$$A = v \begin{bmatrix} \tau & 0 & -\tau & -1 & 1 & 0 \\ 0 & -1 & 0 & \tau & \tau & 1 \\ 1 & \tau & 1 & 0 & 0 & \tau \end{bmatrix}.$$

Now think of the *rows* of this matrix as vectors in a six-dimensional space. If the normalization factor is chosen to be $v = 1/[2(1+\tau^2)]^{\frac{1}{2}}$, they are orthonormal. They lie in a three-dimensional space which we call *p*-space. The matrix

$$P = A^T A = \frac{1}{2\sqrt{5}} \begin{bmatrix} \sqrt{5} & 1 & -1 & -1 & 1 & 1 \\ 1 & \sqrt{5} & 1 & -1 & -1 & 1 \\ -1 & 1 & \sqrt{5} & 1 & -1 & 1 \\ -1 & -1 & 1 & \sqrt{5} & 1 & 1 \\ 1 & -1 & -1 & 1 & \sqrt{5} & 1 \\ 1 & 1 & 1 & 1 & 1 & \sqrt{5} \end{bmatrix}.$$

projects points of the six-dimensional space on to *p*-space. The images of the six coordinate axes are six lines arranged like the fivefold axes of an icosahedron. The image of a unit cube in 6-space, parallel to the cubes of the lattice of points with integer coordinates, is a *rhombic triacontahedron* in *p*-space! If we eliminate from the six-dimensional lattice all the vertices that project to points outside this triacontahedron, we are left with a *slice* of the six-dimensional lattice. It is analogous to the strip of two-dimensional lattice in Figure 14. The three-dimensional space orthogonal to *p*-space will be called *q*-space. Projection on to *q*-space is achieved by the matrix $Q = I - P$. The image in *q*-space of any three-dimensional facet (ordinary cube) of a lattice hypercube is a Kowalewski unit in *q*-space. Moreover, it turns out that the projection on to *q*-space of all the three-dimensional facets contained in the slice gives a Levine-Steinhardt aperiodic pattern in *q*-space!

A similar method exists for computing Penrose rhomb patterns, by projection on to a plane of a five-dimensional cubic lattice. We simply start with a 2×5 matrix A whose columns are the coordinates of the vertices of a regular pentagon centred at the origin.

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Optimum nutrition

Mahtab S. Bamji

Recommended dietary allowances, or dietary standards, are amounts of essential nutrients considered necessary for normal physiology and health of individuals of a defined population. But the question of optimum nutrition is complex, and the current recommendations are at best tentative.

Nutrition has been defined as the 'science of food as it relates to optimal health and performance'[1]. Though mankind has always been interested in food which would ensure health, reproductive vigour and well-being, both of human beings and domestic animals, it was only towards the turn of this century that the subject of nutrition shifted from the realm of mere beliefs and dogmas to a systematic science. Discovery of vitamins and minerals as essential food factors, enzymes as biocatalysts, and the elucidation of metabolic pathways laid the foundation of both the sciences of biochemistry as well as nutrition. Till the middle of the present century, the two sciences were

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indistinguishable and most eminent biochemists were also nutritionists.

The science of nutrition has raised questions such as what, why and how much we should eat; what happens if we eat more or less than what is needed; what are the interactions within nutrients and between nutrients and other chemical and biological factors in the environment, and what should be the strategies for achieving nutritional goals.

What should we eat?

Today we know a great deal about what we should eat. Foods contain 42–44 nutrients, including carbohydrates, amino acids (proteins), fatty acids (fats), vitamins, minerals and other trace elements. The nutritional importance and functions of some of the trace elements like zinc, chromium and selenium have been realized only in recent years and new ones are being added to this list.

The term 'essential' (to describe nutrients which the body needs but cannot synthesize) is misleading. Many of the so-called non-essential nutrients play vital roles in the body. If essential amino acids and fatty acids are important, the non-essential ones are equally important metabolically. In fact, they may be so important that during evolution the capability to synthesize them was conserved.

The term 'essential' is applied even to some (but not all!) nutrients that are synthesized in the body from precursors that are essential. For instance, niacin, which can be synthesized in the body from the amino acid tryptophan, is regarded as an essential nutrient because tryptophan is an essential amino acid. Yet, carnitine, which is also synthesized from an essential amino acid lysine and plays a vital role, is not called a vitamin, albeit the knowledge that lysine is the precursor of carnitine is rather recent[2]. Lipoic acid (speculated to be synthesized from the essential fatty acids arachidonic acid or linoleic acid), choline (synthesized from methionine), taurine (synthesized from methionine) and inositol (synthesized from glucose) fall in the same category. Vitamin D can be synthesized in the skin from 7-dehydrocholesterol on exposure to sunlight. 7-Dehydrocholesterol can be synthesized from cholesterol, yet vitamin D is considered essential. Though glucose can be formed in the body by the process of gluconeogenesis, it would be unwise to give a carbohydrate-free diet and expect the body to derive all its energy from fats and proteins without adverse consequences. Thus, glucose is an essential nutrient. The bias in labelling some substances as essential but not the others may be due to the fact that while the deficiencies of niacin and vitamin D are known to occur in human beings and animals, deficiencies of carnitine, lipoic acid, taurine, choline and inositol are either non-existent or very rare in humans. They occur only in some genetic or metabolic disorders. Menon and Natraj[3] have labelled these nutrients as 'shadow nutrients'. They are absolutely essential for some species but not all. For instance, the cat needs taurine and arginine[4] and the meal-worm *Tenebrio molitor* needs carnitine for growth[5]. Ascorbic acid (vitamin C) is required by only guinea pigs, monkeys, fruit-eating bats and human beings. All other species can synthesize it.

Why should we eat?

This question embodies information on functions of nutrients. Functions have to be understood at various levels—biochemical, molecular, physiological, morphological and finally at the level of overall performance: growth, psychomotor functions and physical activity. The biochemical functions of most nutrients in metabolism are fairly well defined. Yet vast gaps of ignorance persist particularly in our understanding of the biochemical mechanism of action of the fat-soluble vitamins such as vitamins A and E. The understanding of the biochemical role of vitamin K in gamma carboxylation of specific glutamic-acid residues of prothrombin precursors and the bone protein osteocalcin is relatively recent.

Vitamin A is needed for growth, differentiation, reproduction and vision. Of these, only its role in vision (synthesis of the visual pigment rhodopsin) is well defined. Much less is known about its other functions, though interesting hypothesis (synthesis of complex polysaccharides and glycoproteins) backed by some experimental evidence (at times controversial) exists[6].

Functions of vitamin E are even less defined. Being a powerful (lipid-soluble) antiperoxidant and free-radical scavenger, *in vivo* also it is believed to play this role. Though experimental deficiency of vitamin E in animals gives rise to a variety of symptoms, its deficiency in humans is not known because of its ubiquitous presence in natural foods. There are, however, numerous claims (based on subjective experiments or experiences) of the health benefits of vitamin E supplements. Vitamin E is one of the most mysterious and abused amongst the vitamins. Apart from the physiological functions, several nutrients may act as prophylactic drugs at much higher doses[7]. Such pharmacological functions have to be viewed differently.

Amongst the water-soluble vitamins, biochemical functions of B-complex vitamins as coenzymes are clearly defined. However, the biochemical role of vitamin C is less understood. It has been found to stimulate some hydroxylation reactions which require molecular oxygen, Fe^{2+} ion, 2-ketoglutarate and a reducing agent. Such hydroxylation reactions are involved in synthesis of collagen (hydroxyproline and

hydroxylysine) and carnitine[8]. However, the requirement for ascorbate in these reactions is not absolute.

The question of 'how much'

The greyest area in nutrition is the most vital question of 'how much is enough'. The reason we do not have a precise answer to this question is: we do not know what happens if we eat more or less than what we need to. We do not have reliable parameters to judge good health. The World Health Organization defines health as not just absence of disease, but a positive state of well-being. This definition itself is subjective. A woman who is used to doing heavy physical labour can carry out these tasks even with a haemoglobin level of 7 g% (optimum level 14 g%), without realizing that she is on the brink of total health failure. On the other hand, a well-nourished man habituated to sedentary life will get fatigued with little physical exertion. Higher functions like physical performance, endurance and psychomotor performance are determined not only by the health status but also by work experience and training.

In the development of a nutritional-deficiency disease, the first stage is tissue depletion of the nutrients (Figure 1). This may be absolute or relative. In the latter case, the concentration of the nutrient in the tissues may be normal but the cellular requirement may be higher due to greater avidity of some enzymes or carrier proteins for their ligands (cofactor-vitamins, trace-elements). This can lead to a redistribution of the ligand (vitamin or the trace element) between its functions creating pockets of deficiency and excess. Such changes have been described following treatment with steriod hormones—corticosteriods[9] and sex steriods[10].

Absolute or relative deficiency leads to a plethora of biochemical changes such as alterations in enzyme activities which in turn can produce perturbations in the levels of micro- and macromolecules. One or more of these changes can be expected to be the molecular basis of the specific nutritional-deficiency-disease syndrome. Apart from the role of vitamin A in the prevention of night-blindness and that of vitamin K in the prevention of blood-clotting disorders, the links between the biochemical or molecular functions of different nutrients (even where the functions are understood) and nutritional-deficiency diseases like kwashiorkor, marasmus, beri beri, scurvy, pellagra are still not understood.

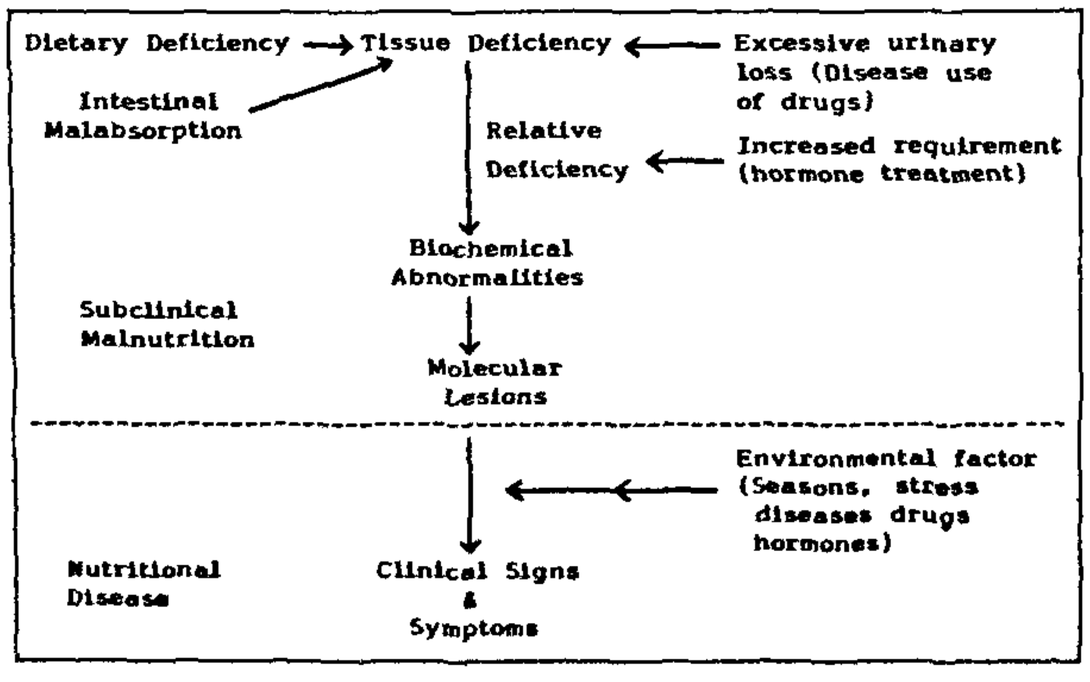

Figure 1. Stages in the development of deficiency diseases.

Disease is a complex entity. Attempts have been made to identify biochemical parameters such as levels of the nutrients or their metabolite(s) in blood or urine or some biochemical functions, which would reveal deficiency at the subclinical stage. Attempts have also been made to assess the level of biochemical insult at which the risk of developing the clinical symptoms of the disease is increased and use this information to derive estimates of nutrient requirements. Such exercises suggest that sharp cut-off values for changes in biochemical indices and health risk seldom exist. In tipping an individual from a state of subclinical deficiency (as revealed by biochemical test) to clinical state, other environmental insults such as infections, stress and other deficiencies are also involved.

Yet another question that has been raised relates to apparent health versus real health. Is an apparently healthy individual really healthy, or hiding some biochemical or other functional deficits like suboptimal psychomotor performance, impaired immune functions, increased susceptibility to infections, reduced learning capacity, and lower physical performance and endurance? Such subtle deficits would not become apparent unless special attempts are made and tests developed to detect them.

With decrease in death rate and increase in longevity, there is new interest in finding out the relationships between nutrition and degenerative diseases like cardiovascular diseases, mental health, immune disorders and cancer. Some nutrients are known to play a positive or negative role in these diseases. Current definition of nutrient requirements does not take these associations into consideration, but in future this new knowledge will have to be considered.

With this preamble about the complexity of the problem of optimum nutrition (or nutrient requirements), we shall now turn to the present-day recommendations for the major nutrients and the basis for deriving them. Even greater complexity arises when information on nutrient requirement has to be translated into recommendations for food, because there are interactions within the nutrients present in foods and between the nutrients and some non-nutrients which may be present in foods. For instance, vitamin C facilitates iron absorption. Thiaminase present in raw fish reduces food thiamin availability.

The concept of recommended dietary allowances

Recommended dietary allowances (RDAs) are a set of dietary standards which when applied to healthy population groups would be expected to keep them in a state of positive health. They do not include extra allowances that may be needed by a person who is in a state of deficiency. Prior to 1920, nutritionists were called upon to provide standards for nutrients and make recommendations for food supplies at least cost, to prevent starvation and illness arising out of famine conditions, economic depression and war. With the scientific knowledge of physiology and nutrition gained during the first two decades of this century, attempts began towards the end of the First World War to make dietary recommendations not just for survival but for achieving positive health. Between 1925 and 1935, the Health Organisation of the League of Nations examined several aspects of food and nutrition and called attention to the need for developing dietary standards, particularly for groups with special needs such as mothers and children. The British Medical Association and the US Department of Agriculture made recommendations for calories and a few nutrients such as proteins, vitamin A, vitamin C, calcium, phosphorus and iron.

In 1940 the Food and Nutrition Board was formed under the National Research Council of the US Academy of Sciences and the first formal RDA report came out in 1941. It was published in 1943. Between 1943 and 1985, the RDA report was revised eight times[11].

Most countries have special committees that examine the available information and make recommendations for dietary allowances. In India, the first set of recommendations for calories, proteins, iron, calcium vitamin A, thiamin, ascorbic acid and vitamin D were made in 1944 by the Indian Research Fund Association, later renamed as the Indian Council of Medical Research (ICMR). These recommendations were updated and new nutrients added with successive revisions in 1958, 1968, 1981 and more recently in 1989.

Several terminologies, such as safe intakes of nutrients, recommended nutrient intake, RDA, recommended dietary intakes (RDI) and simply dietary standards, have been used to mean the same thing. FAO/WHO define safe intakes or RDA as 'the amounts of essential nutrients considered necessary to meet the physiological needs and maintain health in nearly all persons in a specific group'[12]. Except for calories, where the recommendations are made on the basis of physiological requirements (since both more and less are undesirable) for all other nutrients, overages are added to the physiological requirements to ensure safe tissue reserves. RDA takes intó account cooking and processing losses and inter- and intra-individual variations. Generally, the requirement is fixed at a level of mean ± 2 SD; so that it covers 97.5% of the population. While fixing RDA for a nutrient, it is assumed that no other nutrient deficiency exists in the diet.

RDAs are not meant to be used by individuals to judge the adequacy or otherwise of their intakes on a given day. They are derived for and meant to be used for population projections. The nutrient requirements of an individual and dietary recommendations for a group or a population are two different things. The former depends upon the age, the body size and the physiological status of the individual, whereas the latter additionally makes allowance for inter-individual variations, availability from the diet, etc.

Approaches to deriving RDA

Intake observed in healthy populations

Most earlier recommendations of dietary standards were based on diet surveys in healthy population groups where dietary constraints did not exist and nutritional-deficiency diseases were not seen. This approach can underestimate or overestimate the requirement. For instance, the protein requirement was earlier thought to be above 60 g because healthy Europeans and Americans consumed that level. The riboflavin intake of even the well-to-do Indians tends to be suboptimal and they reveal biochemical if not clinical riboflavin deficiency. Also, the availability of nutrients from different foods and diets differ substantially. For instance, the availability of iron (particularly haem iron) from animal foods is much better than that of non-haem iron from cereals, pulses and vegetables where the content of phytates and tannins is high. Hence, the observations made in one population group cannot be applied to another group with different type of diet. Adjustments for differences in quality of the diets have to be made.

Experimental assessment of physiological requirement

Basically four types of approaches have been used to derive the physiological requirements.

Nutrient balance. Minimum intake needed to balance the output of the nutrient and/or its metabolite(s) through urine, faeces and other routes such as sweat, integuments, etc. is derived through actual measurements. Some allowances to provide for positive balance have to be made. This approach has been used for proteins and minerals like calcium. The balance method can be used only for nutrients which are either not metabolized or whose metabolites are well defined and easily measured. Thus, this method is unsuitable for most vitamins.

Factorial method. The minimal loss (obligatory loss) of the concerned nutrient or its metabolic products (e.g. urea for proteins), through normal routes of elimination such as urine, faeces, sweat, are determined on a diet which is devoid of or very low in the concerned nutrient. This is the minimum requirement to replenish the obligatory losses. To this value, the amounts needed for other functions like tissue accretion for growth, requirement for physical activity and requirements for pregnancy and lactation are added. Each of these quantums has to be assessed through actual measurements such as analysis of the component concerned (milk, foetus, body mass) or metabolic costs as in the case of activity. The factorial approach has been used to derive the requirements for calories and the additional requirements of several nutrients for pregnancy, lactation and for growth in children. Earlier, the factorial method was used for deriving protein requirement, but of late, balance studies are preferred for obtaining estimates of protein needs. The factorial method tends to give lower values for protein requirement than the balance studies. Factorial method does not take into account interactions between nutrients in a composite diet and metabolism.

Nutrient turnover. Information on turnover using isotopically labelled nutrient has been used to compute the requirement of vitamin A, vitamin C, iron and vitamin[12]. Safe levels of radioactive isotopes were used in the past, but the present day trend is to use stable isotopes. This is expensive and requires special counters.

Depletion and repletion studies. In this method, the subject is first partially depleted of the nutrient by feeding a diet which is very low in the nutrient and then repleted with graded doses of the nutrient. The body response in terms of tissue (blood, urine), levels of the nutrient, or a metabolite, or a biochemical function such as the activity of an enzyme dependent on the nutrient (B-vitamins) is measured. This method has been used for the water-soluble vitamins. Intakes beyond which the urinary excretion rises rapidly, or the enzyme activity shows linear increase or plateau, or blood cells show signs of saturation are used for assessing RDA.

After habitual high intakes of a vitamin, the enzyme dependent on it (coenzyme) may attain artefactually higher levels. Withdrawal of the vitamin in such a situation may precipitate signs of deficiency, even clinical signs. Such information however cannot be used for deriving RDA, since it will make an overestimate of the requirement.

None of the above methods is easy and hence the number of individuals on whom such measurements can be made is limited. Also, invariably these measurements are made in adults and estimates for other age and physiological groups derived through extrapolation or interpolations. Corrections or additions are made for the inter- and intra-individual variations, the type of diet, cooking losses and the conversion factor in the case of pro-nutrients in foods, e.g. beta carotene to vitamin A and tryptophan to niacin. Thus, both scientific information and an element of judgement are involved in making recommendations for dietary allowances, conducive to optimum nutrition.

Reference man and reference woman

Since RDA by its definition pertains to recommendations for healthy individuals to maintain them in a state of optimum health, one has to define health at least in terms of anthropometric indices such as height and weight for the given age. The question often raised is: Should the values for optimum heights (for age) and weights (for height) attainable be used as reference, or should the heights and weights seen in the community be taken as reference? Are there genetic differences with regard to stature? Is there a functional advantage in attaining optimum genetic potential for stature?

Marked differences in stature between nations and between ethnic groups within a nation are seen. While genetic influences cannot be discounted, it is now believed that environmental factors, including nutrition, are the major determinants of body size. Secular trends show that Japanese brought up in the USA are taller than their parents who grew up in Japan. In Japan also secular trends are observed[13]. Such father–son and mother–daughter trends are also apparent in other populations[13]. In India, the growth of well-to-do children is comparable to the children in USA up to the age of 12–14 years[14]. Adult well-to-do Indians, however, tend to have smaller statures than the adult well-to-do Americans. The reason(s) for this are not clear.

Till recently, the weight for reference Indian adult man aged 20–30 years was fixed at 55 kg and for woman, 45 kg. However, the recent expert committee of ICMR constituted to examine RDAs for Indians has recommended raising these figures to 60 kg and 50 kg respectively, since the currently observed mean heights of well-to-do Indian men and women are 163 cm and 151 cm respectively. The revised standards for weights are those expected for the observed heights.

Information on the relationship between anthropometry and functional capacity, including physical performance, is scanty. Limited data on work output among industrial workers in India suggest that underweight individuals are at a disadvantage[15]. Also, they have to exert more as seen from faster heart rates,

to accomplish the same task. Efficiency however declines beyond a certain body weight.

Energy requirements

Till recently kilocalorie used to be the unit of energy in vogue. The International Union of Sciences and International Union of Nutritional Sciences have now adopted joule as the unit of energy (1 kilocalorie = 4.18 kilojoules). The FAO/WHO/UNU consultation group on energy and protein requirements defined energy requirement of an individual as 'the level of energy intake from food that will balance energy expenditure, when the individual has a body size and composition and a level of physical activity consistent with long term good health and that will allow for maintenance of economically necessary and socially disirable physical activity'[16]. In children and pregnant and lactating women the energy requirement additionally includes the energy needs for deposition of tissues or the secretion of milk at rates consistent with good health.

It has been suggested that energy requirement should be assessed from energy expenditure rather than intake. Energy requirement is a sum of three components: (i) basal metabolism/resting metabolism (BMR); (ii) non-shivering, diet-induced thermogenesis, which is the increase in oxygen consumption over the basal state due to factors such as digestion, absorption and assimilation of food (specific dynamic action of foods); (iii) physical activity. In the case of children and pregnant and lactating women, special needs such as growth in children, tissue accretion in pregnancy and milk production in lactation also have to be considered. Of these, basal metabolism/resting metabolism constitutes the largest (50–60%) component. BMR is mainly influenced by body size, particularly weight, and remains constant over a certain age. Intra-individual variations in BMR are small though inter-individual variations may be as high as 15%. The contribution of thermogenesis to the overall energy requirement is about 10%. Physical activity is the major variable in determining the energy requirement of an individual.

Almost 100% variability in the energy intakes of individuals of the same age and sex has been observed[17]. The question is: can all of this be explained on the basis of differences in body weight and activity or do metabolic adaptations have a role. Edmundson[18] monitored the work output and energy intake of 54 adult East Javanese men on six different days over a period of one year. There seemed to be no correlation between the energy output and energy intake. Edmundson suggested that there must be 'compensatory mechanisms' that allow much greater efficiency of those accustomed to low energy intakes than those with relatively higher intakes. In a subsequent study he observed the BMR of the low energy consumers to be 50% that of the high energy consumers and their energy expenditure 70% of the high energy consumers for the same activity[19]. According to Hegstead[20], the amount of total energy converted into high energy compounds like ATP may not be the same in all individuals and at all times. The more energy-efficient individuals may be wasting lesser amount of energy in the futile cycles. Genetically, obese mice are more efficient users of energy than normal non-obese mice. They have lower levels of Na–K pump activity in their erythrocytes[21]. Obese subjects have also been reported to have lesser number of sodium–potassium pump units in erythrocytes[22].

BMR is known to reduce in chronic energy deficiency. However, 65% of this can be attributed to the shrinkage of the metabolizing mass of cells and only 35% to an actual decrease in cellular metabolic rate.

Most nutritionists feel that the contribution from metabolic adaptation may be minor compared to that from differences in physical activity. Accurate measurement of the quantum of physical activity and its energy cost are very difficult and subject to tremendous errors even in the hands of best investigators.

The energy requirement for pregnancy is an enigma and a subject of considerable interest. The factorial approach in which the additional costs of the increased BMR (known to be associated with pregnancy) and accretion of maternal and foetal tissues are added to the RDA for the non-pregnant state is generally applied[23]. In a recent multicentric study conducted by the Nestle Foundation in five countries, Scotland, Holand, Gambia, Thailand and Philippines, some very interesting observations were made[24]. Except Thailand (where the daily energy intake of the pregnant mother was found to increase by 250 kcal above the non-pregnant state), in none of the other centres did the mothers food intake increase to meet the theoretically expected quantities. The energy cost of the activities like walking was found to be only slightly lower than the non-pregnant state and unlikely to make an overall impact on energy demand. The Gambian women showed much lesser magnitude of rise in BMR during pregnancy compared to the other women. In fact, their BMR tended to decrease in the first trimester. This remarkable observation needs to be confirmed since similar trend was not observed in the other two developing countries where perhaps the magnitude of the dietary deprivation was not as severe as in Gambia and women's body weights above 45 kg. Administration of food supplement to the Gambian women increased their BMR, suggesting that it was not a genetic adaptation but an adaptation to low energy intake.

Durnin is of the opinion that small reductions in energy expenditure which cannot be detected easily must occur in pregnancy to explain the observed deficit

of almost 46,000 kcal (192 MJ) between the total intake and calculated expenditure over the entire period of pregnancy in the Scottish women studied by him. He is also of the view that the extra energy requirements for pregnancy may be much less than 300 kcal currently recommended and may not exceed 100 kcal (420 kJ) (ref. 24).

The question of adaptation to chronic low-calorie intakes is a subject of controversy and of great importance in view of its relevance to the determination of poverty line and projections for food requirements in developing countries like India. Is 'small really beautiful'? The eminent Indian biostatistician Sukhatme holds the view that RDA for energy can be fixed at a level of -2 SD of the mean for the population, because, according to him, substantial autocorrelated intra-individual variations over a period of time can occur in energy and protein intake and a person can get adapted to much lower intakes than now recommended. However, most nutritionists totally disagree with this stand. Since RDAs are not meant for individuals but for population groups, freak observations cannot be used to make recommendations. Adaptation (even if it does occur) should be regarded as body's compromise with a stressful condition. It is neither natural nor normal and certainly cannot be called healthy.

Young and Bier[25] have attempted to differentiate between 'adaptation' and 'accommodation'. While adaptation was defined as the 'integrated metabolic response that maintains the organism in a steady state within a preferred range, which is fully reversible when the environment changes' (implying no significant loss of overall physiologic function), 'accommodation' was defined as a 'metabolic adjustment that occurs when the environment (in this case diet) is more extensively altered and accompanied by changes that are outside of the preferred range, resulting in loss of a physiological function. The limits of normalcy, adaptation and accommodation may be different for different individuals and hence while talking about RDA it is advisable to aim at normalcy and not even adaptation.

Protein

Proteins provide total nitrogen and essential amino acids. They can also be used for providing energy if an adequate supply of energy is not available through carbohydrates and fats, but this is a wasteful strategy since protein-rich foods are expensive. Proteins should be conserved for more important tissue-building and metabolic functions.

The earliest estimates of protein requirement based on the amounts available through the diets of healthy populations, were much higher than the present day estimates based on nitrogen balance studied. The lowest amount of nitrogen intake needed to maintain nitrogen equilibrium is assessed by measuring the intake of nitrogen from diet and losses of nitrogen through urine, faeces and other routes, such as sweat, integuments, etc. A safe value of 2 SD is added to the mean value to cover 97.5% individuals and allowances made for growth, lactation, etc. by the factorial approach.

The nitrogen balance approach has several limitations. For instance, nitrogen balance in adults can be achieved over a range of protein intakes. The overall nitrogen balance in the body may not mean nitrogen equilibrium in all organs and tissues, the rate of protein turnover in different cells being different. The extrapolation of short-term nitrogen balance studies to longer periods of time is open to question. Biochemical abnormalities do show up when the lowest levels seemingly enough to maintain balance over short periods, are continued over longer periods. Recent studies show that energy intake has positive influence on protein requirement and calorie balance seems to have a nitrogen sparing effect[26,27]. Accurate measurements of nitrogen losses, particularly through routes other than urine and faeces, are difficult to make and the factors applied may not be correct for all individuals.

Attempts have been made to obtain estimates of the requirements for each of the 8–10 non-dispensable (essential) amino acids. A consistent pattern between the requirement of essential amino acids and proteins does not seem to emerge. For instance, the total amount of essential amino acids required by adult women has been estimated to be 4 g, which would be supplied through just 8–10 g of high-quality protein[28] (protein quality is determined by the relative amounts of essential amino acids *vis-á-vis* egg protein as standard). The relationship between the level of safe protein intake and essential amino acid requirement differs with age. While in infants, 43% of the RDA for proteins can be attributed to RDA for amino acids, in adults this value drops to 11% (ref. 25).

Young and Bier[25] have examined this phenomenon by measuring the kinetic indices of the metabolism of single amino acids labelled with stable isotopes administered by continuous infusion. These studies indicate that the requirement for the essential amino acids may be much higher than hitherto revealed by the conventional nitrogen balance measurements. However, the inherent assumption that the fate of a single amino acid given by intravenous, or, intragastric route is similar to that of the same amino acid consumed as dietary protein, may not be valid.

The last word with regard to protein requirement has not been said. Like for most other nutrients, lack of a sensitive functional parameter to use as the yardstick for judging adequacy is the basic problem. The question of accommodation or adaptation raised for calories has also been a subject of debate with regard to protein

requirement. Can people who are habituated to low protein intakes get adapted to it and utilize it more efficiently? Obligatory nitrogen losses of different populations subsisting on varying levels of proteins have been found to be remarkably similar[16]. There is, however, one exception. Nigerian farmers habituated to low-protein intakes could achieve balance at protein intakes much lower than the US subjects habituated to much higher protein intakes[29].

During the fifties and the early sixties, it was widely believed that protein deficiency is the major nutritional problem in populations who subsist on cereal-based diets. Frantic efforts were made to identify new sources of proteins and fortify cereals with limiting amino acids. With the downward revision of RDA for protein and the realization that cereal-based diets generally include legumes or small quantities of animal proteins and thus provide reasonably good-quality protein; the emphasis has shifted from protein gap to food gap. Qualitatively these diets are more deficient in micronutrients like vitamins and minerals than proteins.

Fat

Fats provide the most concentrated form of energy in our diet—9 kcal/g, compared to 4 kcal/g from carbohydrates or proteins. They facilitate the absorption and transport of fat-soluble vitamins and provide the essential polyunsaturated fatty acids (PUFA). Dietary fat adds to the flavour of food and increases the feeling of satiety. Fat requirement has thus to be considered at three levels. Minimum needed to meet the essential fatty acid requirement, desirable level for improving the palatability of food and providing satiety, and the maximum safe level which should not be exceeded, since excess fat is injurious to health.

PUFA are of two types, the n-3 (omega-3) and the n-6 (omega-6) series, depending on the position of the double bond closest to the methyl terminal group of the fatty-acid chain. The n-6 fatty acids are derived from linoleic acid (Lin, 18:2 n-6), which has 18 carbon atoms and two double bonds, the first double bond being on the sixth carbon from the methyl group. Its biologically important derivatives are: gamma-linoleic acid (18:3 n-6), dihomogamma-linoleic acid 20:3 n-6), arachidonic acid (18:4 n-6) and docosapentaenoic acid (DPA, 22:5 n-6). Except carnivores like cats, all animals and humans can synthesize these longer-chain, higher-unsaturation fatty acids from Lin. Cats require dietary arachidonic acid[4]. The fatty acids of the n-3 series are derived from linolenic acid (Len, 18:3 n-3). The biologically important derivatives of Len are: eicosapentaenoic acid (EPA, 20:5 n-3), decosapentaenoic acid (DPA, 22:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3). While n-6 PUFA predominate in membranes of most cells, cell membranes in retina and nervous tissue have a higher proportion of n-3 PUFA. The importance of n-3 PUFA in vision and nervous system is recent knowledge of considerable nutritional significance[30]. PUFA are the precursors of prostaglandins and leukotrienes, which have important and varying physiological functions in vascular, immune and renal systems. The longer-chain-length PUFA are more important. A great deal remains to be understood about the interactions and balances between the functions of n-6 and n-3 PUFA at molecular and physiological levels.

Effects of different fatty acids on serum lipids are not uniform[31a]. While the saturated fats raise blood low-density-lipoprotein (LDL) cholesterol (an undesirable effect), all types of PUFA and oleic acid lower it. Saturated fats as well as n-6 PUFA lower high-density-lipoprotein (HDL) cholesterol whereas the serum triglyceride-lowering effect is seen only with n-3 PUFA. Both n-6 and n-3 PUFA reduce platelet aggregation. Thus n-3 PUFA seem to be even more beneficial than n-6 PUFA in maintaining a desirable blood-lipid picture. Fatty-acid requirement has thus to be considered in terms of not just the total PUFA content but also the n-6/n-3 ratio.

The FAO/WHO has suggested 15% total fat calories as optimum for all age groups. The minimum requirement of linoleic acid is estimated to be 3 en% (energy per cent)[32], except during pregnancy (4.5 en%) and lactation (6 en%). Not all of this need be derived from visible fats such as vegetable oils, since our diet does contain a good deal of invisible or bound fat—a fact not appreciated till recently. In fact, almost 50% of the Lin requirement may be met from invisible fat[33]. Taking all requirements into consideration, it is estimated that 22 g vegetable oil in the daily diet would be adequate[31b]. Total fat calories in the diet should not exceed 30%. However, there are populations like the Eskimoes who derive more than 50% of calories through fat and yet have a low incidence of cardiovascular diseases. This is suspected to be due to the beneficial effect of the higher chain length PUFA such as EPA and DHA present in the fish oil.

The requirement for Len has not yet been worked out since its unique importance was not recognized till recently. 0.3–1 en% Len and n-6/n-3 ratio 5–10 appear to be desirable[30]. Ratio above 50 is harmful since it tends to lower serum DHA levels. The significance of ratios between 10 and 50 is not clear.

The new findings regarding the importance of n-3 fatty acids and the ratio of n-6/n-3 raise questions regarding the wisdom of promoting oils such as safflower oil which are very rich in linoleic acid and contains negligible amounts of linolenic acid. Ghafoorunissa[31] has computed the Lin/Len ratio in the diets of Indians of various income groups who use different types of oils. The highest ratio of 39.6 was obtained

with the high-income-group subjects, who use safflower oil as the cooking medium. Ratios 10 and below were seen only with palmolein, and soybean and mustard oil. However, mustard oil has erucic acid, which is an undesirable fatty acid. Green leafy vegetables are a good source of Len.

The subject of fat requirement is of great interest to countries like India, where there is considerable edible-oil shortage on the one hand and increasing incidence of coronary heart disease on the other. For achieving the aim of optimum nutrition, increased production will have to be accompanied by steps to achieve equitable distribution and health education. Otherwise increased supply of edible oil will go to those who need it the least.

Macrominerals and microminerals

Depending on the quantity present in the body and the amount needed through the diet, essential minerals are called macrominerals or microminerals—trace elements. Macrominerals include calcium, phosphorus, magnesium and the electrolytes sodium and potassium. Deficiencies of these macrominerals except calcium are unknown or occur rarely in some diseases or physiological conditions. Dietary calcium deficiency is, however, known to occur in populations whose milk intake is low.

Body can adapt to and remain in positive calcium balance over a wide range of calcium intake by regulating urinary excretion and enterohepatic losses of calcium. Thus the RDA for calcium has varied from 400 mg for Indians[34] to 1200 mg for Americans[35]. Apart from the calcium content of diet, the ratio of calcium to phosphorus is also important to ensure optimum calcium absorption and utilization. Based on studies in experimental animals, it is suggested that the optimum ratio of calcium:phosphorus should be 1:1 except in infancy when it should be 1.5:1. More research is needed to establish the ideal ratio for humans.

There is considerable epidemiological evidence to correlate hypertension with high intakes of sodium. Recommendations for sodium requirement are confounded by the fact that losses through sweat (which are substantial in tropical countries) are hard to estimate. Sodium intake of 40–100 mmol per day for adults is considered safe. Intakes above 100 mmols (2.3 g sodium or 5.8 g NaCl) are undesirable. Natural diet provides about 1 g NaCl. The current intake of 4–15 g salt in most populations thus exceeds this safe level. The ratio of sodium to potassium is also important. The present recommendation is 1:1.

About 15 elements are currently regarded as essential microminerals or trace elements. Of these, the essentiality of iron, iodine, copper, chromium, cobalt, manganese, molebdynum, selenium and zinc is well established and their functions known. The dietary requirement of others such as nickel, silicon, tin, vanadium, arsenic and fluorine has been demonstrated recently in animals, but their functions and nutritional significance in humans are yet to be established.

From the nutritional point of view, iron and iodine are the most important, since their deficiencies are widespread. Zinc deficiency has also been reported from Egypt and the Middle-East[36]. Absence of similar reports from other parts of the world does not rule out its occurrence. Selenium deficiency has been reported from China[37] and is believed to be the cause of Keshan disease—an endemic cardiomyopathy. While RDAs for iron and iodine have been worked out, the requirements of the other trace elements have not been established, though some tentative recommendations based on amounts derived through the diet of healthy individuals have been attempted. The trace-element content of food varies a great deal, being influenced by the environment. At higher levels, some trace elements can be toxic and hence it would be desirable not only to get estimates of the minimum requirement but also the limits of safe intake.

Food iron occurs in non-haem and haem forms. The absorption of the former is adversely affected by dietary factors such as phytates and tannins and is enhanced by dietary ascorbic acid. For instance, the absorption of iron from Continental breakfasts was found to be 7.6% with coffee as the beverage, but only 3% with tea as the beverage perhaps because of the tannins in tea. Inclusion of orange juice in the breakfast with coffee raised the absorption to 16.4% (ref. 38). Haem iron is absorbed better than non-haem iron, but it forms a very small percentage of total food iron. The absorption of iron from cereal–millet diets consumed in developing countries is only 3%. It tends to be higher in pregnancy and in anaemia. Such variations in iron absorption complicate the derivation of the iron requirement, and marked differences in dietary recommendations exist between countries. For instance, recommendations in the US for adult male and female respectively are 10 mg and 18 mg per day whereas the Indian recommendations are 24 mg and 32 mg.

Iodine deficiency in water and food is the major cause of endemic goitre. However, the severity of goitre does not always coincide with the severity of iodine deficiency. Presence of goitrogens like thiocyanates (also formed from hydrocyanic acid, in foods) can also cause goitre. Iodization of salt has been recommended to combat endemic goitre. Some countries do universal iodization of all salt to overcome the problem of dual marketing. However, the safety and wisdom of supplying iodized salt to areas where water and food are not deficient in iodine has been questioned by some. Available information is inadequate to comment on the

safety aspect, particularly in populations where salt intake is high. There are socioeconomic and political dimensions to the issue of universal iodization of salt.

Vitamins

While all the substances categorized as vitamins are essential for humans, dietary deficiencies of only some are known to occur. Dietary deficiencies of vitamins E, K (except in new-born infants), biotin pantothenic acid and even B_{12} are seldom seen.

Some of the dreaded diseases of the past like beri beri (thiamin deficiency) and scurvy (vitamin C deficiency) which used to take a heavy toll of life even 50 years ago, have almost disappeared. Rickets is more a clinical than a public-health problem and its aetiology in tropical countries where there is plenty of sunshine to ensure the synthesis of vitamin D_3 in skin is not clear. Dietary deficiency of calcium and/or protein may be the causative factor in tropical rickets. Pellagra still occurs in pockets, but its aetiology is controversial. Dietary factors which interfere with the conversion of tryptophan to niacin (excess leucine, vitamin B_6 deficiency and toxins) have been implicated[39]. Milder forms of deficiencies of thiamin, niacin and vitamin C, however, do occur and can be detected through biochemical tests. Contrary to the above cited deficiencies, vitamin A deficiency continues to be a major public health problem in the developing countries. Millions of children become blind annually owing to severe deficiency of vitamin A and many more suffer from less severe manifestations like corneal xerosis, night-blindness and bitot spots.

Deficiency of some B-complex vitamins, notably riboflavin, followed by folic acid and pyridoxine in developing countries like India, are rampant, particularly in women and children[40,41]. They do not receive adequate attention because they do not result in crippling disabilities. However, they do lead to functional defects such as reduced psychmotor performance[40] and impaired wound healing[42,43] and hence can be expected to compromise the overall performance of populations where they occur. Folic-acid deficiency impairs haemopoiesis, but its contribution to the causation of anaemia is less compared to that of iron deficiency. Neural-tube defects have been traced to folic-acid deficiency in pregnancy[44].

The values for the RDA of vitamins are based on very limited experimentation. Many of the earlier depletion-repletion studies in humans, which have given valuable information, would not pass through the ethics committees of today. Often recommendations are made and subsequent revisions suggested on the basis of single experiments in adult subjects. For instance, till recently, RDA for vitamin A in adults was 750 μg per day for both men and women. The basis of this figure was a study conducted in the UK called the Sheffield study[45]. Recently, FAO/WHO[16] have reduced this level to 600 μg on the basis of another study conducted by Sauberlich *et al.*[46] on American adults. The additional argument for such a downward revision was the observation that the liver stores of vitamin (which reflect body reserves) in healthy subjects who die in accidents are around 20 μg/g (ref. 47). A factorial computation of intakes needed to ensure this level of storage was made assuming a liver-weight-to-body-weight ratio of 0.03, a fractional catabolic rate of 0.5%, liver stores forming 90% of the body stores, and the efficiency of storage as 50% of the amount ingested. To the resulting figure of 434 μg retinol per day, a coefficient of variation of 20% was added, and the level of 600 μg obtained as RDA for adult men and women. With refinement in stable-isotope techniques to measure vitamin stores and turnover, it may be possible to obtain better estimates of vitamin requirements than in the past.

RDA for infants should ideally be based on amounts obtainable through milk in well-nourished women. Yet the present-day recommendations for many vitamins are much higher, since they are based on a few case studies of infants fed on formula milk or on the basis of amounts required to treat children suffering from deficiency diseases. For instance, vitamin B_6 intake through breast milk can be maximally 100 μg per day. On much lower intakes Indian breast-fed infants were found to have normal vitamin B_6 status[48]. Yet the present recommendation for the vitamin B_6 requirement of infants is 300 μg, and was based on the amount needed to treat children suffering from infantile convulsions.

For vitamins like vitamin A and niacin where part of the requirement is derived through precursors (β-carotene and tryptophan), there is the additional complication of efficiency of conversion and absorption of the precursor as well as the vitamin. Even today there is a controversy about the site of β-carotene cleavage (central or random) by the enzyme β-carotene oxygenase and the number of vitamin A molecules arising from one molecule of β-carotene. Most workers consider the efficiency of conversion of β-carotene to retinol to be 50% and make further allowance for absorption, which can differ substantially depending on the dietary source of β-carotene.

The amounts of a vitamin required to maintain its different functions may differ. Thus the requirement of vitamin C has become a subject of controversy and the recommended daily allowances suggested by different countries have varied from 20 mg to 200 mg. A daily intake of 10 mg ascorbic acid can protect against scurvy but much higher intakes are necessary to saturate the body stores. Is it necessary or wasteful to saturate the

Table 1. Summary of recommended dietary allowances for Indians (1989).

Group		Body wt (kg)	Net energy (kcal/day)	Protein (g/day)	Fat (g/day)	Calcium (mg/day)	Iron (mg/day)	Vit. A. μg/day		Thiamin (mg/day)	Ribo-flavin (mg/day)	Nico-tinic acid (mg/day)	Pyri-doxin (mg/day)	Ascor-bic acid (mg/day)	Folic acid (μg/day)	Vit. B-12 (μg/day)
								Retinol	β-Caro-tene							
Man	Sedentary work		2425							1.2	1.4	16				
	Moderate work	60	2875	60	20	400	28	600	2400	1.4	1.6	18	2.0	40	100	1
	Heavy work		3800							1.6	1.9	21				
Woman	Sedentary work		1875							0.9	1.1	12				
	Moderate work	50	2225	50	20	400	30	600	2400	1.1	1.3	14	2.0	40	100	1
	Heavy work		2925							1.2	1.5	16				
	Pregnant woman	50	+300	+15	30	1000	38	600	2400	+0.2	+0.2	+2	2.5	40	400	1
	Lactation															
	0–6 Months		+550	+25						+0.3	+0.3	+4	2.5	80	150	1.5
	6–12 Months	50	+400	+18	45	1000	30	950	3800	+0.2	+0.2	+3				
Infants	0–6 Months	5.4	108/kg	2.05/kg						55 μg/kg	65 μg/kg	710 μg/kg	0.1	25	25	0.2
	6–12 Months	8.6	98/kg	1.65/kg		500		350	1200	50 μg/kg	60 μg/kg	650 μg/kg	0.4			
Children	1–3 Years	12.2	1240	22			12	400	1600	0.6	0.7	8	0.9		30	
	4–6 Years	19.0	1690	30	25	400	18	400		0.9	10	11		40	40	0.2–1.0
	7–9 Years	26.9	1950	41			26	600	2400	1.0	1.2	13	1.6		60	
Boys	10–12 Years	35.4	2190	54			34			1.1	1.3	15	1.6	40	70	0.2–1.0
Girls	10–12 Years	31.5	1970	57	22	600	19	600	2400	1.0	1.2	13				
Boys	13–15 Years	47.8	2450	70			41			1.2	1.5	16	2.0	40	100	0.2–1.0
Girls	13–15 Years	46.7	2060	65	22	600	28	600	2400	1.0	1.2	14				
Boys	16–18 Years	57.1	2640	78			50			1.3	1.6	17	2.0	40	100	0.2–1.0
Girls	16–18 Years	49.9	20.60	63	22	500	30	600	2400	1.0	1.2	14				

Source: 'Nutrient requirements and recommended dietary allowances for Indians', Indian Council of Medical Research, New Delhi, 1989, p. 129.

body stores? Though the megadose therapy suggested by Cameron and Pauling[49] to fight infections cannot be recommended as the daily allowance, the suggestion that, under certain conditions like ageing, thyrotoxicosis, stress and viral infections, the need for vitamin C may be higher cannot be vaguely dismissed.

Conclusions

The question of optimum nutrition is highly complex and unlikely to be resolved unless precise, non-invasive and safe techniques are developed to measure nutrient turnover and tissue stores in sufficiently large numbers of subjects of different ages, physiological states and dietary habits. Even that information may be inadequate and difficult to interpret when tested for its relationship with positive health. In the absence of objective parameters of optimum health, biochemical indices which reveal the state of tissue saturation will have to be used as guidelines to assess optimum nutrition provided saturation does not call for abnormally high intakes. The current recommendations for dietary intakes (Table 1) are at best tentative.

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Correction

Peroxisomal targeting signals — the end and the beginning. Suresh Subramani, *Curr. Sci.*, 1991, **61**, 28–32.
On page 30, in line 5 of paragraph 'The consensus tripeptide, read 'cysteine' for 'lysine'; lines 4 and 5 should read '... in the first position of the tripeptide serine is as good as alanine but these are better than cysteine....' On page 31, in the last column of table 2, the name W. Kunau has been misspelt.