with sub-clinical VAD. Meta-analyses conducted by three independent groups using data from several randomised trials provide convincing evidence that community-based improvement of the vitamin A status of deficient children 6 months to 6 years of age reduces their risk of dying by 20–30 percent on average (*21,40,41*). Mortality in children who are blind from keratomalacia or who have corneal disease is reported to be from 50 percent to 90 percent (*20,42*) and measles mortality associated with VAD is increased up to 50 percent (*43*). Limited data are available from controlled studies of the possible link between morbidity history and vitamin A status of pregnant and lactating women (*44*).

There are discrepancies in the linkage between incidence and severity of infectious morbidity of various aetiologies and vitamin A status. The weight of evidence supports an association of VAD with severity of an infection once acquired, except for respiratory diseases, which are nonresponsive (*16,37-39,45*). The severity of the pneumonia associated with measles, however, is an exception because it decreases with treatment with vitamin A supplements (*43,46*).

Infectious diseases depress circulating retinol and contribute to vitamin A depletion. Enteric infections may alter absorptive-surface area, compete for absorption-binding sites, and increase urinary loss (*7,47,48*). Febrile systemic infections also increase urinary loss (*6,49*) and metabolic utilisation rates and may reduce apparent retinol stores if fever occurs frequently (*50*). In the presence of latent deficiency, disease occurrence is often associated with precipitating ocular signs (*51,52*). Measles virus infection is especially devastating to vitamin A metabolism, adversely interfering with both efficiencies of utilisation and conservation (*43,52,53*). Severe protein-energy malnutrition affects many aspects of vitamin A metabolism, and even when some retinyl ester stores are still present, malnutrition – often coupled with infection – can prevent transport-protein synthesis, that results in immobilisation of existing vitamin A stores (*54*).

The compromised integrity of the epithelium, together with the possible alteration in hormonal balance at severe levels of deficiency, impairs normal reproductive functions in animals (*9,14,15,25,55,56*). Controlled human studies are, of course, lacking. In animals and Humans, congenital anomalies can result if the foetus is exposed to severe deficiency or large excesses of vitamin A at critical periods early in gestation (first trimester) when foetal organs are being formed (*25,57*). Reproductive performance measured by infant outcomes in one community-based clinical intervention trial, however, was not influenced by vitamin A status (*44*).

The growth of children may be impaired by VAD. Interventions with vitamin A only have not consistently demonstrated improved growth in community studies because VAD seldom occurs in isolation of other nutrient deficiencies that also affect growth and may be more limiting (*58*).

A lack of vitamin A can affect iron metabolism when deficiencies of both nutrients coexist and particularly in environments that favour frequent infections (*59*). Maximum haemoglobin response occurs when iron and vitamin A deficiencies are corrected together (*60*). VAD appears to influence the availability of storage iron for use by haematopoietic tissue (*60,61*). However, additional research is needed to clarify the mechanisms of the apparent interaction.

Units of expression

In blood, tissues, and human milk, vitamin A levels are conventionally expressed in µg/dL or µmol/l of all-*trans* retinol. Except for postprandial conditions, most of the circulating vitamin A is retinol whereas in most tissues (such as the liver), secretions (such as human milk), and other animal food sources it exists mainly as retinyl esters, that are usually hydrolysed before analytical detection.

To express the vitamin A activity of carotenoids in diets on a common basis, a joint FAO/WHO Expert Group (*62*) in 1967 introduced the concept of the retinol equivalent (RE) and established the following relationships among food sources of vitamin A:

1 µg retinol	=	1 RE
1 µg β-carotene	=	0.167 µg RE
1 µg other pro-vitamin A carotenoids	=	0.084 µg RE

These equivalencies were derived from balance studies to account for the less-efficient absorption of carotenoids (thought to be about one-third that of retinol) and their bioconversion to vitamin A (one-half for β-carotene and one-fourth for other pro-vitamin carotenoids). It was recognised at the time that the recommended conversion factors (i.e., 1:6 for vitamin A:β-carotene and 1:12 for vitamin A: all other pro-vitamin carotenoids) were only average estimates for a mixed diet. Recently there has been renewed interest in examining bio-availability factors by using more quantitative stable isotope techniques for measuring whole-body stores in response to controlled intakes (*63-65*) and by following postabsorption carotenoids in the triacylglycerol-rich lipoprotein fraction (*66-68*). The data are inconsistent but in general suggests that revision toward lower bio-availability estimates is likely. Until additional definitive data are available, however, the above conversion factors will be used *(67, 68)*.

Retinol equivalents in a diet are calculated as the sum of the weight of the retinol portion of preformed vitamin A with the weight of β-carotene divided by its conversion factor and with the weight of other carotenoids divided by their conversion factor (*69*). Most recent food composition tables report β-carotene and sometimes other pro-vitamin A carotenoids as μg/g edible portion. However, older food composition tables frequently report vitamin A as international units (IUs). The following applies to determining comparable values as μg:

1 IU retinol	=	0.3 μg retinol
1 IU β-carotene	=	0.6 μg β-carotene
1 IU retinol	=	3 IU β-carotene

It is strongly recommended that weight or molar units replace the use of IU to decrease confusion and overcome limitations in the non-equivalence of the IU values for retinol and beta-carotene.

Dietary sources

Preformed vitamin A is found almost exclusively in animal products, such as human milk, glandular meats, liver and fish liver oils (especially), egg yolk, and whole milk and dairy products. Preformed vitamin A is also used to fortify processed foods, that may include sugar, cereals, condiments, fats, and oils (*70*). Pro-vitamin A carotenoids are found in green leafy vegetables (e.g., spinach, amaranth, and young leaves from various sources), yellow vegetables (e.g., pumpkins, squash, and carrots), and yellow and orange noncitrus fruits (e.g., mangoes, apricots, and papaya). Red palm oil produced in several countries worldwide is especially rich in pro-vitamin A (*71*). Some other indigenous plants also may be unusually rich sources of pro-vitamin A. Such examples are the palm fruit known in Brazil as *buritĺ*, that is found in areas along the Amazon (as well as elsewhere in Latin America) (*72*), and the fruit known as *gac* in Vietnam, that is used to colour rice, particularly on ceremonial occasions (*73*). Foods containing pro-vitamin A carotenoids tend to be less biologically available but more affordable than animal products. It is mainly for this reason that carotenoids provide most of the vitamin A activity in the diets of economically deprived populations.

Dietary intake and patterns

Vitamin A status cannot be assessed from dietary intake alone, but dietary intake assessment can provide evidence of risk of an inadequate status. Quantitative collection of dietary information is fraught with measurement problems. These problems arise both from obtaining representative quantitative dietary histories from individuals, communities, or both and from interpreting these data while accounting for differences in bio-availability, preparation losses, and variations in food composition data among population groups (*70*). This is especially

difficult in populations consuming most of their dietary vitamin A from pro-vitamin carotenoid sources. Simplified guidelines have been developed recently in an effort to improve the obtaining of reliable dietary intake information from individuals and communities (*68,74*).

World and regional supply and patterns

In theory the world's food supply is sufficient to meet global requirements. Great differences exist, however, in the available sources (animal and vegetable) and in per capita consumption of the vitamin among different countries, age categories, and socio-economic groups. VAD as a global public health problem, therefore, is largely due to inequitable food distribution among and within countries and households in relation to need for ample bio-available vitamin A sources (*75,76*).

Earlier FAO global estimates in 1984 indicated that preformed vitamin A constituted about one-third of total dietary vitamin A activity (*69*). World availability of vitamin A for human consumption at that time was approximately 220 µg of preformed retinol per capita daily and 560 µg RE from pro-vitamin carotenoids (about 3400 µg carotenoids for a 6:1 conversion factor) per person per day, for a total of about 790 µg RE. These values are based on supply estimates and not consumption estimates. Losses commonly occur during food storage and processing, both industrially and in the home (*70*).

The estimated available regional supply of vitamin A from a more recent global evaluation (*76*) shown in ***Table 16*** illustrates the variability in amounts and sources of vitamin A. The variability is further complicated by access to the available supply, that varies with household income, poverty being a yardstick for risk of VAD. VAD is most prevalent in Southeast Asia, Africa, and the Western Pacific, where vegetable sources contribute nearly 80 percent or more of the available supply of retinol equivalents. Furthermore, in Southeast Asia the total available supply is about half of that of most other regions and is particularly low in animal sources. In contrast, the Americas, Europe, and Eastern Mediterranean regions have a supply ranging from 800 to 1000 µg RE/day, one-third of which comes from animal sources. Recent national data from the USA Continuing Survey of Food Consumption (*77*) and the National Health and Nutrition Examination Survey (*78*) included mean dietary intakes of children 0–6 years of age of 864 ± 497 and 921 ± 444 µg RE daily. In the Dietary and Nutritional Survey of British Adults (*79*), the median intake of men and women 35-49 years old was 1118 µg RE and 926 µg RE, respectively, which corresponded to serum retinol concentrations of 2.3 µmol/l and 1.8 µmol/l, respectively. In another selected survey in the United Kingdom, median intakes for nonpregnant women who did not consume liver or liver products during the survey week were reported to be 686 µg RE daily (*80*).

The available world supply figures in ***Table 16*** were recently reassessed based on a bio-availability ratio of 1:30 for retinol to other pro-vitamin A carotenoids (*81*). This conversion factor was justified on the basis of one published controlled intervention study conducted in Indonesia (*82*) and a limited number of other studies not yet published in full. Applying the unconfirmed conversion factor to the values in ***Table 16*** would lead to the conclusion that regional and country needs for vitamin A could not be met from predominantly vegetarian diets. This is inconsistent with the preponderance of epidemiologic evidence. Most studies report a positive response when vegetable sources of pro-vitamin A are given under controlled conditions to deficient subjects freed of confounding parasite loads and provided with sufficient dietary fat (*83,84*). Emerging data are likely to justify a lower biologic activity for pro-vitamin A carotenoids because of the mix of total carotenoids found in food sources in a usual meal (*67,68*). This Consultation concluded that the 1:6

bioconversion factor originally derived on the basis of balance studies should be retained until there is firm confirmation from ongoing studies that use more precise methodologies.

Table 16

Available supply of vitamin A by WHO region

Region	Total μg RE/day	Animal sources μg RE/day	Vegetable sources μg RE/day
Africa	775	122	654 (84)[a]
Americas	814	295	519 (64)
Southeast Asia	431	53	378 (90)
Europe	738	271	467 (63)
Eastern Mediterranean	936	345	591 (63)
Western Pacific	997	216	781 (78)
Total	782	212	565 (72)

[a]Numbers in parentheses indicate the percent of total retinol equivalents from carotenoid food sources.
Source: ACC/SCN, 1993 (*76*).

Evidence for making recommendations

Indicators of vitamin A deficiency

Ocular signs of VAD are measured by clinical examination and history and are quite specific in preschool-age children. However, these are rare occurrences that require examination of large populations to obtain incidence and prevalence data. Sub-clinical VAD is more prevalent, requiring smaller sample sizes to obtain valid prevalence estimates (*16*).

A full description of clinical indicators with coloured illustrations for each is found in the WHO Field Guide (*20*). The most frequently occurring is night blindness, which is the earliest manifestation of xerophthalmia. In mild form it is generally noticeable after stress from a bright light that bleaches the rhodopsin (visual purple) found in the retina. VAD prolongs the time to regenerate rhodopsin, thus delays adaptation time in dark environments. Night-blind young children tend to stumble when going from bright to dimly lighted areas and they, as well as night-blind mothers, tend to remain inactive at dusk and at night (*85*). No field-applicable objective tool is currently available for measuring night blindness in children under about 3 years of age. It can be measured by history in certain cultures (*86*). When night blindness is prevalent, many cultures coin a word descriptive of the characteristic symptom that they can reliably recall on questioning, making this a useful tool for assessing the prevalence of VAD (*87*). Questioning for night blindness is not consistently a reliable assessment measure where a local term is absent. In addition, there is no clearly defined blood retinol level that is directly associated with occurrence of the symptom. Vitamin A – related night blindness, however, responds rapidly, usually within 1–2 days, to administration of vitamin A.

Sub-clinical vitamin A deficiency

Direct measurement of concentrations of vitamin A in the liver where it is stored or in the total body pool relative to known specific vitamin A–related functions (e.g., night blindness) would be the indicator of choice for determining requirements. This cannot be done with the methodology now available for population use. There are several practical biochemical methods for estimating sub-clinical vitamin A status but all have limitations (*16,86,88,89*). Each method is useful to identify deficient populations, but no one of these indicators is definitive or directly related quantitatively to disease occurrence. The indicators of choice are

listed in ***Table 17***. These indicators are less specific to VAD than clinical eye signs and less sensitive for measuring sub-clinical vitamin A status. WHO recommends that where feasible at least two sub-clinical biochemical indicators, or one biochemical and a composite of non-biochemical risk factors, should be measured and that both types of indicators should point to deficiency in order to identify populations at high risk of VAD (*16*). Cut-off points given in ***Table 17*** represent the consensus gained from practical experience comparing populations with some evidence of VAD with those without VAD. There are no field studies that quantitatively relate the prevalence of adverse health symptoms (e.g., incidence or prevalence of severe diarrheal disease) and relative levels of biologic indicator cut-off values. Furthermore, each of the biochemical indicators listed is subject to confounding factors, which may be unrelated to vitamin A status (e.g., infections).

Table 17

Biochemical indicators of sub-clinical VAD in mothers and in children 6–71 months of age

Indicator	Cut-off to indicate deficiency
Night blindness (24–71 mo)	≥ 1% report a history of night blindness
Biochemical	
Breast milk retinol	≤1.05 μmol/l (≤8 μg/g milk fat)
Serum retinol	≤0.70 μmol/l
Relative dose response	≥20%
Modified relative dose response	ratio ≥0.06

Source: Adapted from World Health Organization, Geneva, 1996 (*16*).

Although all biochemical indicators currently available have limitations, the biochemical indicator of choice for population assessment is the distribution of serum levels of vitamin A (serum retinol). Only at very low blood levels (<0.35 μmol/l) is there an association with corneal disease prevalence (*90*). Blood levels between 0.35 and 0.70 μmol/l are likely to characterise sub-clinical deficiency (*91*), but sub-clinical deficiency may still be present at levels between 0.70 and 1.05 μmol/l and occasionally above 1.05 μmol/l (*92*). The prevalence of values below 0.70 μmol/l is a generally accepted population cut-off for preschool-age children to indicate risk of inadequate vitamin A status (*16*) and above 1.05 μ mol/l to indicate an adequate status (*93,94*). As noted elsewhere, clinical and sub-clinical infections can lower serum levels of vitamin A on average as much as 25 percent independently of vitamin A intake (*95,96*). Therefore, at levels between about 0.5 and 1.05 μ mol/l, the relative dose response or modified relative dose response test on a subsample of the population can be useful for identifying the prevalence of critically depleted body stores when interpreting the left portion of serum retinol distribution curves.

Determining mean requirements and setting safe levels of intake

Requirement and safe level of intake for vitamin A in this report do not differ significantly from those of the 1988 FAO/WHO Expert Consultation (*69*) except for adapting to the age categories defined in this consultation and during pregnancy. The term safe level of intake used in the 1988 report is retained in this report because the levels in ***Table 18*** do not strictly correspond to the definition of a recommended nutrient intake.

The mean requirement for an individual is defined as the minimum daily intake of vitamin A as presented in μg retinol equivalents (μg RE) to prevent xerophthalmia in the absence of clinical or sub-clinical infection. This intake should account for proportionate bio-availability of preformed vitamin (about 90 percent) and pro-vitamin A carotenoids from a diet that contains sufficient fat (e.g., at least 5–10 g). Bio-availability of carotenoids varies widely by source (e.g., fibrous green leafy vegetables or soft-tissue fruits). The required level of intake is set to prevent clinical signs of deficiency, allow for normal growth, and reduce the risk of vitamin A – related severe morbidity and mortality on a population basis. It does not allow for frequent or prolonged periods of infections or other stresses.

The safe level of intake for an individual is defined as the average continuing intake of vitamin A required to permit adequate growth and other vitamin A–dependent functions and to maintain an acceptable total body reserve of the vitamin. This reserve helps offset periods of low intake or increased need resulting from infections and other stresses. Useful indicators include a plasma retinol concentration above 0.70 μmol/l, that is associated with a relative dose response below 20 percent, or a modified relative dose response below 0.06. For lactating women, breast-milk retinol levels above 1.05 μmol/l (or above 8 μg/g milk fat) are considered to reflect minimal maternal stores because levels above 1.75 μmol/l are common to populations known to be healthy and without evidence of insufficient dietary vitamin A (*25,26*).

Infants and children

Vitamin A requirements for infants are calculated from the vitamin A provided in human milk. During at least the first 6 months of life, exclusive breast-feeding can provide sufficient vitamin A to maintain health, permit normal growth, and maintain sufficient stores in the liver (*97*).

Reported retinol concentrations in human milk varies widely from country to country (0.70–2.45 μmol/l). In some developing countries the vitamin A intake of breast-fed infants who grow well and do not show signs of deficiency is from 120 to 170 μg RE/day (*26,97*). Such intakes are considered adequate to cover infant requirements if the infant's weight is assumed to be at least at the 10th percentile according to WHO standards (*69*). However, this intake is unlikely to build adequate body stores because xerophthalmia is common in preschool-age children in the same communities with somewhat lower intakes. Because of the need for vitamin A to support the growth rate of infancy, which can vary considerably, a requirement estimate of 180 μg RE/day seems appropriate.

The safe level for infants up to 6 months of age is based on observations of breast-fed infants in communities in which good nutrition is the norm. Average consumption of human milk by such infants is about 750 ml/day during the first 6 months (*97*). Assuming an average concentration of vitamin A in human milk of about 1.75 μmol/l, the mean daily intake would be about 375 μg RE, which is therefore the recommended safe level. From 7-12 months, human milk intake averages 650 ml, which would provide 325 μg vitamin A daily. Because breast-fed infants in endemic vitamin A–deficient populations are at increased risk of death

from 6 months onward, the requirement and recommended safe intake are increased to 190 μg and 400 μg, respectively.

The requirement (with allowance for variability) and the recommended intake for older children may be estimated from those derived for late infancy (i.e., 20 and 39 μg RE/kg body weight/day) (*69*). On this basis, including allowances for storage requirements and variability, requirements for preschool children would be in the range of 200–400 μg RE daily. In poor communities where children 1–6 years old are reported to have intakes of about 100–200 μg RE/day, signs of VAD do occur; in southern India these signs were relieved and risk of mortality was reduced when the equivalent of 350–400 μg RE/day was given to children weekly (*98*). In the United States most preschool-age children maintain serum retinol levels of 0.70 μmol/l or higher while consuming diets providing 300–400 μg RE/day (from the databank for the third National Health and Nutrition Examination Survey).

Adults

Estimates for the requirements and recommended safe intakes for adults are also estimated from those derived for late infancy, i.e. 4.8 and 9.3 μg RE/kg body weight/day (*69*). Detailed accounts for arriving at the requirement for vitamin A is provided in the FAO/WHO report of 1988 (*69*) and will not be repeated here because there are no new published studies to indicate a need to revise the assumptions on which the calculations were based. The safe intakes recommended are consistent with the per capita vitamin A content in the food supply of countries that show adequate vitamin A status in all sectors of the population. Additional evidence that the existing safe level of intake is adequate for adults on a population basis is provided by an analysis of dietary data from the 1990 survey of British adults in whom there was no evidence of VAD (*79*). The median intake for another survey of nonpregnant UK women who did not consume liver or liver products during the survey week was 686 μg/day (80). This value is substantially above the estimated mean requirement for pregnant women and falls quite short of the amount in which teratology risk is reported (*99-101*). About one-third of the calculated retinol equivalents consumed by the British women came from pro-vitamin A sources (20 percent from carrots).

Pregnancy

During pregnancy additional vitamin A is needed for the growth and maintenance of the foetus for providing a limited reserve in the foetal liver and for maternal tissue growth. There are no reliable figures available for the specific vitamin A requirements for these processes (*28*).

Newborn infants need around 100 μg retinol daily to meet their needs for growth. During the third trimester the foetus grows rapidly and, although obviously smaller in size than the infant born full term, the foetus presumably has similar needs. Incremental maternal needs associated with pregnancy are assumed to be provided from maternal reserves in populations of adequately nourished healthy mothers. In populations consuming at the basal requirement, an increment of 100 μg/day during the full gestation period should enhance maternal storage during early pregnancy and allow adequate amounts of vitamin A for the rapidly growing foetus in late pregnancy. However, this increment may be minimal for women who normally ingest only the basal requirement level of vitamin A inasmuch as the needs and growth rate of the foetus will not be affected by the mother's initial vitamin A reserves.

A recent study in Nepal (*44*), where night blindness is prevalent in pregnant women, provided 7000 μg RE (about 23,300 IU) weekly to pregnant and lactating women (equivalent to 1000 μg RE/day). This level of intake normalised serum levels of vitamin A and was

associated with a decrease in prevalence of night blindness and a decrease in maternal mortality. The findings of this study need to be confirmed. In the interim period, however, it seems prudent, recognising that a large portion of the world's population of pregnant women lives under conditions of deprivation, to increase by 200 μg the recommended safe level to ensure adequacy of intake during pregnancy. Because therapeutic levels of vitamin A are generally higher than preventive levels, the safe intake level recommended during pregnancy is 800 μg RE/day. Women who are or who might become pregnant should carefully limit their total daily vitamin A intake to a maximum of 3000 μg RE (10 000 IU) to minimise risk of foetal toxicity (*102*).

Lactation

If the amounts of vitamin A recommended for infants are supplied by human milk, mothers should absorb at least as much in their diets to replace maternal losses. Thus, the increments in basal and recommended intakes during lactation are 180 μg RE and 350 μg RE, respectively, for the safe recommended intake per day. After the infant reaches the age of 6 months or when solid foods are introduced, the mother's need for additional amounts of vitamin A lessens.

Elderly

There is no indication that the vitamin A requirements of healthy elderly individuals differs from those of other adults. It should be remembered, however, that diseases that impede vitamin A absorption, storage, and transport might be more common in the elderly than in other age groups.

Recommended safe intakes

Table 18 provides the estimated mean requirements for vitamin A and the recommended safe intakes, taking into account the age and gender differences in mean body weights. For most values the true mean and variance are not known. Values in the table have been rounded. It should be noted that there are no adequate data available to derive mean requirements for any group and, therefore, a recommended nutrient intake cannot be calculated. However, information is available on cures achieved in a few vitamin A–deficient adult men and on the vitamin A status of groups receiving intakes that are low but nevertheless adequate to prevent the appearance of deficiency-related syndromes. The figures for mean dietary requirements are derived from these, with the understanding that the curative dose is higher than the preventive dose. They are at the upper limits of the range so as to cover the mean dietary requirements of 97.5 percent of the population (*69*).

In calculating the safe intake, a normative storage requirement was calculated as a mean for adults equivalent to 434 μg RE/day, and the recommended safe intake was derived in part by using this value + 2 standard deviations. It is doubtful that this value can be applied to deal with growing children. The safe intake for children was compared with the distribution of intakes and comparable serum vitamin A levels reported for children 0–6 years of age from the United States and with distributions of serum levels of vitamin A of children 9–62 months in Australia (*103*), where evidence of VAD is rare.

Table 18

Estimated mean requirement and safe level of intake for vitamin A

Age group	Mean requirement μg RE/day	Recommended safe intake μg RE/day
Infants and children		
0–6 months	180	375
7–12 months	190	400
1–3 years	200	400
4–6 years	200	450
7–years	250	500
Adolescents, 10–18 years	330–400	600
Adults		
Females,19–65 years	270	500
Males, 19–65 years	300	600
65+	300	600
Pregnant women	370	800
Lactating women	450	850

Source: Adapted from FAO/WHO, Rome 1988 (*69*).

Toxicity

Because vitamin A is fat soluble and can be stored, primarily in the liver, routine consumption of large amounts of vitamin A over a period of time can result in toxic symptoms, including liver damage, bone abnormalities and joint pain, alopecia, headaches and vomiting, and skin desquamation. Hypervitaminosis A appears to be due to abnormal transport and distribution of vitamin A and retinoids caused by overloading of the plasma transport mechanisms *(104)*.

The smallest daily supplement associated with liver cirrhosis that has been reported is 7500 μg taken for 6 years (*100,101*). Very high single doses can also cause transient acute toxic symptoms that may include bulging fontanels in infants; headaches in older children and adults; and vomiting, diarrhoea, loss of appetite, and irritability in all age groups. Rarely does toxicity occur from ingestion of food sources of preformed vitamin A. When this occurs, it usually results from very frequent consumption of liver products. Toxicity from food sources of pro-vitamin A carotenoids is not reported except for the cosmetic yellowing of skin.

Infants, including newborns (*105*), administered single doses equivalent to 15 000–30 000 μg retinol (50 000–100 000 IU) in oil generally show no adverse symptoms. However, daily prophylactic or therapeutic doses should not exceed 900 μg, that is well above the mean requirement of about 200 μg daily for infants. An excess of bulging fontanels occurred in infants under 6 months of age in one endemically deficient population given two or more doses of 7500 μg or 15 000 μg preformed vitamin A in oil (*106,107*), but other large-scale controlled clinical trials have not reported excess bulging after three doses of 7500 μg given with diptheria-pertussis-tetanus immunisations at about 6, 10, and 14 weeks of age (*108*). No effects were detected at 3 years of age that related to transient vitamin A–induced bulging that had occurred before 6 months of age (*105,109*).

Most children 1–6 years of age tolerate single oral doses of 60 000 μg (200 000 IU) vitamin A in oil at intervals of 4–6 months without adverse symptoms (*100*). Occasionally diarrhoea or vomiting is reported but is transient with no lasting sequelae. Older children seldom experience toxic symptoms unless they habitually ingest vitamin A in excess of 7500 μg (25 000 IU) for prolonged periods of time (*100*).

When taken by women at early stages of gestation at daily levels of more than 7500 μg (25 000 IU), foetal anomalies and poor reproductive outcomes are reported (*101*). One report suggests an increased risk of teratogenicity at intakes as low as 3000 μg (10 000 IU) but this is not confirmed by other studies (*102*). Women who are pregnant or might become pregnant should avoid taking excessive amounts of vitamin A. A careful review of the latest available information by a WHO Expert Group recommended that daily intakes in excess of 3000 μg (10 000 IU) or weekly intakes in excess of 7500 μg (25 000 IU), should not be taken at any period during gestation (*102*). High doses of vitamin A (60 000 μg, or 200 000 IU) can be safely given to breast-feeding mothers for up to 2 months postpartum and for 6 weeks for women who are not breast-feeding.

Future research

Further research is needed:

- on the interaction of vitamin A and iron with infections, as they relate to serum levels and disease incidence and prevalence;
- on the relation among vitamin A, iron, and zinc and their role in the severity of infections;
- on the nutritional role of 9-*cis* retinoic acid and the mechanism which regulates its endogenous production;
- on the bio-availability of pro-vitamin A carotenoids from different classes of leafy and other green and orange vegetables, tubers, and fruits as typically provided in diets (e.g., relative to level of fat in the diet or meal); and
- to identify a reliable indicator of vitamin A status for use in direct quantification of mean requirements and for relating status to functions.

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Chapter 8
Vitamin D

Summary of the role of vitamin D in human metabolic processes

Vitamin D is required to maintain normal blood levels of calcium and phosphate, that are in turn needed for the normal mineralisation of bone, muscle contraction, nerve conduction, and general cellular function in all cells of the body. Vitamin D achieves this after its conversion to the active form 1,25-dihydroxyvitamin D [1,25-$(OH)_2$D], or calcitriol. This active form regulates the transcription of a number of vitamin D–dependent genes coding for calcium-transporting proteins and bone matrix proteins.

Vitamin D also modulates the transcription of cell cycle proteins, that decrease cell proliferation and increase cell differentiation of a number of specialised cells of the body (e.g., osteoclastic precursors, enterocytes, keratinocytes, etc.). This property may explain the actions of vitamin D in bone resorption, intestinal calcium transport, and skin. Vitamin D also possesses immuno-modulatory properties that may alter responses to infections *in vivo*. The cell differentiating and immuno-modulatory properties underlie the reason why vitamin D derivatives are now used successfully in the treatment of psoriasis and other skin disorders.

Clinical assays measure 1,25-$(OH)_2D_2$ and 1,25-$(OH)_2D_3$, collectively called 1,25-$(OH)_2$D. Similarly, calcidiol is measured as 25-OH-D but it is a mixture of 25-OH-D_2 and 25-OH-D_3. For the purposes of this document, 1,25-$(OH)_2$D and 25-OH-D will be used to refer to calcitriol and calcidiol, respectively.

Overview of the role of vitamin D

Vitamin D, a seco-steroid, can either be made in the skin from a cholesterol-like precursor (7-dehydrocholesterol) by exposure to sunlight or can be provided pre-formed in the diet (*1*). The version made in the skin is referred to as vitamin D_3 whereas the dietary form can be vitamin D_3 or a closely related molecule of plant origin known as vitamin D_2 . Because vitamin D can be made in the skin, it should not strictly be called a vitamin, and some nutritional texts refer to the substance as a prohormone and to the two forms as cholecalciferol (D_3) or ergocalciferol (D_2).

From a nutritional perspective, the two forms are metabolised similarly in humans, are equal in potency, and can be considered equivalent. It is now firmly established that vitamin D_3 is metabolised first in the liver to 25-hydroxyvitamin-D (25-OH-D or calcidiol) (*2*) and subsequently in the kidneys to 1,25-$(OH)_2$D (*3*) to produce a biologically active hormone. The 1,25-$(OH)_2$D, like all vitamin D metabolites, is present in the blood complexed to vitamin D binding protein, a specific α-globulin. The 1,25-$(OH)_2$D is believed to act on target cells similarly to the way a steroid hormone would act. Free hormone crosses the plasma membrane and interacts with a specific nuclear receptor known as the vitamin D receptor, a DNA-binding, zinc-finger protein with a molecular weight of 55,000 (*4*). This ligand-receptor complex binds to a specific vitamin D–responsive element and, with associated transcription factors (e.g., retinoid X receptor), enhances transcription of mRNAs which code for calcium-transporting proteins, bone matrix proteins, or cell cycle–regulating proteins (*5*). As a result of these processes ,1,25-$(OH)_2$D stimulates intestinal absorption of calcium and phosphate and mobilises calcium and phosphate by stimulating bone resorption (*6*). These functions serve

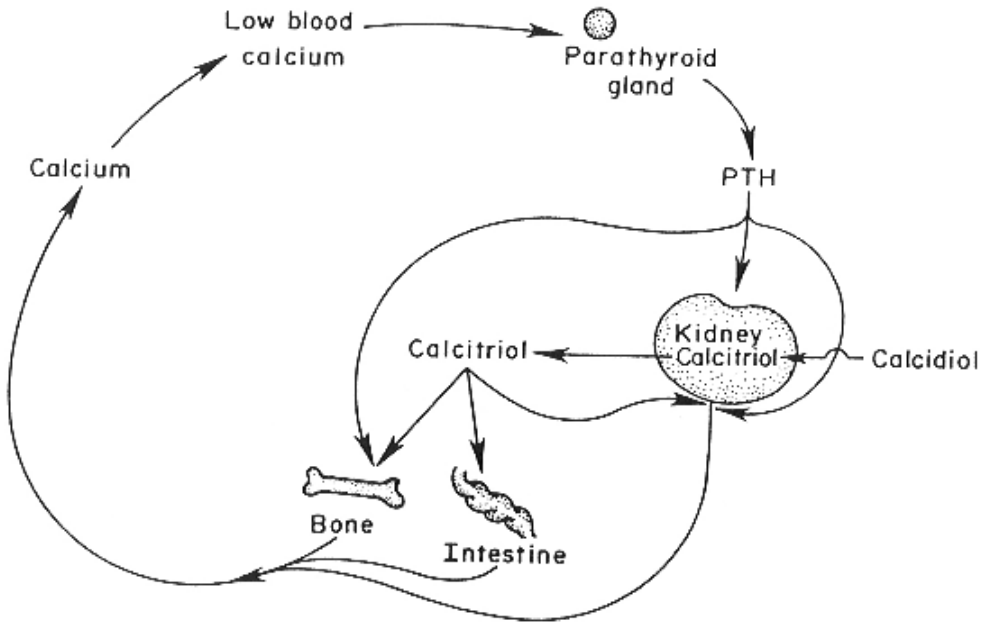
the common purpose of restoring blood levels of calcium and phosphate to normal when concentrations of the two ions are low.

Lately, interest has focused on other cellular actions of 1,25-$(OH)_2$D. With the discovery of 1,25-$(OH)_2$D receptors in many classical non-target tissues such as brain, various bone marrow–derived cells, skin, thymus, etc. (*7*), the view has been expressed that 1,25-$(OH)_2$D induces fusion and differentiation of macrophages (*8, 9*). This effect has been widely interpreted to mean that the natural role of 1,25-$(OH)_2$D is to induce osteoclastogenesis from colony forming units–granulatory monocytes in the bone marrow. The 1,25-$(OH)_2$D also suppresses interleukin 2 production in activated T lymphocytes (*10, 11*), an effect which suggests the hormone might play a role in immuno-modulation *in vivo*. Other tissues (e.g., skin) are directly affected by exogenous administration of vitamin D, though the physiologic significance of these effects is poorly understood. The pharmacologic effects of 1,25-$(OH)_2$D are profound and have resulted in the development of vitamin D analogues, that are approved for use in hyper-proliferative conditions such as psoriasis (*12*).

In calcium homeostasis 1,25-$(OH)_2$D works in conjunction with parathyroid hormone (PTH) to produce its beneficial effects on the plasma levels of ionised calcium and phosphate (*5, 13*). The physiologic loop (***Figure 10***) starts with calcium sensing by the calcium receptor of the parathyroid gland (*14*). When the level of ionised calcium in plasma falls, PTH is secreted by the parathyroid gland and stimulates the tightly regulated renal enzyme 25-OH-D-1-α-hydroxylase to make more 1,25-$(OH)_2$D from the large circulating pool of 25-OH-D. The resulting increase in 1,25-$(OH)_2$D (with the rise in PTH) causes an increase in calcium transport within the intestine, bone, and kidney. All these events raise plasma calcium levels back to normal, that in turn is sensed by the calcium receptor of the parathyroid gland. The further secretion of PTH is turned off not only by the feedback action of calcium, but also by a short feedback loop involving 1,25-$(OH)_2$D directly suppressing PTH synthesis in the parathyroid gland (not shown in figure).

Figure 10

Calcium homeostasis



Source: Adapted from Jones *et al*. (*13*).

Although this model oversimplifies the events involved in calcium homeostasis it is easy to see from it that sufficient 25-OH-D must be available to provide adequate 1,25-$(OH)_2$D synthesis and hence an adequate level of plasma calcium and that vitamin D deficiency will result in inadequate 25-OH-D and 1,25-$(OH)_2$D synthesis, inadequate calcium homeostasis, and a constantly elevated PTH level (termed: secondary hyperparathyroidism).

It becomes evident from this method of presentation of the role of vitamin D that the nutritionist can focus on the plasma levels of 25-OH-D and PTH to gain an insight into vitamin D status. Not shown but also important is the endpoint of the physiologic action of vitamin D, namely adequate plasma calcium and phosphate ions, that provide the raw materials for bone mineralisation.

Populations at risk for vitamin D deficiency

Infants

Infants constitute a population at risk for vitamin D deficiency because of relatively large vitamin D needs brought about by their high rate of skeletal growth. At birth, infants have acquired *in utero* the vitamin D stores that must carry them through the first months of life. A recent survey of French neonates revealed that 64 percent had 25-OH-D values below 30 nmol/l, the lower limit of the normal range (*15*). Breast-fed infants are particularly at risk because of the low concentrations of vitamin D in human milk (*16*). This problem is further compounded in some infants fed human milk by a restriction in exposure to ultraviolet (UV) light for seasonal, latitudinal, cultural, or social reasons. Infants born in the autumn months at extremes of latitude are particularly at risk because they spend the first 6 months of their life indoors and therefore have little opportunity to synthesise vitamin D in their skin during this period. Consequently, although vitamin D deficiency is rare in developed countries, sporadic cases of rickets are still being reported in many northern cities but almost always in infants fed human milk (*17-20*).

Infant formulas are supplemented with vitamin D at levels ranging from 40 international units (IUs) or 1 µg /418.4 kJ to 100 IU or 2.5 µg /418.4 kJ, that provide approximately between 6 µg and 15 µg of vitamin D, respectively. These amounts of dietary vitamin D are sufficient to prevent rickets.

Adolescents

Another period of rapid growth of the skeleton occurs at puberty and increases the need not for the vitamin D itself, but for the active form 1,25-$(OH)_2$D. This need results from the increased conversion of 25-OH-D to 1,25-$(OH)_2$D in adolescents (*21*). Furthermore, unlike infants, adolescents are usually outdoors and therefore usually are exposed to UV light sufficient for synthesising vitamin D for their needs. Excess production of vitamin D in the summer and early fall months is stored mainly in the adipose tissue (*22*) and is available to sustain high growth rates in the winter months that follow. Insufficient vitamin D stores during these periods of increased growth can lead to vitamin D insufficiency (*23*).

Elderly

Over the past 20 years, clinical research studies of the basic biochemical machinery handling vitamin D have suggested an age-related decline in many key steps of vitamin D action (*24*) including rate of skin synthesis, rate of hydroxylation leading to activation to the hormonal form, and response of target tissues (e.g., bone) as well as reduced skin exposure (*25*). Not surprisingly a number of independent studies from around the world have shown that there appears to be vitamin D deficiency in a subset of the elderly population, as characterised by low blood levels of 25-OH-D coupled with elevations of plasma PTH and alkaline

phosphatase (*26*). There is evidence that this vitamin D deficiency contributes to declining bone mass and increases the incidence of hip fractures (*27*). Although some of these studies may exaggerate the extent of the problem by focusing on institutionalised individuals or in-patients with decreased sun exposures, in general they have forced health professionals to re-address the intakes of this segment of society and look at potential solutions to correct the problem. Several groups have found that modest increases in vitamin D intakes (between 10 and 20 µg/day) reduce the rate of bone loss and the fracture rate (*25-29*).

These findings have led agencies and researchers to suggest an increase in recommended vitamin D intakes for the elderly from the suggested 2.5–5 µg /day to a value that is able to maintain normal 25-OH-D levels in the elderly, such as 10–15 µg/day. This vitamin D intake results in lower rates of bone loss and is suggested for the middle-aged (50–70 years) and old-aged (>70 years) populations (*33*). The increased requirements are justified mainly on the grounds of the reduction in skin synthesis of vitamin D, a linear reduction occurring in both men and women, that begins with the thinning of the skin at age 20 years (*24*).

Pregnancy and lactation

Elucidation of the changes in calciotropic hormones occurring during pregnancy and lactation has revealed a role for vitamin D in the former but probably not the latter. Even in pregnancy, the changes in vitamin D metabolism which occur, namely an increase in the maternal plasma levels of 1,25-$(OH)_2$D (*34*) due to a putative placental synthesis of the hormone (*35*), do not seem to impinge greatly on the maternal vitamin D requirements. The concern that modest vitamin D supplementation might be deleterious to the foetus is not justified. Furthermore, because transfer of vitamin D from mother to foetus is important for establishing the newborn's growth rate, the goal of ensuring adequate vitamin D status with conventional prenatal vitamin D supplements probably should not be discouraged.

In lactating women there appears to be no direct role for vitamin D because increased calcium needs are regulated by PTH-related peptide (*36, 37*), and recent studies have failed to show any change in vitamin D metabolites during lactation (*38, 39*). As stated above, the vitamin D content of human milk is low (*16*). Consequently, there is no great drain on maternal vitamin D reserves either to regulate calcium homeostasis or to supply the need of human milk. Because human milk is a poor source of vitamin D, rare cases of nutritional rickets are still found, but these are almost always in breast-fed babies deprived of sunlight exposure (*17-20*). Furthermore, there is little evidence that increasing calcium or vitamin D supplements to lactating mothers results in an increased transfer of calcium or vitamin D in milk (*38*). Thus, the current thinking, based on a clearer understanding of the role of vitamin D in lactation, is that there is little purpose in recommending additional vitamin D for lactating women. The goal for mothers who breast-feed their infants seems to be merely to ensure good nutrition and sunshine exposure in order to ensure normal vitamin D status during the perinatal period.

Table 19

Randomised, controlled trials with dietary vitamin D supplements

Reference	Study Group	n [b]	Age (years) Mean	Age (years) SD	Regimen	Duration (years)	Results[a]
Dawson-Hughes *et al.*, 1991 (*28*)	Healthy, post-menopausal women living independently	249	62	0.5	10 µg vitamin D + 400 mg calcium	1.0	Reduced late wintertime bone loss from vertebrae; net spine BMD; no change in whole-body BMD.
Chapuy *et al.,* 1992 (*29*)	Healthy, elderly women living in nursing homes or in apartments for the elderly	3270	84	6	20 µg vitamin D + 1200 mg calcium	1.5	Hip fractures 43% ; non-vertebral fractures 32%; in subset (*n*=56), BMD of proximal femur 2.7% in vitamin D group and 4.6% in placebo group.
Chapuy *et al.,* 1994 (*30*) [c]						3.0	Hip fractures 29%; non-vertebral fractures 24%.
Dawson-Hughes *et al.,* 1995 (*31*)	Healthy post-menopausal women living independently	261	64	5	2.5 µg or 17.5 µg vitamin D + 500 mg calcium	2.0	Loss of BMD from femoral neck lower in 17.5µg group (-1.06%) than in 2.5 µg group (-2.54%); no difference in BMD at spine.
Lips *et al.,* 1996 (*32*)	Healthy elderly living independently, in nursing homes, or in apartments for the elderly	2578 (1916-Fem.) (662-Male)	80	6	10 µg vitamin D		No difference in fracture incidence; in subset (*n*=248) of women from nursing homes, BMD 2.3% after 2 years.

[a] ↑ Increase; ↓ decrease.
[b] Number of subjects enrolled in the study.
[c] Same study as Chapuy *et al.* (*29*) after further 1.5 years of treatment.
Source: Adapted with permission from Shearer (*25*).

Evidence used for estimating recommended vitamin D intake

Lack of accuracy in estimating dietary intake and skin synthesis

The unique problem of estimating total intake of a substance that can be provided in the diet or made in the skin by exposure to sunlight makes it difficult to estimate adequate total intakes of vitamin D for the general population. Accurate food composition data are not available for vitamin D, accentuating the difficulty for estimating dietary intakes. Whereas this has led two recent US national surveys to avoid attempting this task, the second National Health and Nutrition Examination Survey (NHANES II) estimated vitamin D intakes to be 2.9 µg/day and 2.3 µg/day for younger and older women, respectively. A recent study of elderly women by Kinyamu *et al.* (*40*) concurred with this assessment, finding an intake of 3.53 µ g/day.

Skin synthesis is equally difficult to estimate, being affected by such imponderables as age, season, latitude, time of day, skin exposure, sun screen use, etc. In vitamin D – replete individuals, estimates of skin synthesis are put at around 10 µg /day (*24, 41*), with total intakes estimated at 15 µg/day (*24*).

Use of plasma 25-OH-D as a measure of vitamin D status

Numerous recent studies have used plasma 25-OH-D as a measure of vitamin D status, and there is a strong presumptive relationship of this variable with bone status. Thus, it is not surprising that several nutritional committees (e.g., the Food and Nutrition Board of the US National Academy of Sciences' Institute of Medicine in conjunction with Health Canada) have chosen to use a biochemical basis for estimating required intakes and used these estimates to derive recommended intakes (*33*). The method used involved the estimation of the mean group dietary intake of vitamin D required to maintain the plasma 25-OH-D levels above 27 nmol/l, that is necessary to ensure normal bone health. Previously, many studies had established 27 nmol/l as the lower limit of the normal range (e.g., NHANES III [*41*]). This dietary intake of vitamin D for each population group was rounded to the nearest 50 IU (1.25 µg) and then doubled to cover the needs of all individuals within that group irrespective of sunlight exposure. They termed this amount *adequate intake* (AI) and used it in place of recommended dietary allowance (RDA), that had been used by US agencies since 1941. We are recommending the use of those figures here as recommended nutrient intakes (RNIs) because it is an entirely logical approach to estimating the vitamin D needs for the whole population.

Table 20

Preliminary unweighted results from NHANES III data[a]

Percentile	25-OH-D[b] ng/ml
1st	7.6
5th	10.9
10th	13.2
50th	24.4
90th	40.1
95th	45.9
99th	59.0

[a]Total number of samples used in data analysis: 18,323; mean 25-OH-D value for United States: 25.89 ng/ml (+/- 11.08). Frequency distribution of serum or plasma 25-OH-D. Values for all ages, ethnicity groups, both sexes.[b]High values: four values 90-98 ng/ml, one value of 160.3 ng/ml. Values < 5 ng/ml (lowest standard) entered arbitrarily in the database as "3". Source: NHANES III (*42*).

Because study after study had been recommending increases in vitamin D intakes for the elderly, it might have been expected that the proposed increases in suggested intakes from 5 µg/day (RDAs in the United States [*43*], RNIs in Canada [*44*]) to 10 µg/day or 15 µg/day (AI) would be welcomed. However, a recent editorial in a prominent medical journal attacked the recommendations as being too conservative (*45*). This came on the heels of an article in the same journal (*46*) reporting the level of hypovitaminosis D to be as high as 57 percent in a population of ageing (mean 62 years) medical in-patients in the Boston area.

Of course, such in-patients are by definition sick and should not be used to calculate normal intakes. Indeed, the new NHANES III study (*42)* of 18 323 normal individuals from all regions of the United States suggests that approximately 5 percent had values of 25-OH-D below 27 nmol/l (*47*) (***Table 20***). Although the data are skewed by sampling biases that favour sample collection in the southern states in winter months and northern states in the summer months, even subsets of data collected in northern states in September give the incidence of low 25-OH-D in the elderly in the 6–18 percent range (*47*) as compared with 57 percent in the institutionalized in-patient population (*43*). Ideally, such measurements of the normal population should be made at the end of the winter months and before UV irradiation has reached a strength sufficient to allow skin synthesis of vitamin D. Thus, the NHANES III study may still underestimate the incidence of hypovitaminosis D in a northern elderly population in winter. Nevertheless, in lieu of additional studies of selected human populations, it would seem that the recommendations of the Food and Nutrition Board are reasonable guidelines for vitamin D intakes, at least for the near future. This considered approach allows for a period of time to monitor the potential shortfalls of the new recommendations as well as to assess whether the suggested guidelines can be attained, a point that was repeatedly stated about the RDAs.

Considerations in viewing recommended intakes for vitamin D

In recommending intakes for vitamin D, it must be recognised that in most locations in the world in a broad band around the equator (between latitudes 42°N and 42°S), the most physiologically relevant and efficient way of acquiring vitamin D is to synthesise it endogenously in the skin from 7-dehydrocholesterol by sun (UV) light exposure. In most situations, approximately 30 minutes of skin exposure (without sunscreen) of the arms and face to sunlight can provide all the daily vitamin D needs of the body (*24*). However, skin synthesis of vitamin D is negatively influenced by factors which may reduce the ability of the skin to provide the total needs of the individual (*24*):

- latitude and season – both influence the amount of UV light reaching the skin;
- the ageing process – thinning of the skin reduces the efficiency of this synthetic process;
- skin pigmentation: the presence in the skin of darker pigments interferes with UV light reaching the appropriate layer of the skin;
- clothing – virtually complete covering of the skin for medical, social, cultural, or religious reasons leaves insufficient skin exposed to sunlight; and
- sunscreen use – widespread and liberal use of sun-blockers reduces skin damage by the sun but also deleteriously affects synthesis of vitamin D.

Because not all of these problems can be solved in all geographic locations, particularly during winter at latitudes higher than 42° where synthesis is virtually zero, it is

recommended that individuals not synthesising vitamin D should correct their vitamin D status by consuming the amounts of vitamin D appropriate for their age group (***Table 21***).

Summary of the RNIs for vitamin D by age group

Table 21

RNIs for vitamin D according to age groups

Age group	RNI µg/day
Infants	
0–6 months	5
7–12 months	5
1–3 years	5
4–6 years	5
7–9 years	5
Adolescents, 10–18 years	5
Adults	
19–50 years	5
Older adults, 51–65 years	10
Elderly adults, 65+ years	15
Pregnant women	5
Lactating women	5

[a]Units: for vitamin D, 1 IU = 25 ng, 40 IU = 1 µg, 200 IU = 5 µg, 400 IU = 10 µg, 600 IU = 15 µg, 800 IU = 20 µg; for 25-OH-D, 1 ng/ml = 2.5 nmol/l, 10 ng/ml = 25 nmol/l, 11 ng/ml = 28.5 nmol/l (low limit), 30 ng/ml = 75 nmol/l (normal), 60 ng/ml = 150 nmol/l (upper limit).

Vitamin D toxicity

The adverse effects of high vitamin D intakes – hypercalciuria and hypercalcemia – do not occur at these new recommended intake levels. In fact, it is worth noting that the recommended intakes for all age groups are still well below the lowest observed adverse effect level of 50 µg/day and have not yet even reached the no observed adverse effect level of 20 µg/day (*33, 48*). Outbreaks of idiopathic infantile hypercalcemia in the United Kingdom in the post–World War II era led to the withdrawal of vitamin D fortification from all foods in that country because of concerns that they were due to hypervitaminosis D. There are some suggestions in the literature that these outbreaks of idiopathic infantile hypercalcemia may have been multifactorial with genetic and dietary components and were not just due to technical problems with over-fortification as was assumed (*49,50*). In retrospect, the termination of the vitamin D fortification may have been counter productive because it exposed segments of the UK community to vitamin deficiency and may have discouraged other nations from starting vitamin D fortification programmes (*50*). This is all the more cause for concern because hypovitaminosis D is still a problem worldwide, particularly in developing countries at high latitudes and in countries where skin exposure to sunlight is discouraged (*51*).

Future research

Further research is needed to determine:

- whether vitamin D supplements during pregnancy have any positive effects later in life;
- whether vitamin D has a role in lactation;
- the long-term effects of higher vitamin D intakes;
- whether dietary vitamin D supplements are as good as exposure to UV light; and
- whether vitamin D is only needed for regulation of calcium and phosphate.

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Chapter 9
Vitamin E

Summary of the role of vitamin E in human metabolic processes

A large body of scientific evidence indicates that reactive free radicals are involved in many diseases, including heart disease and cancers (*1*). Cells contain many potentially oxidizable substrates such as polyunsaturated fatty acids (PUFAs), proteins, and DNA. Therefore, a complex antioxidant defence system normally protects cells from the injurious effects of endogenously produced free radicals as well as from species of exogenous origin such as cigarette smoke and pollutants. Should our exposure to free radicals exceed the protective capacity of the antioxidant defence system, a phenomenon often referred to as oxidative stress (*2*), then damage to biologic molecules may occur. There is considerable evidence that disease causes an increase in oxidative stress; therefore, consumption of foods rich in antioxidants, which are potentially able to quench or neutralise excess radicals, may play an important role in modifying the development of such diseases.

Vitamin E is the major lipid-soluble antioxidant in the cell antioxidant defence system and is exclusively obtained from the diet. The term "vitamin E" refers to a family of eight naturally occurring homologues that are synthesised by plants from homogentisic acid. All are derivatives of 6-chromanol and differ in the number and position of methyl groups on the ring structure. The four tocopherol homologues (*d*-α-, *d*-β-, *d*-γ-, and *d*-δ-) have a saturated 16-carbon phytyl side chain, whereas the tocotrienols (*d*-α-, *d*-β-, *d*-γ-, and *d*-δ-) have three double bonds on the side chain. There is also a widely available synthetic form, *dl*-α-tocopherol, prepared by coupling trimethylhydroquinone with isophytol. This consists of a mixture of eight stereoisomers in approximately equal amounts; these isomers are differentiated by rotations of the phytyl chain in various directions that do not occur naturally. For dietary purposes, vitamin E activity is expressed as α-tocopherol equivalents (α-TEs). One α-TE is the activity of 1 mg *RRR*-α-tocopherol (*d*-α-tocopherol). To estimate the α-TE of mixed diet containing natural forms of vitamin E, the number of milligrams of β-tocopherol should be multiplied by 0.5, γ-tocopherol by 0.1, and α-tocotrienol by 0.3. Any of the synthetic all-*rac*-α-tocopherol (*dl*-α-tocopherol) should be multiplied by 0.74. One milligram of the latter compound in the acetate form is equivalent to 1 IU of vitamin E.

Vitamin E is an example of a phenolic antioxidant. Such molecules readily donate the hydrogen from the hydroxyl (-OH) group on the ring structure to free radicals, which then become unreactive. On donating the hydrogen, the phenolic compound itself becomes a relatively unreactive free radical because the unpaired electron on the oxygen atom is usually delocalised into the aromatic ring structure thereby increasing its stability (*3*).

The major biologic role of vitamin E is to protect PUFAs and other components of cell membranes and low-density lipoprotein (LDL) from oxidation by free radicals. Vitamin E is located primarily within the phospholipid bilayer of cell membranes. It is particularly effective in preventing lipid peroxidation, a series of chemical reactions involving the oxidative deterioration of PUFAs. Elevated levels of lipid peroxidation products are associated with numerous diseases and clinical conditions (*4*). Although vitamin E is primarily located in cell and organelle membranes where it can exert its maximum protective effect, its concentration may only be one molecule for every 2000 phospholipid molecules.

This suggests that after its reaction with free radicals it is rapidly regenerated, possibly by other antioxidants (5).

Absorption of vitamin E from the intestine depends on adequate pancreatic function, biliary secretion, and micelle formation. Conditions for absorption are like those for dietary lipid, that is, efficient emulsification, solubilisation within mixed bile salt micelles, uptake by enterocytes, and secretion into the circulation via the lymphatic system (*6*). Emulsification takes place initially in the stomach and then in the small intestine in the presence of pancreatic and biliary secretions. The resulting mixed micelle aggregates the vitamin E molecules, solubilises the vitamin E, and then transports it to the brush border membrane of the enterocyte probably by passive diffusion. Within the enterocyte, tocopherol is incorporated into chylomicrons and secreted into the intracellular space and lymphatic system and subsequently into the blood stream. Tocopherol esters, present in processed foods and vitamin supplements, must be hydrolysed in the small intestine before absorption.

Vitamin E is transported in the blood by the plasma lipoproteins and erythrocytes. Chylomicrons carry tocopherol from the enterocyte to the liver, where they are incorporated into parenchymal cells as chylomicron remnants. The catabolism of chylomicrons takes place in the systemic circulation through the action of cellular lipoprotein lipase. During this process tocopherol can be transferred to high-density lipoproteins (HDLs). The tocopherol in HDLs can transfer to other circulating lipoproteins, such as LDLs and very low-density lipoproteins (VLDLs) (*7*). During the conversion of VLDL to LDL in the circulation, some α-tocopherol remains within the core lipids and thus is incorporated in LDL. Most α-tocopherol then enters the cells of peripheral tissues within the intact lipoprotein through the LDL receptor pathway, although some may be taken up by membrane binding sites recognising apolipoprotein A-I and A-II present on HDL (*8*).

Although the process of absorption of all the tocopherol homologues in our diet is similar, the α form predominates in blood and tissue. This is due to the action of binding proteins that preferentially select the α form over the others. In the first instance, a 30-kDa binding protein unique to the liver cytoplasm preferentially incorporates α-tocopherol in the nascent VLDL (*9*). This form also accumulates in non-hepatic tissues, particularly at sites where free radical production is greatest, such as in the membranes of mitochondria and endoplasmic reticulum in the heart and lungs (*10*).

Hepatic intracellular transport may be expedited by a 14.2-kDa binding protein that binds α-tocopherol in preference to the other homologues (*11*). Other proteinaceous sites with apparent tocopherol-binding abilities have been found on erythrocytes, adrenal membranes, and smooth muscle cells (*12*). These may serve as vitamin E receptors which orient the molecule within the membrane for optimum antioxidant function.

These selective mechanisms explain why vitamin E homologues have markedly differing antioxidant abilities in biologic systems and illustrates the important distinction between the *in vitro* antioxidant effectiveness of a substance in the stabilisation of, for example, a food product and its *in vivo* potency as an antioxidant. From a nutritional perspective, the most important form of vitamin E is α-tocopherol; this is corroborated in animal model tests of biopotency which assess the ability of the various homologues to prevent foetal absorption and muscular dystrophies (***Table 22***).

Plasma vitamin E concentrations vary little over a wide range of dietary intakes. Even daily supplements of the order of 1600 IU/day for 3 weeks only increased plasma levels 2–3 times and on cessation of treatment plasma levels returned to pretreatment levels in 5 days (*13*). Likewise, tissue concentrations only increased by a similar amount when patients undergoing heart surgery were given 300 mg/day of the natural stereoisomer for 2 weeks

preoperatively (*14*). Kinetic studies with deuterated tocopherol (*15*) suggest that there is rapid equilibration of new tocopherol in erythrocytes, liver, and spleen but that turnover in other tissues such as heart, muscle, and adipose tissue is much slower. The brain is markedly resistant to depletion and repletion with vitamin E (*16*). This presumably reflects an adaptive mechanism to avoid detrimental oxidative reactions in this key organ.

The primary oxidation product of α-tocopherol is a tocopheryl quinone that can be conjugated to yield the glucuronate after prior reduction to the hydroquinone. This is excreted in the bile or further degraded in the kidneys to α-tocopheronic acid and hence excreted in the bile. Those vitamin E homologues not preferentially selected by the hepatic binding proteins are eliminated during the process of nascent VLDL secretion in the liver and probably excreted via the bile (*17*). Some vitamin E may also be excreted via skin sebaceous glands (*18*).

Table 22

Approximate biological activity of naturally occurring tocopherols and tocotrienols compared with *d*-α-tocopherol

Common name	Biological activity compared with *d*-α-tocopherol, %
d-α-tocopherol	100
d-β-tocopherol	50
d-γ-tocopherol	10
d-δ-tocopherol	3
d-α-tocotrienol	30
d-β-tocotrienol	5
d-γ-tocotrienol	not known
d-δ-tocotrienol	not known

Defining populations at risk of vitamin E deficiency

There are many signs of vitamin E deficiency in animals most of which are related to damage to cell membranes and leakage of cell contents to external fluids. Disorders provoked, for example, by traces of peroxidized PUFAs in the diets of animals with low vitamin E status are cardiac or skeletal myopathies, neuropathies, and liver necrosis (*19*) (***Table 23***). Muscle and neurological problems are also a consequence of human vitamin E deficiency (*20*). Early diagnostic signs of deficiency include leakage of muscle enzymes such as creatine kinase and pyruvate kinase into plasma, increased levels of lipid peroxidation products in plasma, and increased erythrocyte haemolysis.

The assessment of the vitamin E requirement for humans is confounded by the infrequent occurrence of clinical signs of deficiency because these usually only develop in adults with fat-malabsorption syndromes or liver disease, in individuals with genetic anomalies in transport or binding proteins, and possibly in premature infants (*19, 21*). This suggests that diets contain sufficient vitamin E to satisfy nutritional needs.

Several animal models (*22*) suggest that increasing intakes of vitamin E inhibit the progression of vascular disease by preventing the oxidation of LDL. Evidence suggests that oxidized lipoprotein is a key event in the development of the atheromatous plaque which may ultimately occlude the blood vessel (*23*).

Table 23

Diseases and syndromes in animals associated with vitamin E deficiency and excess intakes of polyunsaturated fatty acids

Syndrome	Affected organ or tissue	Species
Encephalomalacia	Cerebellum	Chick
Exudative diathesis	Vascular	Turkey
Microcytic anaemia	Blood, bone marrow	Chick
Macrocytic anaemia	Blood, bone marrow	Monkey
Pancreatic fibrosis	Pancreas	Chick, mouse
Liver necrosis	Liver	Pig, rat
Muscular degeneration	Skeletal muscle	Pig, rat, mouse
Microangiopathy	Heart muscle	Pig, lamb, calf
Kidney degeneration	Kidney tubules	Monkey, rat
Steatitis	Adipose tissue	Pig, chick
Testicular degeneration	Testes	Pig, calf, chick
Malignant hyperthermia	Skeletal muscle	Pig

Human studies, however, have been less consistent in providing evidence for a role of vitamin E in preventing heart disease. Vitamin E supplements reduce *ex vivo* oxidizability of plasma LDLs but there is no correlation between *ex vivo* lipoprotein oxidizability and endogenous vitamin E levels in an unsupplemented population (*24*). Likewise, the few randomised double-blind, placebo-controlled intervention trials with human volunteers which focused on the relationship between vitamin E and cardiovascular disease have given inconsistent results. There was a marked reduction in non-fatal myocardial infarction in patients with coronary artery disease (as defined by angiogram) who were randomly assigned to take pharmacologic doses of vitamin E (400 and 800 mg/day) or placebo in the Cambridge Heart Antioxidant Study involving 2000 men and women (*25*). However, the incidence of major coronary events in male smokers who received 20 mg/day of vitamin E for approximately 6 years was not reduced in the Alpha-Tocopherol, Beta-Carotene study (*26*).

Epidemiologic studies suggest that dietary vitamin E influences the risk of cardiovascular disease. Gey *et al*. (*27*) reported that lipid-standardized plasma vitamin E concentrations in middle-aged men across 16 European countries predicted 62 percent of the variance in the mortality from ischaemic heart disease. In the United States both the Nurses Health Study (*28*) involving 87000 females in an 8-year follow-up and the Health Professionals Follow-up Study in 40000 men (*29*) concluded that persons taking supplements of 100 mg/day or more of vitamin E for at least 2 years had approximately a 40 percent lower incidence of myocardial infarction and cardiovascular mortality than did those who did not use supplements. However, in US studies there was no influence of dietary vitamin E alone on incidence of cardiovascular disease when those taking supplements were removed from the analyses. A possible explanation for the significant relationship between dietary vitamin E and cardiovascular disease in European countries but not in the United States may be found in the widely differing sources of vitamin E in European countries. It is reported that sunflower seed oil, which is rich in α-tocopherol, tends to be consumed more widely in the southern European countries with the lower cardiovascular disease risk than in northern European countries where soybean oil, which contains more of the γ form, is preferred (*30*) (***Table 24***). However, a study carried out which compared plasma α- and γ-tocopherol concentrations in middle-aged men and women in Toulouse (southern France) with Belfast (Northern Ireland) found that the concentrations of γ-tocopherol in Belfast were twice as high as those in Toulouse; α-tocopherol concentrations were identical in men in both countries but higher in women in Belfast than in Toulouse ($P<0.001$) (*31*).

Table 24

Cross-country correlations between coronary heart disease mortality in men and the supply of vitamin E homologues across 24 European countries

Homologue	Correlation coefficient, *r*
Total vitamin E	-0.386
d-α-tocopherol	-0.753
d-β-tocopherol	-0.345
d-γ-tocopherol	-0.001
d-δ-tocopherol	0.098
d-α-tocotrienol	-0.072
d-β-tocotrienol	-0.329
d-γ-tocotrienol	-0.210

The correlation with *d*-α-tocopherol is highly significant ($P<0.001$) whereas all other correlations do not achieve statistical significance.
Source: Based on reference *30*.

It has also been suggested vitamin E supplementation (200–400 mg/day) may be appropriate therapeutically to moderate some aspects of degenerative diseases such as Parkinson's disease, reduce the severity of neurologic disorders such as tardive dyskinesia, prevent periventricular haemorrhage in pre-term babies, reduce tissue injury arising from ischaemia and reperfusion during surgery, delay cataract development, and improve mobility in arthritis sufferers (*32*). However, very high doses may also induce adverse pro-oxidant effects (*33*), and the long-term advantages of such treatments have not been proven.

Delineation of dietary sources and possible limitations to its availability worldwide

Because vitamin E is naturally present in plant-based diets and animal products and is often added by manufacturers to vegetable oils and processed foods, intakes are probably adequate to avoid overt deficiency in most situations. Exceptions may be during ecologic disasters and cultural conflicts resulting in food deprivation and famine.

Analysis of the Food and Agriculture Organization of the United Nations country food balance sheets indicates that about half the α-tocopherol in a typical northern European diet such as in the United Kingdom is derived from vegetable oils (*30*). Animal fats, vegetables, and meats each contribute about 10 percent to the total *per capita* supply and fruit, nuts, cereals, and dairy products each contribute about 4 percent. Less than 2 percent is each obtained from eggs, fish and pulses.

There are marked differences in *per capita* α-tocopherol supply among different countries ranging from approximately 8-10 mg/head/day (e.g., Iceland, Finland, New Zealand, and Japan) to 20–25 mg/head/day (e.g., France, Greece, and Spain) (*30*). This variation can be ascribed mainly to the type and quantity of dietary oils used in different countries and the proportion of the different homologues in the oils (***Table 25***). For example, sunflower seed oil contains approximately 55 mg α-tocopherol/100 g in contrast to soybean oil that contains only 8 mg/100 ml (*34*). Consumption of these oils varies markedly among countries. Soybean, a rich source of the less biologically active γ form, is most commonly used in northern European countries whereas sunflower seed oils, which mainly contain the α form, are generally used in southern Europe (*30*).

Table 25

Vitamin E content (mg tocopherol/100g) in vegetable oils

Oil	α–tocopherol	γ–tocopherol	δ–tocopherol	α - tocotrienol
Coconut	0.5	0	0.6	0.5
Maize (Corn)	11.2	60.2	1.8	0
Palm	25.6	31.6	7.0	14.3
Olive	5.1	Trace	0	0
Peanut	13.0	21.4	2.1	0
Soybean	10.1	59.3	26.4	0
Wheatgerm	133.0	26.0	27.1	2.6
Sunflower	48.7	5.1	0.8	0

Source: Slover HT, 1971. (34)

Summary of evidence for determining recommended nutrient intakes

In the chapter on antioxidants, it was decided that there was insufficient evidence to enable a recommended nutrient intake (RNI) to be based on the additional health benefits obtainable from nutrient intakes above those usually found in the diet. Even for vitamin E with its important biologic antioxidant properties, there was no consistent evidence for protection against chronic disease from dietary supplements. Nevertheless, the main function of vitamin E appears to be that of preventing oxidation of PUFAs, and this has been used by those bodies proposing RNIs for vitamin E because there is considerable evidence in different animal species that low vitamin E and PUFAs excess gives rise to a wide variety of clinical signs.

There is very little clinical evidence of deficiency disease in humans except in certain inherited conditions where the metabolism of vitamin E is disturbed. Even biochemical evidence of poor vitamin E status in both adults and children is minimal. Meta-analysis of data collected within European countries indicates that optimum intakes may be implied when plasma concentrations of vitamin E exceed 25–30 μmol/L of lipid-standardized α-tocopherol (*35*). However, this approach should be treated with caution, as plasma vitamin E concentrations do not necessarily reflect intakes or tissue reserves because only 1 percent of the body tocopherol may be in the blood (*36*) and the amount in the circulation is strongly influenced by circulating lipid (*37*). Nevertheless, the lipid-standardized vitamin E concentration (e.g., tocopherol-cholesterol ratio) greater that 2.25 (calculated as μmol/mmol) is believed to represent satisfactory vitamin E status (*36, 37*). The erythrocytes of subjects with values below this concentration of vitamin E may show evidence of an increasing tendency to haemolyze when exposed to oxidizing agents and thus such values should be taken as an indication of biochemical deficiency (*38*). However, the development of clinical evidence of vitamin E deficiency (e.g., muscle damage or neurologic lesions) can take several years of exposure to extremely low vitamin E levels (*39*).

The main factor used to assess the adequacy of vitamin E intakes by the US and UK advisory bodies was the dietary intake of PUFAs. PUFAs are very susceptible to oxidation, and their increased intake without a concomitant increase in vitamin E can lead to a reduction in plasma vitamin E concentrations (*40*) and to elevations in some indexes of oxidative damage in human volunteers (*41*). Generally, however, diets high in PUFAs are also high in vitamin E, and to set a dietary recommendation based on extremes of PUFA intake would deviate considerably from median intakes of vitamin E in most Western populations. Hence 'safe' allowances for the United Kingdom (men 10 and women 7 mg/day) (*42*) and 'arbitrary' allowances for the United States (men 10 and women 8 mg/day) (*43*) for vitamin E intakes approximate the median intakes in those countries. It is worth noting that there were only 11 (0.7 percent) subjects out of 1629 adults in the 1986–1987 British Nutrition Survey who had

α-tocopherol – cholesterol ratios <2.25. Furthermore, although the high intake of soybean oil with its high content of γ-tocopherol substitutes for the intake of α-tocopherol in the British diet, a comparison of α-tocopherol-cholesterol ratios found almost identical results in two groups of randomly selected, middle-aged adults in Belfast (Northern Ireland) and Toulouse (France), two countries with very different intakes of α-tocopherol (*34*) and cardiovascular risk (*31*).

It is suggested that when the main PUFA in the diet is linoleic acid, a *d*-α-tocopherol-PUFA ratio of 0.4 (expressed as mg tocopherol per g PUFA) is adequate for adult humans (*44, 45*), and the ratio has been recommended in the United Kingdom for infant formulas (*46*). Use of this ratio to calculate the vitamin E requirements of men and women with energy intakes of 2550 and 1940 kcal/day containing PUFA at 6 percent of the energy intake (approximately 17 and 13 g, respectively) (*42*) produced values of 7 and 5 mg/day of α-TEs, respectively. In both the United States and the United Kingdom, median intakes of α-TE are in excess of these amounts and the α-tocopherol-PUFA ratio is approximately 0.6 (*47*), which is well above the 0.4 ratio which would be considered adequate. The Nutrition Working Group of the International Life Sciences Institute Europe (*48*) has suggested an intake of 12 mg α-tocopherol for a daily intake of 14 g PUFAs to compensate for the high consumption of soya oil in certain countries where over 50 percent of vitamin E intake is accounted for by the less biologically active γ form. As indicated above, however, plasma concentrations in France and Northern Ireland suggest that an increased amount of dietary vitamin E is not necessary to maintain satisfactory plasma concentrations (*31*).

At present, data are not sufficient to formulate recommendations for vitamin E intake for different age groups except for infancy. There is some indication that new-born infants, particularly if born prematurely, are vulnerable to oxidative stress because of low body stores of vitamin E, impaired absorption, and reduced transport capacity resulting from low concentrations at birth of circulating low-density lipoproteins (*49*). However, term infants almost achieve adult plasma vitamin E concentrations in the first week (*50*) and although the concentration of vitamin E in early human milk can be variable, after 12 days it remains fairly constant at 0.32 mg TE/100 ml milk (51). Thus a human-milk-fed infant consuming 850 ml would have an intake of 2.7 mg. It seems reasonable that formula milk should not contain less than 0.3 mg TE/100 ml of reconstituted feed and not less than 0.4 mg TE/g PUFA.

No specific recommendations concerning the vitamin E requirements in pregnancy and lactation have been made by other advisory bodies (*42, 43*) mainly because there is no evidence of vitamin E requirements different from those of other adults and presumably also as the increased energy intake would compensate for the increased needs for infant growth and milk synthesis.

Vitamin E appears to have very low toxicity, and amounts of 100–200 mg of the synthetic all-*rac*-α-tocopherol are consumed widely as supplements (*28, 29*). Evidence of pro-oxidant damage has been associated with the feeding of supplements but usually only at very high doses (e.g., >1000 mg/day) (*33*).

Future research

More investigation is required of the role of vitamin E in biologic processes which do not necessarily involve its antioxidant function. These processes include:

- structural roles in the maintenance of cell membrane integrity;
- anti-inflammatory effects by direct and regulatory interaction with the prostaglandin synthetase complex of enzymes, which participate in the metabolism of arachidonic acid;
- DNA synthesis;

- stimulation of the immune response; and
- regulation of intercellular signalling and cell proliferation through modulation of protein kinase-C.

Similarly, more investigation is required of the growing evidence that inadequate vitamin E status may increase susceptibility to infection particularly by allowing the genomes of certain relatively benign viruses to convert to more virulent strains (*52*).

There is an important need to define optimum vitamin E intakes. Intervention trials with morbidity and mortality endpoints may take years to complete. One approach to circumvent this delay may be to assess the effects of different intakes of vitamin E on biomarkers of oxidative damage to lipids, proteins, and DNA because their occurrence *in vivo* is implicated in many diseases, including vascular disease and certain cancers.

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Chapter 10
Vitamin K

Vitamin K is an essential fat-soluble micronutrient which is needed for a unique post-translational chemical modification in a small group of proteins with calcium-binding properties, collectively known as vitamin K – dependent proteins or Gla-proteins. Thus far, the only unequivocal role of vitamin K in health is in the maintenance of normal coagulation. The vitamin K – dependent coagulation proteins are synthesised in the liver and comprise factors II, VII, IX, and X, which have a haemostatic role (i.e., they are procoagulants that arrest and prevent bleeding), and proteins C and S, which have an anticoagulant role (i.e., they inhibit the clotting process). Despite this duality of function, the overriding effect of nutritional vitamin K deficiency is to tip the balance in coagulation towards a bleeding tendency caused by the relative inactivity of the procoagulant proteins. Vitamin K – dependent proteins synthesised by other tissues include the bone protein osteocalcin and matrix Gla protein; their functions remain to be clarified.

Biological role of vitamin K

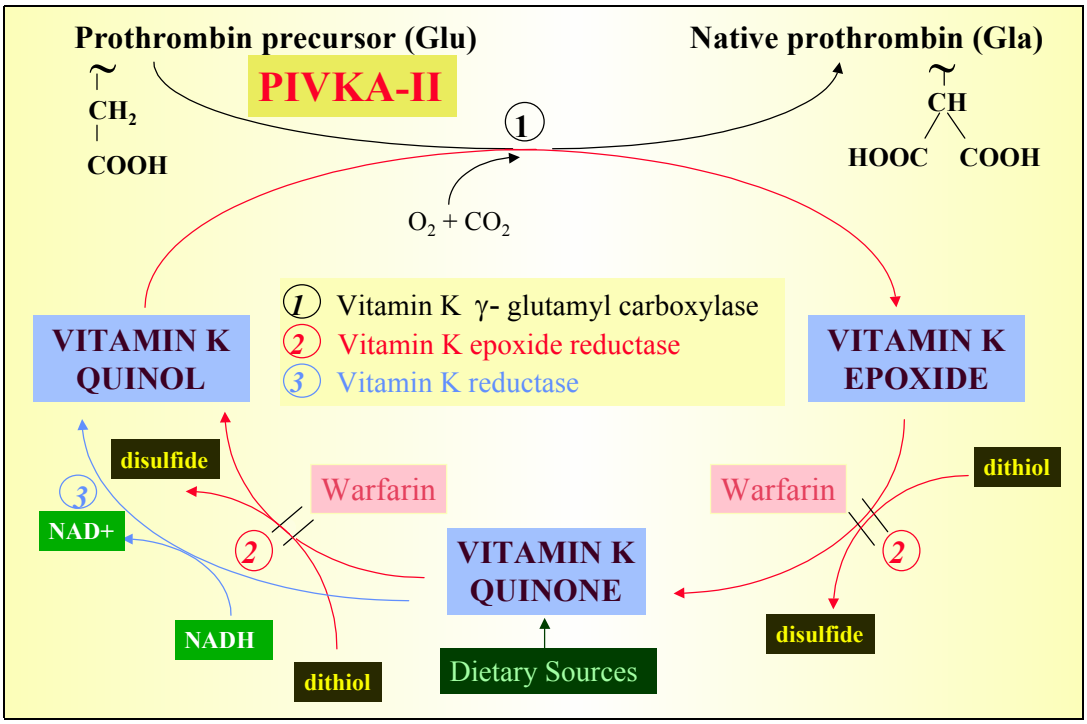
Vitamin K is the family name for a series of fat-soluble compounds, which have a common 2-methyl-1, 4-naphthoquinone nucleus but differ in the structures of a side chain at the 3-position. They are synthesised by plants and bacteria. In plants the only important molecular form is phylloquinone (vitamin K_1), which has a phytyl side chain. Bacteria synthesise a family of compounds called menaquinones (vitamin K_2), which have side chains based on repeating unsaturated 5-carbon (prenyl) units. These are designated menaquinone-n (MK-n) according to the number (n) of prenyl units. Some bacteria also synthesise menaquinones in which one or more of the double bonds is saturated. The compound 2-methyl-1,4-naphthoquinone (common name menadione) may be regarded as a provitamin because vertebrates can convert it to MK-4 by adding a 4-prenyl side chain at the 3-position.

The biologic role of vitamin K is to act as a cofactor for a specific carboxylation reaction that transforms selective glutamate (Glu) residues to γ-carboxyglutamate (Gla) residues (*1,2*). The reaction is catalysed by a microsomal enzyme, γ-glutamyl, or vitamin K – dependent carboxylase, which in turn is linked to a cyclic salvage pathway known as the vitamin K epoxide cycle (***Figure 11***).

Scheme shows the cyclic metabolism of vitamin K in relation to the conversion of glutamate (Glu) to γ-carboxyglutamate (Gla) residues for the coagulation protein prothrombin. A general term for the glutamate precursors of vitamin K-dependent proteins is proteins induced by vitamin K absence, abbreviated PIVKA. For prothrombin (factor II) the glutamate precursor is known as PIVKA-II. The active form of vitamin K needed for carboxylation is the reduced form, vitamin K quinol. Known enzyme reactions are numbered 1, 2, and 3. The carboxylation reaction is driven by a vitamin K-dependent carboxylase activity (*reaction 1*) which simultaneously converts vitamin K quinol to vitamin K 2,3-epoxide. Vitamin K 2,3-epoxide is reduced back to the quinone and then to the quinol by vitamin K epoxide reductase (*reaction 2*). The reductase activity denoted 2 is dithiol dependent and is inhibited by coumarin anticoagulants such as warfarin. Dietary vitamin K may enter the cycle via an NAD(P)H-dependent vitamin K reductase activity (*reaction 3*), which is not inhibited by warfarin.

Figure 11

The vitamin K epoxide cycle



The four vitamin K-dependent procoagulants (factor II or prothrombin, and factors VII, IX, and X) are serine proteases that are synthesised in the liver and then secreted into the circulation as inactive forms (zymogens). Their biologic activity depends on their normal complement of Gla residues, which are efficient chelators of calcium ions. In the presence of Gla and calcium ions these proteins bind to the surface membrane phospholipids of platelets and endothelial cells where, together with other cofactors, they form membrane-bound enzyme complexes. When coagulation is initiated, the zymogens of the four vitamin K-dependent clotting factors are cleaved to yield the active protease clotting factors (*1-3*). Two other vitamin K-dependent proteins called protein C and protein S play a regulatory role in the inhibition of coagulation. The function of protein C is to degrade phospholipid-bound activated factors V and VIII in the presence of calcium. Protein S acts as a synergistic cofactor to protein C by enhancing the binding of activated protein C to negatively charged phospholipids. Yet another vitamin K-dependent plasma protein (protein Z) is suspected to have a haemostatic role but its function is currently unknown.

Apart from the coagulation proteins, several other vitamin K-dependent proteins have been isolated from bone, cartilage, kidney, lungs, and other tissues (*4, 5*). Only two, osteocalcin and matrix Gla protein (MGP), have been well characterised. Both are found in bone but MGP also occurs in cartilage, blood vessel walls, and other soft tissues. There is evidence that protein S is synthesised by several tissues including the vessel wall and bone and may have other functions besides its well-established role as a coagulation inhibitor. It also seems likely that one function of MGP is to inhibit mineralisation (*6*). Thus far, no clear biologic role for osteocalcin has been established despite its being the major non-collagenous bone protein synthesised by osteoblasts (*7-9*). This failure to establish a biologic function for osteocalcin has hampered studies of the possible detrimental effects of vitamin K deficiency on bone health. Evidence of a possible association of a suboptimal vitamin K status with increased fracture risk remains to be confirmed (*7-9*).

Overview of metabolism

Absorption and transport

Dietary vitamin K, mainly as phylloquinone, is absorbed chemically unchanged from the proximal intestine after solubilisation into mixed micelles composed of bile salts and the products of pancreatic lipolysis (*10*). In healthy adults the efficiency of absorption of phylloquinone in its free form is about 80 percent (*10, 11*). Within the intestinal mucosa the vitamin is incorporated into chylomicrons, is secreted into the lymph, and enters the blood via the lacteals (*11, 12*). Once in the circulation, phylloquinone is rapidly cleared (*10*) at a rate consistent with its continuing association with chylomicrons and the chylomicron remnants which are produced by lipoprotein lipase hydrolysis at the surface of capillary endothelial cells (*13*). After an overnight fast, more than half of the circulating phylloquinone is still associated with triglyceride-rich lipoproteins, with the remainder being equally distributed between low-density and high-density lipoproteins (*13*). Phylloquinone is the major circulating form of vitamin K but MK-7 is present in plasma at lower concentrations and has a lipoprotein distribution similar to phylloquinone (*13*). Although phylloquinone in blood must have been derived exclusively from the diet, it is not known whether circulating menaquinones such as MK-7 are derived from the diet, intestinal flora, or a combination of these sources.

Tissue stores and distribution

Until the 1970s, the liver was the only known site of synthesis of vitamin K-dependent proteins and hence was presumed to be the only significant storage site for the vitamin. However, the discovery of vitamin K-dependent processes and proteins in a number of extra-hepatic tissues suggests that this may not be the case.

Human liver stores normally comprise about 90 percent menaquinones and 10 percent phylloquinone (*14, 15*). There is evidence that the phylloquinone liver stores are very labile; under conditions of severe dietary depletion, liver concentrations were reduced to about 25 percent of initial levels after only 3 days (*15*). This high turnover of hepatic reserves of phylloquinone is in accord with the high losses of this vitamer through excretion (*10*). Knowledge of hepatic stores of phylloquinone in different population groups is limited. Adult hepatic stores in a UK study were about 11 pmol/g (*14*) whereas in a study from Japan they were about twofold higher(*15*). Such reserves are about 20 000–40 000-fold lower than those for retinol for relative daily intakes of phylloquinone that are only about 10-fold lower than those of vitamin A (*16*).

The relationship between hepatic and total-body stores of vitamin K is not known. Other sites of storage may be adipose tissue and bone; both are known to be sites where vitamin K-bearing chylomicrons and chylomicron remnants may be taken up. It has been reported that the predominant vitamer in human cortical and trabecular bone is phylloquinone; unlike the situation in liver, no menaquinones higher than MK-8 were detected (*17*).

In contrast to the hepatic preponderance of long-chain menaquinones, the major circulating form of vitamin K is invariably phylloquinone. The menaquinones MK-7 and possibly MK-8 are also present but the common hepatic forms MKs 9–13 are not detectable in blood plasma (*16, 18*). This might be a consequence of a different route of absorption (e.g., the possibility of a portal route for long-chain MKs *versus* the established lymphatic route for phylloquinone) but might suggest that once in the liver, the lipophilic long-chain menaquinones are not easily mobilised (*16, 18, 19*).

Bio-activity

Very little information exists on the relative effectiveness of different hepatic forms of K vitamins for the coagulation function of vitamin K in humans. This information is important because of the preponderance of long-chain menaquinones in human liver. Early bioassay data from rats suggested that long-chain menaquinones (MKs-7, 9, and 10) were more efficient than phylloquinone in reversing vitamin K deficiency when single doses were give parenterally and that their sustained response may be due to their slower hepatic turnover (*18, 19*). A longer duration of the biologic response of MK-9 compared with phylloquinone in vitamin K-deficient rats was also observed by Groenen-van Dooren *et al.* (*20*). On the other hand Will and Suttie (*21*) showed that, when given orally, the dietary requirement of MK-9 for the maintenance of prothrombin synthesis in rats is higher than that for phylloquinone. They also reported that the initial hepatic turnover of MK-9 was two- to three-fold slower than that of phylloquinone.

Suttie (*18*) emphasised that the existence of a large pool of menaquinones in human liver does not necessarily mean that menaquinones make a proportionately greater contribution to the maintenance of vitamin K sufficiency. In humans the development of sub-clinical signs of vitamin K deficiency detected in dietary phylloquinone restriction studies argues against this, especially when placed alongside the lack of change of hepatic menaquinone stores (*15*). One explanation is that much of the hepatic menaquinones is not biologically available to the microsomal γ-glutamyl carboxylase because of a different sub-cellular location, especially location in the mitochondria and possibly other non-microsomal sites (*18*).

Excretion

Vitamin K is extensively metabolised in the liver and excreted in the urine and bile. In tracer experiments it was found that about 20 percent of an injected dose of phylloquinone was recovered in the urine whereas about 40–50 percent was excreted in the faeces via the bile (*10*); the proportion excreted was the same regardless of whether the injected dose was 1 mg or 45 μg. It seems likely, therefore, that about 60–70 percent of the amounts of phylloquinone absorbed from each meal will ultimately be lost to the body by excretion. These results suggest that the body stores of phylloquinone are being constantly replenished.

Two major human excretion products have been identified: carboxylic acids with 5 and 7-carbon sidechains that are excreted in the urine as glucuronide conjugates (*10*). The biliary metabolites have not been clearly identified but are initially excreted as water-soluble conjugates and become lipid soluble during their passage through the gut, probably through deconjugation by the gut flora. There is no evidence for body stores of vitamin K being conserved by an enterohepatic circulation. Vitamin K itself is too lipophilic to be excreted in the bile and the sidechain-shortened carboxylic acid metabolites are not biologically active.

Populations at risk

Vitamin K deficiency bleeding in infants

In infants up to around age 6 months, vitamin K deficiency, although rare, represents a significant public health problem throughout the world (*19, 22, 23*). The deficiency syndrome is traditionally known as haemorrhagic disease of the newborn or more recently, to give a better definition of the cause, vitamin K deficiency bleeding (VKDB).

The time of onset of VKDB is more unpredictable than previously supposed and it is now useful to recognise three syndromes: early, classic, and late (***Table 26***). Until the 1960s, VKDB was considered to be solely a problem of the first week of life. Then, in 1966, came the first reports from Thailand of a new vitamin K deficiency syndrome that typically

presented between 1 and 2 months of life and is now termed late VKDB. In 1977 Bhanchet and colleagues (*24*), who had first described this syndrome, summarised their studies of 93 affected Thai infants, establishing the idiopathic history, preponderance of breast-fed infants (98 percent), and high incidence of intracranial bleeding (63 percent). More reports from South East Asia and Australia followed, and in 1983 McNinch *et al*. (*25*) reported the return of VKDB in the United Kingdom. This increased incidence was ascribed to a decrease in the practice of vitamin K prophylaxis and to an increased trend towards exclusive human milk feeding (*25*). Human milk has lower concentrations of vitamin K than do infant milk formulas (*26*).

Without vitamin K prophylaxis, the incidence of late VKDB (per 100,000 births), based on acceptable surveillance data, has been estimated to be 4.4 in the United Kingdom, 7.2 in Germany, and as high as 72 in Thailand (*27*). Of real concern is that late VKDB, unlike the classic form, has a high incidence of death or severe and permanent brain damage resulting from intracranial haemorrhage (*19, 22, 23*).

Epidemiologic studies worldwide have identified two major risk factors for both classic and late VKDB: exclusive human milk feeding and the failure to give any vitamin K prophylaxis (*19, 22, 23*). The increased risk for infants fed human milk compared with formula milk is probably related to the relatively low concentrations of vitamin K (phylloquinone) in breast milk compared with formula milks (*26, 28, 29*). For classic VKDB, studies using the detection of under-carboxylated prothrombin or proteins induced by vitamin K absence (PIVKA)-II as a marker of sub-clinical vitamin K deficiency have suggested that it is the low cumulative intake of human milk in the first week of life rather than an abnormally low milk concentration *per se* that seems to be of greater relevance (*30, 31*). Thus, classic VKDB may be related, at least in part, to a failure to establish early breast-feeding.

Table 26

Classification of vitamin K deficiency bleeding of the newborn

Syndrome	Time of presentation	Common bleeding sites	Comments
Early VKDB	0–24 hours	Cephalohaematoma, intracranial, intrathoracic, intra-abdominal	Maternal drugs a frequent cause (e.g., Warfarin, anti-convulsants)
Classic VKDB	1–7 days	Gastrointestinal, skin, nasal, circumcision	Mainly idiopathic, maternal drugs
Late VKDB	1–12 weeks	Intracranial, skin, gastrointestinal	Mainly iodiopathic, may be presenting feature of underlying disease (e.g., cystic fibrosis, α-1-antitrypsin deficiency, biliary atresia); some degree of cholestasis often present

[a] VKDB, vitamin K deficiency bleeding.
Source: Shearer (19).

For late VKDB other factors seem to be important because the deficiency syndrome occurs when breast-feeding is well established and mothers of affected infants seem to have normal concentrations of vitamin K in their milk (*31*). Instead some (although not all) infants

who develop late haemorrhagic disease of the newborn are later found to have abnormalities of liver function that may affect their bile acid production and result in a degree of malabsorption of vitamin K. The degree of cholestasis may be mild and its course may be transient and self-correcting, but affected infants will have increased dietary requirements for vitamin K because of a reduced absorption efficiency.

Vitamin K prophylaxis in infants

Because bleeding can occur spontaneously and because no screening test is available, it is now common paediatric practice to protect all infants by giving vitamin K supplements in the immediate perinatal period. Vitamin K prophylaxis has had a chequered history but in recent years has become a high-profile issue of public health in many countries throughout the world. The reasons for this are twofold. First there is now a convincing body of evidence showing that without vitamin K prophylaxis, infants have a small but real risk of dying from or being permanently brain damaged by vitamin K deficiency in the first 6 months of life (*19, 22, 23*). The other, much less certain evidence stems from a reported epidemiologic association between vitamin K given intramuscularly (but not orally) and the later development of childhood cancer (*32*). The debate, both scientific and public, which followed this and other publications has led to an increase in the use of multiple oral supplements instead of the traditional single intramuscular injection (usually of 1 mg of phylloquinone) given at birth. Although most of the subsequent epidemiologic studies have not confirmed any cancer link with vitamin K, the issue is still not resolved (*33, 34*).

Vitamin K in adults

In adults, primary vitamin K–deficient states that manifest as bleeding are almost unknown except when the absorption of the vitamin is impaired as a result of an underlying pathology (*1*).

Dietary sources

High-performance liquid chromatography can be used to accurately determine the major dietary form of vitamin K (phylloquinone) in foods, and food tables are being compiled for Western diets (*16, 35, 36*). Phylloquinone is distributed ubiquitously throughout the diet, and the range of concentrations in different food categories is very wide. In general, the relative values in vegetables confirm the known association of phylloquinone with photosynthetic tissues, with the highest values (normally in the range 400–700 μg/100 g) being found in green leafy vegetables. The next best sources are certain vegetable oils (e.g., soybean, rapeseed, and olive oils) which contain 50–200 μg/100 g. Some vegetable oils, such as peanut, corn, sunflower and safflower oils, have a much lower phylloquinone content (1–10 μg/100 g). The great differences between vegetable oils obviously presents problems for calculating the phylloquinone contents of oil-containing foods when the type of oil (or its storage condition) is not known.

Menaquinones seem to have a more restricted distribution in the diet than does phylloquinone. In the Western diet nutritionally significant amounts of long-chain menaquinones have been found in animal livers and fermented foods such as cheeses. Yeasts do not synthesise menaquinones and menaquinone-rich foods are those with a bacterial fermentation stage. The Japanese food *natto* (fermented soybeans) has a menaquinone content even higher than that of phylloquinone in green leafy vegetables.

The relative dietary importance of MK-4 is more difficult to evaluate because concentrations in foods may well depend on geographic differences in the use of menadione in animal husbandry, menadione from which MK-4 may be synthesised in animal tissues.

Another imponderable factor is the evidence that animal tissues and dairy produce may contain some MK-4 as a product of tissue synthesis from phylloquinone itself (*37*).

Knowledge of the vitamin K content of human milk has been the subject of methodologic controversies with a 10-fold variation in reported values of phylloquinone concentrations of mature human milk (*38*). Where milk sampling and analytical techniques have met certain criteria for their validity, the phylloquinone content of mature milk have generally ranged between 1 and 4 μg/l, with average concentrations near the lower end of this range (*28, 29, 38*). However, there is considerable intra- and inter-subject variation, and levels higher are in colostral milk than in mature milk (*28*). Menaquinone concentrations in human milk have not been accurately determined but appear to be much lower than those of phylloquinone. Phylloquinone concentrations in infant formula milk range from 3 to 16 μg/l in unsupplemented formulas and up to 100 μg/l in fortified formulas (*26*). Nowadays most formulas are fortified; typical phylloquinone concentrations are about 50 μg/l.

Bio-availability of vitamin K from foods

Very little is known about the bio-availability of the K vitamins from different foods. It has been estimated that the efficiency of absorption of phylloquinone from boiled spinach (eaten with butter) is no greater than 10 percent (*39*) compared with an estimated 80 percent when phylloquinone is given in its free form (*10, 11*). This poor absorption of phylloquinone from green leafy vegetables may be explained by its location in chloroplasts and tight association with the thylakoid membrane, where this naphthoquinone plays a role in photosynthesis. In comparison, the bio-availability of MK-4 from butter artificially enriched with this vitamer was more than twofold higher than that of phylloquinone from spinach (*39*). The poor extraction of phylloquinone from leafy vegetables, which as a category represents the single greatest food source of phylloquinone, may place a different perspective on the relative importance of other foods with lower concentrations of phylloquinone (e.g., containing soybean and rapeseed oils) but in which the vitamin is not tightly bound and its bio-availability is likely to be greater. Even before bio-availability was taken into account, fats and oils that are contained in mixed dishes were found to make an important contribution to the phylloquinone content of the US diet (*40*) and in a UK study contributed 30 percent of the total dietary intake (*41*).

No data exist on the efficiency of intestinal absorption of dietary long-chain menaquinones. Because the lipophilic properties of menaquinones are greater than those of phylloquinone, it is likely that the efficiency of their absorption, in the free form, is low, as suggested by animal studies (*18, 21*).

Importance of intestinal bacterial synthesis as a source of vitamin K

Intestinal microflora synthesise large amounts of menaquinones, which are potentially available as a source of vitamin K (*42*). Quantitative measurements at different sites of the human intestine have demonstrated that most of these menaquinones are present in the distal colon (*42*). Major forms produced are MK-10 and MK-11 by *Bacteroides*, MK-8 by *Enterobacter*, MK-7 by *Veillonella,* and MK-6 by *Eubacterium lentum*. It is noteworthy that menaquinones with very long chains (MKs 10–13) are known to be synthesised by members of the anaerobic genus *Bacteroides* and are major inhabitants of the intestinal tract but have not been detected in significant amounts in foods. The widespread presence of MKs 10–13 in human livers at high concentrations (*14, 15*) therefore suggests that these forms, at least, originate from intestinal synthesis (*16*).

It is commonly held that animals and humans obtain a significant fraction of their vitamin K requirement from direct absorption of menaquinones produced by microfloral synthesis (*43*), but hard experimental evidence documenting the site and extent of any absorption is singularly lacking (*18, 19, 23*). The most promising site of absorption is the terminal ileum, where there are some menaquinone-producing bacteria as well as bile salts. The evidence overall suggests that the bio-availability of bacterial menaquinones is poor because they are mostly tightly bound to the bacterial cytoplasmic membrane and the largest pool is present in the colon, which lacks bile salts for their solubilisation (*19, 23*).

Evidence on which recommendations can be based

Assessment of vitamin K status

Conventional coagulation assays are useful for detecting overt vitamin K-deficient states which are associated with a risk of bleeding. However, they offer only a relatively insensitive insight into vitamin K nutritional status and the detection of sub-clinical vitamin K-deficient states. A more sensitive measure of vitamin K sufficiency can be obtained from tests that detect under-carboxylated species of vitamin K-dependent proteins. In states of vitamin K deficiency, under-carboxylated species of the vitamin K-dependent coagulation proteins are released from the liver into the blood; their levels increase with the degree of severity of vitamin K deficiency. These under-carboxylated forms (PIVKA) are unable to participate in the normal coagulation cascade because they are unable to bind calcium. The measurement of under-carboxylated prothrombin (PIVKA-II) is the most useful and sensitive homeostatic marker of sub-clinical vitamin K deficiency. Importantly, PIVKA-II is detectable in plasma before any changes occur in conventional coagulation tests. Several types of assay for PIVKA-II have been developed which vary in their sensitivity (*44*).

In the same way that vitamin K deficiency causes PIVKA-II to be released into the circulation from the liver, a deficit of vitamin K in bone will cause the osteoblasts to secrete under-carboxylated species of osteocalcin (ucOC) into the bloodstream. It has been proposed that the concentration of circulating ucOC reflects the sufficiency of vitamin K for the carboxylation of this Gla protein in bone tissue (*7, 45*). Most assays for ucOC have been indirect because they rely on the differential absorption of carboxylated and under-carboxylated forms to hydroxyapatite and are difficult to interpret (*46*).

Other criteria of vitamin K sufficiency that have been used are plasma measurements of phylloquinone and the measurement of urinary Gla. It is expected and found that the excretion of urinary Gla is decreased in vitamin K deficiency.

Dietary intakes in infants and their adequacy

The average intake of phylloquinone in infants fed human milk during the first 6 months of life has been reported to be less than 1 μg/day; this is approximately 100-fold lower than the intake in infants fed a typical supplemented formula (29). This big disparity between intakes is reflected in plasma levels (***Table 27***).

Using the detection of PIVKA-II as a marker of sub-clinical deficiency, a study from Germany concluded that a minimum daily intake of about 100 ml of colostral milk (that supplies about 0.2-0.3 μg of phylloquinone) is sufficient for normal haemostasis in a baby of about 3 kg during the first week of life (*30, 47*). Similar conclusions were reached in a Japanese study which showed a linear correlation between the prevalence of PIVKA-II and the volume of breast milk ingested over 3 days (*48*); 95 percent of infants with detectable PIVKA-II had average daily intakes of less than about 120 ml, but the marker was not detectable when intakes reached 170 ml/day.

Table 27

Dietary intakes and plasma levels of phylloquinone in human-milk-fed *versus* formula-fed infants aged 0–6 months

Age (weeks)	Phylloquinone intake (μg/day)		Plasma phylloquinone (μg/l)	
	Human milk fed[a]	Formula fed[b]	Human milk fed	Formula fed
6	0.55	45.4	0.13	6.0
12	0.74	55.5	0.20	5.6
26	0.56	52.2	0.24	4.4

[a]Breast-milk concentrations averaged 0.86, 1.14, and 0.87 μg/l of phylloquinone at 6, 12, and 26 weeks, respectively.

[b]All infants were fed a formula containing phylloquinone at 55 μg/l.

Source: Greer FR *et al*. (*29*).

Factors of relevance to classical vitamin K deficiency bleeding

The liver stores of vitamin K in the newborn infant differ both qualitatively and quantitatively from that in adults. First, phylloquinone levels at birth are about one-fifth those in adults and second, bacterial menaquinones are undetectable (*14*). The resistance to placental transport of vitamin K to the human foetus is well-established (*19, 22*). Thereafter, the limited available data suggest that hepatic stores of menaquinones build up gradually, becoming detectable at around the second week of life but only reaching adult concentrations after 1 month of age (*14, 49*). A gradual increase in liver stores of menaquinones may reflect the gradual colonisation of the gut by enteric microflora.

A practical problem in assessing the functional status of vitamin K in the neonatal period is that there are both gestational and postnatal increases in the four vitamin K-dependent procoagulant factors which are unrelated to vitamin K status (*50*). This means that unless the deficiency state is quite severe, it is very difficult to interpret clotting factor activities as a measure of vitamin K sufficiency. The best diagnostic tool of the adequacy of vitamin K stores for neonates is by the detection of PIVKA-II by immunoassays. The use of this marker has clearly shown that there is a temporary dip in the vitamin K status of infants exclusively fed human milk in the first few days after birth (*30, 47, 48, 51, 52*). The fact that the degree of this dip is associated with human-milk intakes (*30, 47, 48*) and is less evident or abolished in infants given artificial feeds (*30, 48, 52*) or prophylactic vitamin K at birth (*48, 51, 52*) shows that the detection of PIVKA-II reflected a dietary lack of vitamin K.

Factors of relevance to late vitamin K deficiency bleeding

The natural tendency for human-milk-fed infants to develop a sub-clinical vitamin K deficiency in the first 2–3 days of life is self-limiting. Comparisons between untreated human-milk-fed infants with others who had received vitamin K or supplementary feeds clearly suggest that this improvement in vitamin K–dependent clotting activity is due to an improved vitamin K status. After the first week, vitamin K–dependent clotting factor increases are more gradual, and it is not possible from clotting factor assays to differentiate between the natural post-natal increase in the synthesis of the core proteins from an improved vitamin K status leading to greater functional activity.

Use of the most sensitive assays for PIVKA-II shows that there is still evidence of suboptimal vitamin K status in infants solely fed human milk between the ages of 1 and 2

months (*52, 53*). Deficiency signs are less common in infants who have received adequate vitamin K supplementation (*52, 53*) or who have been formula fed (*52*).

Dietary intakes in older infants, children, and adults and their adequacy

The only comprehensive national survey of phylloquinone intakes across all age groups (except infants aged 0–6 months) is that of the US Food and Drug Administration Total Diet Study, which was based on the 1987–88 Nationwide Food Consumption Survey (*40*). For infants and children from the age of 6 months to 16 years, average phylloquinone intakes were above the current US recommended dietary allowance (RDA) values for their respective age groups, more so for children up to 10 years than from 10 to 16 years (***Table 28***) (*40*). There have been no studies of intakes in children in relation to functional markers of vitamin K sufficiency in children.

Intakes for adults in The Total Diet Study (***Table 28***) were also close to or slightly higher than the current US RDA values of 80 μg for men and 65 μg for women, although intakes were slightly lower than the RDA in the 25–30 years age group (*54*). There is some evidence from an evaluation of all the US studies that older adults have higher dietary intakes of phylloquinone than do younger adults (*55*).

The US results are very comparable with a detailed, seasonality study in the United Kingdom in which mean intakes in men and women (aged 22–54 years) were 72 and 64 μg/day, respectively; no significant sex or seasonal variations were found (*56*). Another UK study suggested that intakes were lower in manual workers and in smokers, reflecting their lower intakes of green vegetables and high-quality vegetable oil (*57*).

Several dietary restriction and repletion studies have attempted to assess the adequacy of vitamin K intakes in adults (*55, 58*). It is clear from these studies that volunteers consuming less than 10 μg/day of phylloquinone do not show any changes in conventional coagulation tests even after several weeks unless other measures to reduce the efficiency of absorption are introduced. However, a diet containing only 2–5 μg/day of phylloquinone fed for 2 weeks did result in an increase of PIVKA-II and a 70 percent decrease in plasma phylloquinone (*59*). Similar evidence of a sub-clinical vitamin K deficiency together with an increased urinary excretion of Gla was found when dietary intakes of phylloquinone were reduced from about 80 to about 40 μg/day for 21 days (*60*). A repletion phase in this study was consistent with a human dietary vitamin K requirement (for its coagulation role) of about 1 μg/kg body weight/day.

The most detailed and controlled dietary restriction and repletion study in healthy human subjects is that by Ferland *et al*. (*61*). In this study 32 healthy subjects in two age groups (20–40 and 60–80 years) were fed a mixed diet containing about 80 μg/day of phylloquinone, which is the RDA for adult males in the United States (*54*). After 4 days on this baseline diet there was a 13-day depletion period during which the subjects were fed a diet containing about 10 μg/day. After this depletion phase the subjects entered a 16-day repletion period during which, over 4-day intervals, they were sequentially repleted with 5, 15, 25, and 45 μg phylloquinone. The depletion protocol had no effect on conventional coagulation and specific factor assays but did induce a significant increase in PIVKA-II in both age groups. The most dramatic change was in plasma levels of phylloquinone, which fell to about 15 percent of the values on day 1. The drop in plasma phylloquinone also suggested that the average dietary intake of these particular individuals before they entered the study had been greater than the baseline diet of 80 μg/day. The repletion protocol failed to bring the plasma phylloquinone levels of the young subjects back above the lower limit of the normal range (previously established in healthy, free-living adults) whereas the plasma levels in the elderly group only rose slightly above this lower limit in the last 4 days. Another indication of

a reduced vitamin K status in the young group was the fall in urinary output of Gla (90 percent of baseline) that was not seen in the elderly group; this suggested that younger subjects are more susceptible to the effects of an acute deficiency than are older subjects.

Table 28

Mean dietary intakes of phylloquinone from US Food and Drug Administration Total Diet Study based on 1987-88 Nationwide Food Consumption Survey *versus* US RDA

Group	No.[a]	Phylloquinone Intake (μg/day) 1990 TDS[b]	RDA[c]
Infants			
6 months	141	77	10
Children			
2 years	152	24	15
6 years	154	46	20
10 years	119	45	30
Females, 14–16 years	188	52	45-55
Males, 14–16 years	174	64	45-65
Younger adults			
Females, 25–30 years	492	59	65
Males, 25–30 years	386	66	80
Females, 40–45years	319	71	65
Males, 40–45years	293	86	80
Older adults			
Females, 60–65 years	313	76	65
Males, 60–65 years	238	80	80
Females, 70+ years	402	82	65
Males, 70+ years	263	80	80

[a]The number of subjects as stratified by age and/or sex.
[b]Total Diet Study (*40*).
[c]Recommended Dietary Allowance, 1989 (*54*).

One important dietary intervention study measured the carboxylation status of the bone vitamin K-dependent protein, osteocalcin, in response to altered dietary intakes of phylloquinone (*62*). This was a crossover study, which evaluated the effect in young adults of increasing the dietary intake of phylloquinone to 420 μg/day for 5 days from a baseline intake of 100 μg/day. Although total concentrations of osteocalcin were not affected by either of the dietary treatments, ucOC fell dramatically in response to the 420 μg diet and by the end of the 5-day supplementation period was 41 percent lower than the baseline value. After the return to the mixed diet, the ucOC percent rose significantly but after 5 days had not returned to pre-supplementation values. This study suggested that the carboxylation of osteocalcin in bone may require higher dietary intakes of vitamin K than those needed to sustain its haemostatic function.

Recommendations for vitamin K intakes

Table 29

Recommended nutrients intakes for vitamin K

Age group	Recommended Nutrient Intake† μg /day
Infants and children	
0–6 months	5*
7–12 months	10
1–3 years	15
4–6 years	20
7–9 years	25
Adolescents, 10–18 years	
Females	35-55
Males	35-55
Adults	
Females, 19–65 years	55
65+ years	55
Males, 19–65 years	65
65+ years	65
Pregnancy	55
Lactation	55

†The RNI for each age group is based on a daily intake of approximately 1 microgram/body weight of phylloquinone.
*This intake cannot be met by infants who are exclusively breast-fed.

To prevent bleeding due to vitamin K deficiency, the panel recommends that all breast babies should receive vitamin K supplementation at birth according to nationally approved guidelines. Vitamin K formulations and prophylactic regimes differ from country to country. Guidelines range from a single intramuscular injection (usually 1 mg of phylloquinone) given at birth to multiple oral doses given over the first few weeks (***Table 29***).

Infants 0–6 months

Consideration of the requirements of vitamin K for infants up to age 6 months is complicated by the need to prevent a rare but potentially devastating bleeding disorder, which is caused by vitamin K deficiency. To protect the few affected infants, most developed and some developing countries have instituted a blanket prophylactic policy to protect infants at risk. The numbers of infants at risk without such a programme has a geographic component, being more prevalent in the Far East, and a dietary component with solely human-milk-fed babies having the highest risk (*22, 23, 27*). Of the etiologic factors, some of which may still be unrecognised, one factor in some infants is mild cholestasis. The problem of overcoming a variable and, in some infants, inefficient absorption is the likely reason that oral prophylactic regimens, even with two or three pharmacologic doses (1 mg phylloquinone), have occasionally failed to prevent VKDB (*63*). This makes it difficult to design an effective oral prophylaxis regimen that is comparable in efficacy with the previous "gold standard" of 1 mg phylloquinone given by intramuscular injection at birth. In several countries intramuscular prophylaxis fell out of favour after the epidemiologic report and subsequent controversy that this route may be linked to childhood cancer (*32-34*).

Infants who have been entirely fed with supplemented formulas are well protected against VKDB and on intakes of around 50 μg/day have plasma levels that are about 10-fold higher than the adult average of about 1.0 nmol/l (0.5 μg/l) (*29*) (***Table 29***). Clearly then, an optimal intake would lie below an intake of 50 μg/day. Cornelissen *et al*. (*64*) evaluated the effectiveness of giving infants a daily supplement of 25 μg phylloquinone after they had received a single oral dose of 1 mg at birth. This regimen resulted in median plasma levels at ages 4, 8, and 12 weeks of around 2.2 nmol/l (1.0 μg/l) when sampled 20–28 hours after the most recent vitamin K dose; this level corresponds to the upper end of the adult fasting range. In 12-week-old infants supplemented with this regime, the median plasma level was about fourfold higher than that in a control group of unsupplemented infants (1.9 *versus* 0.5 nmol/l). Also none of the 50 supplemented infants had detectable PIVKA-II at 12 weeks compared with 15 of 131 infants (11.5 percent) in the control group. This regime has now been implemented in The Netherlands and surveillance data on late VKDB suggest that it may be as effective as parenteral vitamin K prophylaxis (*63*).

The fact that VKDB is epidemiologically associated with breast feeding means that it is not prudent to base requirements solely on normal intakes of human milk and justifies the setting of a higher value that can only be met by some form of supplementation. The current US RDA for infants is 5 μg/day for the first 6 months (the greatest period of risk for VKDB) and 10 μg/day during the second 6 months (*54*). This is based on the adult RDA of 1 μg/kg body weight/day. However, if the vitamin K content of human milk is assumed to be about 2 μg/l, exclusively breast-fed infants aged 0–6 months may ingest only 20 percent of their presumed daily requirement of 5 μg (*54*). Whether a figure of 5 μg/day is itself safe is uncertain. In the United Kingdom the dietary reference value for infants was set at 10 μg/day, which in relation to body weight (2 μg/kg) is about double the estimate for adults (*65*). It was set with reference to the upper end of possible human milk concentrations plus a further qualitative addition to allow for the absence of hepatic menaquinones in early life and the presumed reliance on dietary vitamin K alone.

The association of VKDB with breast-feeding does not mean that most infants are at risk of developing VKDB, because this is a rare vitamin K deficiency syndrome. In contrast to measurements of PIVKA-II levels, comparisons of vitamin K–dependent clotting activities have shown no detectable differences between infants fed human milk and those fed artifical formula. The detection of PIVKA-II with normal functional levels of vitamin K–dependent coagulation factors does not imply immediate or even future haemorrhagic risk for a particular individual. The major value of PIVKA-II measurements in infants is to assess the prevalence of suboptimal vitamin K status in population studies. However, because of the potential consequences of VKDB, the paediatric profession of most countries agrees that some form of vitamin K supplementation is necessary even though there are widespread differences in actual practice.

Infants (7–12 months), children, and adults

In the past, the requirements for vitamin K only considered its classical function in coagulation; an RDA was given for vitamin K in the United States (*54, 58*) and a safe and adequate intake level was given in the United Kingdom (*65*). In both countries the adult RDA or adequate intake was set at a value of 1 μg/kg body weight/day. Thus in the United States the RDA for a 79-kg man is listed as 80 μg/day and for a 63-kg women as 65 μg/day (*54*).

At the time previous recommendations were set there were few data on dietary intakes of vitamin K (mainly phylloquinone) in different populations. The development of more accurate and wide-ranging food databases is now helping to address this question. The results of several dietary intake studies in the United States and the United Kingdom suggest that the

average intakes for adults are very close to the respective recommendations of each country. In the United States, preliminary intake data also suggest that average intakes of phylloquinone in children and adolescents also exceed the RDA; in 6-month-old infants the intakes exceeded the RDA of 10 μg by nearly eightfold (*40*), reflecting the use of supplemented formula foods. Because there is no evidence of even sub-clinical deficiencies of haemostatic function, a daily intake of 1 μg/kg may still be used as the basis for the recommended nutrient intake (RNI). There is no basis as yet for making different recommendations for pregnant and lactating women.

A relevant question is whether the RNI should be raised to take into account recent evidence that the requirements for the optimal carboxylation of vitamin K–dependent proteins in other tissues are greater than those for coagulation. There is certainly evidence that the γ-carboxylation of osteocalcin can be improved by intakes somewhere between 100 and 420 μg/day (*62*). If an RNI for vitamin K sufficiency is to be defined as that amount necessary for the optimal carboxylation of all vitamin K-dependent proteins, including osteocalcin, then it seems clear that this RNI would lie somewhere above the current intakes of many, if not most, of the population in the United States and the United Kingdom. Because a clearly defined metabolic role and biochemical proof of the necessity for fully γ-carboxylated osteocalcin for bone health is currently lacking, it would be unwise to make such a recommendation.

Toxicity

When taken orally, natural K vitamins seem free of toxic side effects. This safety is illustrated by the common clinical administration of phylloquinone at doses of 10–20 mg or greater. Some patients with chronic fat malabsorption regularly ingest doses of this size without evidence of any harm. However, synthetic preparations of menadione or its salts are best avoided for nutritional purpose, especially for vitamin prophylaxis in the newborn. Besides lacking intrinsic biologic activity, the high reactivity of its unsubstituted 3-position has been associated with neonatal haemolysis and liver damage.

Future research

The following are recommended areas for future research:

- prevalence, causes, and prevention of VKDB in infants in different population groups;
- bio-availability of dietary phylloquinone (and menaquinones) from foods and menaquinones from gut flora;
- significance of menaquinones to human requirements for vitamin K;
- the physiologic roles of vitamin K-dependent proteins in functions other than coagulation; and
- the significance of under-carboxylated vitamin K-dependent proteins and sub-optimal vitamin K status to bone and cardiovascular health.

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Chapter 11
Calcium

It is nearly 30 years since the last FAO/WHO recommendations on calcium intake were published in 1974 (*1*) and nearly 40 years since the experts' meeting in Rome (*2*) on which these recommendations were based. During this generation gap, a paradigm shift has occurred with respect to the involvement of calcium in the aetiology of osteoporosis. The previous reports were written against the background of the Albright paradigm (*3*), according to which osteomalacia and rickets were due to calcium deficiency, vitamin D deficiency, or both, whereas osteoporosis was attributed to failure of new bone formation secondary to negative nitrogen balance, osteoblast insufficiency, or both. The rediscovery of earlier information that calcium deficiency led to the development of osteoporosis (not rickets and osteomalacia) in experimental animals (*4*) resulted in a reexamination of osteoporosis in humans, notably in postmenopausal women. This reexamination yielded evidence in the late 1960s that menopausal bone loss was not due to a decrease in bone formation but rather to an increase in bone resorption (*5-8*), and this has had a profound effect on our understanding of other forms of osteoporosis. Although reduced bone formation may aggravate the bone loss process in elderly people (*9*) and probably plays a major role in corticosteroid osteoporosis (*10*) – and possibly in osteoporosis in men (*11*) – bone resorption is increasingly held responsible for osteoporosis in women and for the bone deficit associated with hip fractures in elderly people of both sexes (*12*). Because bone resorption is also the mechanism whereby calcium deficiency destroys bone, it is hardly surprising that the role of calcium in the pathogenesis of osteoporosis has received increasing attention and that recommended calcium intakes have risen steadily in the past 35 years from the nadir which followed the publication of the report from Rome in 1962 (*13*). The process has been accelerated by the growing realisation that insensible losses of calcium (via skin, hair, nails, etc.) need to be taken into account in the calculation of calcium requirement.

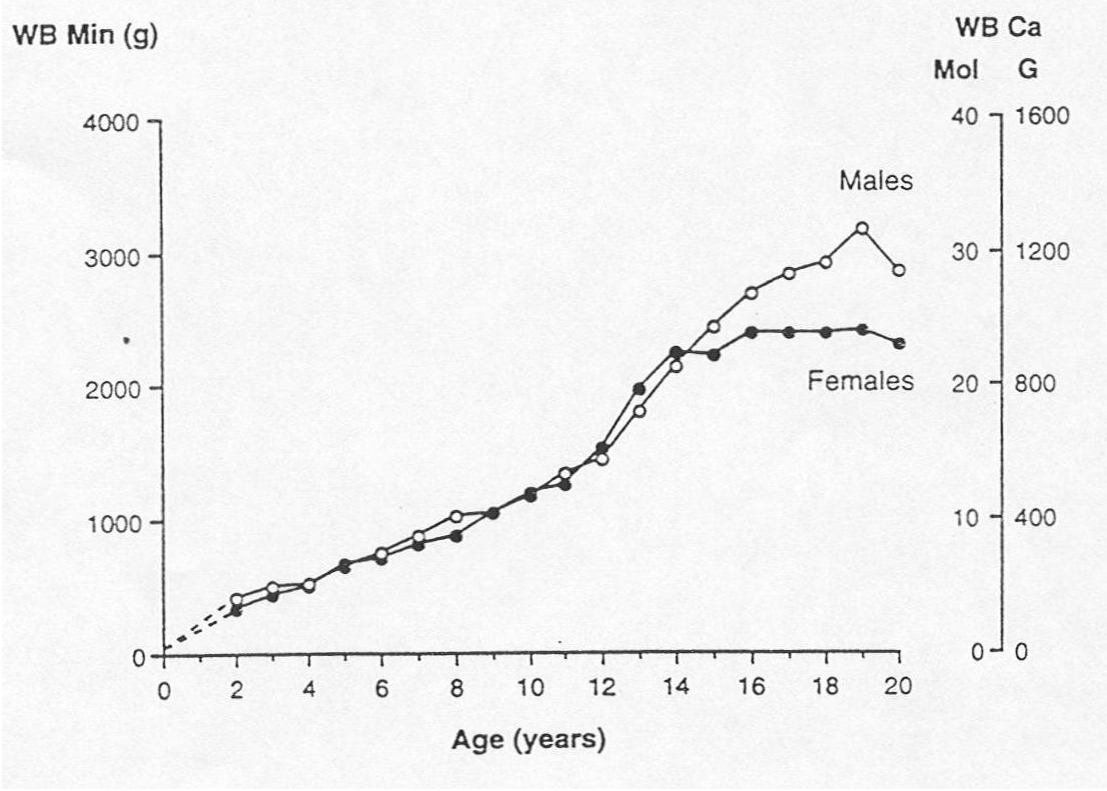
As the calcium allowances recommended for developed nations have been rising – and may still not have reached their peak – the gap between them and the actual calcium intakes in developing countries has widened. The concept that calcium requirement may itself vary from culture to culture for dietary, genetic, lifestyle, and geographical reasons is emerging. This report therefore seeks to make it clear that our main recommendations – like the latest recommendations from USA and Canada (*14*), Great Britain (*15*), the European Union (*16*), and Australia (*17*) – are largely based on data derived from the developed world and are not necessarily applicable to nations with different dietary cultures, different lifestyles, and different environments for which different calculations may be indicated.

Chemistry and distribution of calcium

Calcium is a divalent cation with an atomic weight of 40. In the elementary composition of the human body, it ranks fifth after oxygen, carbon, hydrogen, and nitrogen, and it makes up 1.9 percent of the body by weight (18). Carcass analyses show that it constitutes 0.1–0.2 percent of early foetal fat-free weight, rising to about 2 percent of adult fat-free weight. In absolute terms, this represents a rise from about 24 g (600 mmol) at birth to 1300 g (32.5 mol) at maturity, requiring an average daily positive calcium balance of 180 mg (4.5 mmol) during the 20 years of growth (***Figure 12***).

Figure 12

Whole-body bone mineral (WB Min) (left axis) and calcium (right axis) as a function of age as determined by total-body dual-energy X-ray absorptiometry



Note: Data supplied by Dr Zanchetta, IDIM, Buenos Aires, Argentina).

Ninety-nine percent of the body calcium is located in the skeleton. The remaining 1 percent is equally distributed between the teeth and soft tissues, with only 0.1 percent in the extracellular fluid (ECF). In the skeleton it constitutes 25 percent of the dry weight and 40 percent of the ash weight. The ECF contains ionised calcium at about 4.8 mg/100 ml (1.20 mmol/l) maintained by the parathyroid – vitamin D system as well as complexed calcium at about 1.6 mg/100 ml (0.4 mmol/l). In the plasma there is an additional protein-bound calcium fraction of 3.2 mg/100 ml (0.8 mmol/l). In the cellular compartment the total calcium concentration is comparable with that in the ECF, but the free calcium concentration is lower by several orders of magnitude (*19*).

Biological role of calcium

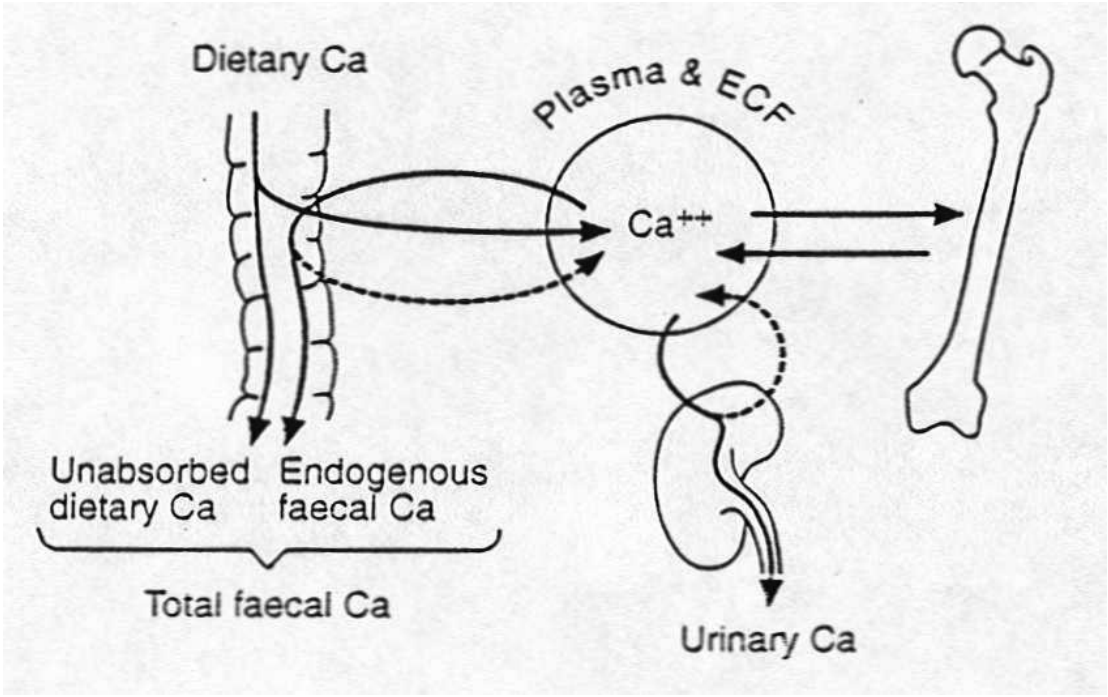
Calcium salts provide rigidity to the skeleton and calcium ions play a role in many if not most metabolic processes. In the primitive exoskeleton and in shells, rigidity is generally provided by calcium carbonate, but in the vertebrate skeleton it is provided by a form of calcium phosphate which approximates hydroxyapatite [$Ca_{10}(OH)_2(PO_4)_6$] and is embedded in collagen fibrils.

Bone mineral serves as the ultimate reservoir for the calcium circulating in the ECF. Calcium enters the ECF from the gut by absorption and from bone by resorption. Calcium leaves the ECF via the gastrointestinal tract, kidneys, and skin and enters into bone via bone formation (***Figure 13***). In addition, calcium fluxes occur across all cell membranes. Many neuromuscular and other cellular functions depend on the maintenance of the ionised calcium concentration in the ECF. Calcium fluxes are also important mediators of hormonal effects on target organs through several intracellular signalling pathways, such as the phosphoinositide and cyclic adenosine monophosphate systems. The cytoplasmic calcium concentration is kept

down by a series of calcium pumps, which concentrate calcium within the intracellular storage sites or extrude from the cells the calcium which flows in by diffusion. The physiology of calcium metabolism is primarily directed towards the maintenance of the concentration of ionised calcium in the ECF. This is protected and maintained by a feedback loop through calcium receptors in the parathyroid glands (*20*), which control the secretion of parathyroid hormone (see ***Figure 10*** of Chapter 8). This hormone increases the renal tubular reabsorption of calcium, promotes intestinal calcium absorption by stimulating the renal production of 1,25-dihyroxycolecaliferol [$1,25(OH)_2D$], and, if necessary, resorbs bone. However, the integrity of the system depends critically on vitamin D status; if there is a deficiency of vitamin D, the loss of its calcaemic action (*21*) leads to a decrease in the ionised calcium and secondary hyperparathyroidism and hypophosphataemia. This is why experimental vitamin D deficiency results in rickets and osteomalacia whereas calcium deficiency gives rise to osteoporosis (*4,22*).

Figure 13

Major calcium movements in the body



Determinants of calcium balance

Calcium intake

In a strictly operational sense, calcium balance is determined by the relationship between calcium intake and calcium absorption and excretion. A striking feature of the system is that relatively small changes in calcium absorption and excretion can neutralise a high intake or compensate for a low one. There is a wide variation in calcium intake among nations, generally following the animal protein intake and depending largely on dairy product consumption. The lowest calcium intakes are in developing countries, particularly in Asia, and the highest are in developed countries, particularly in USA, Canada and Europe (***Table 30***).

Table 30

Protein and calcium intakes in different regions of the world 1987–1989

Region	Protein (g)			Calcium (mg)		
	Total	Animal	Vegetable	Total	Animal	Vegetable
USA and Canada	108.7	72.2	36.5	1031	717	314
Europe	102.0	59.6	42.4	896	684	212
Oceania	98.3	66.5	31.8	836	603	233
Other developed	91.1	47.3	43.8	565	314	251
USSR	106.2	56.1	50.1	751	567	184
All developed	103.0	60.1	42.9	850	617	233
Africa	54.1	10.6	43.5	368	108	260
Latin America	66.8	28.6	38.2	476	305	171
Near East	78.7	18.0	60.7	484	223	261
Far East	58.2	11.0	47.2	305	109	196
Other developing	55.8	22.7	33.1	432	140	292
All developing	59.9	13.3	46.6	344	138	206

Source: Adapted from FAO Yearbook, 1990 (*23*).

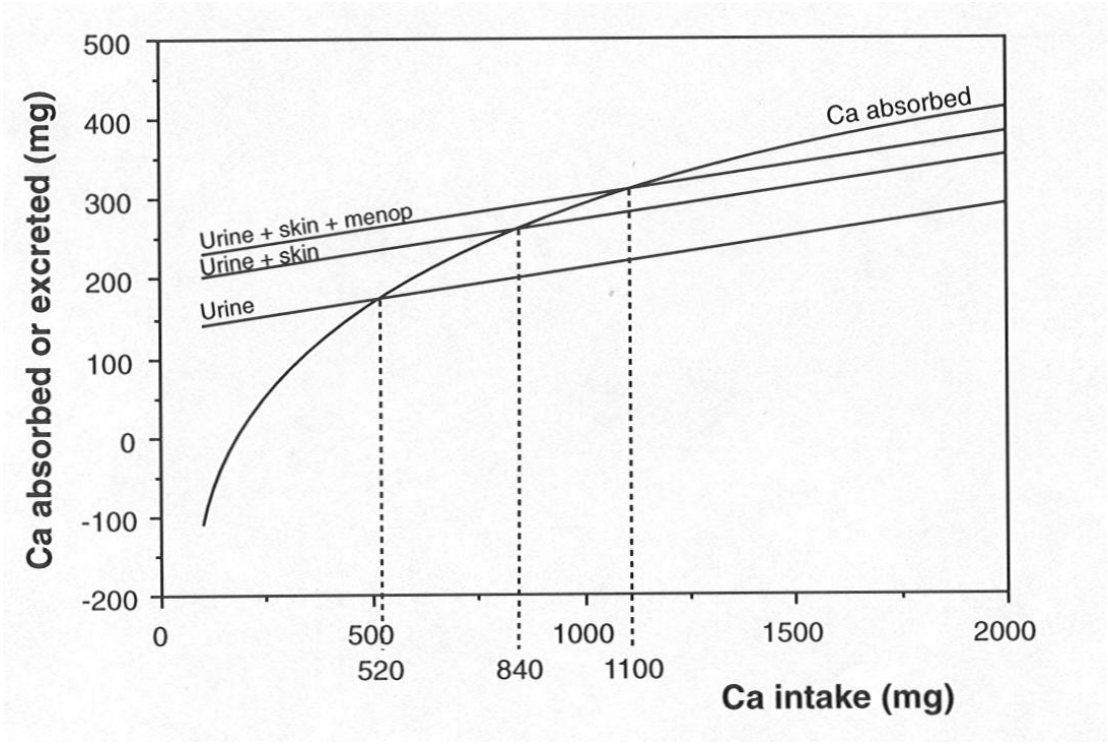
Calcium absorption

Ingested calcium mixes with digestive juice calcium in the proximal small intestine from where it is absorbed by a process, which has an active saturable component and a diffusion component (*24-27*). At low calcium intakes calcium is mainly absorbed by active (transcellular) transport, but at higher intakes an increasing proportion of calcium is absorbed by simple (paracellular) diffusion. The unabsorbed component appears in the faeces together with the unabsorbed component of digestive juice calcium known as endogenous faecal calcium. Thus, the faeces contain unabsorbed dietary calcium and unreabsorbed digestive juice calcium (***Figure 14***).

True absorbed calcium is the total calcium absorbed from the calcium pool in the intestines and therefore contains both dietary and digestive juice components. Net absorbed calcium is the difference between dietary calcium and faecal calcium and is numerically the same as true absorbed calcium minus endogenous faecal calcium. At zero calcium intake, all the faecal calcium is endogenous and represents the digestive juice calcium which has not been reabsorbed; net absorbed calcium at this intake is therefore negative to the extent of about 200 mg (5 mmol) (*28,29*). When the intake reaches about 200 mg (5 mmol), dietary and faecal calcium become equal and net absorbed calcium is zero. As calcium intake increases, net absorbed calcium also increases, steeply at first but then, as the active transport becomes saturated, more slowly until the slope of absorbed on ingested calcium approaches linearity with an ultimate gradient of about 5–10 percent (*24,25,30,31*). The relationship between intestinal calcium absorption and calcium intake, derived from 210 balance studies performed in 81 individuals collected from the literature (*32-39*), is shown in ***Figure 14***.

Figure 14

Relationship between calcium absorption and calcium intake



The relationships between calcium intake and calcium absorbed and excreted calcium calculated from 210 balance experiments in 81 subjects (32-39). Equilibrium is reached at an intake of 520 mg, which rises to 840 mg when skin losses of 60 mg are added and to 1100 mg when menopausal loss is included. The curvilinear relationship between intestinal calcium absorption and calcium intake can be made linear by using the logarithm of calcium intake to yield the equation: Caa = 174 loge Cai -909 ± 71 (SD) mg/day, where Cai represents ingested calcium and Caa net absorbed calcium. The relationship between urinary calcium excretion and calcium intake is given by the equation: Cau = 0.078 Cai + 137 ± 11.2 (SD) mg/day, where Cau is urinary calcium and Cai calcium intake.

True absorption is an inverse function of calcium intake, falling from some 70 percent at very low intakes to about 35 percent at high intakes (***Figure 15***). Percent net absorption is negative at low intakes, becomes positive as intake increases, reaches a peak of about 30 percent at an intake of about 400 mg, and then falls off as the intake increases. The two lines converge as intake rises because the endogenous faecal component (which separates them) becomes proportionately smaller.

Many factors influence the availability of calcium for absorption and the absorptive mechanism itself. The former includes substances, which form insoluble complexes with calcium, such as the phosphate ion. The relatively high calcium-phosphate ratio of 2.2 in human milk compared with 0.77 in cow milk (*18*) may be a factor in the higher absorption of calcium from human milk than cow milk (see below).

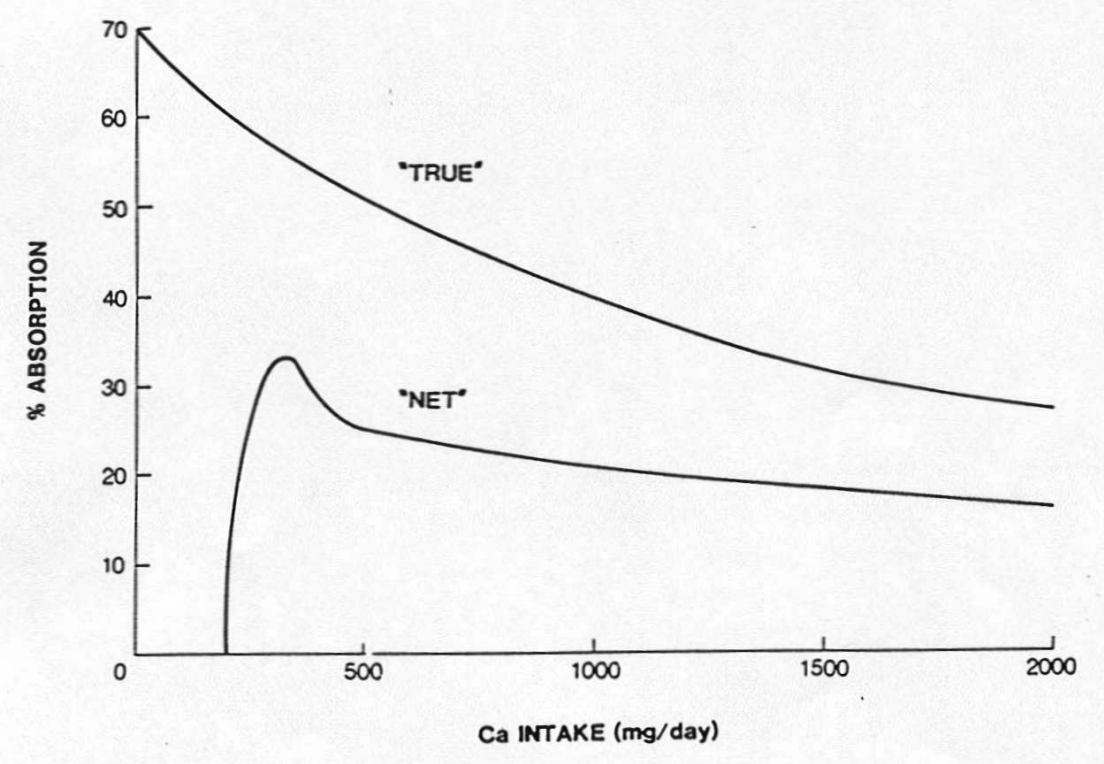
Intestinal calcium absorption is mainly controlled by the serum concentration of $1,25(OH)_2D$ (see ***Chapter 8***). The activity of the 1-α-hydroxylase, which catalyses $1,25(OH)_2D$ production from 25-hydroxycolecalciferol (25OHD) in the kidneys, is negatively related to the plasma calcium and phosphate concentrations and positively to plasma parathyroid hormone (*21*). Thus the inverse relationship between calcium intake and

fractional absorption described above is enhanced by the inverse relationship between dietary calcium and serum $1,25(OH)_2D$ (*21,40,41*).

Phytates, present in the husks of many cereals as well as in nuts, seeds, and legumes, can form insoluble calcium phytate salts in the gastrointestinal tract. Excess oxalates can precipitate calcium in the bowel but are not an important factor in most diets.

Figure 15

True and net calcium absorption as percents of calcium intake



Note: The great differences between these functions at low calcium intakes and their progressive convergence as calcium intake increases.

Urinary calcium

Urinary calcium is the fraction of the filtered plasma water calcium, which is not reabsorbed in the renal tubules. At a normal glomerular filtration rate of 120 ml/min and ultrafiltrable calcium of 6.4 mg/100 ml (1.60 mmol/l), the filtered load of calcium is about 8 mg/min (0.20 mmol/min) or 11.6 g/day (290 mmol/day). Because the usual 24-hour calcium excretion in developed countries is about 160–200 mg (4–5 mmol), it follows that 98–99 percent of the filtered calcium is usually reabsorbed in the renal tubules. However, calcium excretion is extremely sensitive to changes in filtered load. A decrease in plasma water calcium of only 0.17 mg/100 ml (0.043 mmol/l), which is barely detectable, was sufficient to account for a decrease in urinary calcium of 63 mg (1.51 mmol) when 27 subjects changed from a normal- to a low-calcium diet (*42*). This very sensitive renal response to calcium deprivation combines with the inverse relationship between calcium intake and absorption to stabilise the plasma ionised calcium concentration and to preserve the equilibrium between calcium entering and leaving the ECF over a wide range of calcium intakes. However, there is always a significant obligatory loss of calcium in the urine (as there is in the faeces), even on a low calcium intake, simply because maintenance of the plasma ionised calcium and, therefore, of the filtered load, prevents total elimination of the calcium from the urine. The lower limit for urinary calcium in developed countries is about 140 mg (3.5 mmol) but

depends on protein and salt intakes. From this obligatory minimum, urinary calcium increases on intake with a slope of about 5–10 percent (*30,31,43*). In the graph derived from 210 balance studies referred to above (***Figure 14***), the relationship between urinary calcium excretion and calcium intake is represented by the line which intersects the absorbed calcium line at an intake of 520 mg.

Insensible losses

Urinary and endogenous faecal calcium are not the only forms of excreted calcium; losses through skin, hair, and nails need to be taken into account. These are not easily measured, but a combined balance and isotope procedure has yielded estimates of daily insensible calcium losses in the range of 40–80 mg (1–2 mmol), which are unrelated to calcium intake (*44,45*). The addition of a loss of 60 mg (1.5 mmol) as a constant to urinary calcium loss raises the dietary calcium at which absorbed and excreted calcium reach equilibrium from 520 to 840 mg (13 to 21 mmol) (***Figure 14***).

Calcium requirements and recommended intakes

Methodology

Although it is well established that calcium deficiency causes osteoporosis in experimental animals, the contribution that calcium deficiency makes to osteoporosis in humans is much more controversial, not least because of the great variation in calcium intakes across the world (***Table 30***), which does not appear to be associated with any corresponding variation in the prevalence of osteoporosis. This issue is dealt with at greater length below in the section on nutritional factors; in this section we will simply define what is meant by calcium requirement and how it may be calculated.

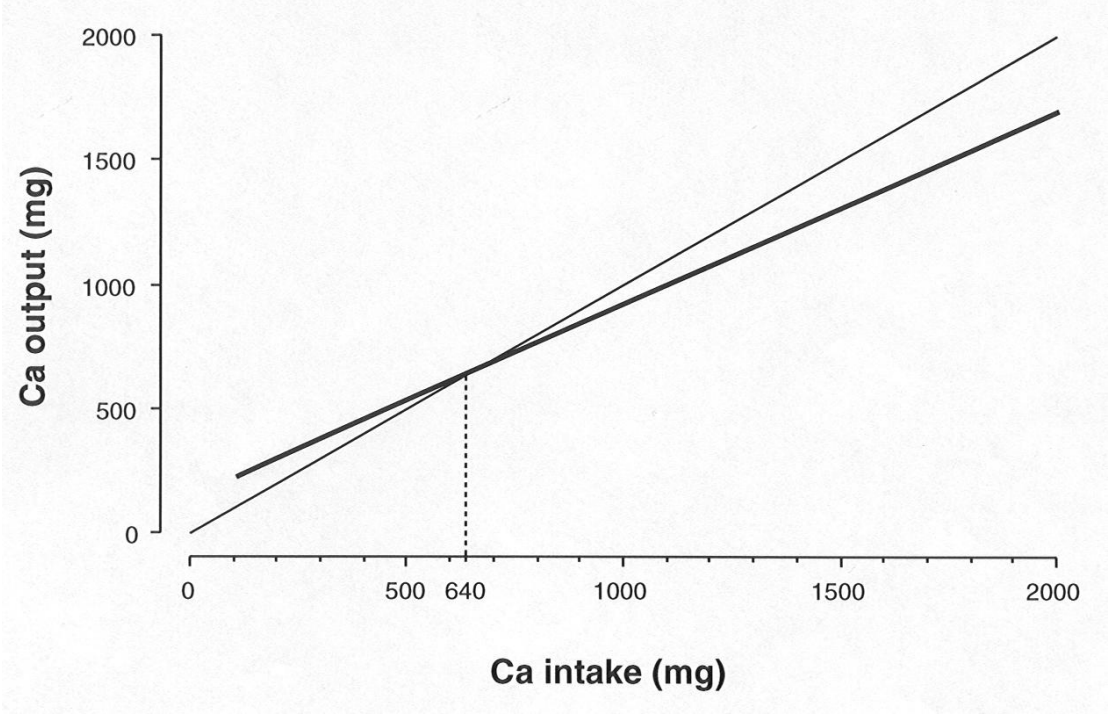
The calcium requirement of an adult is generally recognised to be the intake required to maintain calcium balance and therefore skeletal integrity. The mean calcium requirement of adults is therefore the mean intake at which intake and output are equal, which at present can only be determined by balance studies conducted with sufficient care and over a sufficiently long period to ensure reasonable accuracy and then corrected for insensible losses. The reputation of the balance technique has been harmed by a few studies with inadequate equilibration times and short collection periods, but this should not be allowed to detract from the value of the meticulous work of those who have collected faecal and urinary samples for weeks or months from subjects on well-defined diets. This meticulous work has produced valuable balance data, which are clearly valid; the mean duration of the balances in the 210 studies from eight publications used in this report was 90 days with a range of 6–480 days. (The four 6-day balances in the series used a non-absorbable marker and are therefore acceptable.)

The usual way of determining mean calcium requirement from balance studies has been by linear regression of calcium output (or calcium balance) on intake and calculation of the mean intake at which intake and output are equal (or balance is zero). This was probably first done in 1939 by Mitchell and Curzon (*46*), who arrived at a mean requirement of 9.8 mg/kg/day or about 640 mg (16 mmol) at a mean body weight of 65 kg. The same type of calculation was subsequently used by many other workers who arrived at requirements ranging from 200 mg/day (5 mmol/day) in male Peruvian prisoners (*47*) to 990 mg (24.75 mmol) in premenopausal women (*48*), but most values were about 600 mg (15 mmol) (*31*) without allowing for insensible losses. However, this type of simple linear regression yields a higher mean calcium requirement (640 mg in the same 210 balances) (***Figure 16a***) than the intercept of absorbed and excreted calcium (520 mg) (***Figure 14***) because it tends to underestimate the negative calcium balance at low intake and overestimate the positive balance at high intake. A better reflection of biological reality is obtained by deriving calcium

output from the functions given in the previous section and then regressing that output on calcium intake. This yields the result shown in ***Figure 16b*** where the negative balance is more severe at low intakes and less positive at high intakes than in the linear model and in which zero balance occurs at 520 mg as in ***Figure 14***.

Figure 16a

Calcium output as a linear (A) function of calcium intake calculated from the same balances as Figure 14



Note: The regression line crosses the line of equality at an intake of 640 mg.
The equation is: $Ca_o = 0.779\ Ca_i + 142$ mg where Ca_o is calcium output.

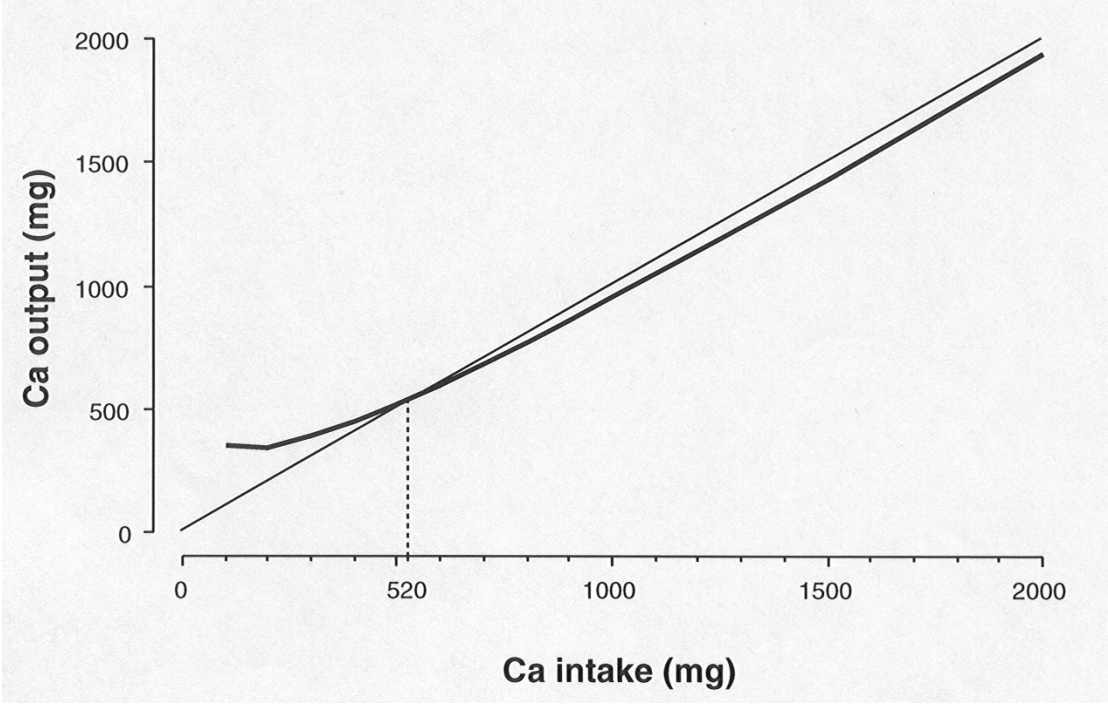
An alternative way of calculating calcium requirement is to determine the intake at which the mean maximum positive balance occurs. This has been done with a two-component, split, linear regression model in which calcium balance is regressed on intake to determine the threshold intake above which no further increase in calcium retention occurs (*49*). This may well be an appropriate way of calculating the calcium requirement of children and adolescents (and perhaps pregnant and lactating women) who need to be in positive calcium balance and in whom the difference between calcium intake and output is therefore relatively large and measurable by the balance technique. However, in normal adults the difference between calcium intake and output at high calcium intakes represents a very small difference between two large numbers, and this calculation therefore carries too great an error to calculate their requirement.

We are inclined to think that the most satisfactory way of calculating calcium requirement from current data is as the intake at which excreted calcium equals net absorbed calcium, which has the advantage of permitting separate analysis of the effects of changes in calcium absorption and excretion. This intercept has been shown in ***Figure 14*** to occur at an intake of about 520 mg, but when insensible losses of calcium of 60 mg (1.5 mmol) (*44,45*) are taken into account, the intercept rises to 840 mg, which we believe is as close as it is possible to get at present to the calcium requirement of adults on Western-style diets. The addition to this excretion line of an additional obligatory urinary calcium of 30 mg (0.75 mmol) at menopause (*50*) raises the amount to about 1100 mg, which we suggest is the mean calcium requirement of postmenopausal women (see below). However, this type of

calculation cannot easily be applied to other high-risk populations (such as children) because there are not sufficient published data from these groups to permit a similar analysis of the relationship among calcium intake, absorption, and excretion. An alternative is to estimate how much calcium each population group needs to absorb to meet obligatory calcium losses and desirable calcium retention and then to calculate the intake required to provide this rate of calcium absorption. This is what has been done in the following section.

Figure 16b

Calcium output as a non-linear function of calcium intake calculated from the same balances as Figure 14



Note: The regression line crosses the line of equality at an intake of 520 mg.
The equation is: $Ca_o = Ca_i - 174\ Log_e\ Ca_I - 909 + 0.078\ Ca_u + 137$ mg.

Populations at risk

It is clear from ***Figure 12*** that a positive calcium balance (i.e., net calcium retention) is required throughout growth, particularly during the first 2 years of life and during puberty and adolescence. These age groups therefore constitute populations at risk for calcium deficiency, as are pregnant women (especially in the last trimester), lactating women, postmenopausal women, and, possibly, elderly men. Our calculations for these groups, ultimately derived from Western European and North American data, are given below.

Recommendations by group

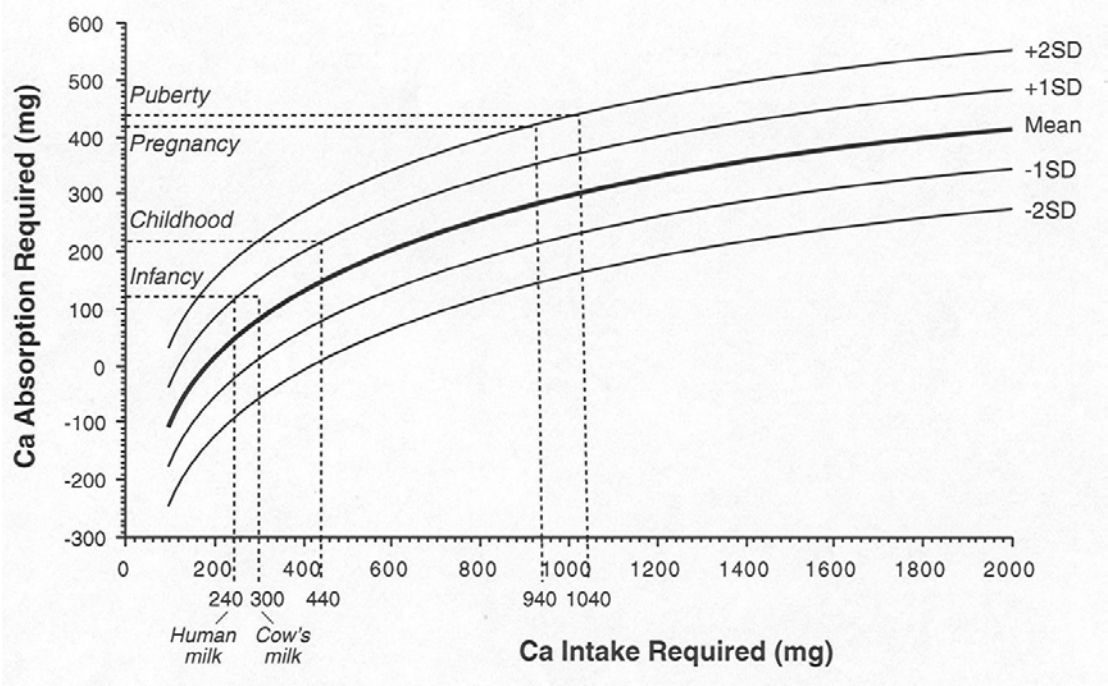
Infancy

In the first 2 years of life, the daily calcium increment in the skeleton is about 100 mg (2.5 mmol) (*51*). The urinary calcium of infants is about 10 mg/day (0.25 mmol/day) and is virtually independent of intake (*52-56*) and insensible losses are perhaps the same. Therefore, infants need to absorb some 120 mg (3 mmol) of calcium daily to allow for normal growth. What this represents in dietary terms can be calculated from calcium absorption studies in newborn infants (*52-56*), which suggest that the absorption of calcium from cow milk by

infants is about 0.5 SD above the normal adult slope and from human milk is more than 1 SD above the normal adult slope. If this information is correct, different recommendations need to be made for infants depending on milk source. With human milk, an absorption of 120 mg (3 mmol) of calcium requires a mean intake of 240 mg (6 mmol) (***Figure 17***) and a recommended intake of say 300 mg (7.5 mmol), which is close to the amount provided in the average daily milk production of 750 ml. With cow milk, calcium intake needs to be about 300 mg (7.5 mmol) to meet the requirement (***Figure 17***) and the allowance should be 400 mg (10 mmol) (***Table 31***).

Figure 17

Calcium intakes required to provide the absorbed calcium necessary to meet calcium requirements at different stages in the lifecycle



Note: The solid lines represent the mean and range of calcium absorption as a function of calcium intake derived from the equation in ***Figure 14***. The interrupted lines represent the estimated calcium requirements based on Western European and North American data.

Childhood

The accumulation of whole-body calcium with skeletal growth is illustrated in ***Figure 12***. It rises from about 120 g (3 mol) at age 2 years to 400 g (10 mol) at age 9 years. These values can be converted into a daily rate of calcium accumulation from ages 2 to 9 of about 120 mg (3 mmol), which is very similar to the amount calculated by Leitch and Aitken (*57*) from growth analyses. Although urinary calcium must rise with the growth-related rise in glomerular filtration rate, a reasonable estimate of the mean value from ages 2 to 9 might be 60 mg (1.5 mmol) (*58*). When this is added to a daily skeletal increment of 120 mg (3 mmol) and a dermal loss of perhaps 40 mg (1.0 mmol), the average daily net absorbed calcium needs to be 220 mg (5.5 mmol) during this period. If the net absorption of calcium by children is 1 SD above that of adults, the average daily requirement during this period is about 440 mg (11 mmol) (***Figure 17***) and the average recommended intake is 600 mg (15 mmol) – somewhat lower in the earlier years and somewhat higher in the later years (***Table 31***).

Table 31

Recommended calcium allowances based on Western European, American and Canadian data

Group	Recommended intake mg/day
Infants and children	
0–6 months	
Human milk	300
Cow milk	400
7–12 months	400
1–3 years	500
4–6 years	600
7–9 years	700
Adolescents, 10–18 years	1300[a]
Adults	
Females	
19 years to menopause	1000
Postmenopause	1300
Males	
19–65 years	1000
65 +	1300
Pregnancy (last trimester)	1200
Lactation	1000

[a] Particularly during the growth spurt.

Puberty and adolescence

As can be seen in ***Figure 12***, a striking increase in the rate of skeletal calcium accretion occurs at puberty – from about ages 10 to 17 years. The peak rate of calcium retention in this period is 300–400 mg (7.5–10 mmol) daily (*57*); it occurs earlier in girls but continues longer in boys. For a target value of 300 mg (7.5 mmol) for the skeleton, 100 mg (2.5 mmol) for urinary calcium (*58*), and insensible losses of 40 mg (1.0 mmol), the net absorbed calcium during at least part of this period needs to be 440 mg (11 mmol) daily. Even with assuming high calcium absorption (+2 SD), this requires an intake of 1040 mg (26.0 mmol) daily (***Figure 17***) and an allowance of 1300 mg (32.5 mmol) during the peak growth phase (***Table 31***). It is difficult to justify any difference between the allowances for boys and girls because, as mentioned above, although the growth spurt starts earlier in girls, it continues longer in boys. This recommended intake (which is close to that derived differently by Matkovic and Heaney [*49,58*]) is not achieved by many adolescents even in developed countries (*59-61*), but the effects of this shortfall on their growth and bone status are unknown.

Adults

As indicated earlier and for the reasons given, we accept that the mean apparent calcium requirement of adults in developed countries is about 520 mg (13 mmol) but that this is increased by insensible losses to some 840 mg (21 mmol) (***Figure 14***). This reasoning forms the basis of our recommended intake for adults of 1000 mg (***Table 31***).

Menopause

The most important single cause of osteoporosis – at least in developed countries – is probably menopause, which is accompanied by an unequivocal and sustained rise in obligatory urinary calcium of about 30 mg (0.75 mmol) daily (*50,62,63*). Because calcium absorption certainly does not increase at this time – and probably decreases (*64,65*) – this extra urinary calcium represents a negative calcium balance which is compatible with the average bone loss of about 0.5–1.0 percent per year after menopause. There is a consensus that these events are associated with an increase in bone resorption but controversy continues about whether this is the primary event, the response to an increased calcium demand, or both. The results of calcium trials are clearly relevant. Before 1997, there had been 20 prospective trials of calcium supplementation in 857 postmenopausal women and 625 control subjects; these trials showed highly significant suppression of bone loss by calcium (*65*). Another meta-analysis covering similar numbers showed that calcium supplementation significantly enhanced the effect of oestrogen on bone (*66*). It is therefore logical to recommend sufficient additional calcium after the menopause to cover at least the extra obligatory loss of calcium in the urine. The additional dietary calcium needed to meet an increased urinary loss of 30 mg (0.75 mmol) is 260 mg/day (6.5 mmol/day) (***Figure 14***), which raises the daily requirement from 840 mg (21 mmol) to 1100 mg (27.5 mmol) and the recommended intake from 1000 to 1300 mg/day (25 to 32.5 mmol/day), which is a little higher than that recommended by the United States and Canada (*14*) (***Table 31*** and ***32***).

Table 32

Current calcium intake recommendations (mg/day)

	Australia 1991 Recommended Dietary Intake	United Kingdom 1991 Reference Nutrient Intake	European Union 1993 Population Reference Intake	United States and Canada 1997 Adequate Intake
Pregnancy (last trimester)	1100	700	700	1000–1300
Lactation	1200	1250	1200	1000–1300
Infancy	300 (human milk) 500 (cow milk)	525	400	210–270
Childhood	530-800	350–550	400-550	500–800
Puberty and adolescence				
Boys	1000–1200	1000	1000	1300
Girls	800–1000	800	800	1300
Maturity				
Males	800	700	700	1000
Females	800	700	700	1000
Later life				
Males >65 years	800	700	700	1200
Postmenopausal women	1000	700	700	1200

Ageing

Not enough is known about bone and calcium metabolism during ageing to enable calculation of the calcium requirements of older men and women with any confidence. Calcium absorption tends to decrease with age in both sexes (*67-69*) but whereas the evidence that calcium requirement goes up at the menopause is strong, corresponding evidence about ageing men is less convincing (*32,36*). Nonetheless, as a precaution we propose an extra allowance of 300 mg/day (7.5 mmol/day) for men over 65 to bring them into line with postmenopausal women (***Table 31***).

Pregnancy

The calcium content of the newborn infant is about 24 g (600 mmol). Most of this calcium is laid down in the last trimester of pregnancy, during which the foetus retains about 240 mg (6 mmol) of calcium daily (*51*). There is some evidence that pregnancy is associated with an increase in calcium absorption [associated with a rise in the plasma $1,25(OH)_2$ D level] (*70-72*). For a maternal urinary calcium of 120 mg (3 mmol) and a maternal skin loss of 60 mg (1.5 mmol), the absorbed calcium should be 420 mg (10.5 mmol) daily. Even at optimal calcium absorption, the corresponding calcium intake would need to be 940 mg (23.5 mmol) (***Figure 17***) and the recommended allowance would need to be 1200 mg (30 mmol) (***Table 31***), which is similar to that proposed by the United States and Canada (*14*) (***Table 32***).

Lactation

The calcium content of human milk is about 36 mg per 100 ml (9 mmol/l) (*18*). A lactating woman produces about 750 ml of milk daily, which represents about 280 mg (7.0 mmol) of calcium. For a maternal urinary calcium of 100 mg/day (2.5 mmol/day) and maternal skin loss of 60 mg/day (1.5 mmol/day), the required absorption is 440 mg/day (11 mmol/day) – the same as at puberty. If calcium absorption efficiency is maximal (i.e., 2 SD above the normal mean) – possibly because of the effect of prolactin on the production of $1,25(OH)_2D$ (*72*) – the requirement would be about 1040 mg (26.0 mmol) and the recommended intake would be about 1300 mg (32.5 mmol). However, although it is known that bone is lost during lactation and restored after weaning (*73,74*), early reports that this bone loss could be prevented by calcium supplementation (*75*) have not been confirmed in controlled studies (*76-78*). The prevailing view now is that calcium absorption does not increase and may decrease during lactation. It is increasingly thought that lactational bone loss is not a nutritional problem but may be due to parathyroid hormone-related peptide secreted by the breast (*79*) and therefore beyond the control of dietary calcium. In view of this uncertainty, we do not at present recommend any extra calcium allowance during lactation; any risk to adolescent mothers is covered by our general recommendation of 1300 mg for adolescents.

Upper limits

Because of the inverse relationship between fractional calcium absorption and calcium intake (***Figure 15***), a calcium supplement of 1000 mg (2.5 mmol) added to a Western-style diet only increases urinary calcium by about 60 mg (1.5 mmol). Urinary calcium also rises very slowly with intake (slope of 5–10 percent) and the risk of kidney stones from dietary hypercalciuria must therefore be negligible. In fact, it has been suggested that dietary calcium may protect against renal calculi because it binds dietary oxalate and reduces oxalate excretion (*80,81*). Toxic effects of a high calcium intake have only been described when the calcium is given as the carbonate in very high doses; this toxicity is caused as much by the alkali as by the calcium and is due to precipitation of calcium salts in renal tissue (milk-alkali syndrome) (*82*). However, in practice we recommend an upper limit on calcium intake of 3 g (75 mmol).

Comparisons with other recommendations

Our recommendations in ***Table 31*** can be compared with the current recommendations of Australia, the United Kingdom, the European Union, and the United States and Canada in ***Table 32***. Our recommendations for adults are very close to those of the United States and Canada but higher than those of the United Kingdom and Australia, which do not take into account insensible losses, and higher than those of the European Union, which assumed 30 percent absorption of dietary calcium. The British and European values make no allowance for ageing or menopause. Recommendations for other high-risk groups are very similar in all five sets of recommendations except for the rather low allowance for infants by the United States and Canada. Nonetheless, and despite this broad measure of agreement, we have some misgivings about the application of these recommendations, all of which rely ultimately on data from developed nations, to developing countries where other dietary constituents – such as animal protein and sodium – and environmental factors may be very different. We shall therefore in the next sections briefly review current knowledge about the prevalence of osteoporosis across racial national boundaries and its relevance to calcium requirement.

Ethnic and environmental variations in the prevalence of osteoporosis

Intakes of calcium have been known for many years to vary greatly from one country to another, as is clearly shown in FAO food balance sheets (***Table 30***). Until fairly recently, it was widely assumed that low calcium intakes had no injurious consequences. This view of the global situation underlay the very conservative adequate calcium intakes recommended by WHO/FAO in 1962 (*2*). At that time, osteoporosis was still regarded as a bone matrix disorder and the possibility that it could be caused by calcium deficiency was barely considered. The paradigm has changed since then. Calcium deficiency is taken more seriously than it was and the apparent discrepancy between calcium intake and bone status across the world has attracted more attention. In general, recent investigations have sought for evidence of low bone density and high fracture incidence in countries where calcium intake is low; rickets has not been looked for, but the low calcium rickets recently reported from Nigeria (*83*) will no doubt attract attention.

This issue can be considered at several levels. The first level is genetic: Is there a genetic (ethnic) difference in the prevalence of osteoporosis between racial groups within a given society? The second level might be termed environmental-cultural (e.g., dietary): Is there a difference in the prevalence of osteoporosis between national groups of similar ethnic composition? The third level is environmental-geographical (e.g., latitude, affluence, and lifestyle): Is there a difference in the prevalence of osteoporosis between countries regardless of ethnic composition? At each of these levels, the prevalence of osteoporosis can in theory be determined in at least two ways – from the distribution of bone density within the population and from the prevalence of fractures, notably hip fractures. In practice, hip fracture data (or mortality from falls for elderly people which has been used as a surrogate [*84*]) are more readily available than bone densitometry.

Ethnicity

Comparisons between racial groups within countries suggest substantial racial differences in the prevalence of osteoporosis. This was probably first noted by Trotter (*85*) when she showed that bone density (weight/volume) was significantly higher in skeletons from black than from Caucasian subjects in the United States. It was later shown that hip fracture rates were lower in blacks than Caucasians in South Africa (*86*) and the United States (*87*). These observations have been repeatedly confirmed (*88,89*) without being fully explained but appear to be genetic in origin because the difference in bone status between blacks and Caucasians in the United States is already apparent in childhood (*90*) and cannot be explained by differences in body size (*91*). The difference in fracture rates between blacks and Caucasians cannot be

explained by differences in hip axis length (*91*); it seems to be largely or wholly due to real differences in bone density. Comparisons between Caucasians and Samoans in New Zealand (*92*) have also shown the latter to have the higher bone densities whereas the lower bone densities of Asians than Caucasians in New Zealand are largely accounted for by differences in body size (*92*). In the United States, fracture rates are lower among Japanese than among Caucasians but may be accounted for by their shorter hip axis length (*93*) and their lower incidence of falls (*94*). Bone density is generally lower in Asians than Caucasians within the United States (*95*) but this again is largely accounted for by differences in body size (*96*). There are also lower hip fracture rates for Hispanics, Chinese, Japanese, and Koreans than Caucasians in the United States (*97,98*). The conclusion must be that there are probably genetic factors influencing the prevalence of osteoporosis and fractures, but it is impossible to exclude the role of differences in diet and lifestyle between ethnic communities within a country.

Geography

There are wide geographical variations in hip fracture incidence, which cannot be accounted for by ethnicity. In the United States, the age-adjusted incidence of hip fracture in Caucasian women aged 65 and over varied with geography but was high everywhere – ranging from 700 to 1000 per 100 000 per year (*99*). Within Europe, the age-adjusted hip fracture rates ranged from 280 to 730 per 100 000 women in one study (*100*) and from 419 to 545 per 100 000 in another study (*97*) in which the comparable rates were 52.9 in Chile, 94.0 in Venezuela, and 247 in Hong Kong. In another study (*101*) age-adjusted hip fracture rates in women in 12 European countries ranged from 46 per 100 000 per year in Poland to 504 per 100 000 in Sweden, with a marked gradient from south to north and from poor to rich. In Chinese populations, the hip fracture rate is much lower in Beijing (87–97 per 100 000) than in Hong Kong (181–353 per 100 000) (*102*), where the standard of living is higher. Thus there are marked geographic variations in hip fracture rates within the same ethnic groups.

Ethnicity, environment, and lifestyle

The conclusion from the above is that there are probably ethnic differences in hip fracture rates within countries but also environmental differences within the same ethnic group which may complicate the story. For international comparisons on a larger scale, it is impossible to separate genetic from environmental factors, but certain patterns emerge which are likely to have biological meaning. The most striking of these is the positive correlation between hip fracture rates and standard of living noted by Hegsted when he observed that osteoporosis was largely a disease of affluent Western cultures (*103*). He based this conclusion on a previously published review of hip fracture rates in 10 countries (*104*), which strongly suggested a correlation between hip fracture rate and affluence. Another review of 19 regions and racial groups (*105*) confirmed this by showing a gradient of age- and sex-adjusted hip fracture rates from 31 per 100 000 in South African Bantu to 968 per 100 000 in Norway. In the analysis of hip fracture rates in Beijing and Hong Kong referred to above (*102*), it was noted that the rates in both cities were much lower than in the United States. Many other publications point to the same conclusion – that hip fracture prevalence (and by implication osteoporosis) is related to affluence and, consequently, to animal protein intake, as Hegsted pointed out, but also and paradoxically to calcium intake.

The calcium paradox

The paradox that hip fracture rates are higher in developed nations where calcium intake is high than in developing nations where calcium intake is low clearly calls for an explanation. Hegsted (*103*) was probably the first to note the close relation between calcium and protein intakes across the world (which is also true within nations [*63*]) and to hint at but dismiss the

possibility that the adverse effect of protein might outweigh the positive effect of calcium on calcium balance. Only recently has fracture risk been shown to be a function of protein intake in American women (*106*). There is also suggestive evidence that hip fracture rates (as judged by mortality from falls in elderly people across the world) are a function of protein intake, national income, and latitude (*107*). The latter is particularly interesting in view of the strong evidence of vitamin D deficiency in hip fracture patients in the developed world (*108-114*) and the successful prevention of such fractures with small doses of vitamin D and calcium (*115,116*) (see ***Chapter 8***). It is therefore possible that hip fracture rates may be related to protein intake, vitamin D status, or both and that either of these factors could explain the calcium paradox. We shall therefore consider how these and other nutrients (notably sodium) affect calcium requirement.

Nutritional factors affecting calcium requirements

The calculations of calcium requirements proposed above were based on data from developed countries (notably the United States and Norway) and can only be applied with any confidence to nations and populations with similar dietary cultures. Other dietary cultures may entail different calcium requirements and call for different recommendations. In particular, the removal or addition of any nutrient that affects calcium absorption or excretion must have an effect on calcium requirement. Two such nutrients are sodium and animal protein, both of which increase urinary calcium and must be presumed therefore to increase calcium requirement. A third candidate is vitamin D because of its role in calcium homeostasis and calcium absorption.

Sodium

It has been known at least since 1961 that urinary calcium is related to urinary sodium (*117*) and that sodium administration raises calcium excretion, presumably because sodium competes with calcium for reabsorption in the renal tubules. Regarding the quantitative relationships between the renal handling of sodium and calcium, the filtered load of sodium is about 100 times that of calcium (in molar terms) but the clearance of these two elements is similar at about 1 ml/min, which yields about 99 percent reabsorption and 1 percent excretion for both (*118*). However, these are approximations, which conceal the close dependence of urinary sodium on sodium intake and the weaker dependence of urinary calcium on calcium intake. It is an empirical fact that urinary sodium and calcium are significantly related in normal and hypercalciuric subjects on freely chosen diets (*119-122*). The slope of urinary calcium on sodium varies in published work from about 0.6 percent to 1.2 percent (in molar terms); a representative figure is about 1 percent – that is, 100 mmol of sodium (2.3 g) takes out about 1 mmol (40 mg) of calcium (*63,120*). The biological significance of this relationship is supported by the accelerated osteoporosis induced by feeding salt to rats on low-calcium diets (*123*) and the effects of salt administration and salt restriction on markers of bone resorption in postmenopausal women (*124,125*). Because salt restriction lowers urinary calcium, it is likely also to lower calcium requirement and, conversely, salt feeding is likely to increase calcium requirement. This is illustrated in ***Figure 18***, which shows that lowering sodium intake by 100 mmol (2.3 g) from, for example, 150 to 50 mmol (3.45 to 1.15 g), reduces the theoretical calcium requirement from 840 mg (21 mmol) to 600 mg (15 mmol). However, the implications of this on calcium requirement across the world cannot be computed because information about sodium intakes is available from very few countries (*126*).

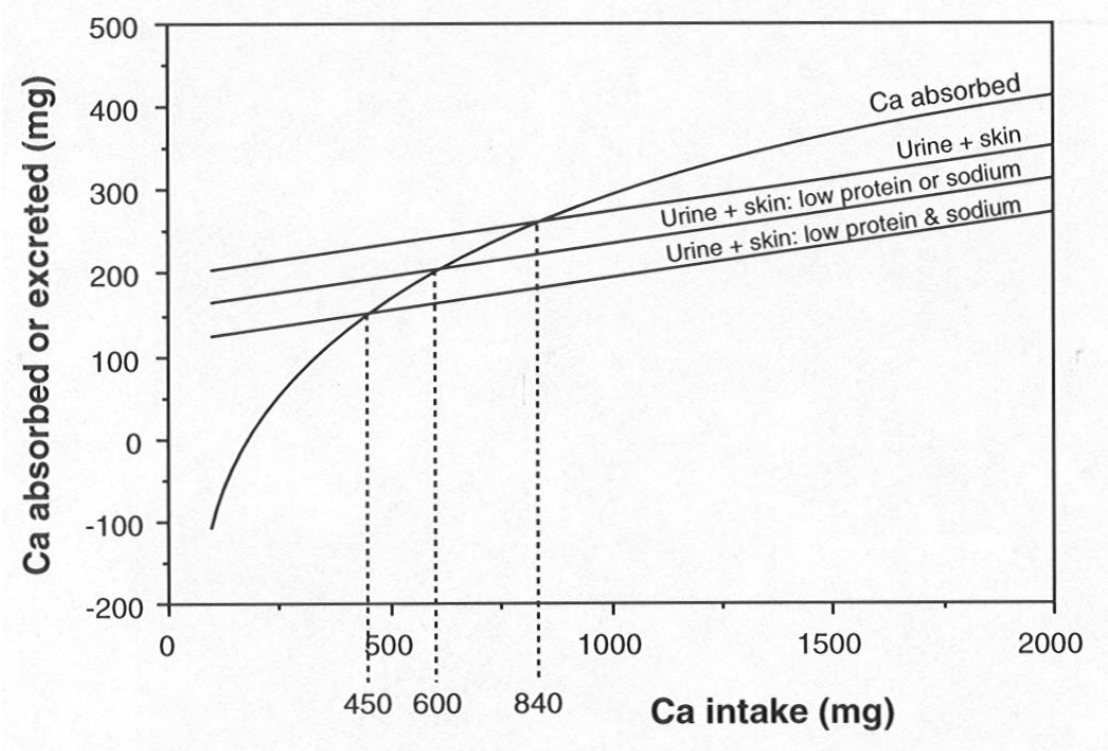
Protein

The positive effect of dietary protein – particularly animal protein – on urinary calcium has also been known at least since the 1960s (*127-129*). One study found that 0.85 mg of calcium

was lost for each gram of protein in the diet (*130*). A meta-analysis of 16 studies in 154 adult humans on protein intakes up to 200 g found that 1.2 mg of calcium was lost in the urine for every 1g rise in dietary protein (*131*). A small but more focussed study showed a rise of 40 mg in urinary calcium when dietary animal protein was raised from 40 to 80 g (i.e., within the physiological range) (*132*). This ratio of urinary calcium to dietary protein ratio (1mg to 1g) is a representative value, which we have adopted. This means that a 40g reduction in animal protein intake from 60 to 20 g (or from the developed to the developing world [***Table 30***]) would reduce calcium requirement by the same amount as a 2.3g reduction in dietary sodium, i.e. from 840 to 600 mg. (***Figure 18***).

Figure 18

The effect of varying protein or sodium intake on theoretical calcium requirement.



Note: In a western-style diet, absorbed calcium matches urinary and skin calcium at an intake of 840 mg as in ***Figure 14***. Reducing animal protein intakes by 40 g reduces the intercept value and requirement to 600 mg. Reducing both sodium and protein reduces the intercept value to 450 mg.

How animal protein exerts its effect on calcium excretion is not fully understood. A rise in glomerular filtration rate in response to protein has been suggested as one factor (*128*) but this is unlikely to be important in the steady state. The major mechanisms are thought to be the effect of the acid load contained in animal proteins and the complexing of calcium in the renal tubules by sulphate and phosphate ions released by protein metabolism (*133,134*). Urinary calcium is significantly related to urinary phosphate (as well as to urinary sodium), particularly in subjects on restricted calcium intakes or in the fasting state, and most of the phosphorus in the urine of people on Western-style diets comes from animal protein in the diet (*63*). Similar considerations apply to urinary sulphate but it is probably less important than the phosphate ion because the association constant for calcium sulphate is lower than that for calcium phosphate (*135*). The empirical observation that each 1 g of protein results in 1 mg of calcium in the urine agrees very well with the phosphorus content of animal protein (about 1 percent by weight) and the observed relationship between calcium and phosphate in the urine (*63*).

Vitamin D

One of the first observations made on vitamin D after it had been identified in 1918 (*136*) was that it promoted calcium absorption (*137*). It is now well established that vitamin D (synthesised in the skin under the influence of sunlight) is converted to 25OHD in the liver and then to $1,25(OH)_2D$ in the kidneys and that the latter metabolite controls calcium absorption (*21*) (see ***Chapter 8***). However, plasma 25OHD closely reflects vitamin D nutritional status and because it is the substrate for the renal enzyme which produces $1,25(OH)_2D$, it could have an indirect effect on calcium absorption. The plasma level of $1,25\text{-}(OH)_2D$ is principally regulated is through increased gene expression of the 1-α-hydroxylase (CYP1α) and not by increased 25OHD levels. This has been seen consistently in animal studies, and the high calcium absorption (138) and high plasma $1,25\text{-}(OH)_2D$ (139) observed in Gambian mothers is consistent with this type of adaptation. However, increasing latitude may compromise vitamin D synthesis to the degree that 25OHD levels are no longer sufficient to sustain adequate $1,25\text{-}(OH)_2D$ levels and efficient intestinal calcium absorption, although this theory remains unproved. Regardless of the mechanism of compromised vitamin D homeostasis, the differences in calcium absorption efficiency have a major effect on theoretical calcium requirement, as illustrated in ***Figure 18***, which shows that an increase in calcium absorption of as little as 10 percent reduces the intercept of excreted and absorbed calcium (and therefore calcium requirement) from 840 to 680 mg. (The figure also shows the great increase in calcium requirement that must result from any impairment of calcium absorption.)

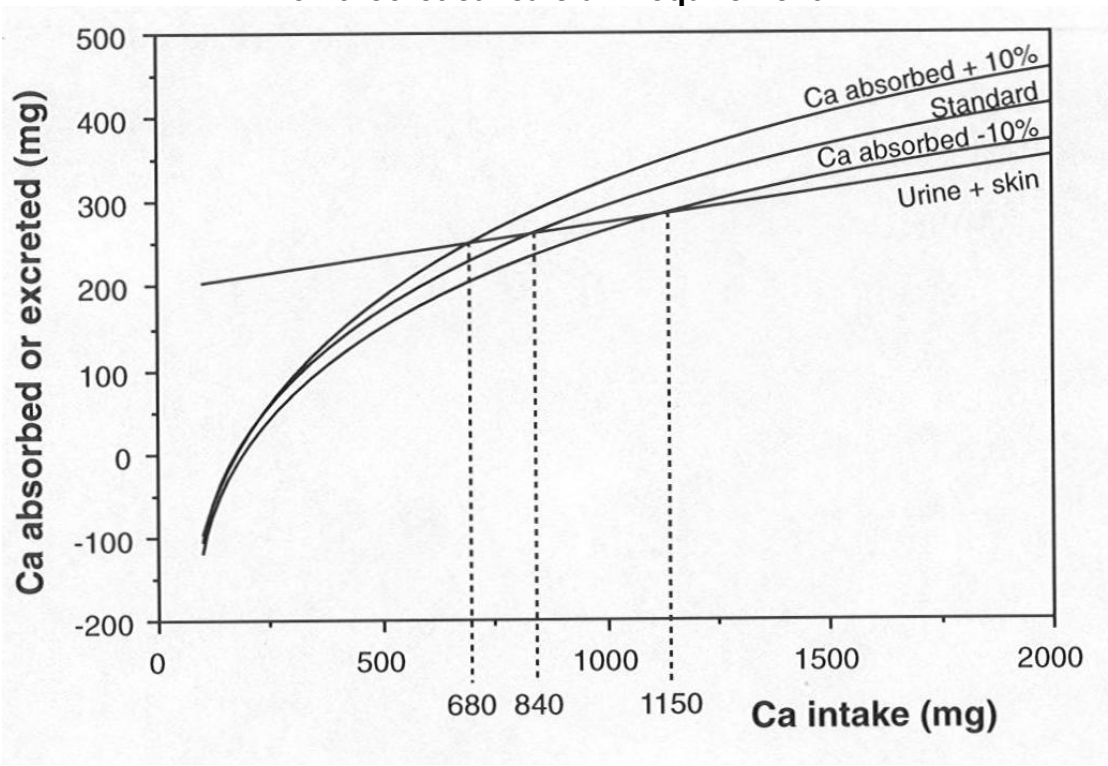
Implications

The major reduction in theoretical calcium requirement which follows animal protein restriction has led us to attempt to show in ***Table 33*** how the calcium allowances recommended in ***Table 31*** could be modified to apply to nations where the animal protein intake per capita is around 20–40 g rather than around the 60–80 g in developed countries. These hypothetical allowances take into account the need to protect children, in whom skeletal needs are much more important determinants of calcium requirement than are urinary losses and in whom calcium supplementation had a beneficial effect in the Gambia (*140*). However, adjustment for animal protein intake has a major effect on the recommended calcium allowances for adults as the table shows. It also brings the allowances nearer to what the actual calcium intakes are in many parts of the world.

If sodium intakes were also lower in developing than developed nations or urinary sodium were reduced for other reasons such as increased sweat losses, the calcium requirement might be even lower, for example, 450 mg (***Figure 18***). This would be reduced still further by any increase in calcium absorption, whether resulting from better vitamin D status because of increased sunlight exposure or for other reasons, as illustrated in ***Figure 19***. Because the increase in calcium absorption in the Gambia is much more than 10 percent (*138*), this is likely to have a major – although not at present calculable – effect on calcium requirement there. However, the adjusted bone mineral density in Gambian women is reported to be some 20 percent lower in the spine (but not in the forearm) than in British women (*141*), which emphasises the need for more data from developing countries.

Figure 19

The effect of varying calcium absorptive efficiency on theoretical calcium requirement



Note: At normal calcium absorption, the intercept of urinary plus skin calcium meets absorbed calcium at an intake of 840 mg as in ***Figure 14***. A 10 percent reduction in calcium absorption raises the intercept and requirement to 1150 mg and a 10 percent increase in calcium absorption reduces it to 680 mg.

Table 33

Theoretical calcium allowances based on an animal protein intake of 20–40 g

Group	Recommended intake mg/day
Infants and children	
0–6 months	
Human milk	300
Cow milk	400
7–12 months	450
1–3 years	500
4–6 years	550
7–9 years	700
Adolescents, 10–18 years	1000[a]
Adults	
Females	
19 years to menopause	750
Postmenopause	800
Males	
19–65 years	750
65 +	800
Pregnancy (last trimester)	800
Lactation	750

[a] Particularly during the growth spurt.

Conclusions

Calcium is an essential nutrient that plays a vital role in neuromuscular function, many enzyme-mediated processes, blood clotting, and providing rigidity to the skeleton by virtue of its phosphate salts. Its non-structural roles require the strict maintenance of ionised calcium concentration in tissue fluids at the expense of the skeleton if necessary and it is therefore the skeleton which is at risk if the supply of calcium falls short of requirement.

Calcium requirements are essentially determined by the relationship between absorptive efficiency and excretory rate – excretion being through the bowel, kidneys, skin, hair, and nails. In adults, the rate of calcium absorption from the gastrointestinal tract needs to match the rate of all losses from the body if the skeleton is to be preserved; in children and adolescents, an extra input is needed to cover the requirements of skeletal growth.

Compared with that of other minerals, calcium economy is relatively inefficient. On most intakes, only about 25–30 percent of dietary calcium is effectively absorbed and obligatory calcium losses are relatively large. Absorbed calcium has to match these obligatory losses and the dietary intake has to be large enough to ensure this rate of absorption if skeletal damage is to be avoided. The system is subject to considerable inter-individual variation in both calcium absorption and excretion for reasons that are not fully understood but which include vitamin D status, sodium and protein intake, age, and menopausal status in women. Although it needs to be emphasised that calcium deficiency and negative calcium balance must sooner or later lead to osteoporosis, this does not mean that all osteoporosis can be attributed to calcium deficiency. On the contrary, there may be more osteoporosis in the world from other causes. Nonetheless, it would probably be generally agreed that any form of osteoporosis must inevitably be aggravated by negative external calcium balance. Such negative balance – even for short periods – is prejudicial because it takes so much longer to rebuild bone than to destroy it. Bone that is lost, even during short periods of calcium deficiency, is only slowly replaced when adequate amounts of calcium become available.

In seeking to define advisable calcium intakes on the basis of physiologic studies and clinical observations, nutrition authorities have to rely largely on data from developed nations living at relatively high latitudes. Although it is now possible to formulate recommendations that are appropriate to different stages in the life cycle of the populations of these nations, extrapolation from these figures to other cultures and nutritional environments can only be tentative and must rely on what is known of nutritional and environmental effects on calcium absorption and excretion. Nonetheless, we have made an attempt in this direction, knowing that our speculative calculations may be incorrect because of other variables not yet identified.

No reference has been made in this account to the possible beneficial effects of calcium in the prevention or treatment of pre-eclampsia (*142*), colon cancer (*143*), or hypertension (*144*) and no attempt has been made to use these conditions as endpoints on which to base calcium intakes. In each of the above conditions, epidemiologic data suggested an association with calcium intake, and experimentation with increased calcium intakes has now been tried. In each case the results have been disappointing, inconclusive, or negative (*145-147*) and have stirred controversy (*148-150*). Because there is no clear consensus about optimal calcium intake for prevention or treatment of these conditions and also no clear mechanistic ideas on how dietary calcium intakes affect them, it is not possible to allow for the effect of health outcomes in these areas on our calcium recommendations. However, although the anecdotal information and positive effects of calcium observed in these three conditions cannot influence our recommendations, they do suggest that generous calcium allowances may confer other benefits besides protecting the skeleton. Similarly, no reference has been made to the effects of physical activity, alcohol, smoking, or other known risk

factors on bone status because the effects of these variables on calcium requirement are beyond the realm of simple calculation.

Future research

Future research should:

- recognise that there is an overwhelming need for more studies of calcium metabolism in developing countries;
- investigate further the cultural, geographical, and genetic bases for differences in calcium intakes in different groups in developing nations;
- establish the validity of different recommended calcium intakes based on animal protein and sodium intakes;
- clarify the role of dietary calcium in pre-eclampsia, colon cancer, and hypertension; and
- investigate the relationship of latitude, sun exposure, and synthesis of vitamin D with intestinal calcium absorption in different geographical locations.

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Chapter 12
Iodine

Summary of the human metabolic processes requiring iodine

At present, the only physiologic role known for iodine in the human body is in the synthesis of thyroid hormones by the thyroid gland. Therefore, the dietary requirement of iodine is determined by normal thyroxine (T_4) production by the thyroid gland without stressing the thyroid iodide trapping mechanism or raising thyroid stimulating hormone (TSH) levels.

Iodine from the diet is absorbed throughout the gastrointestinal tract. Dietary iodine is converted into the iodide ion before it is absorbed. The iodide ion is bio-available and absorbed totally from food and water. This is not true for iodine within thyroid hormones ingested for therapeutic purposes. Iodine enters the circulation as plasma inorganic iodide, which is cleared from circulation by the thyroid and kidney. The iodide is used by the thyroid gland for synthesis of thyroid hormones, and the kidney excretes iodine with urine. The excretion of iodine in the urine is a good measure of iodine intake. In a normal population with no evidence of clinical iodine deficiency either in the form of endemic goitre or endemic cretinism, urinary iodine excretion reflects the average daily iodine requirement. Therefore, for determining the iodine requirements, the important indexes are serum T_4 and TSH levels (indicating normal thyroid status) and urinary iodine excretion. The simplified diagram of metabolic circuit of iodine is given in ***Figure 20*** (*1*).

Overview of significant scientific information

All biologic actions of iodide are attributed to the thyroid hormones. The major thyroid hormone secreted by the thyroid gland is T_4 (tetra-iodo-thyronine). T_4 in circulation is taken up by the cells and is de-iodinated by the enzyme 5' prime-mono-de-iodinase in the cytoplasm to convert it into tri-iodo-thyronine (T_3), the active form of thyroid hormone. T_3 traverses to the nucleus and binds to the nuclear receptor. All the biologic actions of T_3 are mediated through the binding to the nuclear receptor, which controls the transcription of a particular gene to bring about the synthesis of a specific protein.

The physiologic actions of thyroid hormones can be categorised as 1) growth and development and 2) control of metabolic processes in the body. Thyroid hormones play a major role in the growth and development of brain and central nervous systems in humans from the 15th week of gestation to age 3 years. If iodine deficiency exists during this period and results in thyroid hormone deficiency, the consequence is derangement in the development of brain and central nervous system. These derangements are irreversible, the most serious form being that of cretinism. The effect of iodine deficiency at different stages of life is given in ***Table 34*** (*2*).

The other physiologic role of thyroid hormone is to control several metabolic processes in the body. These include carbohydrate, fat, protein, vitamin, and mineral metabolism. For example, thyroid hormone increases energy production, increases lipolysis, and regulates neoglucogenesis, and glycolysis.

Figure 20

Simplified diagram of the metabolic circuit of iodine

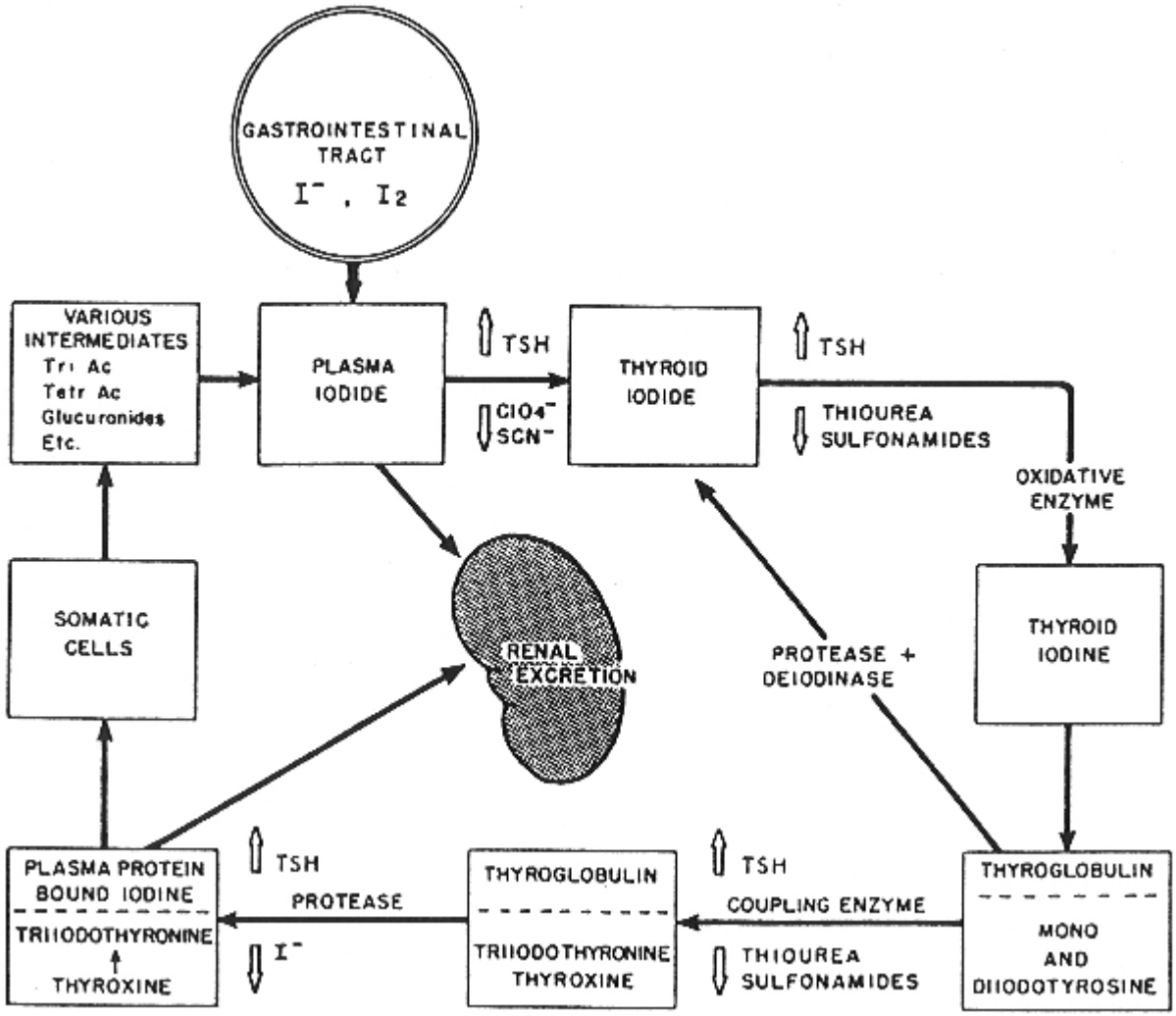


Table 34

The spectrum of iodine deficiency disorders

Life stage	Effects
Foetus	Abortions Stillbirths Congenital anomalies Increased perinatal mortality Increased infant mortality Neurological cretinism: mental deficiency, deaf mutism, spastic diplegia, and squint Myxedematous cretinism: mental deficiency and dwarfism Psychomotor defects
Neonate	Neonatal goitre Neonatal hypothyroidism
Child and Adolescent	Goitre Juvenile hypothyroidism Impaired mental function Retarded physical development
Adult	Goitre with its complications Hypothyroidism Impaired mental function

Population at risk

Iodine deficiency affects all stages of human life, from the intra-uterine stage to old age, as shown in ***Table 34***. However, pregnant women, lactating women, women of reproductive age, and children younger than 3 years are considered to be at high risk (*3*). During foetal and neonatal growth and development, iodine deficiency leads to irreversible damage to the brain and central nervous system.

Dietary sources

The iodine content of food depends on the iodine content of the soil in which it is grown. The iodine present in the upper crust of earth is leached by glaciation and repeated flooding and is carried to the sea. Sea water is, therefore, a rich source of iodine (*4*). The seaweed located near coral reefs has an inherent biologic capacity to concentrate iodine from the sea. The reef fish which thrive on seaweed are rich in iodine. Thus, a population consuming seaweed and reef fish has a high intake of iodine, as the case in Japan. The amount of iodine intake by the Japanese is in the range of 2–3 mg/day (*4*). In several areas of Asia, Africa, Latin America, and parts of Europe, iodine intake varies from 20 to 80 μg/day. In the United States and Canada and some parts of Europe, the intake is around 500 μg/day. The average iodine content of foods (fresh and dry basis) as reported by Koutras *et al.* (*4*) is given in ***Table 35.***

Table 35

Average iodine content of foods (in µg/g)

Food	Fresh basis		Dry basis	
	Mean	Range	Mean	Range
Fish (fresh water)	30	17–40	116	68–194
Fish (marine)	832	163–3180	3715	471–4591
Shellfish	798	308–1300	3866	1292–4987
Meat	50	27–97	—	—
Milk	47	35–56	—	—
Eggs	93	—	—	—
Cereal grains	47	22–72	65	34–92
Fruits	18	10–29	154	62–277
Legumes	30	23–36	234	223–245
Vegetables	29	12–201	385	204–1636

The iodine content of food varies with geographic location because there is a large variation in the iodine content of the inorganic world (***Table 36***) (*4*). Thus, the average iodine content of foods shown in ***Table 35*** can not be used universally for estimating iodine intake.

Recommended intake

The daily intake of iodine recommended by the National Research Council of the US National Academy of Sciences in 1989 was 40 µg/day for young infants (0–6 months), 50 µg/day for older infants (6–12 months), 60–100 µg/day for children (1–10 years), and 150 µg/day for adolescents and adults (*5*). These values approximate 7.5 µg/kg/day for age 0–12 months, 5.4 µg/kg/day for age 1–10 years, and 2 µg/kg/day for adolescents and adults. These amounts are proposed to allow normal T_4 production without stressing the thyroid iodide trapping mechanism or raising TSH levels.

Iodine requirements in infancy

The US recommendation of 40 µg/day for infants aged 0–6 months (or 8 µg/kg/day, 7 µg/100 kcal, or 50 µg/l milk) is probably derived from the observation that until the late 1960s the iodine content of human milk was approximately 50 µg/l and from the concept that nutrition of the human-milk-fed infant growing at a satisfactory rate has been the standard against which nutrition requirements have been set (6, 7). However, more recent data indicate that the iodine content of human milk varies markedly as a function of the iodine intake of the population. For example, it ranges from 20 to 330 µg/l in Europe and from 30 to 490 µg/l in the United States (6, 8). It is as low as 12 µg/l under conditions of severe iodine deficiency (6, 8). An average human-milk intake of 750 ml/day would give an intake of iodine of about 60

µg/day in Europe and 120 µg/day in the United States. The upper US value (490 µg/l) would provide 368 µg/day or 68 µg/kg/day for a 5-kg infant. Positive iodine balance in the young infant, which is required for the increasing iodine stores of the thyroid, is achieved only when the iodine intake is at least 15 µg/kg/day in full-term infants and 30 µg/kg/day in pre-term infants (9). The iodine requirement of pre-term infants is twice that of term infants because of a 50 percent lower retention of iodine by pre-term infants. This corresponds approximately to an iodine intake of 90 µg/day. (This is probably based on the assumption of average body weight of 6 kg for a child of 6 months, the mid-age of an infant.) This value is twofold higher than the US recommendations.

On the basis of these considerations, a revision is proposed for the earlier World Health Organization (WHO), United Nations Children's Fund (UNICEF), and International Council for the Control of Iodine Deficiency Disorders (ICCIDD) recommendations (10): an iodine intake of 90 µg/day from birth onwards is suggested. To reach this objective, and based on an intake of milk of about 150 ml/kg/day, the iodine content of formula milk should be increased from 50 to 100 µg/l for full-term infants and to 200 µg/l for pre-term infants.

For a urine volume of about 4–6 dl/day from 0 to 3 years, the urinary concentration of iodine indicating iodine repletion should be in the range of 150–220 µg/l (1.18-1.73µmol/l) in infants aged 0–36 months. Such values have been observed in iodine-replete infants in Europe (11), Canada (12), and the United States (12). Under conditions of moderate iodine deficiency, as seen in Belgium, the average urinary iodine concentration is only 50–100 µg/l (0.39-0.79µmol/l) in this age group. It reaches a stable normal value of 180–220 µg/l (1.41-1.73µmol/l) only after several months of daily iodine supplementation with a physiologic dose of 90 µg/day (***Figure 21***).

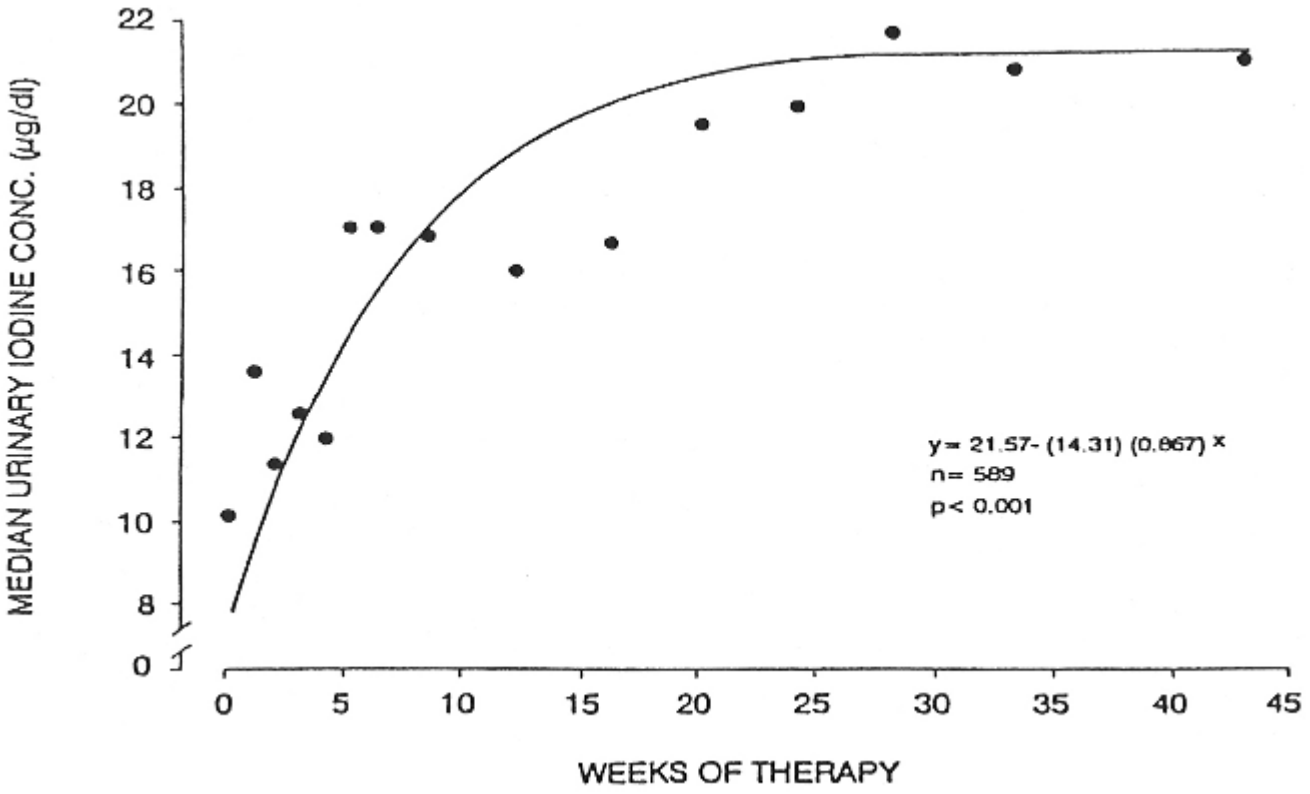
Table 36

Iodine content of the inorganic world

Location	Iodine content
Terrestrial air	1.0 µg/l
Marine air	100.0 µg/l
Terrestrial water	5.0 µg/l
Sea water	50.0 µg/l
Igneous rocks	500.0 µg/kg
Soils from igneous rocks	9000.0 µg/kg
Sedimentary rocks	1500.0 µg/kg
Soils from sedimentary rocks	4000.0 µg/kg
Metamorphic rocks	1600.0 µg/kg
Soils from the metamorphic rocks	5000.0 µg/kg

Figure 21

Changes over time for the median urinary concentration of iodine in healthy Belgian infants aged 6–36 months and supplemented with iodine at 90 µg/kg/day for 44 weeks



Each point represents 32–176 iodine determinations (13).

When the urinary iodine concentration in neonates and young infants is below a threshold of 50-60 µg/l (0.39-0.47µmol/l), corresponding to an intake of 25–35 µg/day, there is a sudden increase in the prevalence of neonatal serum TSH values in excess of 50 mU/ml, indicating sub-clinical hypothyroidism and eventually complicated by transient neonatal hypothyroidism (14). When the urinary iodine concentration is in the range of 10–20 µg/l (0.08-0.16µmol/l), as observed in severe endemic goitre regions, up to 10 percent of the neonates have overt severe hypothyroidism, with serum TSH levels above 100 mU/mL and serum T_3 values below 30 µg/l (39 nmol/L) (14). Untreated, these infants progress to myxedematous endemic cretinism (15).

Thus, the iodine requirement of the young infant approximates 15 µg/kg/day (30 µg/kg/day in pre-term infants). Hyperthyrotropinemia (high levels of serum TSH), indicating sub-clinical hypothyroidism with the risk of brain damage, occurs when the iodine intake is about one-third of this value, and dramatic neonatal hypothyroidism resulting in endemic cretinism occurs when the intake is about one-tenth of this value.

Iodine requirements in children

The daily iodine need on a body weight basis decreases progressively with age. A study by Tovar and colleagues (16) correlating 24-hour thyroid radioiodine uptake and urinary iodine excretion in 9–13-year-old schoolchildren in rural Mexico suggested that an iodine intake in excess of 60 μg/day is associated with a 24-hour thyroidal radioiodine uptake below 30 percent. Lower excretion values are associated with higher uptake values. This would approximate 3 μg/kg/day in an average size 10-year-old child (approximate body weight of 20 kg), so that an intake of 60–100 μg/day for child of 1–10 years seems appropriate. These requirements are based on the body weight of Mexican children who participated in this study. The average body weight of a 10-year-old child, as per the Food and Agriculture Organization references, is 25 kg. Thus, the iodine requirement for a 1–10-year-old child would be 90–120 μg/day.

Iodine requirements in adults

Iodine at 150 μg/day for adolescents and adults is justified by the fact that it corresponds to the daily urinary excretion of iodine and to the iodine content of food in non-endemic areas (areas where iodine intake is adequate) (5). It also provides the iodine intake necessary to maintain the plasma iodide level above the critical limit of 0.10 μg/dl, which is the average level likely to be associated with the onset of goitre (17). Moreover, this level of iodine intake is required to maintain the iodine stores of the thyroid above the critical threshold of 10 mg, below which an insufficient level of iodisation of thyroglobulin leads to disorders in thyroid hormone synthesis (18).

Data reflecting either iodine balance or its effect on thyroid physiology can help to define optimal iodine intake. In adults and adolescents in equilibrium with their nutritional environment, most dietary iodine eventually appears in the urine, so the urinary iodine concentration is a useful measure for assessing iodine intake. For this, casual samples are sufficient if enough are collected and if they accurately represent a community (19). A urinary iodine concentration of 100 μg/L corresponds to an intake of about 150 μg/day in the adult. Median urinary iodine concentrations below 100 μg/l in a population are associated with increases in median thyroid size and in serum TSH and thyroglobulin values. Correction of the iodine deficiency will bring all these measures back into the normal range. Recent data from the Thyro-Mobil project in Europe have confirmed these relations by showing that the largest thyroid sizes are associated with the lowest urinary iodine concentrations (20). Once a median urinary iodine excretion of about 100 μg/L is reached, the ratio of thyroid size to body size remains fairly constant. Moulopoulos *et al.* (21) reported that a urinary iodine excretion between 151 and 200 μg/g creatinine (1.18-1.57 μmol/g creatinine), corresponding to a concentration of about 200 μg/l (1.57 μmol/l), gave the lowest values for serum TSH in a non-goitrous population. Similar recent data from Australia show that the lowest serum TSH and thyroglobulin values were associated with urine containing 200–300 μg iodine/g creatinine (1.57-2.36μmol/g creatinine) (22).

Other investigations followed serum TSH levels in subjects without thyroid glands who were given graded doses of T_4 and found that euthyroidism established in adults with an average daily dose of 100 μg T_4 would require at least 65 μg of iodine with maximal efficiency of iodine use by the thyroid. In practice such maximal efficiency is never obtained and therefore considerably more iodine is necessary. Data from controlled observations associated a low urinary iodine concentration with a high goitre prevalence, high radioiodine uptake, and low thyroidal organic iodine content (23). Each of these measures reached a steady state once the urinary iodine excretion was 100 μg/l (0.78 μmol/l)or greater.

Iodine requirements in pregnancy

The iodine requirement during pregnancy is increased to provide for the needs of the foetus and to compensate for the increased loss of iodine in the urine resulting from an increased renal clearance of iodine during pregnancy (24). These requirements have been derived from studies of thyroid function during pregnancy and in the neonate under conditions of moderate iodine deficiency. For example, in Belgium, where the iodine intake is estimated to be 50–70 µg/day (25), thyroid function during pregnancy is characterised by a progressive decrease of the serum concentrations of thyroid hormones and an increase in serum TSH and thyroglobulin. Thyroid volume progressively increases and is above the upper limit of normal in 10 percent of the women by the end of pregnancy. Serum TSH and thyroglobulin are still higher in the neonates than in the mothers (26). These abnormalities are prevented only when the mother receives a daily iodide supplementation of 161 µg/day during pregnancy (derived from 131 µg potassium iodide and 100 µg T_4 given daily) (27). T_4 with iodine was probably administered to the pregnant women to rapidly correct sub-clinical hypothyroidism, which would not have occurred if iodine had been administered alone. These data indicate that the iodine intake required to prevent the onset of sub-clinical hypothyroidism of mother and foetus during pregnancy, and thus to prevent the possible risk of brain damage of the foetus, is approximately 200 µg/day.

On the basis of the considerations reviewed above for the respective population groups to meet the daily iodine requirements, revisions of the current recommendations for daily iodine intake by WHO, UNICEF, and ICCIDD (10) are proposed; these proposed revisions are presented in ***Table 37***.

Table 37

Proposed revision for daily iodine intake recommendations of 1996 by the World Health Organization, United Nations Children's Fund, and International Council for the Control of Iodine Deficiency Disorders

Population sub-groups	Total iodine intake µg/day	Iodine µg/kg/day
Infants (first 12 months)	90[a]	15.0
Children (1–6 years)	90	6.0
Schoolchildren (7–12 years)	120	4.0
Adults (12+ years)	150	2.0
Pregnant and lactating women	200	3.5

[a] Revised to 90 µg from the earlier recommendation of 50 µg.

Upper limit of iodine intake for different age groups

An iodine excess also can be harmful to the thyroid of infants by inhibiting the process of synthesis and release of thyroid hormones (Wolff-Chaikoff effect) (28). The threshold upper limit of iodine intake (the intake beyond which thyroid function is inhibited) is not easy to define because it is affected by the level of iodine intake before exposure to iodine excess. Indeed, long-standing moderate iodine deficiency is accompanied by an accelerated trapping of iodide and by a decrease in the iodine stores within the thyroid (18). Under these conditions, the critical ratio between iodide and total iodine within the thyroid, which is the starting point of the Wolff-Chaikoff effect, is more easily reached during iodine depletion

than under normal conditions. In addition, the neonatal thyroid is particularly sensitive to the Wolff-Chaikoff effect because the immature thyroid gland is unable to reduce the uptake of iodine from the plasma to compensate for increased iodine ingestion (29). For these reasons transient neonatal hypothyroidism or transient hyperTSHemia after iodine overload of the mother, especially after the use of povidone iodine, has been reported more frequently in European countries such as in Belgium, France, and Germany, which have prevailing moderate iodine deficiency (30-33).

Iodine intake in areas of moderate iodine deficiency

In a study in Belgium, iodine overload of mothers (cutaneous povidone iodine) increased the milk iodine concentration and increased iodine excretion in the term newborns (mean weight about 3 kg). Mean milk iodine concentrations of 18 and 128 µg/dl were associated with average infant urinary iodine excretion levels of 280 and 1840 µg/l (2.20-14.48 µmol/l), respectively (30). Estimated average iodine intakes would be 112 and 736 µg/day, or 37 and 245 µg/kg/day, respectively. The lower dose significantly increased the peak TSH response to exogenous thyroid releasing hormone but did not increase the (secretory) area under the TSH response curve. The larger dose increased both the peak response and secretory area as well as the baseline TSH concentration. Serum T_4 concentrations were not altered, however. Thus, these infants had a mild and transient, compensated hypothyroid state. Non-contaminated mothers secreted milk containing 9.5 µg iodine/dl, and the mean urinary iodine concentration of their infants was 144 µg/l (1.13 µmol/l). These data indicate that modest iodine overloading of term infants in the neonatal period in an area of relative dietary iodine deficiency (Belgium) also can impair thyroid hormone formation.

Similarly, studies in France indicated that premature infants exposed to cutaneous povidone iodine or fluorescinated alcohol-iodine solutions and excreting iodine in urine in excess of 100 µg/day manifested decreased T_4 and increased TSH concentrations in serum (32). The extent of these changes was more marked in premature infants with less than 34 weeks gestation than in those with 35–37 weeks gestation. The full-term infants were not affected. These studies suggest that in Europe the upper limit of iodine intake, which predisposes to blockage of thyroid secretion in premature infants (about 200 µg/day) is 2 to 3 times the average intake from human milk and about equivalent to the upper range of intake.

Iodine intake in areas of iodine sufficiency

Similar studies have not been conducted in the United States, where transient hypothyroidism is rarely seen perhaps because iodine intake is much higher. For example, urinary concentrations of 50 µg/dl and above in neonates, which can correspond to a Wolff-Chaikoff effect in Europe, are frequently seen in healthy neonates in North America (11, 12).

The average iodine intake of infants in the United States in 1978, including infants fed whole cow milk, was estimated by the market-basket approach (34) to be 576 µg/day (standard deviation [SD] 196); that of toddlers was 728 µg/day (SD 315) and of adults was 952 µg/day (SD 589). The upper range for infants (968 µg/day) would provide a daily intake of 138 µg/kg for a 7-kg infant, and the upper range for toddlers (1358 µg/day) would provide a daily intake of 90 µg/kg for a 15-kg toddler.

Table 38 summarises the recommended dietary intake of iodine for age and approximate level of intake which appear not to impair thyroid function in the European studies of Delange in infants, in the loading studies of adults in the United States, or during ingestion of the highest estimates of dietary intake (just reviewed) in the United States (34). Except for the values for premature infants, these probably safe limits are 15–20 times more than the recommended intakes. These data refer to all sources of iodine intake. The average iodine content of infant formulas is approximately 5 µg/dl. The upper limit probably should

be one that provides a daily iodine intake of no more than 100 µg/kg. For this limit and with the assumption that the total intake is from infant formula, with a daily intake of 150 ml/kg (100 kcal/kg), the upper limit of the iodine content of infant formula would be about 65 µg/dl. The current suggested upper limit of iodine in infant formulas of 75 µg/100 kcal (89µg/500 kJ or 50 µg/dl), therefore, seems reasonable.

Table 38

Recommended dietary intakes of iodine and probable safe upper limits

Group	Recommended µg/kg/day	Upper limit[a] µg/kg/day
Premature infants	30	100
Infants 0–6 months	15	150
Infants 7–12 months	15	140
Children 1–6 years	6	50
School children 7–12 years	4	50
Adolescents and adults (12+ years)	2	30
Pregnancy and lactation	3.5	40

[a] Probably safe.

Excess iodine intake

Excess iodine intake is more difficult to define. Many people regularly ingest huge amounts of iodine – in the range 10–200 mg/day – without apparent adverse effects. Common sources are medicines (e.g., amiodarone contains 75 mg iodine per 200-mg capsule), foods (particularly dairy products), kelp (eaten in large amounts in Japan), and iodine-containing dyes (for radiologic procedures). Excess consumption of salt has never been documented to be responsible for excess iodine intake. Occasionally each of these may have significant thyroid effects, but generally they are tolerated without difficulty. Braverman *et al*. (35) showed that people without evidence of underlying thyroid disease almost always remain euthyroid in the face of large amounts of excess iodine and escape the acute inhibitory effects of excess intra-thyroidal iodide on the organification (i.e., attachment of `oxidized iodine' species to throsyl residues in the thyroid gland for the synthesis of thyroid hormones) of iodide and on subsequent hormone synthesis (escape from or adaptation to the acute Wolff-Chaikoff effect). This adaptation most likely involves a decrease in thyroid iodide trapping, perhaps corresponding to a decrease in the thyroid sodium-iodide transporter recently cloned (36). Some people, especially those with long-standing nodular goitre who live in iodine-deficient regions and are generally ages 40 years or older, may develop iodine-induced hyperthyroidism after ingestion of excess iodine in a short period of time.

Iodine fortification

Iodine deficiency is present in almost all parts of the developed and developing world, and environmental iodine deficiency is the main cause of iodine deficiency disorders. Iodine is irregularly distributed over the earth's crust, resulting in acute deficiencies in areas such as mountainous regions and flood plains. The problem is aggravated by accelerated deforestation

and soil erosion. Thus, the food grown in iodine-deficient regions can never provide enough iodine for the people and livestock living there. The iodine deficiency results from geologic rather than social and economic conditions. It cannot be eliminated by changing dietary habits or by eating specific kinds of foods but must be corrected by supplying iodine from external sources. It has, therefore, been a common practice to use common salt as a vehicle for iodine fortification for the past 75 years. Salt is consumed at approximately the same level throughout the year by the entire population of a region. Universal salt iodisation is now a widely accepted strategy for preventing and correcting iodine deficiency disorders.

There are areas where consumption of goitrogens in the staple diet (e.g., cassava) affects the proper utilisation of iodine by the thyroid gland. For example, in Congo, Africa, as a result of cassava diets there is an overload of thiocyanate (37). To overcome this problem, appropriate increases in salt iodisation are required to ensure the recommended dietary intake. The iodisation of salt is done either by spraying potassium iodate or potassium iodide in amounts that ensure a minimum of 150 μg iodine/day. Both of these forms of iodine are absorbed as iodide ions and are completely bio-available. Other methods of iodine prophylaxis are also used: iodised oil (capsule and injections), iodised water, iodised bread, iodised soya sauce, iodoform compounds used in dairy and poultry, and certain food additives (38).

Iodine loss occurs as a result of improper packaging, Humidity and moisture, and transport in open trucks and railway wagons exposed to sunlight. To compensate for these losses, higher levels of iodine are used during the production of iodised salt. Losses during the cooking process vary from 20 percent to 40 percent depending on the type of cooking used (39).

To ensure the consumption of recommended levels of iodine, the iodine content of salt at the production level should be monitored with proper quality assurance programmes. Regular evaluation of the urinary iodine excretion pattern in the population consuming iodised salt or exposed to other iodine prophylactic measures would help the adjusting of iodine intake (40).

Recommendations

Recommendations for future research:

- elaborate the role of T_4 in brain development at the molecular level;
- investigate the relation between selenium and iodine deficiency, which has been reported in certain areas of Africa; and
- investigate the possible interference of infections and other systemic illnesses with iodine or thyroid hormone use (such interference has not been reported on a population basis).

Recommendations for future actions:

- establish quality assurance procedures at iodised salt production sites;
- track the progress of iodine deficiency disease elimination through the implementation of cyclic monitoring, which involves division of the country into five zones and carrying out the assessment in one zone each year; and
- develop and validate quantitative testing kits for iodised salt.

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Chapter 13
Iron

The role of iron in human metabolic processes

Iron has several vital functions in the body. It serves as a carrier of oxygen to the tissues from the lungs by red blood cell haemoglobin, as a transport medium for electrons within cells, and as an integrated part of important enzyme systems in various tissues. The physiology of iron has been extensively reviewed (*1-6*).

Most of the iron in the body is present in the erythrocytes as haemoglobin, a molecule composed of four units, each containing one heme group and one protein chain. The structure of haemoglobin allows it to be fully loaded with oxygen in the lungs and partially unloaded in the tissues (e.g., in the muscles). The iron-containing oxygen storage protein in the muscles, myoglobin, is similar in structure to haemoglobin but has only one heme unit and one globin chain. Several iron-containing enzymes, the cytochromes, also have one heme group and one globin protein chain. These enzymes act as electron carriers within the cell and their structures do not permit reversible loading and unloading of oxygen. Their role in the oxidative metabolism is to transfer energy within the cell and specifically in the mitochondria. Other key functions for the iron-containing enzymes (e.g., cytochrome P450) include the synthesis of steroid hormones and bile acids; detoxification of foreign substances in the liver; and signal controlling in some neurotransmitters, such as the dopamine and serotonin systems in the brain. Iron is reversibly stored within the liver as ferritin and hemosiderin whereas it is transported between different compartments in the body by the protein transferrin.

Iron requirements

Basal iron losses

Iron is not actively excreted from the body in urine or in the intestines. Iron is only lost with cells from the skin and the interior surfaces of the body – intestines, urinary tract, and airways. The total amount lost is estimated at 14 μg/kg body weight/day (*7*). In children, it is probably more correct to relate these losses to body surface. A non-menstruating 55-kg women loses about 0.8 mg Fe/day and a 70-kg man loses about 1 mg. The range of individual variation has been estimated to be ±15 percent (*8*).

Earlier studies suggested that sweat iron losses could be considerable, especially in a hot, Humid climate. However, new studies which took extensive precautions to avoid the interference of contamination of iron from the skin during the collection of total body sweat have shown that these sweat iron losses are negligible (*9*).

Growth

The newborn term infant has an iron content of about 250–300 mg (75 mg/kg body weight). During the first 2 months of life, haemoglobin concentration falls because of the improved oxygen situation in the newborn infant compared with the intrauterine foetus. This leads to a considerable redistribution of iron from catabolised erythrocytes to iron stores. This iron will cover the needs of the term infant during the first 4–6 months of life and is why iron requirements during this period can be provided by human milk, that contains very little iron. Because of the marked supply of iron to the foetus during the last trimester of pregnancy, the iron situation is much less favourable in the premature and low-birth-weight infant than in the

term infant. An extra supply of iron is therefore needed in these infants even during the first 6 months of life.

In the full-term infant, iron requirements will rise markedly after age 4–6 months and amount to about 0.7–0.9 mg/day during the remaining part of the first year. These requirements are therefore very high, especially in relation to body size and energy intake (***Table 39***) (*10*).

Table 39

Iron intakes required for growth under the age of 18 years, median basal iron losses, menstrual losses in women, and total absolute iron requirements

Group	Age	Body weight	Required Iron intakes for Growth	Basal Iron losses	Menstrual losses		Total Absolute Requirements †	
	(Years)	Mean (kg)	(mg/day)	Median (mg/day)	Median (mg/day)	95th percentile (mg/day)	Median (mg/day)	95th percentile (mg/day)
Children	0.5–1	9	0.55	0.17			0.72	0.93
	1–3	13.3	0.27	0.19			0.46	0.58
	4–6	19.2	0.23	0.27			0.50	0.63
	7–10	28.1	0.32	0.39			0.71	0.89
Males	11–14	45	0.55	0.62			1.17	1.46
	15–17	64.4	0.60	0.90			1.50	1.88
	18+	75		1.05			1.05	1.37
Females	11–14 [b]	46.1	0.55	0.65			1.20	1.40
	11–14	46.1	0.55	0.65	0.48 [c]	1.90 [c]	1.68	3.27
	15–17	56.4	0.35	0.79	0.48 [c]	1.90 [c]	1.62	3.10
	18+	62		0.87	0.48 [c]	1.90 [c]	1.46	2.94
Post-menopausal		62		0.87			0.87	1.13
Lactating		62		1.15			1.15	1.50

† Total Absolute Requirements = Requirement for growth + basal losses + menstrual losses (females only)

[a]Based in part on a 1988 report from Food and Agriculture Organization of the United Nations (FAO)/World Health Organizaiton (WHO) (*8*) and in part on new calculations of the distribution of iron requirements in menstruating women. Because of the very skewed distribution of iron requirements in these women, dietary iron requirements are calculated for four levels of dietary iron bio-availability (***Table 40***).

[b]Non-menstruating.

[c]Effect of the normal variation in haemoglobin concentration not included in this figure.

In the first year of life, the full-term infant almost doubles its total iron stores and triple its body weight. The change in body iron during this period occurs mainly during the first 6–12 months of life. Between 1 and 6 years of age, the body iron content is again doubled. The requirements for absorbed iron in infants and children are very high in relation to their energy requirements. For example, in infants 6–12 months of age, about 1.5 mg of iron need to be absorbed per 4.184 MJ and about half of this amount is required up to age 4 years.

Table 40

The recommended nutrient intakes for iron based on varying dietary iron bio-availabilities

Group	Age (years)	Mean Body weight (kg)	Recommended Nutrient Intake[a] (mg/day) % Dietary Iron Bio-availability			
			15	**12**	**10**	**5**
Children	0.5–1	9	[6.2][b]	[7.7][b]	[9.3][b]	[18.6][b]
	1–3	13.3	3.9	4.8	5.8	11.6
	4–6	19.2	4.2	5.3	6.3	12.6
	7–10	28.1	5.9	7.4	8.9	17.8
Males	11–14	45	9.7	12.2	14.6	29.2
	15–17	64.4	12.5	15.7	18.8	37.6
	18+	75	9.1	11.4	13.7	27.4
Females	11–14[c]	46.1	9.3	11.7	14	28
	11–14	46.1	21.8	27.7	32.7	65.4
	15–17	56.4	20.7	25.8	31	62
	18+	62	19.6	24.5	29.4	58.8
Post-menopausal		62	7.5	9.4	11.3	22.6
Lactating		62	10	12.5	15	30

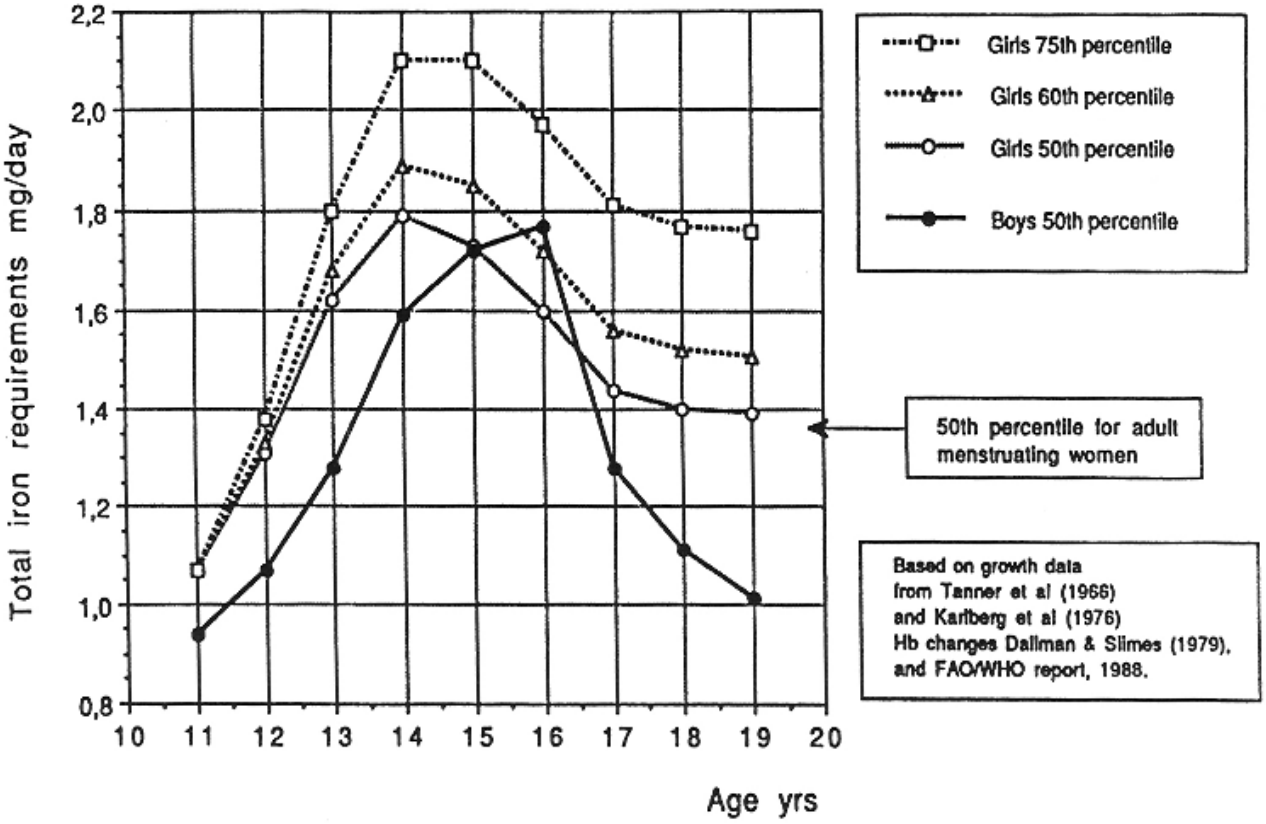
[a]Based in part on a 1988 report from the FAO/WHO (*8*) and in part on new calculations of the distribution of iron requirements in menstruating women. Because of the very skewed distribution of iron requirements in these women, dietary iron requirements are calculated for four levels of dietary iron bio-availability.
[b]Bio-availability of dietary iron during this period varies greatly.
[c]Non-menstruating.

In the weaning period, the iron requirements in relation to energy intake are the highest of the lifespan except for the last trimester of pregnancy, when iron requirements to a large extent have to be covered from the iron stores of the mother (see section on iron and pregnancy). The rapidly growing weaning infant has no iron stores and has to rely on dietary iron. It is possible to meet these high requirements if the diet has a consistently high content of meat and foods rich in ascorbic acid. In most developed countries today, infant cereal products are the staple foods for that period of life. Commercial products are regularly fortified with iron and ascorbic acid, and they are usually given together with fruit juices and solid foods containing meat, fish, and vegetables. The fortification of cereal products with iron and ascorbic acid is important in meeting the high dietary needs, especially considering the importance of an optimal iron nutrition during this phase of brain development.

Iron requirements are also very high in adolescents, particularly during the period of rapid growth (*11*). There is a marked individual variation in growth rate and the requirements may be considerably higher than the calculated mean values given in ***Table 39***. Girls usually have their growth spurt before menarche, but growth is not finished at that time. Their total iron requirements are therefore considerable. In boys during puberty there is a marked increase in haemoglobin mass and concentration, further increasing iron requirements to a level above the average iron requirements in menstruating women (***Figure 22***). (The calculations in ***Figure 22*** are based on references *8* and *12–16.*)

Figure 22

Iron requirements of boys and girls

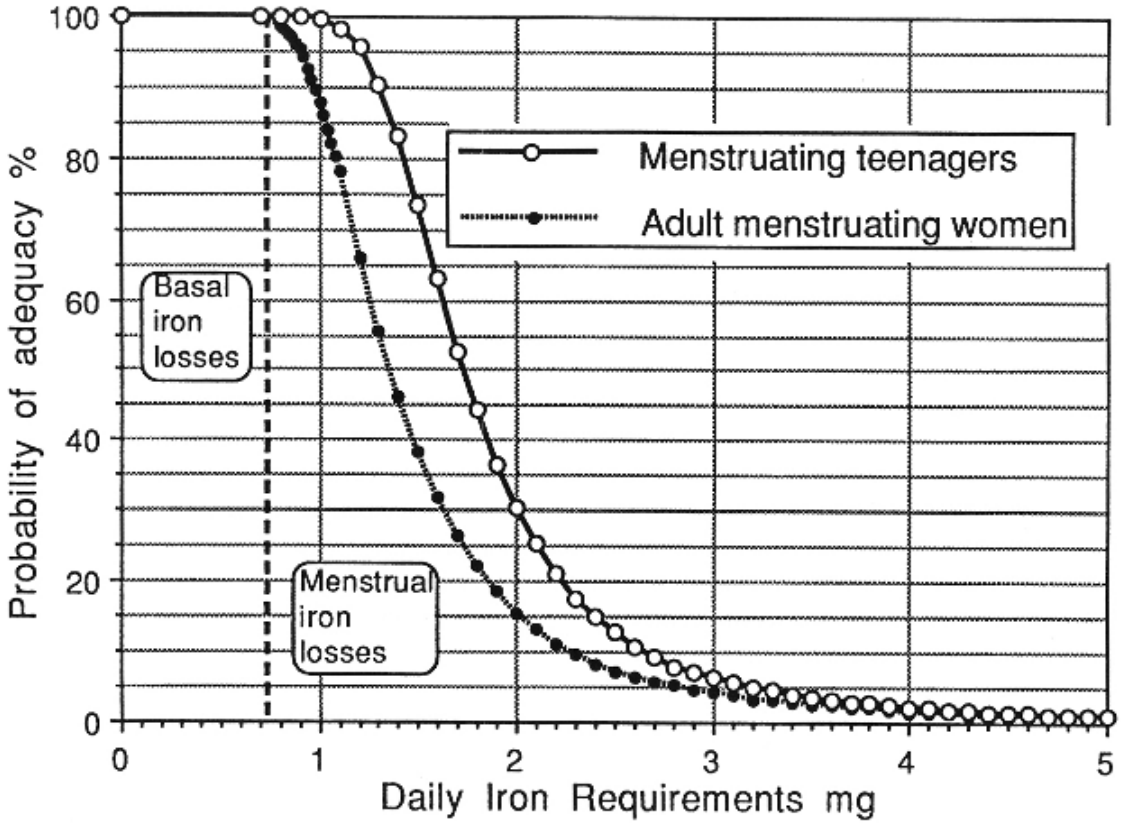


Menstrual iron losses

Menstrual blood losses are very constant from month to month for an individual but vary markedly from one woman to another (*16*).The main part of this variation is genetically controlled by the content of fibrinolytic activators in the uterine mucosa even in populations which are geographically widely separated (Burma, Canada, China, Egypt, England, and Sweden) (*17, 18*). These findings strongly suggest that the main source of variation in iron status in different populations is not related to a variation in iron requirements but to a variation in the absorption of iron from the diets. (This statement disregards infestations with hookworms and other parasites.) The mean menstrual iron loss, averaged over the entire menstrual cycle of 28 days, is about 0.56 mg/day. The frequency distribution of physiologic menstrual blood losses is highly skewed. Adding the average basal iron loss (0.8 mg) and its variation allows the distribution of the total iron requirements in adult women to be calculated as the convolution of the distributions of menstrual and basal iron losses (***Figure 23***). The mean daily total iron requirement is 1.36 mg. In 10 percent of women it exceeds 2.27 mg and in 5 percent it exceeds 2.84 mg (*19*). In 10 percent of menstruating (still-growing) teenagers, the corresponding daily total iron requirement exceeds 2.65 mg, and in 5 percent of the girls it exceeds 3.2 mg/day. The marked skewness of menstrual losses is a great nutritional problem because personal assessment of the losses is unreliable. This means that women with physiologic but heavy losses cannot be identified and reached by iron supplementation. The choice of contraceptive method greatly influences menstrual losses. The methods of calculating iron requirements in women and their variation were recently re-examined (*19*).

Figure 23

Distribution of daily iron requirements in menstruating adult women and teenagers: the probability of adequacy at different amounts of iron absorbed



Note: Left: basal obligatory losses that amount to 0.8 mg; right: varying menstrual iron losses. This graph illustrates that growth requirements in teenagers vary considerably at different age and between girls.

In postmenopausal women and in physically active elderly people, the iron requirements per unit of body weight are the same as in men. When physical activity decreases as a result of ageing, blood volume and haemoglobin mass also diminish, leading to a shift of iron from haemoglobin and muscle to iron stores. This implies a reduction of the daily iron requirements. Iron deficiency in the elderly is therefore seldom of nutritional origin but is usually caused by pathologic iron losses. The absorbed iron requirements in different groups are given in ***Table 39***. Dietary iron requirements will be discussed below. The iron situation during pregnancy and lactation are dealt with separately below.

Iron absorption

With respect to the mechanism of absorption, there are two kinds of dietary iron: heme iron and non-heme iron (*20*). In the human diet the primary sources of heme iron are the haemoglobin and myoglobin from consumption of meat, poultry, and fish whereas non-heme iron is obtained from cereals, pulses, legumes, fruits, and vegetables. The average absorption of heme iron from meat-containing meals is about 25 percent (*21*) The absorption of heme iron can vary from about 40 percent during iron deficiency to about 10 percent during iron repletion (*22*). Heme iron can be degraded and converted to non-heme iron if foods are cooked at a high temperature for too long. Calcium (see below) is the only dietary factor that

negatively influences the absorption of heme iron and does so to the same extent that it influences non-heme iron (***Table 41***) (*23*).

Table 41

Factors influencing dietary iron absorption

HEME IRON ABSORPTION	
Iron status of subject Amount of dietary heme iron, especially as meat Content of calcium in meal (e.g., milk, cheese) Food preparation (time, temperature)	
NON-HEME IRON ABSORPTION	
Iron status of subjects Amount of potentially available non-heme iron (adjustment for fortification iron and contamination iron) * Balance between enhancing and inhibiting factors	
Enhancing factors	**Inhibiting factors**
Ascorbic acid (e.g., certain fruit juices, fruits, potatoes, and certain vegetables) Meat, chicken, fish and other seafood Fermented vegetables (e.g., sauerkraut), fermented soy sauces, etc.	Phytates and other inositol phosphates (e.g., bran products, bread made from high-extraction flour, breakfast cereals, oats, rice [especially unpolished rice], pasta products, cocoa, nuts, soya beans, and peas) Iron-binding phenolic compounds (e.g., tea, coffee, cocoa, certain spices, certain vegetables, and most red wines) Calcium (e.g., milk, cheese) Soy proteins

Non-heme iron is the main form of dietary iron. The absorption of non-heme iron is influenced by individual iron status and by several factors in the diet. Dietary factors influencing iron absorption are outlined in ***Table 41***. Iron compounds used for the fortification of foods will only be partially available for absorption. Once iron is dissolved, its absorption from fortificants and food contaminants is influenced by the same factors as the iron native to the food substance (*24, 25*). Iron originating from the soil (e.g., from various forms of clay) is sometimes present in considerable amounts on the surface of foods as a contaminant originating from dust on air-dried foods or from water used in irrigation. Even if the fraction of iron that is available is often small, contamination iron may still be nutritionally important because of the great amounts present (*26, 27*).

Reducing substances (i.e., substances that keep iron in the ferrous form) must be present for iron to be absorbed (*28*). The presence of meat, poultry, and fish in the diet enhance iron absorption. Other foods contain factors (ligands) that strongly bind ferrous ions, that subsequently inhibit absorption. Examples are phytates and certain iron-binding polyphenols.

Inhibition of iron absorption

Phytates are found in all kinds of grains, seeds, nuts, vegetables, roots (e.g., potatoes), and fruits. Chemically, phytates are inositol hexaphosphate salts and are a storage form of phosphates and minerals. Other phosphates have not been shown to inhibit non-heme iron absorption. In North American and European diets, about 90 percent of phytates originate from cereals. Phytates strongly inhibit iron absorption in a dose-dependent fashion and even small amounts of phytates have a marked effect (*29, 30*).

Bran has a high content of phytate and strongly inhibits iron absorption. Whole-wheat flour, therefore, has a much higher content of phytates than does white wheat flour (*31*). In bread some of the phytates in bran are degraded during the fermentation of the dough. Fermentation for a couple of days (sourdough fermentation) can therefore almost completely degrade the phytate and increase the bio-availability of iron in bread made from whole-wheat flour (*32*). Oats strongly inhibit iron absorption because of their high phytate content, that results from native phytase in oats being destroyed by the normal heat process used to avoid rancidity (*33*). Sufficient amounts of ascorbic acid can counteract this inhibition (*34*). By contrast, non-phytate-containing dietary fibre components have almost no influence on iron absorption.

Almost all plants contain phenolic compounds as part of their defence system against insects, animals, and humans. Only some of the phenolic compounds (mainly those containing galloyl groups) seem to be responsible for the inhibition of iron absorption (*35*). Tea, coffee, and cocoa are common plant products that contain iron-binding polyphenols (*36-39*). Many vegetables, especially green leafy vegetables (e.g., spinach), and herbs and spices (e.g., oregano) contain appreciable amounts of galloyl groups, that strongly inhibit iron absorption. Consumption of betel leaves, common in areas of Asia, also has a marked negative effect on iron absorption.

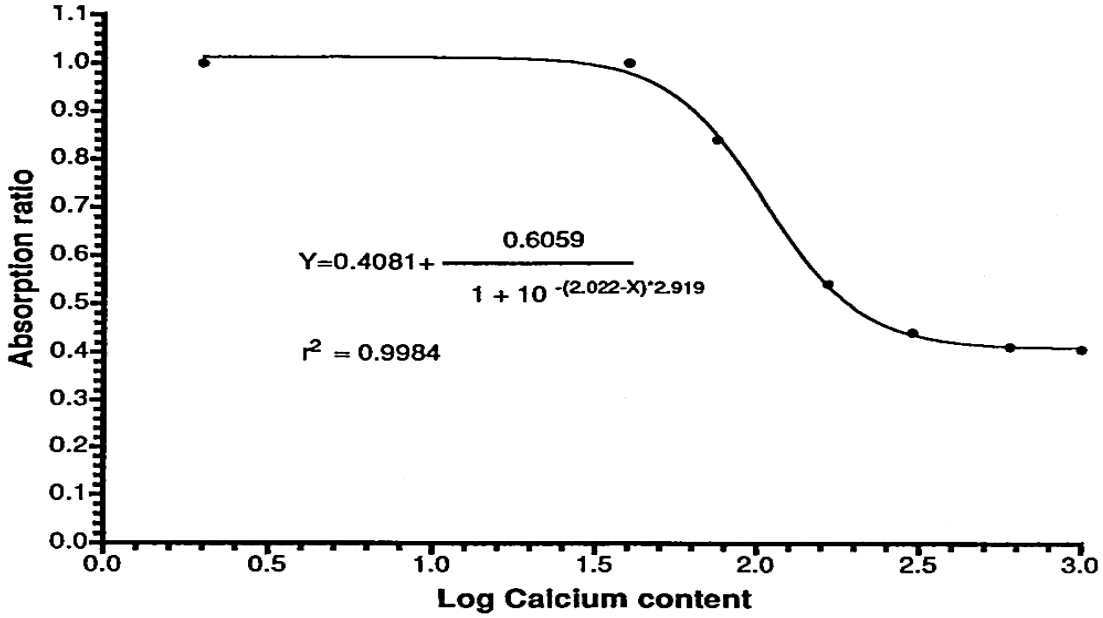
Calcium, consumed as a salt or in dairy products interferes significantly with the absorption of both heme and non-heme iron *(40-42*). Because calcium and iron are both essential nutrients, calcium cannot be considered to be an inhibitor in the same way as phytates or phenolic compounds. The practical solution for this competition is to increase iron intake, increase its bio-availability, or avoid the intake of foods rich in calcium and foods rich in iron at the same meal (*43*).

The mechanism of action for absorption inhibition is unknown, but the balance of evidence strongly suggest that the inhibition is located within the mucosal cell itself at the common final transfer step for heme and non-heme iron. Recent analyses of the dose-effect relationship show that no inhibition is seen from the first 40 mg of calcium in a meal. A sigmoid relationship is then seen, reaching a 60 percent maximal inhibition of iron absorption by 300–600 mg calcium. The form of this curve suggests a one-site competitive binding of iron and calcium (***Figure 24***). This relationship explains some of the seemingly conflicting results obtained in studies on the interaction between calcium and iron (*44*).

For unknown reasons, the addition of soy protein to a meal reduces the fraction of iron absorbed (*45-48*). This inhibition is not solely explained by the high phytate content of soy protein. However, because of the high iron content of soy proteins, the net effect on iron absorption of an addition of soy products to a meal is usually positive. In infant foods containing soy proteins, the inhibiting effect can be overcome by the addition of sufficient amounts of ascorbic acid. Some fermented soy sauces, however, have been found to enhance iron absorption (*49, 50*).

Figure 24

Effect of different amounts of calcium on iron absorption



Enhancement of iron absorption

Ascorbic acid is the most potent enhancer of non-heme iron absorption (*34, 51-53*). Synthetic vitamin C increases the absorption of iron to the same extent as the native ascorbic acid in fruits, vegetables, and juices. The effect of ascorbic acid on iron absorption is so marked and essential that this effect could be considered as one of vitamin C's physiologic roles (*54*). Each meal should preferably contain at least 25 mg of ascorbic acid and possibly more if the meal contains many inhibitors of iron absorption. Therefore, a requirement of ascorbic acid for iron absorption should be taken into account when establishing the requirements for vitamin C, that are set only to prevent vitamin C deficiency (especially scurvy).

Meat, fish, and seafood all promote the absorption of non-heme iron *(55-58*). The mechanism for this effect has not been determined. It should be pointed out that meat also enhances the absorption of heme iron to about the same extent (*21*). Meat promotes iron nutrition in two ways: it stimulates the absorption of both heme and non-heme iron and it provides the well-absorbed heme iron. Epidemiologically, the intake of meat has been found to be associated with a lower prevalence of iron deficiency.

Organic acids, such as citric acid, have in some studies been found to enhance the absorption of non-heme iron (*29).* This effect is not observed as consistently as is the effect of ascorbic acid *(47, 52*). Sauerkraut (*59*) and other fermented vegetables and even some fermented soy sauces (*49, 50*) enhance iron absorption. The nature of this enhancement has not yet been determined.

Iron absorption from meals

The pool concept (see above) in iron absorption implies that there are two main pools in the gastrointestinal lumen – one pool of heme iron and another pool of non-heme iron – and that iron absorption takes place independently from these two pools (*24*). The pool concept also implies that the absorption of iron from the non-heme iron pool results from all ligands

present in the mixture of foods included in a meal. The absorption of non-heme iron from a certain meal not only depends on its iron content but also, and to a marked degree, on the composition of the meal (i.e., the balance among all factors enhancing and inhibiting the absorption of iron). The bio-availability can vary more than 10-fold among meals with a similar content of iron, energy, protein, fat, etc. (*20*). Just the addition of certain spices (e.g., oregano) or a cup of tea may reduce the bio-availability by one-half or more. However, the addition of certain vegetables or fruits containing ascorbic acid may double or even triple iron absorption, depending on the other properties of the meal and the amounts of ascorbic acid present.

Iron absorption from the whole diet

There is limited information about the total amounts of iron absorbed from the diet because no simple method is available to measure iron absorption from the whole diet. It has been measured by chemical balance studies using long balance periods or by determining the haemoglobin regeneration rate in subjects with induced iron deficiency anaemia and a well-controlled diet over a long period of time.

A method was recently developed to measure iron absorption from the whole diet. In the first studies all non-heme iron in all meals over periods of 5–10 days was homogeneously labelled to the same specific activity with an extrinsic inorganic radioiron tracer (*43*, *60*). Heme iron absorption was then estimated. In a further study, heme and non-heme iron were separately labelled with two radioiron tracers as biosynthetically labelled haemoglobin and as an inorganic iron salt (*22*). New information could be obtained, for example, about the average bio-availability of dietary iron in different types of diets, overall effects of certain factors (e.g., calcium) on iron nutrition, and regulation of iron absorption in relation to iron status. Iron absorption from the whole diet is the sum of the absorption of iron from the single meals included in the diet. It has been suggested that the iron absorption of single meals may exaggerate the absorption of iron from the diet (*61*, *62*). Iron absorption from single meals can never represent iron absorption from the whole diet, but iron absorption from a single meal was the same when the meal was served in the morning after an overnight fast or at lunch or supper (*63*). The same observation was made in another study when a hamburger meal was served in the morning or 2–4 hours after a breakfast (*42*).

Because energy expenditure and energy intake set the limit for the amount of food eaten and for meal size, it is practical to relate the bio-availability of iron in different meals to energy content (bio-available nutrient density). The use of bio-available nutrient density is a feasible way to compare different meals, construct menus, and calculate recommended intakes (*64*).

Intake of energy and essential nutrients such as iron was probably considerably higher for early humans than it is today (*65-67*). The present low iron intake associated with a low-energy lifestyle implies that the interaction between different factors influencing iron absorption, will be more critical. For example, the interaction between calcium and iron absorption probably had no importance in the nutrition of early humans, who had a diet with ample amounts of both iron and calcium.

Iron balance and regulation of iron absorption

The body has three unique mechanisms for maintaining iron balance and preventing iron deficiency and iron overload. The first is the continuous re-utilisation of iron from catabolised erythrocytes in the body. When an erythrocyte dies after about 120 days, it is usually degraded by the macrophages of the reticular endothelium. The iron is released and delivered to transferrin in the plasma, which brings the iron back to red blood cell precursors in the

bone marrow or to other cells in different tissues. Uptake and distribution of iron in the body is regulated by the synthesis of transferrin receptors on the cell surface. This system for internal iron transport not only controls the rate of flow of iron to different tissues according to their needs but also effectively prevents the appearance of free iron and the formation of free radicals in the circulation.

The second mechanism is the access of the specific storage protein, ferritin, which can store and release iron to meet excessive iron demands. This iron reservoir is especially important in the third trimester of pregnancy.

The third mechanism involves the regulation of absorption of iron from the intestines, with an increased iron absorption in the presence of decreasing body iron stores and a decreased iron absorption when iron stores increase. Iron absorption decreases until an equilibrium is established between absorption and requirements. For a given diet this regulation of iron absorption, however, can only balance losses up to a certain critical point beyond which iron deficiency will develop (*68*). About half of the basal iron losses are from blood, primarily in the gastrointestinal tract. Both these losses and the menstrual iron losses are influenced by the haemoglobin level; during the development of an iron deficiency, menstrual and basal iron losses will successively decrease when the haemoglobin level decreases. In a state of more severe iron deficiency, skin iron losses may also decrease. Iron balance (absorption equals losses) may be present not only in normal subjects but also during iron deficiency and iron overload.

The three main factors that affect iron balance are absorption (intake and bio-availability of iron), losses, and amount in stores. The interrelationship among these factors was recently been described in mathematical terms, making it possible to predict, for example, the amount of stored iron when iron losses and bio-availability of dietary iron are known (69). With increasing iron requirements or decreasing bio-availability, the regulatory capacity to prevent iron deficiency is limited (68). However, to prevent iron overload with increasing dietary iron intake or bio-availability, the regulatory capacity seems to be extremely good (69).

Iron deficiency

Populations at risk for iron deficiency

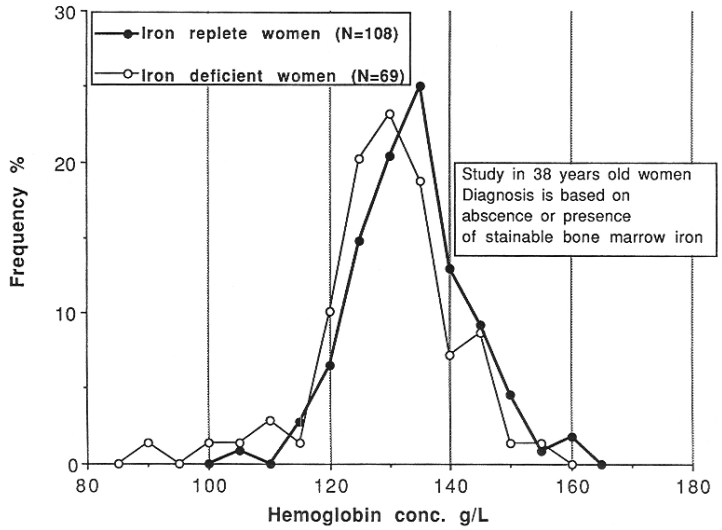
Worldwide, the highest prevalence of iron deficiency is found in infants, children, adolescents, and women of childbearing age, especially pregnant women. The weaning period in infants is especially critical because of the very high iron requirements in relation to energy requirements. Thanks to better information and access to fortified cereals for infants and children, the iron situation has markedly improved in these groups in most industrialized countries where the highest prevalences of iron deficiency today are observed in menstruating and pregnant women and adolescents of both sexes. In developing countries, however, the iron situation is very critical in many groups, especially in the weaning period. Iron nutrition is of great importance for the adequate development of the brain and other tissues such as muscles, which are finally differentiated early in life.

Iron deficiency and iron deficiency anaemia are often incorrectly used as synonyms. A definition of these terms may clarify some confusion about different prevalence figures given in the literature (*70*). The cause of the problem is the very wide distribution of the haemoglobin concentration in healthy, fully iron-replete subjects (in women, 120–160 g/l; in men, 140–180g/l [*71*]). During the development of a negative iron balance in subjects with no mobilisable iron from iron stores (no visible iron in technically perfect bone marrow smears or a serum ferritin concentration <15 µg/l), there will be an immediate impairment in the production of haemoglobin with a resulting decrease in haemoglobin and different erythrocyte

indexes (e.g., mean corpuscular haemoglobin and mean corpuscular volume). In turn this will lead to an overlap of the distributions of haemoglobin in iron-deficient and iron-replete women (***Figure 25***). The extent of overlap depends on the prevalence and severity of iron deficiency. In populations with more severe iron deficiency, for example, the overlap is much less marked.

Figure 25

Distribution of haemoglobin concentration in a sample of 38-year-old women with and without stainable bone marrow iron



Note: The main fraction (91 percent) of the iron-deficient women in this sample had haemoglobin levels above the lowest normal level for the population: 120 g/l (mean ± 2 SD) (*68*). The degree of overlap of the two distributions depends on the severity of anaemia in a population.

In women, anaemia is defined as a haemoglobin <120 g/l. For a woman who has her normal homeostatic value set at 150 g/l, haemoglobin level must decrease to 119 g/l (by 26 percent) before she is considered to be anaemic, whereas for a woman who has her normal haemoglobin set at 121 g/l, haemoglobin level must only decrease by 1.5 percent to 119 g/l. Iron deficiency anaemia is a rather imprecise concept for evaluating the single subject and has no immediate physiologic meaning. By definition, this implies that the prevalence of iron deficiency anaemia is less frequent than iron deficiency and that the presence of anaemia in a subject is a statistical rather than a functional concept. The main use of the cut-off value is in comparisons between population groups (*72*). In practical work, iron deficiency anaemia should be replaced by the functional concept of iron deficiency. Anaemia per se is mainly important when it becomes so severe that oxygen delivery to tissues is impaired. An iron deficiency anaemia which develops slowly in otherwise healthy subjects with moderately heavy work output will not give any symptoms until the haemoglobin level is about 80 g/l or lower (*71*). The reason for the continued use of the concept of iron deficiency anaemia is the ease of determining haemoglobin. Therefore, in clinical practice, knowledge of previous haemoglobin values in a subject is of great importance for evaluating the diagnosis.

Iron deficiency being defined as an absence of iron stores combined with signs of an iron-deficient erythropoiesis implies that in a state of iron deficiency there is an insufficient supply of iron to various tissues. This occurs at a serum ferritin level <15 µg/l. Iron can then no longer be mobilised from iron stores and insufficient amounts of iron will be delivered to

transferrin, the circulating transport protein for iron. The binding sites for iron on transferrin will therefore contain less and less iron. This is usually described as a reduction in transferrin saturation. When transferrin saturation drops to a certain critical level, erythrocyte precursors, which continuously need iron for the formation of haemoglobin, will get an insufficient supply of iron. At the same time, the supply of iron by transferrin to other tissues will also be impaired. Liver cells will get less iron, more transferrin will be synthesised, and the concentration of transferrin in plasma will then suddenly increase. Cells with a high turnover rate are the first ones to be affected (e.g., intestinal mucosal cells with a short life span). The iron-transferrin complex is bound to transferrin receptors on cell surfaces and the whole complex is then taken up by special receptors on the surface of various cells and tissues. The uptake of iron seems to be related both to transferrin saturation and the number of transferrin receptors on the cell surface (*73*, *74*). There is a marked diurnal variation in the saturation of transferrin because the turnover rate of iron in plasma is very high. This fact makes it difficult to evaluate the iron status from single determinations of transferrin saturation.

Indicators of iron deficiency

The absence of iron stores (iron deficiency) can be diagnosed by showing that there is no stainable iron in the reticuloendothelial cells in bone marrow smears or more easily by a low concentration of ferritin in serum (≤15 µg/l). Even if an absence of iron stores per se may not necessarily be associated with any immediate adverse effects, it is a reliable and good indirect indicator of iron-deficient erythropoiesis and of an increased risk of a compromised supply of iron to different tissues.

Even before iron stores are completely exhausted, the supply of iron to the erythrocyte precursors in the bone marrow is compromised, leading to iron-deficient erythropoiesis (*70*). A possible explanation is that the rate of release of iron from stores is influenced by the amount of iron remaining. As mentioned above it can then be assumed that the supply of iron to other tissues needing iron is also insufficient because the identical transport system is used. During the development of iron deficiency haemoglobin concentration, transferrin concentration, transferrin saturation, transferrin receptors in plasma, erythrocyte protoporphyrin, and erythrocyte indexes are changed. All these methods, however, show a marked overlap between normal and iron-deficient subjects, that makes it impossible to identify the single subject with mild iron deficiency by using any of these methods. Therefore, these tests have been used in combination (e.g., for interpreting results from the second National Health and Nutrition Examination Survey in the United States of America [*75*, *76*]). The diagnostic specificity then increases but the sensitivity decreases, and thus the true prevalence of iron deficiency is markedly underestimated if multiple diagnostic criteria are used. By definition in screening for iron deficiency, the more tests that are used the higher is the diagnostic specificity but the lower is the sensitivity of the procedure. Fortunately, a low serum ferritin, ≤15µg/l is always associated with an iron-deficient erythropoiesis. The use of serum ferritin alone as a measure will also underestimate the true prevalence of iron deficiency but to a lesser degree than when the combined criteria are used.

A diagnosis of iron deficiency anaemia can be suspected if anaemia is present in subjects who are iron-deficient as described above. Preferably, to fully establish the diagnosis, the subjects should respond adequately to iron treatment. The pitfalls with this method are the random variation in haemoglobin concentrations over time and the effect of the regression towards the mean when a new measurement is made.

The use of serum ferritin has improved the diagnostic accuracy of iron deficiency. It is the only simple method available to detect early iron deficiency. Its practical value is somewhat reduced, however, by the fact that serum ferritin is a very sensitive acute-phase

reactant and may be increased for weeks after a simple infection with fever for a day or two (*77*). Several other conditions, such as use of alcohol (*78*, *79*), liver disease, and collagen diseases, may also increase serum ferritin concentrations. Determination of transferrin receptors in plasma has also been recommended in the diagnosis of iron deficiency. Its advantage is that it is not influenced by infections. Its main use is in subjects who are already anaemic and it is not sensitive for the early diagnosis of iron deficiency. The use of a combination of determinations of serum ferritin and serum transferrin receptors has also been suggested (*80*).

Causes of iron deficiency

Nutritional iron deficiency implies that the diet cannot cover physiologic iron requirements. Worldwide this is the most common cause of iron deficiency. In many tropical countries, infestations with hookworms lead to intestinal blood losses that may be considerable. The severity of the infestations with hookworms varies considerably between subjects and regions. The average blood loss can be well estimated by egg counts in stools. Usually the diet in these populations is also limited with respect to iron content and availability.

In clinical practice a diagnosis of iron deficiency must always lead to a search for pathologic causes of blood loss (e.g., tumours in the gastrointestinal tract or uterus, especially if uterine bleedings have increased or changed in regularity). Patients with achlorhydria absorb dietary iron less well (a reduction of about 50 percent) and patients who have undergone gastric surgery, especially if the surgery was extensive, may eventually develop iron deficiency because of impaired iron absorption. Gluten enteropathy is another possibility to consider, especially in young patients.

Prevalence of iron deficiency

Iron deficiency is probably the most frequent nutritional deficiency disorder in the world. A recent estimate based on World Health Organization (WHO) criteria indicated that around 600-700 million people worldwide have a marked iron deficiency anaemia (*81*). In industrialized countries, the prevalence of iron deficiency anaemia is much lower and usually varies between 2 percent and 8 percent. However, the prevalence of iron deficiency, including both anaemic and non-anaemic subjects (see definitions above), is much higher. In industrialized countries, for example, an absence of iron stores or subnormal serum ferritin values is found in about 20–30 percent of women of fertile age. In adolescent girls the prevalence is even higher.

It is difficult to determine the prevalence of iron deficiency more exactly because representative populations for clinical investigation are hard to obtain. Laboratory methods and techniques for blood sampling need careful standardization. One often neglected source of error, for example, when materials in different regions or at different times are compared, is the fact that there are still reagent kits on the market for determining serum ferritin which are not adequately calibrated at different concentrations against the international WHO standards. In addition, seasonal variations in infection rates influence the sensitivity and specificity of most methods used.

Worldwide, the highest prevalence figures for iron deficiency are found in infants, children, teenagers, and women of childbearing age. Thanks to better information and access to fortified cereals for infants and children, the iron situation has markedly improved in these groups in most industrialized countries, where the highest prevalence today is observed in menstruating women and adolescents of both sexes.

In developing countries, where the prevalence of iron deficiency is very high and the severity of anaemia is marked, studies on the distribution of haemoglobin in different

population groups can provide important information as a valuable basis for action programmes (*72*). A more detailed analysis of subsamples may then give excellent information for the planning of more extensive programmes.

Effects of iron deficiency

Studies in animals have clearly shown that iron deficiency has several negative effects on important functions in the body (*3*). Physical working capacity in rats has been shown to be significantly reduced in iron deficiency, that is especially valid for endurance activities (*82*, *83*). This negative effect seems to be less related to the degree of anaemia than to impaired oxidative metabolism in the muscles with an increased formation of lactic acid, that in turn is due to a lack of iron-containing enzymes which are rate limiting for the oxidative metabolism (*84*).

The relationship between iron deficiency and brain function is of great importance for the choice of strategy in combating iron deficiency (*85-88*). Several structures in the brain have a high iron content of the same magnitude as observed in the liver. Of great importance is the observation that the lower iron content of the brain in iron-deficient growing rats cannot be increased by giving iron later on. This fact strongly suggests that the supply of iron to brain cells takes place during an early phase of brain development and that, as such, early iron deficiency may lead to irreparable damage to brain cells.

In humans about 10 percent of brain iron is present at birth; at the age of 10 years the brain has only reached half its normal iron content, and optimal amounts are first reached at the age of 20-30 years.

In populations with long-standing iron deficiency, a reduction of physical working capacity has been demonstrated by several groups with improvement in working capacity after iron administration (*84*).

Iron deficiency also negatively influences the normal defence systems against infections. The cell-mediated immunologic response by the action of T lymphocytes is impaired as a result of a reduced formation of these cells. This in turn is due to a reduced DNA synthesis depending on the function of ribonucleotide reductase, which requires a continuous supply of iron for its function. The phagocytosis and killing of bacteria by the neutrophil leukocytes is an important component of the defence mechanism against infections. These functions are impaired in iron deficiency. The killing function is based on the formation of free hydroxyl radicals within the leukocytes, the respiratory burst, and results from the activation of the iron-sulphur enzyme NADPH oxidase and probably also cytochrome b (a heme enzyme) (*89*).

The impairment of the immunologic defence against infections that was found in animals is also regularly found in humans. Administration of iron normalises these changes within 4–7 days. It has been difficult to demonstrate, however, that the prevalence of infections is higher or that their severity is more marked in iron-deficient subjects than in control subjects. This may well be ascribed to the difficulty in studying this problem with an adequate experimental design.

A relationship between iron deficiency and behaviour such as attention, memory, and learning, has been demonstrated in infants and small children by several groups. In the most recent well-controlled studies, no effect was noted from the administration of iron. This finding is consistent with the observations in animals. Therapy-resistant behavioural impairment and the fact that there is an accumulation of iron during the whole period of brain growth should be considered strong arguments for the more active and effective combating of iron deficiency. This is valid for women, especially during pregnancy, for infants and children, and up through the period of adolescence and early adulthood. In a recent well-

controlled study, administration of iron to non-anaemic but iron-deficient adolescent girls improved verbal learning and memory (*90*).

Well-controlled studies in adolescent girls show that iron-deficiency without anaemia is associated with reduced physical endurance (*91*) and changes in mood and ability to concentrate (*92*). A recent careful study showed that there was a reduction in maximum oxygen consumption in non-anaemic women with iron deficiency that was unrelated to a decreased oxygen-transport capacity of the blood (*93*).

Iron during pregnancy and lactation

Iron requirements during pregnancy are well established (***Table 42***). Most of the iron required during pregnancy is used to increase the haemoglobin mass of the mother, which occurs in all healthy pregnant women who have sufficiently large iron stores or who are adequately supplemented with iron. The increased haemoglobin mass is directly proportional to the increased need for oxygen transport during pregnancy and is one of the important physiologic adaptations that occurs in pregnancy (*94*, *95*). A major problem for iron balance in pregnancy is that iron requirements are not equally distributed over its duration. The exponential growth of the foetus implies that iron needs are almost negligible in the first trimester and that more than 80 percent relates to the last trimester. The total daily iron requirements, including the basal iron losses (0.8 mg), increase during pregnancy from 0.8 mg to about 10 mg during the last 6 weeks of pregnancy.

Iron absorption during pregnancy is determined by the amount of iron in the diet, its bio-availability (meal composition), and the changes in iron absorption that occur during pregnancy. There are marked changes in the fraction of iron absorbed during pregnancy. In the first trimester there is a marked, somewhat paradoxical, decrease in the absorption of iron, which is closely related to the reduction in iron requirements during this period as compared with the non-pregnant state (see below). In the second trimester iron absorption is increased by about 50 percent, and in the last trimester it may increase by up to about four times. Even considering the marked increase in iron absorption, it is impossible for the mother to cover her iron requirements from diet alone, even if its iron content and bio-availability are very high. It can be calculated that with diets prevailing in most industrialized countries, there will be a deficit of about 400–500 mg in the amount of iron absorbed during pregnancy (***Figure 26***).

An adequate iron balance can be achieved if iron stores of 500 mg are available. However, it is uncommon for women today to have iron stores of this size. It is therefore recommended that iron supplements in tablet form, preferably together with folic acid, be given to all pregnant women because of the difficulties in correctly evaluating iron status in pregnancy with routine laboratory methods. In the non-anaemic pregnant woman, daily supplements of 100 mg of iron (e.g., as ferrous sulphate) given during the second half of pregnancy are adequate. In anaemic women higher doses are usually required.

Table 42

Iron requirements during pregnancy

	Iron requirements (mg)
IRON REQUIREMENTS DURING PREGNANCY	
Foetus	300
Placenta	50
Expansion of maternal erythrocyte mass	450
Basal iron losses	240
Total iron requirement	1040
NET IRON BALANCE AFTER DELIVERY	
Contraction of maternal erythrocyte mass	+450
Maternal blood loss	-250
Net iron balance	+200
Net iron requirements for pregnancy if sufficient maternal iron stores are present (1040 - 200 = 840)	840

During the birth process, the average blood loss corresponds to about 250 mg iron. At the same time, however, the haemoglobin mass of the mother is gradually normalised, which implies that about 200 mg iron from the expanded haemoglobin mass (150–250 mg) is returned to the mother. To cover the needs of a woman after pregnancy, a further 300 mg of iron must be accumulated in the iron stores in order for the woman to start her next pregnancy with about 500 mg of stored iron. Such a restitution is not possible with present types of diets.

There is an association between low haemoglobin values and prematurity. An extensive study (*96*) showed that a woman with a hematocrit of 37 percent had twice the risk of having a premature birth as did a woman with a hematocrit between 41 percent and 44 percent ($P \leq 0.01$). A similar observation was reported in another extensive study in the United States of America (*97*). These materials were examined retrospectively and the cause of the lower hematocrit was not examined.

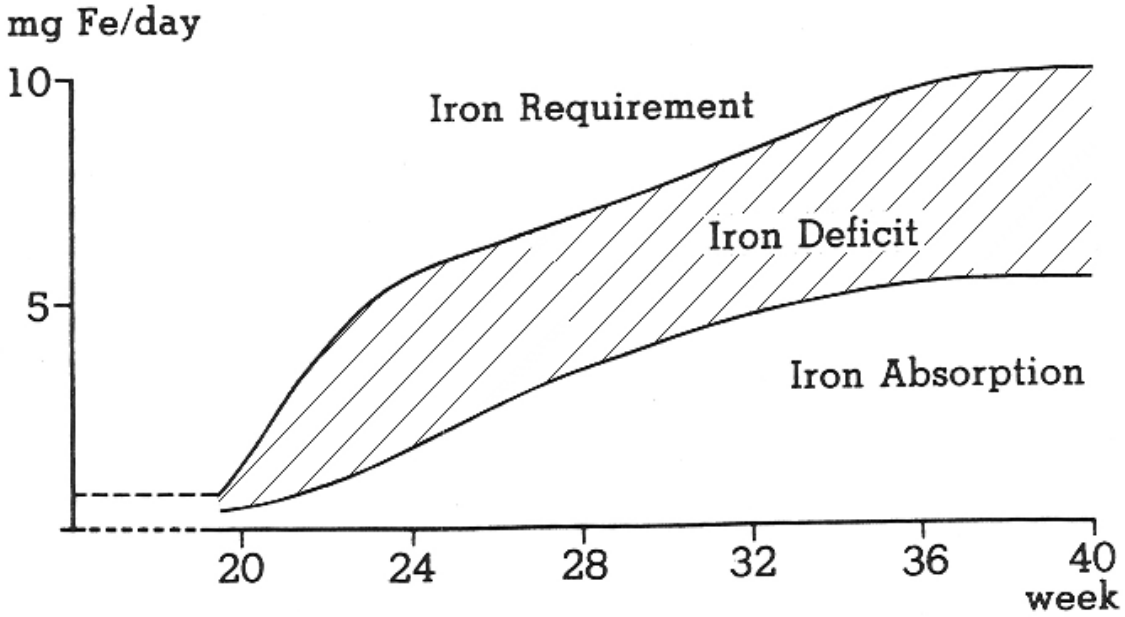
In lactating women, the daily iron loss in milk is about 0.3 mg. Together with the basal iron losses of 0.8 mg, the total iron requirements during the lactation period amount to 1.1 mg/day.

Early in pregnancy there are marked hormonal, haemodynamic, and haematologic changes. There is, for example, a very early increase in the plasma volume, which has been used to explain the physiologic anaemia of pregnancy observed also in iron-replete women. The primary cause of this phenomenon, however, is more probably an increased ability of the haemoglobin to deliver oxygen to the tissues (foetus). This change is induced early in pregnancy by increasing the content of 2, 3-diphospho-D-glycerate in the erythrocytes, which shifts the hemoglobin-oxygen dissociation curve to the right. The anaemia is a consequence of

this important adaptation and is not primarily a desirable change, for example, to improve placental blood flow by reducing blood viscosity.

Figure 26

Daily iron requirements and daily dietary iron absorption in pregnancy



Note: The hatched area represents the deficit of iron that has to be covered by iron from stores or iron supplementation.

Another observation has likewise caused some confusion about the rationale of giving extra iron routinely in pregnancy. In extensive studies of pregnant women, there is a U-shaped relationship between various pregnancy complications and the haemoglobin level (i.e., there are more complications at both low and high levels). There is nothing to indicate, however, that high haemoglobin levels (within the normal non-pregnant range) per se have any negative effects. The haemoglobin increase is caused by pathologic hormonal and hemodynamic changes induced by an increased sensitivity to angiotensin II that occurs in some pregnant women, leading to a reduction in plasma volume, hypertension, and toxaemia of pregnancy.

Pregnancy in adolescents presents a special problem because iron is needed to cover the requirements of growth. In countries with very early marriage, a girl may get pregnant before menstruating. The additional iron requirements for growth of the mother are then very high and the iron situation is very serious.

In summary, the marked physiologic adjustments occurring in pregnancy are not sufficient to balance its very marked iron requirements, and the pregnant woman has to rely on her iron stores, if present. The composition of the diet has not been adjusted to the present low-energy-demanding lifestyle in industrialized countries. This is probably the main cause of the critical iron-balance situation in pregnancy today, that is due to absent or insufficient iron stores in women before they get pregnant. The unnatural necessity to give extra nutrients such as iron and folate to otherwise healthy pregnant women should be considered in this perspective.

Iron supplementation and fortification

The prevention of iron deficiency has become even more urgent in recent years with the accumulation of evidence strongly suggesting a relationship between even mild iron deficiency and brain development and especially with the observation that functional defects affecting learning and behaviour cannot be reversed by giving iron later on. As mentioned, iron deficiency is common both in developed and in developing countries. Great efforts have been made by WHO to develop methods to combat iron deficiency.

Iron deficiency can generally be combated by one or more of the following three strategies: 1) iron supplementation (i.e., giving iron tablets to certain target groups such as pregnant women and preschool children); 2) iron fortification of certain foods, such as flour; and 3) food and nutrition education to improve the amount of iron absorbed from the diet by increasing the intake of iron and especially by improving the bio-availability of the dietary iron.

Several factors determine the feasibility and effectiveness of different strategies, such as the health infrastructure of a society, the economy, access to suitable vehicles for iron fortification, etc. The solutions are therefore often quite different in developing and developed countries. There is an urgency to obtain knowledge about the feasibility of different methods to improve iron nutrition and to apply present knowledge. In addition, initiation of local activities should be stimulated while actions from governments are awaited.

The evidence for estimating the recommended nutrient intake for iron

To translate physiologic iron requirements, given in ***Table 30***, into dietary iron requirements, the bio-availability of iron in different diets must be calculated. It is therefore necessary to choose an iron status where the supply of iron to the erythrocyte precursors and other tissues starts to be compromised. A state of iron-deficient erythropoiesis occurs when iron can no longer be mobilised from iron stores; iron can no longer be mobilised when stores are almost completely empty. A reduction then occurs, for example, in the concentration of haemoglobin and in the average content of haemoglobin in the erythrocytes (a reduction in mean corpuscular haemoglobin). At the same time the concentration of transferrin in the plasma increases because of an insufficient supply of iron to liver cells. These changes were recently shown to occur rather suddenly at a level of serum ferritin of ≤15 µg/l (*68*, *70*). A continued negative iron balance will further reduce the level of haemoglobin. Symptoms related to iron deficiency are less related to the haemoglobin level and more to the fact that there is a compromised supply of iron to tissues.

The bio-availability of iron in meals consumed in countries with a Western-type diet has been measured by using different methods. Numerous single-meal studies have shown absorption of non-heme iron ranging from 5 percent to 40 percent (*59*, *98*, *99*). Attempts have also been made to estimate the bio-availability of dietary iron in populations consuming Western-type diets by using indirect methods (e.g., calculation of the coverage of iron requirements in groups of subjects with known dietary intake). Such estimations suggest that in borderline iron-deficient subjects the bio-availability from good diets may reach a level around 14–16 percent (15 percent relates to subjects who have a serum ferritin value of <15µg/l or a reference dose absorption of 56.5 percent) (*19*).

Recently, direct measurements were made of the average bio-availability of iron in different Western-type diets (*22*, *43*, *60*). Expressed as total amounts of iron absorbed from the whole diet, it was found that 53.2 µg Fe/kg/day could be absorbed daily from each of the two main meals of an experimental diet which included ample amounts of meat or fish. For a body weight of 55 kg and an iron intake of 14 mg/day, this corresponds to a bio-availability of 21 percent in subjects with no iron stores and an iron-deficient erythropoiesis. A diet common

among women in Sweden contained smaller portions of meat and fish, higher phytates, and some vegetarian meals each week was found to have a bio-availability of 12 percent. Reducing the intake of meat and fish further will reduce the bio-availability to about 10 percent (25µg Fe/kg/day). In vegetarians the bio-availability is usually low because of the absence of meat and fish and a high intake of phytate and polyphenols. An average good Western-type whole diet has a bio-availability of about 15 percent but for common diets, especially among women, the bio-availability is around 12 percent or even 10 percent. In countries or for certain groups in a population with a very high meat intake, the bio-availability may rather be around 18 percent. In Western countries, a high bio-availability is mainly associated with a high meat intake, a high intake of ascorbic acid with meals, a low intake of phytate-rich cereals, and no coffee or tea within 2 hours of the main meals (*38*). ***Table 43*** shows examples of diets with different iron bio-availability.

Table 43

Examples of diet with different iron bio-availability

Type of diet	Bio-availability µg/kg/day
Preagricultural ancestors Plant/animal subsistence: 65/35	
Very high meat and ascorbic acid	150
Very high meat in 2 main meals daily and high ascorbic acid (theoretical)	75
High meat/fish in 2 main meals daily	66.7
Moderate meat/fish in 2 main meals daily	53.2
Moderate meat/fish in 2 main meals daily, low phytate and calcium	42.3
Meat/fish in 60% of 2 main meals daily, high phytate and calcium	31.4
Lower meat intake, high phytate. Often one main meal.	25
Meat/fish negligible, high phytate. High tannin, low ascorbic acid.	15

Table 44

Translation of bio-availability expressed as amount of iron absorbed into percent absorbed for two levels of iron intake

Bio-availability, µg/kg/day	Absorption as mg Fe at no iron stores in women of 55 kg body weight	Bio-availability %	
		15 mg	17 mg
150	8.25	55	48.8
75	4.13	27.5	24.4
66.7	3.67	24.5	21.8
53.2	2.93	19.5	17
42.3	2.32	15.5	13.5
31.4	1.73	11.5	10
25	1.38	9.2	8.2
15	0.83	5.5	4.7

Iron absorption data (***Table 44***) are also available from several population groups in Africa (*100*), South America (*101*), India, and Southeast Asia. The bio-availability of different Indian diets after an adjustment to a reference dose absorption of 56.5 percent was 1.7–1.8 percent for millet-based diets, 3.5–4.0 percent for wheat-based diets, and 8.3-10.3 percent for rice-based diets (*102*). In Southeast Asia, iron absorption data has been reported from Burma and Thailand. In Burma, iron absorption from a basal rice-based meal was 1.7

percent, when the meal contained 15 g of fish the bio-availability of iron was 5.5 percent, and with 40 g of fish it was 10.1 percent (*103*). In Thailand, iron absorption from a basal rice-based meal was 1.9 percent; adding 100 g of fresh fruit increased absorption to 4.8 percent and adding 80 g of lean meat increased non-heme iron absorption to 5.4 percent (*104*, *105*). In three other studies serving basal meals with vegetables rich in ascorbic acid, the absorption figures were 5.9 percent, 10 percent, and 10.8 percent, respectively (*106*). In a further study in Thailand, 60 g of fish were added to the same meal, which increased absorption to 21.6 percent. An even more realistic field study was done in Central Thailand to examine the reproducibility of dietary iron absorption measurements under optimal field conditions for 20 farmers and labourers (16 men, 4 women). The subjects had a free choice of foods (rice, vegetables, soup, a curry, and fish). All foods consumed were weighed and the rice was labelled with an extrinsic radioiron tracer. The mean absorption was 20.3 percent (adjusted to reference dose absorption of 56.5 percent) (*107*).

It is obvious that absorbed iron requirements need to be adjusted to different types of diets, especially in vulnerable groups. The Food and Agriculture Organization of the United Nations (FAO) and WHO recommended, for didactic reasons, three bio-availability levels of 5 percent, 10 percent, and 15 percent (8). For developing countries, it may be realistic to use the figures of 5 percent and 10 percent. In populations consuming more Western-type diets, two levels would be adequate – 12 percent and 15 percent – mainly depending on meat intake.

The amount of dietary iron absorbed is mainly determined by the amount of body stores of iron and by the properties of the diet (iron content and bio-availability). In anaemic subjects the rate of erythrocyte production also influences iron absorption. In a 55-kg woman with average iron losses who consumes a diet with an iron bio-availability of 15 percent, the mean iron stores would be about 120 mg. Under these circumstances approximately 10–15 percent of women would have no iron stores. When a diet with a bio-availability of 12 percent is consumed by a 55-kg woman, iron stores would be approximately 75 mg and about 25–30 percent of women would have no iron stores at all. When the bio-availability of iron decreases to 10 percent, mean iron stores are reduced to about 25 mg and about 40–50 percent of women consuming this diet would have no iron stores. Those consuming diets with an iron bio-availability of 5 percent have no iron stores and they are iron deficient. These calculations are based on a recent study (*69*).

Recommendations for iron intake for infants, children, younger and older adults, and pregnant and lactating women

Tables 39 and 40 showed both the physiologic absorbed iron requirements and the dietary iron requirements. All these figures are for the 95th percentile of iron requirements. The figures are given for women with a body weight of 55 kg and men with a body weight of 70 kg. For example, women with a body weight of 45 kg and men with a body weight of 55 kg have iron requirements that are 20 percent lower than those given in ***Table 39***.

No figures are given for dietary iron requirements in pregnant women because the iron balance in pregnancy depends not only on the properties of the diet but also and especially on the amounts of stored iron.

Future research

- Acquire knowledge of the content of phytate and iron-binding polyphenols in food, condiments, and spices. Produce new food tables, which include such data.
- Acquire knowledge about detailed composition of common meals and their usual variation in composition to examine the feasibility to make realistic recommendations

about changes in meal composition, taking into consideration the effect of such changes on other nutrients (e.g., vitamin A).

- Give high priority to systematic research. The very high iron requirements, especially in relation to energy requirements, in the weaning period make it difficult to develop foods and give recommendations that are effective and realistic. Alternatives such as home fortification of weaning foods should also be considered.
- Critically analyse the effectiveness of iron compounds used for fortification.
- Study models for improving iron supplementation, from the distribution of iron tablets to the increase of motivation to take iron supplements, especially during pregnancy.

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Chapter 14
Magnesium

Tissue distribution and functions of magnesium

The human body contains about 760 mg of magnesium at birth, approximately 5 g at age 4–5 months, and 25 g when adult (*1–3*). Of the body's magnesium, 30–40 percent is found in muscles and soft tissues, 1 percent is found in extracellular fluid, and the remainder is in the skeleton, where it accounts for up to 1 percent of bone ash (*4, 5*).

Soft tissue magnesium functions as a co-factor of many enzymes involved in energy metabolism, protein synthesis, RNA and DNA synthesis, and maintenance of the electrical potential of nervous tissues and cell membranes. Of particular importance with respect to the pathologic effects of magnesium depletion is the role of this element in regulating potassium fluxes and its involvement in the metabolism of calcium (*6–8*). Magnesium depletion depresses both cellular and extracellular potassium and exacerbates the effects of low-potassium diets on cellular potassium content. Muscle potassium becomes depleted as magnesium deficiency develops, and tissue repletion of potassium is virtually impossible unless magnesium status is restored to normal. Low plasma calcium develops frequently as magnesium status declines. It is not clear whether this occurs because parathyroid hormone release is inhibited or, more probably, because of a reduced sensitivity of the bone to parathyroid hormone, thus restricting withdrawal of calcium from the skeletal matrix.

Between 50 percent and 60 percent of body magnesium is located within bone, where it is thought to form a surface constituent of the hydroxyapatite (calcium phosphate) mineral component. Initially much of this magnesium is readily exchangeable with serum and therefore represents a moderately accessible magnesium store, which can be drawn on in times of deficiency. However, the proportion of bone magnesium in this exchangeable form declines significantly with increasing age (*9*).

Significant increases in bone mineral density of the femur have been associated positively with rises in erythrocyte magnesium when the diets of subjects with gluten-sensitive enteropathy were fortified with magnesium (*10*). Little is known of other roles for magnesium in skeletal tissues.

Origins and effects of magnesium deficiency

Pathologic effects of primary nutritional deficiency of magnesium occur infrequently in infants (*11*) but are even less common in adults unless a relatively low magnesium intake is accompanied by prolonged diarrhoea or excessive urinary magnesium losses (*12*). Susceptibility to the effects of magnesium deficiency rises when demands for magnesium increase markedly with the resumption of tissue growth during rehabilitation from general malnutrition (*6, 13*). Studies have shown that a decline in urinary magnesium excretion during protein-energy malnutrition (PEM) is accompanied by a reduced intestinal absorption of magnesium. The catch-up growth associated with recovery from PEM is achieved only if magnesium supply is increased substantially (*6, 14*).

Most of the early pathologic consequences of magnesium depletion are neurologic or neuromuscular defects (*12, 15*), some of which probably reflect the influence of the element on potassium flux within tissues. Thus, a decline in magnesium status produces anorexia,

nausea, muscular weakness, lethargy, staggering, and, if deficiency is prolonged, weight loss. Progressively increasing with the severity and duration of depletion are manifestations of hyperirritability, hyperexcitability, muscular spasms, and tetany, leading ultimately to convulsions. An increased susceptibility to audiogenic shock is common in experimental animals. Cardiac arrhythmia and pulmonary oedema frequently have fatal consequences (*12*). It has been suggested that a sub-optimal magnesium status may be a factor in the aetiology of coronary heart disease and hypertension but additional evidence is needed (*16*).

Dietary sources, absorption, and excretion of magnesium

Dietary deficiency of magnesium of a severity sufficient to provoke pathologic changes is rare. Magnesium is widely distributed in plant and animal foods, and geochemical and other environmental variables rarely have a major influence on its content in foods. Most green vegetables, legume seeds, peas, beans, and nuts are rich in magnesium, as are some shellfish, spices, and soya flour, all of which usually contain more than 500 mg/kg fresh weight. Although most unrefined cereal grains are reasonable sources, many highly refined flours, tubers, fruits, and fungi and most oils and fats contribute little dietary magnesium (<100 mg/kg fresh weight) (*17–19*). Corn flour, cassava and sago flour, and polished rice flour have an extremely low magnesium content. ***Table 45*** presents representative data for the dietary magnesium intakes of infants and adults.

Table 45

Typical daily intakes of magnesium by infants (6 kg) and adults (65 kg)

Group and source of intake (reference)	Magnesium intake, mg/day [a]
Infants: 750 ml liquid milk or formula as sole food source	
Human milk	
Finland (*17*)	24 (23-25)
United States (*11, 20*)	23 (18-30)
United Kingdom (*21, 22*)	21 (20-23)
India (*23*)	24 ± 0.9
Formula	
United States (*11, 20*)	30–52
United Kingdom (whey based) (*24*)	30–52
United Kingdom (soya based) (*24*)	38–60
Adults: conventional diets	
France, males (*25*)	369 ± 106
France, females (*25*)	280 ± 84
United Kingdom, males (*26*)	323
United Kingdom, females (*26*)	237
United States, males (*27, 28*)	329
United States, females (*27, 28*)	207
India (*29*)	300–680
China, females (*30*)	190 ± 59
	232 ± 62
	333 ± 103

[a] Mean ± SD or mean (range).

Stable isotope studies with ^{25}Mg and ^{26}Mg indicate that between 50 percent and 90 percent of the labelled magnesium from maternal milk and infant formula can be absorbed by infants (*11, 20*). Studies with adults consuming conventional diets show that the efficiency of magnesium absorption can vary greatly depending on magnesium intake (*31, 32*). In one study 25 percent of magnesium was absorbed when magnesium intake was high compared with 75 percent when intake was low (*33*). During a 14-day balance study a net absorption of 52 ± 8 percent was recorded for 26 adolescent females consuming 176 mg magnesium daily (*34*). Although this intake is far below the US recommended dietary allowance (RDA) for this age group (280 mg/day), magnesium balance was still positive and averaged 21 mg/day. This provided one of several sets of data illustrating the homeostatic capacity of the body to adapt to a wide variety of ranges in magnesium intake (*35, 36*). Magnesium absorption appears to be greatest within the duodenum and ileum and occurs by both passive and active processes (*37*).

High intakes of dietary fibre (40–50 g/day) lower magnesium absorption. This is probably attributable to the magnesium-binding action of phytate phosphorus associated with the fibre (*38-40*). However, consumption of phytate- and cellulose-rich products (usually containing high concentrations of magnesium) increases magnesium intake, which often compensates for the decrease in absorption. The effects of dietary components such as phytate on magnesium absorption are probably critically important only at low magnesium intake. There is no consistent evidence that modest increases in the intake of calcium (*34-36*), iron, or manganese (*22*) affect magnesium balance. In contrast, high intakes of zinc (142 mg/day) decrease magnesium absorption and contribute to a shift toward negative balance in adult males (*41*).

The kidney has a very significant role in magnesium homeostasis. Active reabsorption of magnesium takes place in the loop of Henle in the proximal convoluted tubule and is influenced by both the urinary concentration of sodium and probably by acid-base balance (*42*). The latter relationship may well account for the observation from Chinese studies that dietary changes which result in increased urinary pH and decreased titratable acidity also reduce urinary magnesium output by 35 percent despite marked increases in dietary magnesium input for vegetable protein diets (*30*). Several studies have now shown that dietary calcium intakes in excess of 2600 mg/day (*37*), particularly if associated with high sodium intakes, contribute to a shift toward negative magnesium balance or enhance its urinary output (*42, 43*).

Criteria for assessing magnesium requirements and allowances

In 1996 Shils and Rude (*44*) published a constructive review of past procedures used to derive estimates of magnesium requirements. They questioned the arguments of many authors that metabolic balance studies are probably the only practicable, non-invasive techniques for assessing the relationships of magnesium intake to magnesium status. At the same time, they emphasised the great scarcity of data on variations in urinary magnesium output and on magnesium levels in serum, erythrocytes, lymphocytes, bone, and soft tissues. Such data are needed to verify current assumptions that pathologic responses to a decline in magnesium supply are not likely occur to if magnesium balance remains relatively constant.

In view of the recent conclusion that many estimates of dietary requirements for magnesium were "based upon questionable and insufficient data" (*44*), a closer examination is needed of the value of biochemical criteria for defining the adequacy of magnesium status (*13*). Attention could be paid to the effects of changes in magnesium intake on urinary magnesium-creatinine ratios (*45*), the relationships between serum magnesium-calcium and magnesium-potassium concentrations (*7, 8*), and other functional indicators of magnesium status.

Estimated allowances of magnesium

The scarcity of studies from which to derive estimates of dietary allowances for magnesium has been emphasised by virtually all the agencies faced with this task. One United Kingdom agency commented particularly on the scarcity of studies with young subjects, and circumvented the problem of discordant data from work with adolescents and adults by restricting the range of studies considered (*21*). Using experimental data virtually identical to those used for a detailed critique of the basis for US estimates (27), the Scientific Committee for Food of the European Communities (*46*) did not propose magnesium allowances (or population reference intakes, PRIs) because of inadequate data. Instead, they offered an acceptable range of intakes for adults of 150–500 mg/day and described a series of quasi-PRI values for specific age groups, including an increment of 30 percent to allow for individual variations in growth. Statements of acceptable intakes leave uncertainty as to the extent of overestimation of derived recommended intakes.

It is questionable whether more reliable estimates of magnesium requirements can be made until data from balance studies are supported by the use of biochemical indexes of adequacy that could reveal the development of manifestations of sub-optimal status. Such indexes have been examined, for example, by Nichols *et al*. (*14*) in their studies of the metabolic significance of magnesium depletion during PEM. A loss of muscle and serum magnesium resulted if total body magnesium retention fell below 2 mg/kg/day and was followed by a fall in the myofibrillar nitrogen-collagen ratio of muscle and a fall in muscle potassium content. Repletion of tissue magnesium status preceded a threefold increase in muscle potassium content. It accelerated by 7–10 days at the rate of recovery of muscle mass and composition initiated by restitution of nitrogen and energy supplies to infants previously deficient.

Neurologic signs such as hyper-irritability, apathy, tremors, and occasional ataxia accompanied by low concentrations of potassium and magnesium in skeletal muscle and strongly negative magnesium balances were reported by many other studies of protein calorie deficiency in infants (*47–49*). Particularly noteworthy is evidence that all these effects are ameliorated or eliminated by increased oral magnesium, as were specific anomalies in the electrocardiographic T-wave profiles of such malnourished subjects (*49*). Evidence that the initial rate of growth at rehabilitation is influenced by dietary magnesium intake indicates the significance of this element for those involved in the aetiology of the PEM syndromes (*31, 50*).

Regrettably, detailed studies have yet to be carried out to define the nature of changes resulting from a primary deficiency of dietary magnesium. Definition of magnesium requirements must continue to be based on the limited information provided by balance techniques, which give little or no indications of responses to inadequacy in magnesium supply which may induce covert pathologic changes. Reassurance must thus be sought from the application of dietary standards for magnesium in communities consuming diets differing widely in magnesium content (*29*). The inadequate definition of lower acceptable limits of magnesium intakes raises concern in communities or individuals suffering malnutrition or from a wider variety of nutritional or other diseases which influence magnesium metabolism adversely (*12, 51, 52*).

Derivation of allowances for magnesium

The infrequency with which magnesium deficiency develops in human-milk-fed infants implies that the content and physiologic availability of magnesium in human milk meets the infants' requirements. The intake of maternal milk from exclusively human-milk-fed infants 1–10 months of age ranges from 700 to 900 g/day in both industrialized and developing

countries (*53*). If the magnesium content of milk is assumed to be 29 mg/l (*11, 54, 55*), the intake from milk is 20–26 mg/day, or approximately 0.04 mg/kcal.

The magnesium in human milk is absorbed with substantially greater efficiency (about 80–90 percent) than that of formula milks (about 55–75 percent) or solid foods (about 50 percent) (*56*), and such differences must be taken into account when comparing differing dietary sources. For example, a daily intake of 23 mg from maternal milk probably yields 18 mg available magnesium, a quantity similar to that of the 36 mg or more suggested as meeting the requirements of young infants given formula or other foods (***Table 46***).

An indication of a likely requirement for magnesium at other ages can be derived from studies of magnesium-potassium relationships in muscle (*58*) and the clinical recovery of young children rehabilitated from malnutrition with or without magnesium fortification of therapeutic diets. Nichols *et al.* (*14*) showed that 12 mg magnesium/day was not sufficient to restore positive magnesium balances, serum magnesium content, or the magnesium and potassium contents of muscle of children undergoing PEM rehabilitation. Muscle potassium was restored to normal by 42 mg magnesium/day but higher intakes of dietary magnesium, up to 160 mg/day, were needed to restore muscle magnesium to normal. Although these studies show clearly that magnesium synergized growth responses resulting from nutritional rehabilitation, they also indicated that rectification of earlier deficits of protein and energy was a pre-requisite to initiation of this effect of magnesium.

Similar studies by Caddell *et al.* (*49, 50*) also illustrate the secondary significance of magnesium accelerating clinical recovery from PEM. They indicate that prolonged consumption of diets low in protein and energy and with a low ratio (<0.02) of magnesium (in milligrams) to energy (in kilocalories) can induce pathologic changes which respond to increases in dietary magnesium supply. It is noteworthy that of the balance trials intended to investigate magnesium requirements, none has yet included treatments with magnesium-energy ratios of <0.04 or induced pathologic responses.

The relationship Mg = (kcal x 0.0099) - 0.0117 (SE ± 0.0029) holds for many conventional diets (*59*). Some staple foods in common use have very low magnesium contents; cassava, sago, corn flour or cornstarch, and polished rice all have low magnesium-energy ratios (0.003–0.02) (*18*). Their use in bulk merits appraisal of total dietary magnesium content.

It has been reported, with increasing frequency, that high percents (e.g., <70 percent) (*25*) of individuals from some communities in Europe have magnesium intakes substantially lower than estimates of magnesium requirements derived principally from US and UK sources (*21, 27*). Such reports emphasise the need for reappraisal of estimates for reasons previously discussed (*44*).

The estimates submitted by this Consultation must be regarded as provisional. Until additional data become available, these estimates reflect consideration of anxieties that previous recommendations for magnesium are overestimates. They make greater allowance for developmental changes in growth rate and in protein and energy requirements. In reconsidering data cited in previous reports (*21, 27, 46*), particular attention has been paid to balance data suggesting that the experimental conditions established have provided reasonable opportunity for the development of equilibrium during the investigation (*34, 60-62*).

Recommended magnesium intakes are presented in ***Table 46*** together with indications of the relationships of each recommendation to relevant estimates of the average requirements for dietary protein, and energy (*19*).

Table 46

Recommended nutrient intakes for magnesium (Mg) in milligrams (mg)

Age Group[a]	Assumed body weight kg[b]	RNI mg/day	Relative intake ratios Mg/kg	Mg/g protein	Mg/kcal/day
Infants and children					
0–6 months					
Human-milk fed	6	26		2.5	0.05
Formula fed	6	36	6.0	2.9	0.06
7–12 months	9	54	6.0	3.9	0.06
1–3 years	12	60	5.5	4.0	0.05
4–6 years	19	76	4.0	3.9	0.04
7–9 years	25	100	4.0	3.7	0.05
Adolescents, 10–18 years					
Females	49	220	4.5	5.2	0.10
Males	51	230	3.5	5.2	0.09
Adults, 19–65 years					
Females	55	220	4.0	4.8	0.10
Males	65	260	4.0	4.6	0.10
65+ years					
Females	54	190	3.5	4.1	0.10
Males	64	224	3.5	4.1	0.09

[a] No increment for pregnancy; 50 mg/day increment for lactation.
[b] Assumed body weights of age groups derived by interpolation (*57*).
[c] Intake per gram of recommended protein intake for age of subject (*21*).
[d] Intake per kilocalorie estimated average requirement (*21*).

The detailed studies of magnesium economy during malnutrition and subsequent therapy, with or without magnesium supplementation, provide reasonable grounds that the dietary magnesium recommendations derived herein for young children are realistic. Data for other ages are more scarce and are confined to magnesium balance studies. Some have paid little attention to the influence of variations in dietary magnesium content and of the effects of growth rate before and after puberty on the normality of magnesium-dependent functions.

It is assumed that during pregnancy the foetus accumulates 8 mg and foetal adnexa accumulate 5 mg magnesium. If it is assumed that this dietary magnesium is absorbed with 50 percent efficiency, the 26 mg required over a pregnancy of 40 weeks (0.09 mg/day) can probably be accommodated by adaptation. A lactation allowance of 50–55 mg/day for dietary magnesium is made for the secretion of milk containing 25–28 mg magnesium (*21 ,64*). An absorption efficiency of 50 percent is assumed for all solid diets; data are not sufficient to allow for the adverse influence of phytic acid on magnesium absorption from high-fibre diets or from diets with a high content of pulses. Not surprisingly, few of the representative dietary analyses presented in ***Table 45*** fail to meet these allowances. The few exceptions, deliberately selected for inclusion, are the marginal intakes (232 ± 62 mg) of the 168 women of Changle county and the lower intake (190 ± 59 mg) of 147 women surveyed from Tuoli county China (*30*).

Upper tolerable limits of magnesium intake

Magnesium from dietary sources is relatively innocuous. Contamination of food or water supplies with magnesium salt has been known to cause hypermagnesemia, nausea, hypotension, and diarrhoea. Intakes of 380 mg magnesium as magnesium chloride have

produced such signs in women. Upper limits of 65 mg for children ages 1–3 years, 110 mg for 4–10 years, and 350 mg for adolescents and adults are suggested as tolerable limits for the content of soluble magnesium in foods and drinking water (*63*).

Relationships to previous estimates

The recommended intakes for infants ages 0–6 months take account of differences in the physiologic availability of magnesium from maternal milk as compared with infant formulas or solid foods. With the exception of the Canadian RNI estimates, which are 20 mg/day for ages 0–4 months and 32 mg/day for ages 5–12 months (*64*), other national estimates recommend intakes as RDAs or RNIs which substantially exceed the capacity of the lactating mother to supply magnesium for her offspring.

Recommendations for other ages are based subjectively on the absence of any evidence that magnesium deficiency of nutritional origin has occurred after consumption of a range of diets sometimes supplying considerably less than the US RDA or the UK RNI recommendations based on estimates of average magnesium requirements of 3.4–7 mg/kg body weight. The recommendations submitted herewith assume that demands for magnesium plus a margin of approximately 20 percent (to allow for methodologic variability) are probably met by allowing approximately 3.5–5 mg/kg from pre-adolescence to maturity. This assumption yields estimates virtually identical to those for Canada. Expressed as magnesium allowance (in milligrams) divided by energy allowance (in kilocalories) (the latter based upon energy recommendations from UK estimates (*21*), all the recommendations of ***Table 46*** exceed the provisionally estimated critical minimum ratio of 0.02.

It is appreciated that magnesium demand probably declines in late adulthood as requirements for growth diminish. However, it is reasonable to expect that the efficiency with which magnesium is absorbed declines in elderly subjects. It may well be that the recommendations are overgenerous for elderly subjects, but data are not sufficient to support a more extensive reduction than that indicated.

Future research

There is need for closer investigation of the biochemical changes that develop as magnesium status declines. The responses to magnesium intake which influence the pathologic effects resulting from disturbances in potassium utilisation caused by low magnesium should be studied. They may well provide an understanding of the influence of magnesium status on growth rate and neurologic integrity.

Closer investigation of the influence of magnesium status on the effectiveness of therapeutic measures during rehabilitation from PEM is needed. The significance of magnesium in the aetiology and consequences of PEM in children needs to be clarified. Claims that restoration of protein and energy supply aggravates the neurologic features of PEM if magnesium status is not improved merit priority of investigation. Failure to clarify these aspects may continue to obscure some of the most important pathologic features of a nutritional disorder in which evidence already exists for the involvement of a magnesium deficit.

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Chapter 15
Selenium

The role of selenium in human metabolic processes

Our understanding of the significance of selenium in the nutrition of human subjects has grown rapidly during the past 20 years (*1, 2*). Demonstrations of its essentiality to rats and farm animals were followed by appreciation that the development of selenium-responsive diseases often reflected the distribution of geochemical variables which restricted the entry of the element from soils into food chains. Such findings were the stimulus to in-depth investigations of the regional relevance of selenium in human nutrition (*3*). These studies have now yielded an increased understanding of the complex metabolic role of this trace nutrient. Selenium has been implicated in the protection of body tissues against oxidative stress, maintenance of defences against infection, and modulation of growth and development.

The selenium content of normal adult humans can vary widely. Values from 3 mg in New Zealanders to 14 mg in some Americans reflect the profound influence of the natural environment on the selenium contents of soils, crops, and human tissues. Approximately 30 percent of tissue selenium is contained in the liver, 15 percent in kidney, 30 percent in muscle, and 10 percent in blood plasma. Much of tissue selenium is found in proteins as seleno-analogues of sulphur amino acids; other metabolically active forms include selenotrisulphides and other acid-labile selenium compounds. At least 15 selenoproteins have now been characterised. Examples are given in ***Table 47***.

Table 47

A selection of characterised selenoproteins

Protein	Selenocysteine residues	Tissue distribution
Cytosolic glutathione peroxidase (GSHPx)	1	All, including thyroid
Phospholipid hydroperoxide GSHPx	1	All, including thyroid
Gastrointestinal GSHPx	1	Gastrointestinal tract
Extracellular GSHPx	1	Plasma, thyroid
Thioredoxin reductase	1 or 2	All including thyroid
Iodothyronine-deiodinase (type 1)	1	Liver, kidneys, and thyroid
Iodothyronine-deiodinase (type 2)	1	Central nervous system (CNS), and pituitary
Iodothyronine-deiodinase (type 3)	1	Brown adipose tissue, CNS, and placenta
Selenoprotein P	10	Plasma
Selenoprotein W	1	Muscle
Sperm capsule selenoprotein	3	Sperm tail

Functionally, there appear to be at least two distinct families of selenium-containing enzymes. The first includes glutathione peroxidases (*4*) and thioredoxin reductase (*5*), which are involved in controlling tissue concentrations of highly reactive oxygen-containing metabolites. These metabolites are essential at low concentrations for maintaining cell-mediated immunity against infections but highly toxic if produced in excess. The role of selenium in the cytosolic enzyme glutathione peroxidase (GSHPx) was first illustrated in 1973. During stress, infection, or tissue injury, selenoenzymes may protect against the damaging effects of hydrogen peroxide or oxygen-rich free radicals. This family of enzymes catalyses the destruction of hydrogen peroxide or lipid hydroperoxides according to the following general reactions:

$$H_2O_2 + 2GSH \rightarrow 2H_2O + GSSG$$

$$ROOH + 2GSH \rightarrow ROH + H_2O + GSSG$$

where GSH is glutathione and GSSG is its oxidized form. At least four forms of GSHPx exist; they differ both in their tissue distribution and in their sensitivity to selenium depletion (*4*). The GSHPx enzymes of liver and blood plasma fall in activity rapidly at early stages of selenium deficiency. In contrast, a form of GSHPx associated specifically with phospholipid-rich tissue membranes is preserved against selenium deficiency and is believed to have broader metabolic roles (e.g., in prostaglandin synthesis) (*6*). In concert with vitamin E, selenium is also involved in the protection of cell membranes against oxidative damage (see ***Chapter 6***, ***Chapter 9***, and ***Chapter 17***).

The selenoenzyme thioredoxin reductase is involved in disposal of the products of oxidative metabolism (*5*). It contains two selenocysteine groups per molecule and is a major component of a redox system with a multiplicity of functions, among which is the capacity to degrade locally excessive and potentially toxic concentrations of peroxide and hydroperoxides likely to induce cell death and tissue atrophy (*6*).

Another group of selenoproteins is essential in the conversion of thyroxin, or tetraiodothyronine (T_4), to its physiologically active form, triiodothyronine (T_3) (*7*). Three types of these iodothyronine deiodinases, differing both in tissue distribution and sensitivity to selenium deficiency, have been characterised. The consequences of a low selenium status on physiologic responses to a shortage of iodine are complex. The influence of a loss of selenium-dependent iodothyronine deiodinase differs in its severity depending on whether a target tissue needs a preformed supply of T_3 (e.g., via plasma) or whether, as with the brain, pituitary gland, and placenta, it can rely upon local synthesis of T_3 from T_4. Despite this, marked changes in the T_3-T_4 ratio as a consequence of a reduced selenium status (when iodine supplies are also marginal) indicate the modifying influence of selenium on thyroid hormone balance in both animal models and human subjects. Their possible significance can be anticipated from the fact that whereas thyroid weights increase typically by 50 percent in rats offered an iodine-deficient diet, thyroid weight is increased 154 percent by diets concurrently deficient in both selenium and iodine.

Between 60 percent and 80 percent of selenium in human plasma is accounted for by a well-characterised fraction designated selenoprotein P, the function of which has yet to be determined. It is thought to be a selenium storage protein because there is limited evidence that it also has an antioxidant role. At least 10 other selenoproteins exist, including one which is a component of the mitochondrial capsule of sperm cells, damage to which may account for the development of sperm abnormalities during selenium deficiency. Other aspects of the function and metabolism of selenium are reviewed elsewhere (*8*, *9*).

Selenium deficiency

Non-endemic deficiencies of selenium

Biochemical evidence of selenium depletion (e.g., a decline in blood GSHPx activity) is not uncommon in subjects maintained on parenteral or enteral feeding for long periods. Blood selenium values declining to one-tenth of normal values have been reported when the selenium content of such preparations has not been maintained by fortification (*10, 11*). Low selenium contents of some commercial formulas for infants resulting in a fall in daily selenium intake to approximately 0.5 µg/day have been shown to strongly exacerbate the fall in serum selenium and GSHPx activity normally experienced from 2 to 8 months of age even in human-milk-fed infants typically receiving threefold higher selenium intakes (*12, 13*). The importance of maintaining trace element levels in such preparations was reviewed elsewhere (*14*).

Clinical manifestations of deficiency arising from such situations are uncommon and poorly defined. They include muscular weakness and myalgia with, in several instances, the development of congestive heart failure. In at least one instance such pathologic signs have developed as a consequence of a generally inadequate diet providing selenium at less than 10 µg/day. The 2-year-old subject recovered rapidly after selenium administration (*15*). With this last exception, virtually all of the above reports describe observations with subjects under close medical supervision. This may well be relevant to the scarcity of consistent pathologic findings (*16*).

Keshan disease

Keshan disease was first described in Chinese medical literature more than 100 years ago, but not until 40 years after its widespread occurrence in 1935 was it discovered that selenium deficiency was an important factor in its aetiology (*3*). Endemic in children aged 2–10 years and in women of childbearing age, this disease has a geographic distribution covering localities from northeast to southwest China. Typical manifestations are fatigue after even mild exercise, cardiac arrhythmia and palpitations, loss of appetite, cardiac insufficiency, cardiomegaly, and congestive heart failure. Pathologic changes include a multifocal myocardial necrosis and fibrosis. The coronary arteries are essentially unaffected. Ultrastructural studies show that membranous organelles, such as mitochondria or sarcolemma, are affected earliest. The disease has a marked seasonal fluctuation in incidence (*3*) and may appear after only 3 months exposure to conditions in localities known to be associated with a high risk of myocarditis (*3, 8*). Once the disease is established, selenium is of little or no therapeutic value. Prophylaxis consisting of oral administration of selenium 3 months before the periods of highest anticipated risk is highly effective.

Although geographic similarities in the distribution of Keshan disease and the selenium and vitamin E–responsive white muscle disease in animals first prompted successful investigation of the relevance of a low selenium status, evidence has grown steadily that the disease is multifactorial in origin. The strongest suspicions have fallen on the development of a viral myocarditis probably attributable to enhancement of the virulence of a coxsackievirus during its passage through selenium-deficient host tissues (*17*). Although other nutritional variables such as a marginal vitamin E status may also be involved, the finding of extremely low selenium contents in staple crops of affected areas and convincing demonstrations of the prophylactic effectiveness of selenium administration leave no doubt that selenium deficiency is the primary factor (*3*, *18*).

Recent studies indicate that geochemical variables have an important influence on the distribution of Keshan disease. Acid soils high in organic matter and iron oxide content appear to be responsible for fixing selenium in forms which are poorly absorbed by staple

crops which, in the instance of cereal grains, typically have a selenium content of less than 0.01 μg/g (*19*). A similar geochemical background is believed to be associated with reports of selenium-responsive disorders resembling Keshan disease in the Transbaikalia region of south Siberia. Dietary intakes of selenium are inadequate to maintain blood GSHPx activity; biochemical indicators of tissue peroxidative damage are elevated until selenium therapy is initiated (*8*).

Kaschin-beck disease

A selenium-responsive bone and joint disease (osteoarthropathy) has been detected in children aged 5–13 years in China and less extensively in south-east Siberia. The disease is characterised by joint necrosis – epiphyseal degeneration of the arm and leg joints resulting in structural shortening of the fingers and long bones with consequent growth retardation and stunting (*3*, *20*). Although not identical to Keshan disease, Kaschin-Beck disease also occurs in areas where the availability of soil selenium for crop growth is low. The selenium contents of hair and of whole blood are abnormally low and the blood content of GSHPx is reduced. Although it is ameliorated by selenium therapy, other factors such as the frequent presence of mycotoxins in cereal grains grown in the area may be involved. A spontaneous decrease in incidence from 1970 (44 percent) to 1980 (14 percent) to 1986 (1 percent) has been attributed to general improvements in the nutritional status of Chinese rural communities (*20*).

Selenium status and susceptibility to infection

As stated earlier, the expressions of the cardiac lesions of Keshan disease probably involve not only the development of selenium deficiency but also the presence of a Coxsackie virus (BA) infection. Animal studies have confirmed that selenium-deficient mice infected with Coxsackie virus (CVB/0) were particularly susceptible to the virus. These studies also illustrated that passage of the virus through selenium-deficient subjects enhanced its virulence (*17*). Myocarditic virulence developed even in strains such as CVB/0 which normally were not myopathogenic. The enhancement of virulence in this RNA virus involves modifications to the nucleotide sequence of the phenotype. These modifications were maintained and expressed even during subsequent passage through animals with normal selenium status (*21*).

Enhancing the virulence of a virus with a selenium deficiency (resulting either from a nutritional challenge or an increased metabolic demand on tissue selenium depots) appears not to be unique to the Coxsackie viruses. The early pre-clinical stages of development of human immunodeficiency virus (HIV) infection are accompanied by a very marked decline in plasma selenium. Sub-clinical malnutrition assumes increased significance during the development of acquired immune deficiency syndrome (AIDS). However, for the nutrients affected, there are strong indications that only the extent of the decline in selenium status has predictive value with respect to both the rate of development of AIDS and its resulting mortality (*22-25*). The virulence of other RNA viruses such as hepatitis B and those associated with the development of haemolytic anaemias are enhanced similarly by a decline in selenium status. The mechanisms underlying these effects are not yet resolved. There are indications that the loss of protective antioxidant functions dependent on selenium and vitamin E are both involved and that the resulting structural changes in viral nucleotide sequences are reproducible and appear to provoke additional selenoprotein synthesis (*26*). It is suspected that this further depletes previously diminished pools of physiologically available selenium and accelerates pathologic responses (*27-29*).

Whatever mechanisms are involved, further understanding is needed of the influence of selenium status on susceptibility to viral diseases ranging from cardiomyopathies to haemolytic anaemias. The relationship already illustrates the difficulty of defining nutritional essentiality for nutrients which may primarily maintain defences against infection. Studies of

the effects of selenium deficiency in several experimental animal species have shown that the microbicidal activity of blood neutrophils is severely impaired even though phagocytic activity remains unimpaired (*30, 31*). The complexity of species differences in the influence of selenium status on the effectiveness of cell-mediated immune processes is summarised elsewhere (*8*).

The possibility that increased intakes of selenium might protect against the development of cancer in humans has generated great interest (*32*). However, a number of epidemiologic studies have now been reported which show no relationship between selenium and cancer risk (*33*). Moreover, an analysis of the relationship between selenium and cancer suggests that "the question of whether selenium protects against cancer is still wide open" (*34*). An increased intake of selenium appears to stimulate tumorigenesis in some animal models of pancreatic and skin cancer. In contrast, the protective effect of higher exposures to selenium observed in several animal studies, together with small but statistically significant differences in selenium blood plasma levels detected in some retrospective-prospective studies of subgroups of people developing cancer, explains the continuing interest in the anticarcinogenic potential of selenium. However, the results of prospective-retrospective studies had no predictive value for individuals and could have reflected non-specific influences on groups. The association between low selenium intake and high cancer risk, although clearly of some interest, is in need of further investigation before a conclusion can be reached.

Although a biochemical mechanism can be postulated whereby selenium could protect against heart disease by influencing platelet aggregation (through an effect on the prostacyclin-thromboxane ratio), the epidemiologic evidence linking selenium status and risk of cardiovascular disease is still equivocal (*33*).

Selenium and thyroid hormones

The importance of selenium for thyroid hormone metabolism (*35, 36*) is evident from changes in the T_3-T_4 ratio which develop after relatively mild selenium depletion in infants and elderly (65+ years) subjects. Decreases in the T_3-T_4 ratio indicative of decreased thyroid hormone balance have been detected when serum selenium falls below 0.9 μmol/l (*37*). In a recent Scottish study these decreases were correlated with a decline in dietary and plasma selenium after the replacement of selenium-rich wheat from Canada and the United States with selenium-deficient wheat from European sources (*38*).

Communities noted for a high incidence of myxedematous cretinism have been found to have low plasma selenium status and GSHPx activity in addition to having low iodine status (*39*) and being exposed to high thiocyanate intakes from cassava. Restoration of iodine supply, particularly if excessive, tends to induce a high peroxidative stress through the action of iodide peroxidase, the first step in iodine utilisation by the thyroid. It is postulated that necrosis and thyroid fibrosis leading to irreversible hypothyroidism result if a concurrent deficiency of selenium limits peroxide destruction by the protective action of the selenium-dependent enzymes, GSHPx and, more probably, thioredoxin reductase (*40*). In areas where myxedematous cretinism is endemic and characterised by persistent hypothyroidism, dwarfism, and stunting, it has been recommended that attempts to introduce iodine therapy for mildly affected individuals should be preceded by an assessment of selenium status and rectification of any observed deficit (*39*). Although this suggestion is compatible with pathologic observations on hypothyroid rats differing in selenium status, its validity has yet to be assessed adequately in humans (*41*, *42*).

The influence of diet on selenium status

Environmental conditions and agricultural practices have a profound influence on the selenium content of many foods. ***Tables 48, 49*** *and* ***50*** illustrate the wide range of selenium content of the principal food groups and the variability in the selenium content of individual foods. This variability is exceeded only by that found in the iodine content of foods. Geographic differences in the content and availability of selenium from soils to food crops and animal products have a marked effect on the selenium status of entire communities. For example, the distribution of Keshan disease and Kaschin-Beck disease in China reflects the distribution of soils from which selenium is poorly available to rice, maize, wheat, and pasture grasses (***Table 48***). Cereal crop selenium contents of 3–7 ng/g are not uncommon (*3*) and it has been suggested that <10 ng/g for grain selenium and <3 ng/g for water-soluble soil selenium could be used as indexes to define deficient areas (*19*). Fluctuations in the selenium status of many communities in northern Europe reflect the intrinsically low selenium content of its glacial soils and the extent to which selenium supplementation of fertilisers has been successful in increasing the selenium content of cereal grains, milk, and other animal products. Deliberate importation of cereals from areas with relatively high available selenium in soil has also occurred or been recommended in some areas of Finland, New Zealand, and the United Kingdom after steady declines in the selenium status of some communities were noted. Conversely, low-selenium grains are being selected in China, India, and Venezuela to reduce the risks of selenosis.

Comprehensive data summarising the selenium contents of staple foods are available elsewhere (e.g., *45*). Reports from the Food and Agriculture Organization of the United Nations (FAO) and the International Atomic Energy Agency (IAEA) provide representative data on daily total selenium intakes for more than 40 countries (see reference *8*, p 215–217). The great influence of dietary and geographic variables on selenium status is evident from recent summaries of data describing national and regional differences for the selenium content of human and formula milks, diets, and human serum (***Tables 48, 49, 50*** *and* ***51***).

Absorption and bio-availability

Selenium compounds are generally very efficiently absorbed by humans, and selenium absorption does not appear to be under homeostatic control (*80*). For example, absorption of the selenite form of selenium is greater than 80 percent whereas that of selenium as selenomethionine or as selenate may be greater than 90 percent (*80, 81*). Therefore, the rate-limiting step determining the overall availability of dietary selenium is not likely to be its absorption but rather its conversion within tissues to its metabolically active forms (e.g., its incorporation into GSHPx or 5'-deiodinase) (*40*). A number of depletion-repletion experiments have been carried out on animals to estimate the bio-availability of selenium in human foods (*82*). Based on the restoration of GSHPx activity in depleted rats, the bio-availability of selenium in wheat is quite good, usually 80 percent or better. The selenium in Brazil nuts and beef kidney also appears readily available (90 percent or more by most criteria). The selenium in tuna seems of lesser availability (perhaps only 20–60 percent of that from selenite) whereas the availability of selenium from certain other seafoods (shrimp, crab, and Baltic herring) is high. The selenium in a variety of mushrooms appears to be of uniformly low availability to rats.

Data on the nutritional bio-availability of selenium to humans are sparse. A supplementation study carried out on Finnish men of relatively low selenium status showed that selenate selenium was as effective as the selenium in seleniferous wheat in increasing platelet GSHPx activity (*83*). The wheat selenium, however, increased plasma selenium levels more than did selenate selenium and once the supplements were withdrawn, platelet GSHPx activity declined less in the group given wheat. This study showed the importance of

estimating not only short-term availability but also long-term retention and the convertibility of tissue selenium stores into biologically active forms.

Table 48

The selenium contents of foods and diets

A. Typical ranges of selenium concentrations (ng/g fresh wt) in food groups.

Food group	India (*43*)	United States (*44*)	International compilation (*8*)
Cereals and cereal products	5–95	10–370	10–550
Meat, meat products, and eggs	40–120	100–810	10–360
Fish and marine	280–1080	400–1500	110–970
Fish and freshwater	—	—	180–680
Pulses	10–138	—	—
Dairy products	5–15	10–130	1–170
Fruits and vegetables	1–7	1–60	1–20

B. Typical distribution of selenium in dietary constituents (µg/day) in selected countries.

	China Keshan disease area (*18*)	China Disease-free area (*18*)	India Low-income vegetarian diets (*43*)	India Low-income conventional diets (*43*)	Finland (*45*)	United Kingdom (*46*)
Total diet	7.7	16.4	27.4	52.5	30	31
Food group						
Cereals and cereal products	5.4	11.6	15.7	21.1	2.8	7
Pulses	—	—	3.9	3.6	1.1	—
Meat and eggs			—	3.7	9.2	10
Fish	>0.6	> 2.2	—	18.4	9.5	4
Dairy products			6.9	4.8	6.5	3
Fruits and vegetables	1.7	2.6	0.9	0.9	0.5	6
Other	—	—	—	—	1.1	3

Table 49

Geographic differences in the selenium intakes of infants

Region or country	Selenium intake (μg/day)[a]	Reference
Human milk		
China: Keshan disease area	2.0	*18*
Burundi	4.7 ± 0.8	*48*
New Zealand: south island	5.3	*49*
Former Yugoslavia	6.0 ± 1.3	*50*
Finland	4.0–7.6	*51*
New Zealand: north island	8.1–10.2	*52*
Belgium	8.4	*53*
Austria	8.8–9.8	*13*
Australia	9.4 ± 3.6	*54*
Hungary	9.6 ± 3.7	*50*
Sweden	10.6 ± 2.3	*50*
United States, east coast	8.8–11.4	*55*
United States, unspecified	12.3	*56*
Zaire	12.3 ± 3.6	*50*
Chile	14.1 ± 2.6	*50*
India	14.1 ± 3.6	*50*
Germany	19.3	*57*
Philippines	22.9 ± 4.1	*50*
China (seleniferous area)	199	*18*
International reference value	13.0 ± 18.2	*58*
Infant formula		
Belgium	2.0	*53*
New Zealand	3.3	*59*
Austria	3.6	*13*
United Kingdom	4.9 (2.3–8.2)	*53*
Spain	6.6	*53*
United States, 1982	5.9 (4.2–8.1)	*60*
Germany	6.5-6.8	*57*
New Zealand (selenium fortified)	11.3	*59*
United States, 1997	11.7–18.3	*61*

[a] Mean ± standard deviation (SD) or range.
Assumed age 6 months; assumed human milk or infant formula intake 750 ml (*47*).

Table 50

Geographic differences in the selenium intakes (µg/day) of adults

Region or country	Selenium intake [a]	Reference
China, Keshan disease area	3–11	*62, 63*
China, Kaschin -Beck disease area	2.6–5.0	*20*
Sweden, vegans	10	*64*
New Zealand, low-selenium area	11 ± 3	*64, 65*
China, disease-free area	13.3 ± 3.1	*18*
South Sweden, conventional diets	40 ± 4	*66*
India,vegan low income	27	*43*
Finland, before selenium fertilization	26	*67–69*
Finland, after selenium fertilization	56	*67–69*
Slovakia	27 ± 8	*70*
United Kingdom, 1974	60	*38*
United Kingdom, 1985	43	*38*
United Kingdom, 1994	32	*38*
United Kingdom, 1995	33	*46*
Italy	41	*64*
Germany	38–48	*71*
France	47	*72*
India, conventional diet	48	*43*
United States, all	80 ± 37	*73*
Males	90 ± 14	*73*
Females	74 ± 12	*73*
Canada	98–224	*74*
United States, seleniferous area	216	*64*
Venezuela	80–500	*75*
China, seleniferous area	1338	*64*

[a] Mean ± standard error or range.

Table 51

Representative mean serum selenium concentration (µmol/l) in specific studies[a]

Pathologic investigations	Sample Serum Selenium Concentrations[b]
Keshan disease (China)	0.15–0.25
Kaschin-Beck disease (China)	0.22 ± 0.03
Myxoedematous cretins (Zaire)	0.26 ± 0.12
HIV and AIDS[c]	0.36–0.54
Data for normal subjects	
Serbia and Croatia	0.63–0.85
Bulgaria	0.66–0.72
New Zealand	0.69
Hungary	0.71 ± 0.13
Norway	1.52–1.69
United States, Maryland	1.69–2.15
United States, South Dakota	2.17–2.50
Proposed reference ranges for healthy subjects	0.5–2.5 0.67– 2.04

[a] References 8, 18, 23, 25, 44, 76–79.
[b] Ranges of means or mean ± standard error.
[c] HIV, Human immunodeficiency virus; AIDS, acquired immune deficiency syndrome.

Criteria for assessing selenium requirements

Levander (*84*) convincingly illustrated the impracticability of assessing selenium requirements from input-output balance data because the history of selenium nutrition influences the proportion of dietary selenium absorbed, retained, or excreted. The changing equilibria when selenium intake is varied experimentally yield data which are of limited value for estimating minimal requirements. Examples are cited of estimates of selenium requirement for adults of 7.4 and 80 µg/day derived from Chinese and United States studies, respectively. Such discrepancies reflect differences in the usual daily selenium intakes of the experimental subjects and the extent to which this was changed experimentally. This situation, not unique to selenium, emphasises the importance of basing requirement estimates on functional criteria derived from evidence describing the minimum levels of intake which, directly or indirectly, reflect the normality of selenium-dependent processes.

New opportunities for the development of biochemical indexes of selenium adequacy such as those listed in ***Table 47*** have yet to be exploited. Until this is done, the most suitable alternative is to monitor changes in the relationship of serum selenium to dietary selenium supply, taking advantage of its relatively constant proportionality to the fraction of serum selenium in functionally significant GSHPx (*85*).

A detailed review of 36 reports describing serum selenium values in healthy subjects indicated that they ranged from a low of 0.52 µmol/l in Serbia to a high of 2.5 µmol/l in Wyoming and South Dakota in the United States (*76*). It was suggested that mean values within this range derived from 7502 apparently healthy individuals should be regarded tentatively as a standard for normal reference. This survey clearly illustrated the influence of crop management on serum selenium level; in Finland and New Zealand, selenium fortification of fertilisers for cereals increased serum selenium from 0.6 to 1.5 µmol/l. A summary of these data in ***Table 51*** also includes representative mean serum selenium values within the range of 0.15–0.55 µmol/l reported for specific diseases known to be associated

with disturbances in selenium nutrition or metabolism. These include reports from studies of Keshan disease, Kaschin-Beck disease, and specific studies of cretinism, hypothyroidism, and HIV and AIDS where clinical outcome or prognosis has been related to selenium status.

This report and the report by the World Health Organization (WHO), FAO, and IAEA (*86*) use virtually identical approaches to derive their estimates of basal requirements for selenium (Se_R^{basal}). As yet there are no published reports suggesting that these basal estimates using Se or GSHPx activity as criteria of adequacy are invalid. Some modification is necessary however to estimate population minimum intakes with adequate allowance for the variability (CV) associated with estimates of the average selenium intakes from the typical diets of many communities. In the WHO-FAO-IAEA report (*86*) a CV of 16 percent was assumed for the selenium conventional diets and 12.5 percent for the milk-based diets of infants to limit the risks of inadequacy arising from unexpectedly low selenium contents. More recent studies suggest that the variability of selenium intake from diets for which the selenium content has been predicted rather than measured may be substantially greater than estimated previously (**Table *49*** [*47*] and ***Table 50***).

Recommended selenium intakes

Because balance techniques were shown to be inappropriate for determining selenium requirements, the WHO-FAO-IAEA report (*86*) presented requirement estimates based on of epidemiologic evidence derived from areas of China endemic or non-endemic for Keshan disease (*18*). These comprehensive biochemical and clinical studies showed that Keshan disease did not occur in regions where the mean intake of selenium by adult males or females was greater than 19.1 or greater than 13.3 µg/day, respectively. Although these intakes were sufficient to eliminate clinical evidence of myocarditis and other signs of Keshan disease, other studies showed that they were inadequate to restore erythrocyte or plasma selenium concentrations or GSHPx activities to levels indicative of reserves.

Studies with adult male subjects initially of low selenium status given a carefully monitored diet providing selenium at 11 µg/day together with supplements of selenomethionine given orally which provided 0, 10, 30, 60, or 90 µg/day. Starting at frankly deficient levels, total daily selenium intakes of above 41 µg/day were found sufficient increase plasma GSHPx substantially and to saturate plasma activity in 60-kg male subjects within 5–8 months. It was estimated that satisfactory levels of plasma selenium (>80 µmol/l) and of GSHPx (>0.3 mmol NADPH oxidized/min/l; approximately two-thirds of plasma saturation activity) indicative of adequate selenium reserves would be attained after intakes of approximately 27 µg/day by 65-kg male subjects (*86*). Such criteria satisfying the definition of average normative requirements for selenium ($Se_R^{normative}$) have been used as the basis for calculating recommended nutrient intake (RNI) values in this report after interpolating estimates of average requirements by allowing for differences in weight and basal metabolic rate of age groups to up to 65 years and adding a 25 percent increase (2 x assumed SD) to allow for individual variability in the estimates of RNI (***Table 52***).

Table 52

Recommended nutrient intakes of selenium (µg/day)

Age Group	Assumed Weight	Average normative requirement $Se_R^{normative}$ (kg/day)	Average normative requirement $Se_R^{normative}$ (total/day)	RNI[c], µg/day
Infants and children				
0–6 months	6	0.85	5.1	6
7–12 months	9	0.91	8.2	10
1–3 years	12	1.13	13.6	17
4–6 years	19	0.92	17.5	22
7–9 years	25	0.68	17.0	21
Adolescents				
Female,10–18 years	49	0.42	20.6	26
Male, 10–18 years	51	0.50	22.5	32
Adults				
Female,19–65 years	55	0.37	20.4	26
Male, 19–65 years	65	0.42	27.3	34
Female, 65+ years	54	0.37	20.2	25
Male, 65+ years	64	0.41	26.2	33
Pregnancy				
2nd trimester				28
3rd trimester				30
Lactation				
0–6 months post-partum				35
7–12 months post-partum				42

[a] Weight (kg) interpolated from FAO/WHO (reference *86*, page 8, Table 2.1).

[b] Derived from WHO-FAO-IAEA values (reference *86*, page 116, Table 6.1, by interpolation).

[c] Recommended nutrient intake (RNI) derived from average $Se_R^{normative}$ + 2 x assumed standard error (of 12.5 percent)

The estimates of RNI for infants (***Table 52***) are compatible with estimates of the international reference range of the selenium content of breast milk (18.5 µg /l; ***Table 49***), with data from an extensive international survey of breast milk selenium (WHO-IAEA [*50*]) and with WHO data (*47*) on the milk consumption of exclusively human-milk-fed infants in developed and developing countries. Data from the WHO-IAEA (*50*) survey from six countries suggest that the human milk from all countries met the RNI for infants aged 0–6 months. In two of six countries, Hungary and Sweden, the human milk selenium was marginal with respect to the RNI for infants aged 7–12 months.

Data from Germany (*13, 88*), Austria (*12*), the United States (*89*), and elsewhere suggest that infant formula may contain selenium in amounts insufficient to meet the RNI or recommended dietary allowance for infants. Lombeck *et al.* (*13*) in an extensive study showed that cow-milk-based formula could provide less than one-third of the selenium of human milk. Estimates of selenium intake by 2-month-old infants were 7.8 µg/day from formula compared with 22.4 µg/day from human milk. Levander (*89*) has suggested that infant formulas should provide a minimum of 10 µg/day and not more than 45 µg/day. This recommendation may well have been implemented judging from recent increases in the selenium content of infant formulas (*61*).

Selenium requirements during pregnancy and lactation

Data from balance experiments are not sufficiently consistent for defining the increase of selenium needed to support foetal growth and development during pregnancy. For this reason the European Union Scientific Committee for Food (*90*), the UK Committee on Medical Aspects of Food Policy (*91*), and the Netherlands Food and Nutrition Council (*92*) have suggested that the component of selenium needed for human pregnancy is obtained by an adaptive increase in the efficiency of absorption of dietary selenium rather than by an increased dietary demand.

Others, contesting this view, have attempted to predict the increase of dietary selenium needed for pregnancy by factorial estimation of the likely quantity of selenium incorporated into the tissues of the foetus (*47, 86*). Such estimates have assumed that the total products of conception amount to 4.6–6 kg lean tissue with a protein content of approximately 18.5–20 percent. If, as appears to be a reasonable assumption, the selenium content of this protein resembles that of a skeletal muscle, growth of these tissues could account for between 1.0 and 4.5 µg/day of selenium depending on whether the analyses reflect consumption of diets from a low-selenium (but non-pathogenic) environment (e.g., New Zealand [*49, 52*]) or from a region with relatively high selenium intakes (e.g., United States, ***Table 50*** [73]). Typically such estimates have assumed an 80 percent absorption and utilisation of dietary selenium from which it would appear reasonable to estimate that allowing for a variability of estimates (CV 12.5 percent) an increase of 2 µg/day would be appropriate for the second trimester and 4 µg/day would be appropriate for the third trimester of pregnancy (***Table 52***).

As is evident from ***Table 49*** the selenium content of human milk is sensitive to changes in maternal dietary selenium. The increase of maternal dietary selenium needed to meet requirements for lactation has been estimated from the estimated RNI for infants aged 0–6 months and 7–12 months. It is assumed that the selenium of maternal milk is used with an efficiency of 80 percent, and a SD of 12.5 percent is assumed. For the period 0–6 months it is estimated that the infant must receive 6 µg/day from human milk. The increase of maternal dietary selenium required to produce this will be 6 x $\frac{100}{80}$ + (2 x SD) = 9 µg/day. The corresponding increase needed to meet the infant RNI of 10 µg/day for ages 7–12 months will be 16 µg/day. Added to the non-pregnancy maternal RNI of 26 µg/day, the total RNI for lactation during the first 6 months post-partum will be 35 µg/day and for months 7–12 will be 42 µg/day (***Table 52***).

As implied by the data in ***Tables 48, 49*** *and* ***50***, agricultural growing practices, geologic factors, and social deprivation enforcing the use of an abnormally wide range of dietary constituents may significantly modify the variability of dietary selenium intakes. If accumulated experience suggests that the CV of selenium intake may be 40 percent or more and tabulated rather than analysed data are used to predict the dietary content of selenium, the selenium allowance may have to be increased accordingly or assessed by using the WHO-FAO-IAEA technique (*86*).

Upper tolerable nutrient level for selenium

A comprehensive account of the clinically significant biochemical manifestations of chronic and acute intoxication from selenium arising from high concentrations in food, drinking water, and the environment were published jointly by WHO and the United Nations Environment Programme and the International Labour Organisation (*80*). This report (*44*) stresses that the signs and symptoms of human overexposure to selenium are not well defined. Common clinical features are hair loss and structural changes in the keratin of hair and of nails, the development of icteroid skin, and gastrointestinal disturbances (*93, 94*). An increased incidence of nail dystrophy has been associated with consumption of high-selenium

foods supplying more than 900 μg/day. These foods were grown in selenium-rich (seleniferous) soil from specific areas in China (*95*). A positive association between dental caries and urinary selenium output under similar circumstances was reported (*96*, *97*).

Sensitive biochemical markers of impending selenium intoxication have yet to be developed. In their absence it is suggested that the upper tolerable nutrient intake level (UL) for selenium should be set, provisionally, at 400 μg/day for adults. It is noteworthy that a maximum tolerable dietary concentration of 2 mg/kg dry diet was suggested for all classes of domesticated livestock and has proved satisfactory in use (*98*). This suggests that the proposed UL of 400 μg/day for human subjects provides a fully adequate margin of safety. The UL for children and for pregnant or lactating women has yet to be determined.

Comparison with previous estimates

Compared with WHO-FAO-IAEA (*86*), US (*87*), UK (*91*), and European Union (*90*) recommendations, the present proposals represent a significant decrease in the suggested need for selenium. Reasons for this are:

- the need to derive recommendations which are applicable for a proportionally lower weight range than for most Western and developed communities;
- the decision, also accepted by WHO, FAO, and IAEA (*86*), that it is neither essential nor desirable to maintain selenium status at a level which fully saturates blood GSHPx activity when, on current evidence, this is not an advantage for health; and
- the decision to present estimates as RNIs which, although including an allowance for individual variability, do not provide for the possibility that foods may often differ widely in selenium content according to their geographic sources.

The reduced estimates presented in this report are physiologically justifiable and will only give rise to concern if there are grounds for serious uncertainty as to the predictability of dietary selenium intake.

Food commodity inputs are changing rapidly and in some instances, unpredictably. Under most circumstances it will be unreasonable to expect that the often marked influence of geographic variability on the supply of selenium from cereals and meats can be taken into account. Changes in trade patterns with respect to the sources of cereals and meats are already having significant influences on the selenium nutrition of consumer communities (*38, 66*). Such evidence fully justifies the warning to allow for a high intrinsic variability of dietary selenium content when estimating selenium requirements of populations for which the principal sources of this microelement are unknown.

Future research

Relationships between selenium status and pathologically relevant biochemical indexes of deficiency merit much closer study with the object of providing more reliable and earlier means of detecting a suboptimal status.

Indications that a suboptimal selenium status may have much wider significance in influencing disease susceptibility must be pursued. Such studies must cover both the impact of selenium deficiency on protection against oxidative damage during tissue trauma and its genetic implication for viral virulence.

We lack knowledge of the influence of soil composition on the selenium content of cereals and animal tissues. Chinese experience with respect to the dramatic influence of soil iron and low pH on selenium availability may well be relevant to extensive tracts of lateritic soils in Africa and elsewhere. There are grounds for the belief that factors in common for

selenium and iodine may influence their supply and availability from soils into the human food chain. FAO should be encouraged to develop studies relevant to the influence of soil conditions on the supply of these two metabolically interdependent elements which affect human health.

The early detection of selenium toxicity (selenosis) is hindered by a lack of suitable biochemical indicators. Effective detection and control of selenosis in many developing countries awaits the development of improved specific diagnostic techniques.

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Chapter 16
Zinc

Role of zinc in human metabolic processes

Zinc is present in all body tissues and fluids. The total body zinc content has been estimated to be 30 mmol (2 g). Skeletal muscle accounts for approximately 60 percent of the total body content and bone mass, with a zinc concentration of 1.5–3 μmol/g (100-200 μg/g), for approximately 30 percent. Zinc concentration of lean body mass is approximately 0.46 μmol/g (30 μg/g). Plasma zinc has a rapid turnover rate and it represents only about 0.1 percent of total body zinc content. This level appears to be under close homeostatic control. High concentrations of zinc are found in the choroid of the eye 4.2 μmol/g (274 μg/g) and in prostatic fluids 4.6-7.7 mmol/l (300-500 mg/l) (*1*).

Zinc is an essential component of a large number (>300) of enzymes participating in the synthesis and degradation of carbohydrates, lipids, proteins, and nucleic acids as well as in the metabolism of other micronutrients. Zinc stabilises the molecular structure of cellular components and membranes and contributes in this way to the maintenance of cell and organ integrity. Furthermore, zinc has an essential role in polynucleotide transcription and thus in the process of genetic expression. Its involvement in such fundamental activities probably accounts for the essentiality of zinc for all life forms.

Zinc plays a central role in the immune system, affecting a number of aspects of cellular and Humoral immunity (*2*). The role of zinc in immunity was reviewed extensively by Shanglar *et al*. (*2*).

The clinical features of severe zinc deficiency in humans are growth retardation, delayed sexual and bone maturation, skin lesions, diarrhoea, alopecia, impaired appetite, increased susceptibility to infections mediated via defects in the immune system, and the appearance of behavioural changes (*1*). The effects of marginal or mild zinc deficiency are less clear. A reduced growth rate and impairments of immune defence are so far the only clearly demonstrated signs of mild zinc deficiency in humans. Other effects, such as impaired taste and wound healing, which have been claimed to result from a low zinc intake, are less consistently observed.

Zinc metabolism and homeostasis

Zinc absorption is concentration dependent and occurs throughout the small intestine. Under normal physiologic conditions, transport processes of uptake are not saturated. Zinc administered in aqueous solutions to fasting subjects is absorbed efficiently (60–70 percent), whereas absorption from solid diets is less efficient and varies depending on zinc content and diet composition (*3*).

Zinc is lost from the body through the kidneys, skin, and intestine. The endogenous intestinal losses can vary from 7 μmol/day (0.5 mg/day) to more than 45 μmol/day (3 mg/day), depending on zinc intake (*4*). Urinary and skin losses are of the order of 7-10 μmol/day (0.5–0.7 mg/day) each and depend less on normal variations in zinc intake (*4*). Starvation and muscle catabolism increase zinc losses in urine. Strenuous exercise and elevated ambient temperatures could lead to losses by perspiration.

The body has no zinc stores in the conventional sense. In conditions of bone resorption and tissue catabolism, zinc is released and may be re-utilised to some extent. Human experimental studies with low-zinc diets 2.6–3.6 mg/day (40-55 μmol/day) have shown that circulating zinc levels and activities of zinc-containing enzymes can be maintained within normal range over several months (*5*, *6*), which highlights the efficiency of the zinc homeostasis mechanism. Controlled depletion-repletion studies in humans have shown that changes in the endogenous excretion of zinc through the kidneys, intestine, and skin and changes in absorptive efficiency are how body zinc content is maintained (*7-10*). The underlying mechanisms are poorly understood.

Sensitive indexes for assessing zinc status are unknown at present. Static indexes, such as zinc concentration in plasma, blood cells, and hair, and urinary zinc excretion are decreased in severe zinc deficiency. A number of conditions that are unrelated to zinc status can affect all these indexes, especially zinc plasma levels. Infection, stress situations such as fever, food intake, and pregnancy lower plasma zinc concentrations whereas, for example, long-term fasting increases it (*11*). However, on a population basis, reduced plasma zinc concentrations seem to be a marker for zinc-responsive growth reductions (*12*, *13*). Experimental zinc depletion studies suggest that changes in immune response occur before reductions in plasma zinc concentrations are apparent (*14*). So far, it has not been possible to identify zinc-dependent enzymes which could serve as early markers for zinc status.

A number of functional indexes of zinc status have been suggested, for example, wound healing, taste acuity, and dark adaptation (*11*). Changes in these functions are, however, not specific to zinc and these indexes have so far not been proven useful for identifying marginal zinc deficiency in humans.

The introduction of stable isotope techniques in zinc research (*15*) has created possibilities for evaluating the relationship between diet and zinc status and is likely to lead to a better understanding of the mechanisms underlying the homeostatic regulations of zinc. Estimations of turnover rates of administered isotopes in plasma or urine have revealed the existence of a relatively small rapidly exchangeable body pool of zinc of about 1.5-3 mmol (100-200 mg) (*16-19*). The size of the pool seems to be correlated to habitual dietary intake and it is reduced in controlled depletion studies (*18*). The exchangeable zinc pool was also found to be correlated to endogenous faecal excretion of zinc (*19*) and to total daily absorption of zinc. These data suggest that the size of the exchangeable pool depends on recently absorbed zinc and that a larger exchangeable pool results in larger endogenous excretion. Changes in endogenous intestinal excretion of zinc seem to be more important than changes in absorptive efficiency for maintenance of zinc homeostasis (*19*).

Dietary sources and availability of zinc

Lean red meat, whole-grain cereals, pulses, and legumes provide the highest concentrations of zinc 25-50 mg/kg (380-760 μmol/kg) raw weight. Processed cereals with low extraction rates, polished rice, and lean meat or meat with high fat content have a moderate zinc content 10-25 mg/kg (150-380 μmol/kg). Fish, roots and tubers, green leafy vegetables, and fruits are only modest sources of zinc <10 mg/kg (<150 μmol/kg) (*20*). Separated fats and oils, sugar, and alcohol have a very low zinc content.

The utilisation of zinc depends on the overall composition of the diet. Experimental studies have identified a number of dietary factors as potential promoters or antagonists of zinc absorption (*21)*. Soluble low-molecular-weight organic substances, such as amino and hydroxy acids, facilitate zinc absorption. In contrast, organic compounds forming stable and poorly soluble complexes with zinc can impair absorption. In addition, competitive interactions between zinc and other ions with similar physicochemical properties can affect

the uptake and intestinal absorption of zinc. The risk for competitive interactions seems mainly to be related to high doses in the form of supplements or in aqueous solutions. However, at levels present in food and at realistic fortification levels, zinc absorption appears not to be affected, for example, by iron and copper (*21*).

Isotope studies with human subjects have identified two factors which together with the total zinc content of the diet are major determinants of absorption and utilisation of dietary zinc. The first is the content of inositol hexaphosphate (phytate) and the second is the level and source of dietary protein. Phytates are present in whole-grain cereals and legumes and in smaller amounts in other vegetables. They have a strong potential for binding divalent cations and their depressive effect on zinc absorption has been demonstrated in humans (*21)*. The molar ratio between phytates and zinc in meals or diets is a useful indicator of the effect of phytates in depressing zinc absorption. At molar ratios above the range of 6–10, zinc absorption starts to decline; at ratios above 15 absorption is typically less than 15 percent (*20*). The effect of phytate is, however, modified by the source and amount of dietary proteins consumed. Animal proteins improve zinc absorption from a phytate-containing diet. Zinc absorption from some legume-based diets is comparable with that from animal-protein-based diets despite a higher phytate content in the former. High dietary calcium potentiated the antagonistic effects of phytates on zinc absorption in experimental studies. The results from human studies are less consistent and any effects seem to depend on the source of calcium and the composition of the diet (*22*).

Some examples of recently published absorption studies illustrate the effect of zinc content and diet composition on fractional zinc absorption (***Table 53***) (*19, 23- 25*). The results from the total diet studies, where all main meals of a day's intake have been extrinsically labelled, show a remarkable consistency in fractional absorption despite relatively large variations in meal composition and zinc content. Thus, approximately twice as much zinc was absorbed from a non-vegetarian or high-meat diet (*24*, *25*) than from a diet in rural China based on rice and wheat flour (*20*). Data are lacking on zinc absorption from typical diets of developing countries, which usually have a high phytate content.

The availability of zinc from the diet can be improved by reductions in the phytate content and inclusion of animal protein sources. Lower extraction rates of cereal grains will result in lower phytate content but at the same time the zinc content is reduced, so that the net effect on zinc supply is limited. The phytate content can be reduced by activating the phytase present in most phytate-containing foods or through the addition of microbial or fungal phytases. Phytases hydrolyse the phytate to lower inositol phosphates, resulting in an improved zinc absorption (*26*, *27*). The activity of phytases in tropical cereals such as maize and sorghum is lower than that in wheat and rye (*28*). Germination of cereals and legumes increases phytase activity and addition of some germinated flour to ungerminated maize or sorghum followed by soaking at ambient temperature for 12–24 hours can reduce the phytate content substantially (*28*). Additional reduction can be achieved by the fermentation of porridge for weaning foods or doughs for bread making. Commercially available phytase preparations could also be used but may not be economically accessible in many populations.

Populations at risk for zinc deficiency

The central role of zinc in cell division, protein synthesis, and growth makes infants, children, adolescents, and pregnant women especially at risk for an inadequate zinc intake. Zinc-responsive stunting has been identified in several studies (*29*), and a more rapid body weight gain in malnourished children supplemented with zinc was reported. Other studies have failed to show a growth-promoting effect of zinc supplementation (*13*). A recent meta-analysis of 25 intervention trials comprising 1834 children under 13 years of age, with a mean duration of approximately 7 months and a mean dose of zinc of 14 mg/day (214 μmol/day),

showed a small but significant positive effect of zinc supplementation on height and weight increases (*13*). The initial presence of stunting was significantly associated with an effect of zinc supplementation on height, whereas initial low plasma zinc concentrations were associated with a more pronounced effect on weight gain.

Results from zinc supplementation studies suggest that a low zinc status in children not only affects growth but is also associated with an increased risk of severe infectious diseases (*30*). Episodes of acute diarrhoea with shorter duration and less severity and reductions in incidence of diarrhoea in zinc-supplemented groups have been reported. Other studies indicate that the incidence of acute lower respiratory tract infections and malaria may also be reduced by zinc supplementation. Prevention of sub-optimal zinc status and zinc deficiency in children by an increased intake and availability of zinc could consequently have a significant effect on child health in developing countries.

The role of maternal zinc status on pregnancy outcome is still unclear. Positive as well as negative associations between plasma zinc concentration and foetal growth or labour and delivery complications have been reported (*31*). Results of zinc supplementation studies also remain inconclusive (*31*). Interpretation of plasma zinc concentrations in pregnancy is complicated by the effect of hemodilution, and low plasma zinc levels may reflect other metabolic disturbances (*11*). Zinc supplementation studies of pregnant women have been performed mainly in relatively well-nourished populations, which may be one of the reasons for the mixed results (*31*). A recent study in low-income American women with plasma zinc concentrations below the mean at enrolment in prenatal care showed that a zinc intake of 25 mg/day resulted in greater infant birth weights and head circumferences and a reduction in very low birth weights among non-obese women compared with the placebo group (*12*).

Table 53

Examples of fractional zinc absorption from total diets measured by isotope techniques

Subject characteristics *(ref.)*	Diet/meal characteristics	Isotope technique	Zinc content μmol (mg)	Phytate-zinc molar ratio	Zinc absorption, %(x±SD)[a]
Young adults (n=8) *(22)*	High-fibre diet	Radioisotopes	163 (10.7)	7	27±6
Young women (n=10) *(19)*	Habitual diet	Stable isotopes	124 (8.1)	10	34±9
Women (20-42years) (n=21) *(24)*	Lacto-ovo vegetarian	Radioisotopes	139 (9.1)	14	26 [b]
	Non-vegetarian	Radioisotopes	169 (11.1)	5	33 [b]
Women (20-42years) (n=21) *(24)*	Low meat	Radioisotopes	102 (6.7)	—	30 [c]
Postmenopausal women (n-14) *(25)*	High meat	Radioisotopes	198 (13.0)	—	28 [c]

[a] SD, standard deviation.
[b] Pooled SD=5.[c] Pooled SD=4.6.
Source: Adapted from FAO/WHO Trace Mineral Report(*32*)

Zinc requirements

The lack of specific and sensitive indexes for zinc status limits the possibilities for evaluating zinc requirements from epidemiologic observations. In the FAO/IAEA/WHO 1996 report (*32*), zinc requirements were estimated by using the factorial technique (i.e., by adding the requirements for tissue growth, maintenance, metabolism, and endogenous losses). Experimental zinc repletion studies with low zinc intakes have clearly shown that the body has a pronounced ability to adapt to different levels of zinc intakes by changing the endogenous zinc losses through the kidneys, intestine, and skin (*5-9,33*). The normative requirement for absorbed zinc was defined as the obligatory loss during the early phase of zinc depletion before adaptive reductions in excretion take place and was set at 1.4 mg/day for men and 1.0 mg/day for women. To estimate the normative maintenance requirements for other age groups, the respective basal metabolic rates were used for extrapolation. In growing individuals the rate of accretion and zinc content of newly formed tissues were used to obtain the data required for tissue growth. Similarly, the retention of zinc during pregnancy and the zinc concentration in milk at different stages of lactation were used to estimate the physiologic requirements in pregnancy and lactation (*32*).

The translation of these estimates of absorbed zinc to requirements for dietary zinc involves several considerations. First, the nature of the diet (i.e., its content of promoters and inhibitors of zinc absorption) determines the fraction of the dietary zinc that is potentially absorbable. Second, the efficiency of absorption of potentially available zinc is inversely related to the content of zinc in the diet. The review of available data from experimental zinc absorption studies of single meals or total diets resulted in a division of diets into three categories – high, moderate, and low zinc bio-availability – as detailed in ***Table 54*** (*32*). It was then discovered that the relationship between efficiency of absorption and zinc content differed for these diets (*32*). Algorithms were developed (*32*) and applied to the estimates of requirements for absorbed zinc to achieve a set of figures for the average individual dietary zinc requirements (***Table 55***). The fractional absorption figures applied for the three diet categories were 50 percent, 30 percent, and 15 percent, respectively. From these estimates and from the evaluation of data from dietary intake studies, mean population intakes were identified which were deemed sufficient to ensure a low prevalence of individuals at risk of inadequate zinc intake (*32*).

Infants, children, and adolescents

Endogenous losses of zinc in human-milk-fed infants were assumed to be 20 μg/kg/day (0.31μmol/kg/day) whereas 40 μg/kg/day (0.6 μmol/kg/day) was assumed for infants fed formula or weaning foods (*32*). For other age groups an average loss of 0.002 μmol/basal kJ (0.57 μg/basal kcal) was derived from the estimates in adults. Estimated zinc increases for infant growth were set at 120 and 140 μg/kg/day (1.83–2.14 μmol/kg/day) for female and male infants, respectively, for the first 3 months (*32*). These values decrease to 33 μg/kg/day (0.50 μmol/kg/day) for ages 6–12 months. For ages 1–10 years the requirements for growth were based on the assumption that new tissue contains 30 μg/g (0.46 μmol zinc/g) (*32*). For adolescent growth, a zinc content of 23 μg/g (0.35 μmol/g) increase in body weight was assumed. Pubertal growth spurts increase physiologic zinc requirements substantially. Growth of adolescent males corresponds to an increase in body zinc requirement of about 0.5 mg/day (7.6 μmol/day) (*32*).

Pregnancy

The total amount of zinc retained during pregnancy has been estimated to be 1.5 mmol (100 mg) (*34)*. During the third trimester the physiologic requirement of zinc is approximately twice as high as that in women who are not pregnant (*32*).

Lactation

Zinc concentrations in human milk are high in early lactation, 2-3 mg/l (31-46 μmol/l) in the first month, and fall to 0.9 mg/l (14 μmol/l) after 3 months (*35*). From data on maternal milk volume and zinc content, it was estimated that the daily output of zinc in milk during the first 3 months of lactation could amount to 1.4 mg/day (21.4 μmol/l), which would theoretically triple the physiologic zinc requirements in lactating women compared with non-lactating, non-pregnant women. In setting the estimated requirements for early lactation it was assumed that part of this requirement was covered by postnatal involution of the uterus and from skeletal resorption (*32*).

Elderly

Requirements for the elderly are estimated to be the same as those for other adults. A lower absorptive efficiency has been reported in the elderly, which could justify a higher dietary requirement. On the other hand, endogenous losses seem to be lower in the elderly. Because of the suggested role of zinc in infectious diseases, an optimal zinc status in the elderly could have a significant public health effect and is an area of zinc metabolism requiring further research.

Inter-individual variations in zinc requirements and recommended nutrient intakes

The studies (*6-10*) used to estimate the average physiologic requirements with the factorial technique have considered a relatively small number of subjects and do not allow any estimate of inter-individual variations in obligatory losses of zinc at different intakes. Because zinc requirements are related to tissue turnover rate and growth, it is reasonable to assume that variations in physiologic zinc requirements are of the same magnitude as variations in protein requirements (*36*) and that the same figure (12.5 percent) for the inter-individual coefficient of variation (CV) could be adopted. However, the estimates of dietary zinc requirements involve an estimate of absorption. Consequently, variations in absorptive efficiency, not relevant in relation to estimates of protein requirements, may have to be taken into account in the estimates of the total inter-individual variation in zinc requirements. Systematic studies of the inter-individual variations in zinc absorption under different conditions are few. In small groups of healthy well-nourished subjects, the reported variations in zinc absorption from a defined meal or diet are of the order of 20–40 percent and seem to be independent of age, sex, or diet characteristics. How much these variations, besides being attributable to methodologic imprecision, reflect variations in physiologic requirement, effects of preceding zinc intake, *etc*. is not known. From the available data from zinc absorption studies (*19, 20, 23-27*) it is tentatively suggested that the variation in dietary zinc requirements, which covers variation in requirement for absorbed zinc (i.e., variations in metabolism and turnover rate of zinc) and variation in absorptive efficiency, corresponds to a CV of 25 percent. The recommended nutrient intakes derived from the estimates of average individual dietary requirements (***Table 55***) with the addition of 50 percent (2 standard deviations) are given in ***Table 56***.

Table 54

Criteria for categorising diets according to the potential availability of their zinc

Nominal category	Principal dietary characteristics
High availability	Refined diets low in cereal fibre, low in phytic acid content, and with phytate-zinc (molar) ratio <5; adequate protein content principally from non-vegetable sources, such as meats, fish. Includes semisynthetic formula diets based on animal protein.
Moderate availability	Mixed diets containing animal or fish protein. Lacto-ovo, ovovegetarian, or vegan diets not based primarily on unrefined cereal grains or high-extraction-rate flours. Phytate-zinc molar ration of total diet within the range 5–15 or not exceeding 10 if more than 50% of the energy intake is accounted for by unfermented, unrefined cereal grains and flours whereas the diet is fortified with inorganic calcium salts (>1 g Ca^{2+}/ day). Availability of zinc improves when the diet includes animal or protein sources or milks.
Low availability	Diets high in unrefined, unfermented, and ungerminated cereal grain,[a] especially when fortified with inorganic calcium salts and when intake of animal protein is negligible. Phytate-zinc molar ration of total diet exceeds 15.[b] High-phytate soya-protein products constitute the primary protein source. Diets in which, singly or collectively, approximately 50% of the energy intake is accounted for by the following high-phytate foods: high-extraction-rate (90% +) wheat, rice, maize, grains and flours, oatmeal, and millet; chapatti flours and *tanok*; and sorghum, cowpeas, pigeon peas, grams, kidney beans, blackeye beans, and groundnut flours. High intakes of inorganic calcium salts (>1 g Ca^{2+}/ day), either as supplements or as adventitious contaminants (e.g., from calcareous geophagia), potentiate the inhibitory effects; low intakes of animal protein exacerbate these effects.

[a]Germination of such grains or fermentation (e.g., leavening) of many flours can reduce antagonistic potency; the diet should then be classified as moderate availability.

[b]Vegetable diets with phytate-zinc ratios exceeding 30 are not unknown; for such diets, an assumption of 10 percent availability of zinc or less may be justified, especially if the intake of protein is low, calcium salts is excessive, or both (e.g., calcium salts providing >1.5 g Ca^{2+}/day).

Table 55

Average individual normative requirements for zinc (μg/kg body weight/day) from diets differing in zinc bio-availability[a]

Age range years	High bio-availability[b]	Moderate bio-availability[c]	Low bio-availability[d]
	μg/kg body weight/day		
Infants and Children			
Females, 0–0.25	175[e]	457[f]	1067[g]
Males, 0–0.25	200[e]	514[f]	1200[g]
0.25–0.5	79[e]	204[f]	477[g]
0.5–1	66[e]	—	—
0.5–1	186	311	621
1–3	138	230	459
3-6	114	190	380
6-10	90	149	299
Adolescents			
Females, 10–12	68	113	227
Males, 10–12	80	133	267
Females, 12–15	64	107	215
Males, 12–15	76	126	253
Females, 15–18	56	93	187
Males, 15–18	61	102	205
Adults			
Females, 18–60+	36	59	119
Males, 18–60+	43	72	144

Source: Adapted from FAO/IAEA/WHO (*32*).

[a] For information on diets, see ***Table 54***

[b] Assumed bio-availability of dietary zinc 50 percent.

[c] Assumed bio-availability of dietary zinc 30 percent.

[d] Assumed bio-availability of dietary zinc 15 percent.

[e] Applicable exclusively to infants fed maternal milk alone for which the bio-availability of zinc is with no allowance for storage.

[f] Applicable to infants partly human-milk-fed or fed whey-adjusted cow milk formula or milk plus low-phytate solids. No allowance for storage.

[g] Applicable to infants receiving phytate-rich vegetable-protein-based infant formula with or without whole-grain cereals. No allowance for storage.

Upper limits of zinc intake

Only a few occurrences of acute zinc poisoning have been reported. The toxicity signs are nausea, vomiting, diarrhoea, fever, and lethargy and have been observed after ingestion of 4-8 g (60-120 mmol) zinc. Long-term zinc intakes higher than the requirements could, however, interact with the metabolism of other trace elements. Copper seems to be especially sensitive to high zinc doses. A zinc intake of 50 mg/day (760 μmol) affects copper status indexes, such as CuZn-superoxide dismutase in erythrocytes (*37, 38*). Low copper and ceruloplasmin levels and anaemia have been observed after higher zinc intakes 450-660 mg/day (6.9-10 mmol/day) (*39, 40*). Changes in serum lipid pattern and in immune response have also been observed in zinc supplementation studies (*41, 42*). Because copper also has a central role in immune defence, these observations call for caution before large-scale zinc supplementation programmes are undertaken. Any positive effects of zinc supplementation on growth or

infectious diseases could be disguised or counterbalanced by negative effects on copper-related functions.

The upper level of zinc intake for an adult man is set at 45 mg/day (690 μmol/day) and extrapolated to other groups in relation to basal metabolic rate. For children this extrapolation means an upper limit of intake of 23–28 mg/day (350–430 μmol/day), which is close to what has been used in some of the zinc supplementation studies. Except for excessive intakes of some types of seafood, such intakes are unlikely to be attained with most diets. Adventitious zinc in water from contaminated wells and from galvanized cooking utensils could also lead to high zinc intakes.

Table 56

Recommended nutrient intakes (RNIs) for dietary zinc (mg/day) to meet the normative storage requirements from diets differing in zinc bio-availability[a]

Age group	Assumed body weight, kg	High bio-availability	Moderate bio-availability	Low bio-availability
Infants and children				
0–6 months	6	1.1[b]	2.8[c]	6.6[d]
7–12 months	9	0.8[b]	–	–
7–12 months	9	2.5[e]	4.1	8.4
1–3 years	12	2.4	4.1	8.3
4–6 years	17	2.9	4.8	9.6
7–9 years	25	3.3	5.6	11.2
Adolescents				
Females, 10–18 years,	47	4.3	7.2	14.4
Males, 10–18 years	49	5.1	8.6	17.1
Adults				
Females, 19– 65 years	55	3.0	4.9	9.8
Males, 19–65 years	65	4.2	7.0	14.0
Females, 65+ years	55	3.0	4.9	9.8
Males, 65+ years	65	4.2	7.0	14.0
Pregnant Women				
First trimester	–	3.4	5.5	11.0
Second trimester	–	4.2	7.0	14.0
Third trimester	–	6.0	10.0	20.0
Lactating women				
0–3 months	–	5.8	9.5	19.0
3–6 months	–	5.3	8.8	17.5
6–12 months	–	4.3	7.2	14.4

[a]For information on diets, see ***Table 54***. Unless otherwise specified, the intra-individual variation of zinc requirements is assumed to be 25 percent. Weight data interpolated from FAO/WHO, 1988 (*36*).

[b]Human-milk-fed infants receiving maternal milk only; assumed coefficient of variation (CV) 12.5 percent; assumed availability 80 percent.

[c]Formula-fed infants: moderate bio-availability for whey-adjusted milk formula and for infants partly human-milk-fed or given low-phytate feeds supplemented with other liquid milks; assumed CV 12.5 percent.

[d]Formula-fed infants; low bio-availability applicable to phytate-rich vegetable-protein-based formula with or without whole-grain cereals; CV 12.5 percent.

[e]Not applicable to infants consuming human milk only.

Adequacy of zinc intakes in relation to requirement estimates

The risk for inadequate zinc intakes in children has been evaluated by using the suggested estimates of zinc requirements (*32*) and by using data available on food composition and dietary intake in different parts of the world (*43*). For this assessment it was assumed that the distribution of zinc requirements is Gaussian with a CV of 15 percent and that the correlation between intake and requirement is very low. Zinc absorption from diets in Malawi, Kenya, Mexico, and Guatemala was estimated to be 15 percent based on the high phytate-zinc molar ratio (*37-42*) in these diets, whereas an absorption of 30 percent was assumed for diets in Ghana, Guatemala, Egypt, and Papua New Guinea. Fermented maize and cassava products (*kenkey*, *banku*, and *gari*) in Ghana, yeast leavened wheat-based bread in Egypt, and the use of sago with a low phytate content as the staple in the New Guinean diets were assumed to result in a lower phytate-zinc molar ratio and a better availability. With this approach 68–94 percent of the children were estimated to be at risk for zinc deficiency in these populations, with the exception of Egypt where the estimate was 36 percent (*43*). The average daily zinc intakes of these children were 3.7-6.9 mg (56-105 μmol). Most of the zinc supplementation studies have not provided dietary intake data, which could be used to identify the zinc intake critical for growth effects. In a recent study in Chile, positive effects on height gain in boys after 14 months of zinc supplementation was noted (*44*). The intake in the placebo group at the start was 6.3 ± 1.3 mg/day (96 ± 20 μmol/day) (n=49). Because only 15 percent of the zinc intake of the Chilean children was derived from flesh foods, availability was assumed to be relatively low.

Krebs et al (*45*) observed no effect of zinc supplementation on human-milk zinc content or on maternal status of a group of lactating women and judged their intake sufficient to maintain adequate zinc status through 7 months or more of lactation. The mean zinc intake of the non-supplemented women was 13.0 ± 3.4 mg/day (199 ± 52 μmol/day).

The efficient homeostatic mechanisms for maintaining body zinc content at low intakes, which formed the basis for the estimates of physiologic requirements in the FAO/IAEA/WHO 1996 report (*32*), as well as the presumed negative impact of a high-phytate diet on zinc status, were confirmed in recent experimental studies (*10, 44, 46, 47*). Reductions in urinary and faecal losses maintained normal plasma zinc concentrations over 5 weeks in 11 men with intakes of 2.45 mg zinc/day (37 μmol/day) or higher in a diet with a presumably high availability (*10*). In a similar repletion-depletion study with 15 men, an intake of 4 mg/day (61 μmol/day) from a diet with a molar phytate-zinc ratio of 58 for 7 weeks resulted in a reduction of urinary zinc excretion from 0.52 ± 0.18 to 0.28 ± 0.15 mg/day (7.9 ± 2.8 μmol/day to 4.3 ± 2.3 μmol/day) (*46*). A significant reduction of plasma zinc concentrations and changes in cellular immune response were observed. Effects on immunity were also observed when a zinc-restricted diet with a high phytate content (molar ratio approximately 20) was consumed by five young male volunteers for 20–24 weeks (*14*). Sub-optimal zinc status has also been documented in pregnant women consuming diets with high phytate-zinc ratios (>17) (*47*). Frequent reproductive cycling and high malaria prevalence seemed to contribute to the impairment of zinc status.

Conclusion

In conclusion, the approach used for derivation of average individual requirements of zinc used in the FAO/IAEA/WHO 1996 report (32) and the resulting estimates still seem valid and useful for assessment of the adequacy of zinc intakes in population groups and for planning diets for defined population groups.

Future research

As already indicated in the FAO/IAEA/WHO 1996 report (*32*), there is an urgent need to characterise the early functional effects of zinc deficiency and to define their relation to pathologic changes. This knowledge is especially needed for understanding the role of zinc deficiency in the aetiology of stunting and impaired immunocompetence.

For a better understanding of the relationship between diet and zinc supply, there is a need for further research to carefully evaluate the availability of zinc from diets typical of developing countries. The research should include an assessment of the effect of availability of adopting realistic and culturally accepted food preparation practises such as fermentation, germination, soaking, and inclusion of inexpensive and available animal protein sources in plant-food-based diets.

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Chapter 17

Dietary antioxidants: a consideration of factors influencing requirements

Nutrients with an antioxidant role

The potential beneficial effects from antioxidants in protecting against disease have been used as an argument for recommending increasing intakes of several nutrients above those derived by conventional methods. If it is possible to quantify such claims, antioxidant properties should be considered in decisions concerning the daily requirements of these nutrients. This section examines metabolic aspects of the most important dietary antioxidants – vitamins C and E, the carotenoids, and several minerals – and tries to define the populations which may be at risk of inadequacy to determine whether antioxidant properties *per se* should be and can be considered in setting a requirement. In addition, pro-oxidant metabolism and the importance of iron are also considered.

Members of the Food and Nutrition Board of the National Research Council in the United States, recently defined a dietary antioxidant as a substance in foods which significantly decreases the adverse effects of reactive oxygen species, reactive nitrogen species, or both on normal physiologic function in humans (*1*). It is recognised that this definition is somewhat narrow because maintenance of membrane stability is also a feature of antioxidant function (*2*) and an important antioxidant function of both vitamin A (*3*) and zinc (*4*). However, it was decided to restrict consideration of antioxidant function in this document to nutrients which were likely to interact more directly with reactive species.

The need for biologic antioxidants

It is now well established that free radicals, especially superoxide ($O_2^{\cdot -}$), nitric oxide ($NO^{\cdot}$), and other reactive species such as H_2O_2, are continuously produced *in vivo* (*5-7*). Superoxide in particular is produced by leakage from the electron transport chains within the mitochondria and microsomal P450 systems (*8*) or formed more deliberately, for example, by activated phagocytes as part of the primary immune defence in response to foreign substances or to combat infection by micro-organisms (*9*). Nitric oxide is produced from L-arginine by nitric oxide synthases, and these enzymes are found in virtually every tissue of the mammalian body, albeit at widely different levels (*7*). Nitric oxide is a free radical but is believed to be essentially a beneficial metabolite and indeed it may react with lipid peroxides and function as an antioxidant (*10*). Nitric oxide also serves as a mediator whereby macrophages express cytotoxic activity against micro-organisms and neoplastic cells (*11*). If nitric oxide is at a sufficiently high concentration, it can react rapidly with superoxide in the absence of a catalyst to form peroxynitrite. Peroxynitrite is a potentially damaging nitrogen species which can react through several different mechanisms, including the formation of an intermediate with the reactivity of the hydroxyl radical (*12*).

To cope with potentially damaging reactive oxidant species (ROS), aerobic tissues contain endogenously produced antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase and several exogenously acquired radical-scavenging substances such as vitamins E and C and the carotenoids (*13*). Under normal conditions the high concentrations of SOD maintains superoxide concentrations too low to

allow the formation of peroxynitrite. It is also important to mention the antioxidant reduced glutathione (GSH). GSH is ubiquitous in aerobic tissues, and although it is not a nutrient, it is synthesised from sulfhydryl-containing amino acids and is highly important in intermediary antioxidant metabolism (*14*).

Integrated antioxidant defences protect tissues and are presumably in equilibrium with continuously generated ROS to maintain tissues metabolically intact most of the time. Disturbances to the system occur when production of ROS is rapidly increased, for example, by excessive exercise, high exposure to a xenobiotic compounds (such as an anaesthetic, pollutants, or unusual food), infection, or trauma. Superoxide production is increased by activation of NADPH oxidases in inflammatory cells or after the production of xanthine oxidase, which follows ischaemia. The degree of damage resulting from the temporary imbalance depends on the ability of the antioxidant systems to respond to the oxidant or pro-oxidant load. Fruits and vegetables are good sources of many antioxidants, and it is reported that diets rich in these foods are associated with a lower risk of the chronic diseases of cancer (*15*) and heart disease (*16*). Hence, it is believed that a healthful diet maintains the exogenous antioxidants at or near optimal levels thus reducing the risk of tissue damage. The most prominent representatives of dietary antioxidants are vitamin C, tocopherols, carotenoids, and flavonoids (*17-19*). Requirements for flavonoids are not being considered at this time and work on this subject is still very much in its infancy. In contrast, several intervention studies have been carried out to determine whether supplements of the other nutrients can provide additional benefits against such diseases.

The components in biologic tissues make an ideal mixture of substrates for oxidation. Polyunsaturated fatty acids (PUFAs), oxygen, and transition metals are present in abundance but are prevented from reaction by cellular organisation and structure. PUFAs are present in membranes but are always found with vitamin E. Transition metals, particularly iron, are bound to both transport and storage proteins; abundant binding sites on such proteins prevent overloading the protein molecule with metal ions. Tissue structures, however, break down during inflammation and disease, and free iron and other transition metals have been detected (*20, 21*). Potentially damaging metabolites can arise from interactions between transition metals and the ROS described above. In particular the highly reactive hydroxyl radical can be formed by the Fenton (*reaction 1*) and Haber-Weiss reactions (*reaction 2*; with an iron-salt catalyst) (*22*). Pathologic conditions greatly increase the concentrations of both superoxide and nitric oxide, and the formation of peroxynitrite has been demonstrated in macrophages, neutrophils, and cultured endothelium (*reaction 3*) (*12, 23*). Peroxynitrite can react through several different mechanisms, including the formation of an intermediate with the reactivity of the hydroxyl radical (12).

$$Fe^{2+} + H_2O_2 = Fe^{3+} + OH^{\cdot} + OH^{-} \qquad 1$$

$$O_2^{-\cdot} + H_2O_2 = O_2 + OH^{\cdot} + OH^{-} \qquad 2$$

$$NO + O_2^{-\cdot} = ONOO^{\cdot} \qquad 3$$

During inflammation or other forms of stress and disease, new measures are adopted by the body to counter potential pro-oxidant damage. The body alters the transport and distribution of iron by blocking iron mobilisation and absorption and stimulating iron uptake from plasma by liver, spleen, and macrophages (*3, 24, 25*). Nitric oxide has been shown to play a role in the coordination of iron traffic by mimicking the consequences of iron starvation and leading to the cellular uptake of iron (*26*). The changes accompanying disease are generally termed the acute-phase response and are, generally, protective (*27*). Some of the changes in plasma acute-phase reactants which affect iron at the onset of disease or trauma are shown in ***Table 57***.

Table 57

Systems altered in disease which reduce risk of autoxidation

System	Changes in plasma	Physiologic objectives
Mobilisation and metabolism of iron	Decrease in transferrin Increase in ferritin Increase in lactoferrin Increase in haptoglobin Decrease in iron absorption Movement of plasma iron from blood to storage sites.	Reduce levels of circulating and tissue iron to reduce risk of free radical production and pro-oxidant damage. Reduce level of circulating iron available for microbial growth.
Positive acute phase proteins	Increase in antiproteinases Increase in fibrinogen	Restriction of inflammatory damage to diseased area.
White blood cells	Variable increase in white blood cells of which 70% are granulocytes.	Production of reactive oxygen species to combat infection. Scavenge vitamin C to prevent interaction of vitamin C with free iron.
Vitamin C metabolism	Uptake of vitamin C from plasma by stimulated granulocytes. Reduction of plasma vitamin C in acute and chronic illness or stress-associated conditions. Temporary fall in leukocyte vitamin C associated with acute stress.	Reduce levels of vitamin C in the circulation – because it is a potential pro-oxidant in inflamed tissue – or where free iron may be present. Facilitate movement of vitamin C to tissues affected by disease (e.g., lungs in smokers). Protect granulocytes and macrophages from oxidative damage.

Source: Modified from Koj (*28*) and Thurnham (*3, 29, 30*).

Pro-oxidant activity of biologic antioxidants

Most biologic antioxidants are antioxidants because when they accept an unpaired electron, the free radical intermediate formed has a relatively long half-life in the normal biologic environment. The long half-life means that these intermediates remain stable for long enough to interact in a controlled fashion with intermediates which prevent autoxidation, and the excess energy of the surplus electron is dissipated without damage to the tissues. Thus it is believed that the tocopheroxyl radical formed by oxidation of α-tocopherol is sufficiently stable to enable its reduction by vitamin C or GSH to regenerate the quinol (*31, 32*) rather than oxidizing surrounding PUFAs. Likewise the oxidized forms of vitamin C, the ascorbyl free radical and dehydroascorbate, may be recycled back to ascorbate by GSH or the enzyme dehydroascorbate reductase (*13*). The ability to recycle these dietary antioxidants may be an indication of their physiologic essentiality to function as antioxidants.

Carotenoids are also biologic antioxidants but their antioxidant properties very much depend on oxygen tension and concentration (*33, 34*). At low oxygen tension β-carotene acts as a chain-breaking antioxidant whereas at high oxygen tension it readily autoxidizes and exhibits pro-oxidant behaviour (*33*). Palozza (*34*) reviewed much of the evidence and suggests that β-carotene has antioxidant activity between 2 and 20 mmHg of oxygen tension, but at the oxygen tension in air or above (>150 mmHg) it is much less effective as an antioxidant and can show pro-oxidant activity as the oxygen tension increases. Palozza (*34*) also suggests that autoxidation reactions of β-carotene may be controlled by the presence of other antioxidants (e.g., vitamins E and C) or other carotenoids. There is some evidence that large supplements of fat-soluble nutrients such as β-carotene and other carotenoids may compete with each other during absorption and lower plasma concentrations of other nutrients derived from the diet. However, a lack of other antioxidants is unlikely to explain the increased incidence of lung cancer in the α-tocopherol β-carotene intervention study, because there was no difference in cancer incidence between the group which received both β-carotene and α-tocopherol and the groups which received one treatment only (*35*).

The free radical formed from a dietary antioxidant is potentially a pro-oxidant as is any other free radical. In biologic conditions which might deviate from the norm, there is always the potential for an antioxidant free radical to become a pro-oxidant if a suitable receptor molecule is present to accept the electron and promote the autoxidation (*36*). Mineral ions are particularly important pro-oxidants. For example, vitamin C will interact with both copper and iron to generate cuprous or ferrous ions, respectively, both of which are potent pro-oxidants (*29, 37*). Fortunately, mineral ions are tightly bound to proteins and are usually unable to react with tissue components unless there is a breakdown in tissue integrity. Such circumstances can occur in association with disease and excessive phagocyte activation, but even under these circumstances there is rapid metabolic accommodation in the form of the acute-phase response to minimise the potentially damaging effects of an increase in free mineral ions in extra-cellular fluids (***Table 57***).

Nutrients associated with endogenous antioxidant mechanisms

Both zinc and selenium are intimately involved in protecting the body against oxidant stress. Zinc combined with copper is found in the cytoplasmic form of SOD whereas zinc and magnesium occur in the mitochondrial enzyme. SOD occurs in all aerobic cells and is responsible for the dismutation of superoxide (*reaction 4*):

$$O_2^{\cdot -} + O_2^{\cdot -} + 2H^+ = H_2O_2 + O_2 \qquad 4$$

Hydrogen peroxide produced as a product of dismutation reaction is removed by GPx of which selenium is an integral component (*reaction 5*). To function effectively, this enzyme also needs a supply of hydrogen, which it obtains from GSH. Cellular concentrations of GSH are maintained by the riboflavin-dependent enzyme glutathione reductase.

$$H_2O_2 + 2GSH = GSSG + 2H_2O \qquad 5$$

Four forms of selenium-dependent GPx have been described which have different activities in different parts of the cell (*38*). In addition, a selenium-dependent thyrodoxin reductase was recently characterised in human thyrocytes. Thyrodoxin reductase may be particularly important to the thyroid gland because it can cope with higher concentrations of peroxide and hydroperoxides generated in the course of thyroid hormone synthesis better than can GPx (*39*). It is suggested that in combination with iodine deficiency, the inability to remove high concentrations of hydrogen peroxide may cause atrophy in the thyroid gland, resulting in myxedematous cretinism (*39*).

SOD and GPx are widely distributed in aerobic tissues and, if no catalytic metal ions are available, endogenously produced superoxide and hydrogen peroxide at physiologic concentrations may have limited, if any, damaging effects (*36*). SOD and GPx are of fundamental importance to the life of the cell, and their activity is not readily reduced by deficiencies in dietary intake of these nutrients. In contrast, enzyme activity can be stimulated by increased oxidant stress (e.g., ozone) (*40*). Activities of zinc-dependent enzymes have been shown to be particularly resistant to the influence of dietary zinc (*41*), and although erythrocyte GPx activity correlates with selenium when the intake is below 60–80 μg/day (*42*), there is no evidence of impaired clinical function at low GPx activities found in humans. Nevertheless, one selenium intervention study reported remarkably lower risks of several cancers after 4.5 years of selenium at 200 μg/day (*43*). The effects were so strong on total cancer mortality that the study was stopped prematurely. However, the subjects were patients with a history of basal or squamous cell carcinomas and were not typical of the general population. In addition, a prospective analysis of serum selenium in cancer patients (*44*) (1.72 μmol/L) found very little difference from concentrations in matched controls (1.63 μmol/L) although the difference was significant (*45*). Furthermore, areas with high selenium intakes have a lower cancer incidence than do those with low intakes, but the high selenium areas were the least industrialized (*45*).

Nutrients with radical-quenching properties

Vitamins C and E are the principal nutrients which possess radical-quenching properties. Both are powerful antioxidants, and the most important difference between these two compounds stems from their different solubility in biologic fluids. Vitamin C is water soluble and is therefore especially found in the aqueous fractions of the cell and in body fluids whereas vitamin E is highly lipophilic and is found in membranes and lipoproteins.

Vitamin E

Vitamin E falls into the class of conventional antioxidants which are generally phenols or aromatic amines (see ***Chapter 9***). In the case of the four tocopherols that together constitute vitamin E, the initial step involves a very rapid transfer of phenolic hydrogen to the recipient free radical with the formation of a phenoxyl radical from vitamin E. The phenoxyl radical is resonance stabilised and is relatively unreactive towards lipid or oxygen. It does not therefore continue the chain (*33, 46*). However, the phenoxyl radical is no longer an antioxidant and to maintain the antioxidant properties of membranes, it must be recycled or repaired – that is,

reconverted to vitamin E – because the amount of vitamin E present in membranes can be several thousand-fold less than the amount of potentially oxidizable substrate (*47*). Water-soluble vitamin C is the popular candidate for this role (*31*), but thiols and particularly GSH can also function *in vitro* (*32, 48-50*).

There are eight possible isomers of vitamin E, but α-tocopherol (5,7,8 trimethyl tocol) is the most biologically important antioxidant *in vivo* (*46*). In plasma samples, more than 90 percent is present as α-tocopherol but there may be approximately 10 percent of γ-tocopherol. In foods such as margarine and soy products the γ form may be predominant and palm oil products are rich in the tocotrienols.

Vitamin E is found throughout the body in both cell and sub-cellular membranes. It is believed to be orientated with the quinol ring structure on the outer surface (i.e., in contact with the aqueous phase) to enable it to be maintained in its active reduced form by circulating reductants such as vitamin C (*31*). Within biologic membranes, vitamin E is believed to intercalate with phospholipids and provide protection to PUFAs. PUFAs are particularly susceptible to free radical–mediated oxidation because of their methylene-interrupted double-bond structure. The amount of PUFAs in the membrane far exceeds the amount of vitamin E, and the tocopherol-PUFAs ratios are highest in tissues where oxygen exposure is greatest and not necessarily where the PUFAs content is highest (*47*).

Oxidation of PUFAs leads to disturbances in membrane structure and function and is damaging to cell function. Vitamin E is highly efficient at preventing the autoxidation of lipid and it appears as if the primary, and possibly only, role in biologic tissues is to provide this function (*46*). Autoxidation of lipid is initiated by a free radical abstracting hydrogen from PUFA to form a lipid radical (*reaction 6*) which is followed by a rearrangement of the double-bond structure to form a conjugated diene. *In vitro* the presence of minute amounts of peroxides and transition metals will stimulate the formation of the initial radical. Oxygen adds to the lipid radical to form a lipid peroxide (*reaction 7*) which then reacts with another lipid molecule to form a hydroperoxide and a new lipid radical (*reaction 8*). This process is shown in general terms below for the autoxidation of any organic molecule (RH), where the initial abstraction is caused by a hydroxyl radical ($OH^{\cdot}$).

$$RH + OH^{\cdot} = R^{\cdot} + H_2O \qquad 6$$

$$R^{\cdot} + O_2 = ROO^{\cdot} \qquad 7$$

$$ROO^{\cdot} + RH = ROOH + R^{\cdot} \qquad 8$$

Autoxidation or lipid peroxidation is represented by reactions 6 and 7. The process stops naturally when reaction between two radicals (*reaction 9*) occurs more frequently than does reaction 8.

$$ROO^{\cdot} + ROO^{\cdot} = \text{non-radical products} \qquad 9$$

The presence of the chain-breaking antioxidant, vitamin E (ArOH), reacts in place of RH shown in reaction 8 and donates the hydrogen from the chromanol ring to form the hydroperoxide (*reaction 10*). The vitamin E radical ($ArO^{\cdot}$, tocopheroxyl radical) which is formed is fairly stable and therefore stops autoxidation. Hydroperoxides formed by lipid peroxidation can be released from membrane phospholipids by phospholipase A2 and then degraded by GPx in the cell cytoplasm (*see* ***Chapter 15***).

$$ROO^{\cdot} + ArOH = ArO^{\cdot} + ROOH \qquad 10$$

Vitamin C

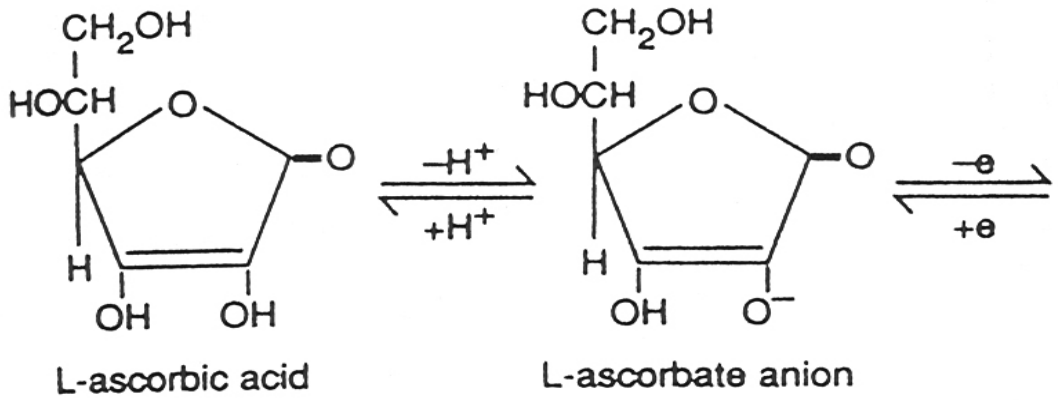
Many, if not all of the biologic properties of vitamin C are linked to its redox properties (see ***Chapter 6***). For example, essential defects in scurvy such as the breakdown of connective tissue fibres (*51*) and muscular weakness (*52*) are both linked to hydroxylation reactions in which ascorbate maintains loosely bound iron in the ferrous form to prevent its oxidation to the ferric form, which makes the hydroxylase enzymes inactive (*53*). Ascorbate exhibits similar redox functions in catecholamine biosynthesis (*53*) and in microsomal cytochrome P450 enzyme activity, although the latter may only be important in young animals (*54*). In the eye, vitamin C concentrations may be 50 times higher than in the plasma and may protect against the oxidative damage of light (*55*). Vitamin C is also present in the gonads, where it may play a critical role in sperm maturation (*56*). Spermatogenesis involves many more cell divisions than does oogenesis, resulting in an increased risk of mutation. Fraga *et al.* (57) reported that levels of sperm oxidized nucleoside 8-OH-2'-deoxyguanosine (an indicator of oxidative damage to DNA) varied inversely with the intake of vitamin C (5-250 mg/day). No apparent effects on sperm quality were noted. Frei (*58*) also showed that vitamin C was superior to all other biologic antioxidants in plasma in protecting lipids exposed *ex vivo* to a variety of sources of oxidative stress. The importance of vitamin C in stabilising various plasma components such as folate, homo-cysteine, proteins, other micronutrients, *etc*. has not been properly evaluated. When blood plasma is separated from erythrocytes, vitamin C is the first antioxidant to disappear.

Vitamin C is a powerful antioxidant because it can donate a hydrogen atom and form a relatively stable ascorbyl free radical (***Figure 27***). As a scavenger of ROS, ascorbate has been shown to be effective against the superoxide radical anion, hydrogen peroxide, the hydroxyl radical, and singlet oxygen (*59, 60*). Vitamin C also scavenges reactive nitrogen oxide species to prevent nitrosation of target molecules (*61*). The ascorbyl free radical can be converted back to reduced ascorbate by accepting another hydrogen atom or it can undergo further oxidation to dehydroascorbate. Dehydroascorbate is unstable but is more fat soluble than ascorbate and is taken up 10–20 times more rapidly by erythrocytes, where it will be reduced back to ascorbate by GSH or NADPH from the hexose monophosphate shunt (*56*).

Thus, mechanisms exist to recycle vitamin C similarly to those for vitamin E. The existence of a mechanism to maintain plasma ascorbate in the reduced state means that the level of vitamin C necessary for optimal antioxidant activity is not absolute because the turnover will change in response to oxidant pressure. Recycling of vitamin C will depend on the reducing environment which exists in metabolically active cells. In atrophic tissues or tissues exposed to inflammation, cell viability may fail and with it the ability to recycle vitamin C. In such an environment, the ability of newly released granulocytes (*62*) or macrophages (*63*) to scavenge vitamin C from the surrounding fluid may be invaluable for conservation of an essential nutrient as well as reducing the risk of ascorbate becoming a pro-oxidant through its ability to reduce iron (*37*).

Figure 27

Vitamin C can donate a hydrogen atom and form a relatively stable ascorbyl free radical



β-*Carotene and other carotenoids*

Many hundreds of carotenoids are found in nature but relatively few are found in human tissues, the five main ones being β-carotene, lutein, lycopene, β-cryptoxanthin, and α-carotene (*17, 18, 64*). β-Carotene is the main source of pro-vitamin A in the diet. There are approximately 50 carotenoids with pro-vitamin A activity, but β-carotene is the most important and is one of the most widely distributed carotenoids in plant species (*64*). Approximately 2–6 mg β-carotene is consumed by adults daily in developed countries (*65, 66*) and similar amounts of lutein (*67*) and lycopene (*66*) are probably also consumed. Smaller amounts may be consumed in the developing world (*68, 69*). β-Cryptoxanthin is a pro-vitamin A carotenoid which is found mainly in fruits (*66*). Consumption is small but bio-availability of carotenoids may be greater from fruit than vegetable, so its contribution to dietary intake and vitamin A status may be higher than the amount in the diet would predict.

β-Carotene has the general structure of this group of compounds and has two 6-membered carbon rings (β-ionone rings) separated by 18 carbon atoms in the form of a conjugated chain of double bonds. The latter is responsible for the antioxidant properties of the molecule (*33, 70, 71*). β-Carotene is unique in possessing two β-ionone rings in its molecule which are essential for vitamin A activity. The chemical properties of the carotenoids closely relate to the extended system of conjugated double bonds, which occupies the central part of carotenoid molecules, and various functional groups on the terminal ring structures. The ROS scavenged by carotenoids are singlet oxygen and peroxyl radicals (*33, 72-74*). Carotenoids in general and lycopene specifically are very efficient at quenching singlet oxygen (*72, 73*). In this process the carotene absorbs the excess energy from singlet oxygen and then releases it as heat. Singlet oxygen is generated during photosynthesis; therefore, carotenoids are important for protecting plant tissues, but there is limited evidence for this role in humans. However, ß-carotene has been used in the treatment of erythropoietic protoporphyria (*75*), which is a light-sensitive condition which in some persons respond to treatment with amounts of β-carotene (in excess of 180 mg/day) (*76*). It has been suggested that large amounts of dietary carotenes may provide some protection against solar radiation but results are equivocal. No benefit was reported when large amounts of β-carotene were

used to treat persons with a high risk of non-melanomatous skin cancer (*77*). However, two carotenoids – lutein (3,3'-dihydroxy α-carotene) and zeaxanthin (the 3,3'-dihydroxylated form of β-carotene) – are found specifically associated with the rods and cones in the eye (*78*) and may protect the retinal pigment epithelium against the oxidative effects of blue light (*79, 80*).

Burton and Ingold (*33*) were the first to draw attention to the radical-trapping properties of β-carotene. Using *in vitro* studies, they showed that β-carotene was effective in reducing the rate of lipid peroxidation at the low oxygen concentrations found in tissues. Because all carotenoids have the same basic structure, they should all have similar properties. Indeed, several authors suggest that the hydroxy-carotenoids are better radical-trapping antioxidants than is β-carotene (*81, 82*). It has also been suggested that because the carotenoid molecule is long enough to span the bilayer lipid membrane (*83*), the presence of oxy functional groups on the ring structures may facilitate similar reactivation of the carotenoid radical in a manner similar to that of the phenoxyl radical of vitamin E (*33*).

There is some evidence for an antioxidant role for β-carotene in immune cells. Bendich (84) suggested that β-carotene protects phagocytes from auto-oxidative damage; enhances T and B lymphocyte proliferative responses; stimulates effector T cell function; and enhances macrophage, cytotoxic T cell, and natural killer cell tumoricidal capacity. Some data are in conflict with evidence of protective effects on the immune system (*85, 86*) and other data have found no effect (*87*). An explanation for the discrepancy may reside in the type of subjects chosen. Defences may be boosted in those at risk but it may not be possible to demonstrate any benefit in healthy subjects (*88*).

A requirement for antioxidant nutrients

Free radicals are a product of tissue metabolism, and the potential damage which they can cause is minimised by the antioxidant capacity and repair mechanisms within the cell. Thus in a metabolically active tissue cell in a healthy subject with an adequate dietary intake, damage to tissue will be minimal and most of the damage occurring will be repaired (*36*). An important dietary source of antioxidant nutrients is the intake of fruit and vegetables, and it is now well established that persons consuming generous amounts of these foods have a lower risk of chronic disease than do those whose intake is small (*15, 16, 89*). These observations suggest that the antioxidant nutrient requirements of the general population can be met by a generous consumption of fruit and vegetables and the slogan "5 portions a day" has been promoted to publicize this idea (*90*).

Occasionally, damage may occur which is not repaired and the risk of this happening may increase in the presence of infection or physical trauma. Such effects may exacerbate an established infection or may initiate irreversible changes leading to a state of chronic disease (e.g., a neoplasm or atherosclerotic lesions). Can such effects also be minimised by a generous intake of dietary antioxidants in the form of fruit and vegetables or are supplements needed?

It is generally recognised that certain groups of people have an increased risk of free radical - initiated damage. Premature infants, for example, are at increased risk of oxidative damage because they are born with immature antioxidant status (*91-93*) and this may be inadequate for coping with high levels of oxygen and light radiation. People who smoke are exposed to free radicals inhaled in the tobacco smoke and have an increased risk of many diseases. People abusing alcohol need to develop increased metabolic capacity to handle the extra alcohol load. Similar risks may be faced by people working in environments where there are elevated levels of volatile solvents (e.g., petrol and cleaning fluids, in distilleries, chemical plants, *etc*.). Car drivers and other people working in dense traffic may be exposed to elevated

levels of exhaust fumes. Human metabolism can adapt to a wide range of xenobiotic substances, but metabolic activity may be raised with the consequent production of more ROS which are potentially toxic to cell metabolism.

Of the above groups, smokers are the most widely accessible people and this has made them a target for several large antioxidant-nutrient intervention studies. In addition, smokers often display low plasma concentrations of carotenoids and vitamin C. However, no obvious benefits to the health of smokers have emerged from these studies and, in fact, β-carotene supplements were associated with an increased risk of lung cancer in two separate studies (*35, 94*) and with more fatal cardiac events in one of them (*95*). Other risk groups identified by their already having had some non-malignant form of cancer, such as non-melanomatous skin cancer (*77*) or a colorectal adenoma (*96*), showed no effect on subsequent recurrences after several years of elevated intakes of antioxidant nutrients. The use of β-carotene (77) or vitamin E alone or in combination with vitamin C (96) showed no benefits. Thus, the results of these clinical trials do not support the use of supplementation with antioxidant micronutrients as a means of reducing cancer or even cardiovascular rates although in the general population, toxicity from such supplements is very unlikely.

Some intervention trials however have been more successful in demonstrating a health benefit. Stitch and colleagues (*97, 98*) gave large quantities of β-carotene and sometimes vitamin A to chewers of betel quids in Kerala, India, and to Canadian Inuits with pre-malignant lesions of the oral tract and showed reductions in leukoplakia and micronuclei from the buccal mucosa. Blot and colleagues (*99*) reported a reduction (13 percent) in gastric cancer mortality in people living in Linxian Province, People's Republic of China, after a cocktail of β-carotene, vitamin E, and selenium. These studies are difficult to interpret because the subjects may have been marginally malnourished at the start and the supplements may have merely restored nutritional adequacy. However, correcting malnutrition is unlikely to be the explanation for the highly successful selenium supplementation study of US patients with a history of basal or squamous cell cancers of the skin (*43*). Interestingly, the intervention with 200 μg/day of selenium for an average of 4.5 years had no effect on the recurrence of the skin neoplasms (relative risk [RR]1.10, confidence interval 0.95–1.28). However, analysis of secondary endpoints showed significant reductions in total cancer mortality (RR 0.5) and incidence (RR 0.63) and in the incidences of lung, colorectal, and prostate cancers. The mean age of this group was 63 years and obviously they were not a normal adult population, but results of further studies are awaited with keen interest. Lastly, results of the Cambridge Heart Antioxidant Study should be mentioned because they provide some support for a beneficial effect of vitamin E in persons who have had a myocardial infarction (*100*). Recruits to the study were randomly assigned to receive vitamin E (800 or 400 mg/day) or placebo. Initial results of the trial suggested a significant reduction in non-fatal myocardial infarctions but a non-significant excess of cardiovascular deaths (*100*).The trial officially ended in 1996, but mortality has continued to be monitored and the authors now report significantly fewer deaths in those who received vitamin E for the full trial (*101*) (*see* ***Chapter 9***).

In conclusion, some studies have shown that health benefits can be obtained by some people with increased risk of disease from supplements of antioxidant nutrients. The amounts of supplements used have, however, been large and the effect possibly has been pharmacologic. Further work is needed to show whether more modest increases in nutrient intakes in healthy adult populations will delay or prevent the onset of chronic disease. The evidence available regarding health benefits to be achieved by increasing intakes of antioxidant nutrients does not assist in setting nutrient requirements.

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APPENDIX 1

Table 1 **Recommended nutrient intakes – minerals***

Age	Calcium (c) *mg/day*	Magnesium *mg/day*	Selenium *µg/day*	Zinc High bioavail-ability *mg/day*	Zinc Moderate bioavail-ability *mg/day*	Zinc Low bioavail-ability *mg/day*	Iron (i) 15% bio-availability *mg/day*	Iron (i) 12% bio-availability *mg/day*	Iron (i) 10% bio-availability *mg/day*	Iron (i) 5% bio-availability *mg/day*	Iodine (o) *µg/day*
Infants											
Premature											30 **(p)** *µg/kg/day*
0 - 6 months	300 **(a)** 400 **(b)**	26 **(a)** 36 **(b)**	6	1.1 **(e)**	2.8 **(f)**	6.6 **(g)**	**(k)**	**(k)**	**(k)**	**(k)**	15 **(p)***µg/kg/day*
7-11 months	400	53	10	0.8 **(e)** 2.5 **(h)**	4.1 **(h)**	8.3 **(h)**	[6] **(l)**	[8] **(l)**	[9] **(l)**	[19] **(l)**	135
Children											
1-3 years	500	60	17	2.4	4.1	8.4	4	5	6	12	75
4-6 years	600	73	21	3.1	5.1	10.3	4	5	6	13	110
7-9 years	700	100	21	3.3	5.6	11.3	6	7	9	18	100
Adolescents											
Males 10 - 18 years	1,300 **(d)**	250	34	5.7	9.7	19.2	10 (10-14 yrs) 12 (15-18 yrs)	12 (10-14 yrs) 16 (15-18 yrs)	15 (10-14 yrs) 19 (15-18 yrs)	29 (10-14 yrs) 38 (15-18 yrs)	135 (10-11 yrs) 110 (12 + yrs)
Females 10 - 18 years	1,300 **(d)**	230	26	4.6	7.8	15.5	9 (10-14 yrs) **(m)** 22 (10-14 yrs) 21 (15-18 yrs)	12 (10-14 yrs) **(m)** 28 (10-14 yrs) 26 (15-18 yrs)	14 (10-14 yrs) **(m)** 33 (10-14 yrs) 31 (15-18 yrs)	28 (10-14 yrs) **(m)** 65 (10-14 yrs) 62 (15-18 yrs)	140 (10-11 yrs) 100 (12 + yrs)
Adults											
Males 19 - 65 years	1,000	260	34	4.2	7.0	14.0	9	11	14	27	130
Females											
19 - 50 years (pre-menopausal)	1,000	220	26	3.0	4.9	9.8	20	24	29	59	110
51 - 65 years (menopausal)	1,300	220	26	3.0	4.9	9.8	8	9	11	23	110
Older adults											
Males 65 + years	1,300	230	34	4.2	7.0	14.0	9	11	14	27	130
Females 65 + years	1,300	190	26	3.0	4.9	9.8	8	9	11	23	110
Pregnancy											
First trimester		220		3.4	5.5	11.0	**(n)**	**(n)**	**(n)**	**(n)**	200
Second trimester		220	28	4.2	7.0	14.0	**(n)**	**(n)**	**(n)**	**(n)**	200
Third trimester	1,200	220	30	6.0	10.0	20.0	**(n)**	**(n)**	**(n)**	**(n)**	200
Lactation											
0-3 months	1,000	270	35	5.8	9.5	19.0	10	12	15	30	200
4-6 months	1,000	270	35	5.3	8.8	17.5	10	12	15	30	200
7-12 months	1,000	270	42	4.3	7.2	14.4	10	12	15	30	200

* For the purposes of the composite tables of RNI values, the body weights used were derived from the 50th percentile of NCHS data until adult weights of 55 kg for females and 65 kg for males were reached. The weights used are the following: 0-6 mo = 6 kg; 7–12 mo = 8.9 kg; 1-3 yo = 12.1 kg; 4-6 yo = 18.2 kg; 7-9 yo = 25.2 kg; 10-11 yo M = 33.4 kg; 10-11 yo F = 34.8 kg; 12-18 yo M = 55.1 kg; 12-18 yo F = 50.6 kg; 10-18 yo M = 55.1 kg; 10-18 yo F = 50.6 kg; 19-65 yo M = 65 kg; 19-65 yo F = 55 kg

Notes - Minerals

(a) Human breast milk.
(b) Infant formula.

Calcium:

(c) The data used in developing calcium RNIs originate from developed countries, and there is controversy as to their appropriateness for developing countries. This notion also holds true for most nutrients, but based on current knowledge, the impact appears to be most marked for calcium.
(d) Particularly during the growth spurt.

Zinc:

(e) Human-milk fed infants only.
(f) Formula-fed infants, moderate zinc bio-availability.
(g) Formula-fed infants, low zinc bio-availability due to infant consumption of phytate rich cereals and vegetable protein based formula.
(h) Not applicable to infants consuming human milk only**.**

Iron:

(i) There is evidence that iron absorption can be significantly enhanced when each meal contains a minimum of 25 mg of Vitamin C, assuming three meals per day. This is especially true if there are iron absorption inhibitors in the diet such as phytate or tannins.
(k) Neonatal iron stores are sufficient to meet the iron requirement for the first six months in full term infants. Premature infants and low birth weight infants require additional iron.
(l) Bio-availability of dietary iron during this period varies greatly.
(m) Non-menstruating adolescents.
(n) It is recommended that iron supplements in tablet form be given to all pregnant women because of the difficulties in correctly evaluating iron status in pregnancy. In the non-anaemic pregnant woman, daily supplements of 100 mg of iron (e.g., as ferrous sulphate) given during the second half of pregnancy are adequate. In anaemic women higher doses are usually required.

Iodine

(o) Data expressed on a per kg body weight basis is sometimes preferred, and this data is as follows:

premature infants = 30 µg/kg/day	infants 0-12 months = 19 µg/kg/day
children 1 - 6 years = 6 µg/kg/day	children 7 - 11 = 4 µg/kg/day
adolescents and adults 12 + years = 2 µg/kg/day	pregnancy and lactation = 3.5 µg/kg/day

(p) In view of the high variability in body weights at these ages the RNIs are expressed as µg/kg body weight/day.

(**NCHS data source:** WHO, Measuring Change in Nutritional Status. Guidelines for Assessing the Nutritional Impact of Supplementary Feeding Programmes for Vulnerable Groups, World Health Organization, 1983)

Table 2 **Recommended nutrient intakes (g) (h) – water and fat soluble vitamins***

	WATER-SOLUBLE VITAMINS									FAT-SOLUBLE VITAMINS			
Age	**Thiamin** *mg/day*	**Riboflavin** *mg/day*	**Niacin (a)** *mg NE/day*	**Vit. B_6** *mg/day*	**Panto-thenate** *mg/day*	**Biotin** *µg/day*	**Folate (c)** *µg DFE/day*	**Vit. B_{12}** *µg/day*	**Vit. C (d)** *mg/day*	**Vit. A (f) (g)** *µg RE/day*	**Vit. D** *µg/day*	**Vit. E (acceptable intakes) (h)** *mg α-TE/ day*	**Vit. K (l)** *µg/day*
Infants													
0 - 6 months	0.2	0.3	2 **(b)**	0.1	1.7	5	80	0.4	25	375	5	2.7 **(i)**	5 **(m)**
7-11 months	0.3	0.4	4	0.3	1.8	6	80	0.5	30	400	5	2.7 **(i)**	10
Children													
1-3 years	0.5	0.5	6	0.5	2	8	160	0.9	30	400	5	5 **(k)**	15
4-6 years	0.6	0.6	8	0.6	3	12	200	1.2	30	450	5	5 **(k)**	20
7-9 years	0.9	0.9	12	1.0	4	20	300	1.8	35	500	5	7 **(k)**	25
Adolescents													
10-18 years													
Males	1.2	1.3	16	1.3	5	25	400	2.4	40	600	5	10	35-65
Females	1.1	1.0	16	1.2	5	25	400	2.4	40	600	5	7.5	35-55
Adults													
Males	1.2	1.3	16	1.3 (19-50 yrs)	5	30	400	2.4	45	600	5 (19-50 yrs)	10	65
19 - 65 years				1.7 (50 + yrs)							10 (50 + yrs)		
Females													
19-50 years (pre- menopausal)	1.1	1.1	14	1.3	5	30	400	2.4	45	500	5	7.5	55
51-65 years (menopausal)	1.1	1.1	14	1.5	5	30	400	2.4	45	500	10	7.5	55
Older adults,													
65 + years													
Males	1.2	1.3	16	1.7	5		400	2.4	45	600	15	10	65
Females	1.1	1.1	14	1.5	5		400	2.4	45	600	15	7.5	55
Pregnancy	1.4	1.4	18	1.9	6	30	600	2.6	55	800	5	**(i)**	55
Lactation	1.5	1.6	17	2.0	7	35	500	2.8	70 **(e)**	850	5	**(i)**	55

* For the purposes of these composite tables of RNI values, the body weights used were derived from the 50th percentile of NCHS data until adult weights of 55 kg for females and 65 kg for males were reached. The weights used are the following: 0-6 mo = 6 kg; 7–12 mo = 8.9 kg; 1-3 yo = 12.1 kg; 4-6 yo = 18.2 kg; 7-9 yo = 25.2 kg; 10-11 yo M = 33.4 kg; 10-11 yo F = 34.8 kg; 12-18 yo M = 55.1 kg; 12-18 yo F = 50.6 kg; 10-18 yo M = 55.1 kg; 10-18 yo F = 50.6 kg; 19-65 yo M = 65 kg; 19-65 yo F = 55 kg

Notes - Vitamins

Niacin

(a) NE = niacin equivalents, 60-to-1 conversion factor for tryptophan to niacin.

(b) Preformed niacin.

Folate

(c) DFE = dietary folate equivalents; μg of DFE provided = [μg of food folate + (1.7 x μg of synthetic folic acid)].

Vitamin C

(d) An RNI of 45 mg was calculated for adult men and women and 55 mg recommended during pregnancy. It is recognised however that larger amounts would promote greater iron absorption if this can be achieved.

(e) An additional 25 mg is needed for lactation.

Vitamin A:

(f) Vitamin A values are "recommended safe intakes" instead of RNIs. This level of intake is set to prevent clinical signs of deficiency, allow normal growth, but does not allow for prolonged periods of infections or other stresses.

(g) Recommended safe intakes as μg RE/day; 1 μg retinol=1 μg RE; 1 μg β-carotene=0.167 μg RE; 1 μg other provitamin A carotenoids=0.084 μg RE.

Vitamin E:

(h) Data were considered insufficient to formulate recommendations for this vitamin so that "acceptable intakes" are listed instead. This represents the best estimate of requirements, based on the currently acceptable intakes that support the known function of this vitamin.

(i) For pregnancy and lactation there is no evidence of requirements for vitamin E that are any different from those of older adults. Increased energy intake during pregnancy and lactation is expected to compensate for increased need for infant growth and milk synthesis. Breast milk substitutes should not contain less than 0.3 mg α-tocopherol equivalents (TE)/100 ml of reconstituted product, and not less than 0.4 mg TE/g PUFA. Human breast milk vitamin E is fairly constant at 2.7 mg for 850 ml of milk.

(k) Values based on a proportion of the adult acceptable intakes.

Vitamin K:

(l) The RNI for each age group is based on a daily intake of 1 μg/kg/day of phylloquinone, the latter being the major dietary source of Vitamin K.

(m) This intake cannot be met by infants who are exclusively breast-fed. To prevent bleeding due to vitamin K deficiency, all breast fed babies should receive vitamin K supplementation at birth according to nationally approved guidelines.

(**NCHS data source:** WHO, Measuring Change in Nutritional Status. Guidelines for Assessing the Nutritional Impact of Supplementary Feeding Programmes for Vulnerable Groups, World Health Organization, 1983)