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PROTEIN AND AMINO ACID REQUIREMENTS IN HUMAN NUTRITION

Report of a Joint
WHO/FAO/UNU Expert Consultation

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Members*

Professor J. Amaya-Farfán, Department of Food and Nutrition Planning, Faculty of Food Engineering, Campinas State, University, São Paulo, Brazil

Professor B. Beaufrère, Head, Department of Human Nutrition, National Institute for Agricultural Research, Clermont-Ferrand, France (*Co-Rapporteur*)

Professor N.F. Butte, Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, Houston, TX, USA

Dr M.I.Z. Cabrera, Supervising Science Research Specialist, Nutritional Biochemistry Division, Department of Science and Technology, Food and Nutrition Research Institute, Metro Manila, Philippines (*Vice-Chair*)

Professor P. Garlick, Director of Surgical Research, Department of Surgery, Stony Brook University, Stony Brook, NY, USA (*Chair*)

Dr G.S. Gilani, Senior Research Scientist, Nutrition Research Division, Health Products and Food Branch, Health Canada, Ottawa, Ontario, Canada

Professor A. Jackson, Institute of Human Nutrition, University of Southampton, Southampton General Hospital, Southampton, England

Professor K. Kishi, Nutrition Physiology, Department of Nutrition, School of Medicine, University of Tokushima, Tokushima, Japan

* Unable to attend: Professor P. Fürst, Institute for Nutritional Sciences, University of Bonn, Bonn, Germany.

Professor A.V. Kurpad, Dean, Population Health Research Institute, St John's Medical College, Bangalore, India (*Co-Rapporteur*)

Professor J. Millward, Director, Centre for Nutrition and Food Safety, School of Biomedical and Life Sciences, University of Surrey, Guildford, England (*Vice-Chair*)

Professor P. Pencharz, Division of Gastroenterology and Nutrition, University of Toronto, The Hospital for Sick Children, Toronto, Ontario, Canada

Professor W.M. Rand, Department of Family Medicine and Community Health, Tufts University School of Medicine, Boston, MA, USA

Professor D. Tomé, Professor in Human Nutrition, Paris-Grignon National Agricultural Institute, Group for Research and Study of Human Biology and Nutrition, Paris, France

Professor B. Torún, Scientist Emeritus, Institute of Nutrition of Central America and Panama, Guatemala, Guatemala

Professor J. Waterlow, London, England

Professor V.R. Young, Laboratory of Human Nutrition, School of Science, Massachusetts Institute of Technology, Cambridge, MA, USA

Dr R.M. Zulu, Senior Scientific Officer, National Institute for Scientific and Industrial Research, Food Technology Research Unit, Lusaka, Zambia

Representatives of other organizations

International Atomic Energy Agency (IAEA)

Dr V. Iyengar, Head, Nutritional and Health-Related Environmental Studies Section, Division of Human Health, Department of Nuclear Sciences and Applications, IAEA, Vienna, Austria

United Nations Administrative Committee on Coordination (UNACC)

Dr S. Rabeneck, Technical Secretary, Sub-Committee on Nutrition, UN Administrative Committee on Coordination, Geneva, Switzerland

Secretariat[†]

Dr G.A. Clugston, Director, Nutrition for Health and Development, WHO, Geneva, Switzerland

Dr I. Darnton-Hill, Institute of Human Nutrition, Columbia University, New York, NY, USA (*Technical Adviser*)

Dr S. Khanum, Medical Officer, Nutrition for Health and Development, WHO, Geneva, Switzerland (*Secretary*)

Dr J. Maskeliunas, Food Standards Officer, Joint FAO/WHO Food Standards Programme, Food and Nutrition Division, FAO, Rome, Italy

Dr P. Shetty, Chief, Nutrition Planning, Assessment and Evaluation Service, Food and Nutrition Division, FAO, Rome, Italy

Dr R. Weisell, Nutrition Officer, Nutrition Planning, Assessment and Evaluation Service, Food and Nutrition Division, FAO, Rome, Italy

[†] Unable to attend: Dr B. Burlingame, Senior Officer, Nutrition Planning, Assessment and Evaluation Service, FAO, Rome, Italy; Professor C. Garza, Director, Food and Nutrition Program, United Nations University, Ithaca, NY, USA; Dr K. Tontisirin, Director, Food and Nutrition Division, FAO, Rome, Italy.

1. Introduction

Addressing the energy and nutrient needs of populations has been a long-standing activity of FAO and WHO – perhaps the longest existing technical activity. The first FAO Expert Committee on requirements met in Washington, DC, in 1949, only four years after the establishment of the United Nations and its technical agencies, and the topic at that time was "Calories". The second FAO Expert Consultation was six years later and focused on protein requirements, followed a year later by the second Expert Consultation on Calorie Requirements. In 1963, protein was again reviewed; this marked the beginning of the collaborative work between FAO and WHO on protein requirements. The 1971 Joint FAO/WHO ad hoc Expert Committee on Energy and Protein Requirements was unique in that energy and protein were considered together for the first time. The United Nations University became part of this joint initiative in 1981.

This report arises from the Joint WHO/FAO/UNU Expert Consultation on Protein and Amino Acid Requirements in Human Nutrition, held at WHO headquarters from 9 to 16 April 2002. It builds on the work of several earlier consultations and meetings. The previous landmark Joint FAO/WHO/UNU Expert Consultation was on both energy and protein requirements, and took place in Rome from 5 to 17 October 1981. The report of that consultation was published in 1985 as No. 724 of the WHO Technical Report Series (*1*), and has been referred to extensively. Scientific knowledge and field experience have now moved far enough forward that a review of the expert opinion is warranted. This time it was felt that there was a need for separate consultations on protein requirements and energy requirements. The Joint FAO/WHO/UNU Expert Consultation on Human Energy Requirements was held in Rome in October 2001 and the subsequent report was published in 2004 (*2*).

In its report (*3*), the 1971 Committee reviewed the principles on which groups of experts in the past had based their recommendations on energy and protein requirements (*4*). It has been consistently stated that estimates of nutrient needs are concerned with groups and not with individuals. In confirming that assertion, the 1971 Committee emphasized two additional points: that estimates of requirements are derived from individuals rather than groups;

and that the nutrient requirements of comparable individuals often vary. Consequently, application of recommendations to any one individual for clinical purposes may lead to errors of diagnosis and management. The methodological basis, analytical aspects and statistical aspects have continued to be refined, and are also addressed further in this current report.

Ideas on the assessment of protein requirements have progressed in a rather different way from considerations of energy, which is one reason why separate consultations have been held, despite aspects of overlap and synergy. It is, for example, largely accepted that nitrogen balance reflects both protein and energy intake from the diet. The FAO Committee on Protein Requirements (*4*), which met in 1955, placed particular emphasis on the pattern of human amino acid requirements and the definition of requirements in terms of a reference protein with an "ideal" amino acid composition. Quantitative estimates of protein requirements were based on information available at that time on the needs for indispensable amino acids. In 1963, a Joint FAO/WHO Expert Group on Protein Requirements (*5*) introduced the new concept that the requirement for protein is determined by the rate of obligatory nitrogen loss from the body (principally in the urine, but also in faeces and through the skin) when the diet contains no protein. Measurement of these losses should provide an estimate of requirement, with correction for protein quality.

The 1971 Ad Hoc Expert Committee made advances in two directions. First, it recognized that, even with protein of high biological value, the minimum nitrogen intake needed to ensure balance, which has generally been used as the criterion for the maintenance requirement, is larger than the so-called obligatory nitrogen loss. An attempt was made, in the light of the information available at the time, to determine the magnitude of this difference. The second advance was the clear recognition that in estimating requirements for groups, the principles are not the same for energy as for protein. For energy, an individual's intake must match his or her output if that individual is to remain in a steady state, and it is accepted that physiological mechanisms exist by which this balance is normally maintained, albeit not on a day-by-day basis or even over longer periods. For protein, in contrast, there is no evidence for a regulatory mechanism that matches intake to requirement. However, there is also no reason to suppose that an intake moderately larger than the individual's physiological need will be harmful, at least within fairly wide limits. Together, these considerations led to an approach that described on the one hand an *average* requirement for energy and, on the other hand, a *safe level of intake* for protein. The safe level for a population was defined as the average protein requirement of the individuals in the population, plus twice the standard deviation (SD). There was little information about the variability of individual requirements, and the 1971 Committee accepted an

estimate of 15% for the coefficient of variation. These considerations were further developed in the 1985 report, and again during the 2002 consultation.

In 1975, WHO and FAO, unusually, convened an informal gathering of experts (*6*) to consider problems that had arisen in the application of the report of the 1971 Committee. They considered a number of situations in which it was thought that the 1973 report had been misused or was incomplete. They also recognized that the emphasis placed by previous groups of experts (*4–6*) on specifying nutrient requirements for healthy populations was an ideal. They began to tackle some of the problems that arise in reconciling this ideal with reality. Of particular importance are two questions relating to children: adjustment of requirements for deficits in growth and for the effects of frequent infections. One of the recurring themes in the 2002 meeting was the need to address again, and more directly, the requirements of children and adults for protein and amino acids in populations with a high disease burden. Another major area that continues to need attention is requirements and demands in catch-up growth.

In October 1977, a further informal meeting of experts continued the review process begun in 1975 (*7*). This group identified five main areas of uncertainty relating to protein requirements. These areas were addressed, and recommendations were made in the 1985 report (*1*). With a view to reconsidering these matters, as well as incorporating new research results and national experience, the 2002 Consultation again discussed these same topics, among others (in particular, quality of protein and labelling). The following areas of uncertainty remain.

- It continues to be questioned whether the 1973 and subsequent higher recommendations on adult protein requirements, based largely on data from healthy, well-nourished individuals, are realistic for developing countries, especially as they might be extrapolated to apply to children.
- Since 1971, a number of studies have re-emphasized the important relationship between energy intake and nitrogen balance, and it has been suggested that protein requirements determined from balance measurements at high levels of energy intake may have been set erroneously low. The 2002 Consultation felt that the previously suggested requirements for amino acids were lower than they should be and, in the light of new evidence, duly revised the requirements upwards.
- The 2002 Consultation considered that previous reports had given too little attention to the requirements of women, adolescents, and older children, and that further review was needed of the requirements for pregnant and lactating women and for the elderly. It is somewhat disappointing that there seems to have been so little new research since 1981 on the amino acid

requirements of infants and children. Such research is also important with regard to the elderly, in view of their increasing numbers in both industrialized and developing countries.

- More information continues to be needed on the ability of some local diets to meet protein needs, and on the extent to which amino acid scores and biological assays in rats give realistic estimates of the protein values of human diets. The 1985 Consultation concluded that few natural diets provide insufficient amounts of indispensable amino acids, except those of infants and preschool children. Nevertheless, it was apparent that more attention should be given to the digestibility of the proteins in a mixed diet, especially in the diets of people in developing countries. It is clear that the availability of dietary protein for all age groups can be significantly affected by digestibility, and that protein requirements should be appropriately adjusted for increased faecal and dermal losses of nitrogen.
- A preliminary attempt was made in the 1977 informal meeting to estimate the extra protein and energy requirements for compensatory growth in malnourished children and for recovery from frequent infections. The 2002 Consultation identified this as an area needing more attention.
- The question continued to be raised of whether or not adaptation to low protein intake involves any disadvantages, provided that the intake is sufficient to achieve balance and normal growth.

The 1981 Consultation concluded that a further full-scale expert consultation, along with further research, was essential. It identified key issues as needing to be addressed by an expert consultation, which the present report partially does. The mandate of the 2002 Consultation was to revise and update the conclusions and recommendations of the 1981 Consultation. In preparation for the 2002 Consultation, well-known scientists were asked to examine and review extant literature and their own experience, and write background papers on various topics that required revision. Several of the authors and other leading scientists met in Rome from 27 June to 05 July 2001 to discuss and analyse critically the contents of the background papers, which were subsequently modified as required. The modified papers, together with other documents and the conclusions of the discussion, were provided to all members of the Expert Consultation for analysis and consideration in their deliberations, and much of the present report is based on those background documents, many of which have been subsequently published (*8*).

Recommendations on protein and amino acid requirements are essential to support the following activities:

- determining food and nutrition adequacy of population food intakes;
- setting of national food and nutrition guidelines by countries worldwide;
- determining nutrient needs, and evaluating and ensuring the adequacy of ration quality and quantity for vulnerable groups such as refugees or displaced populations, in emergency situations, conflicts, time of food shortage and the like (of particular concern to the World Food Programme, the Office of the United Nations High Commissioner for Refugees, and non-governmental organizations active in relief work);
- guidance to the Codex Alimentarius Commission, particularly with respect to labelling and in drawing up regulations on the protein and amino acid content of industrially processed foods;
- providing information to manufacturers of infant formula and processed complementary foods, concerning protein and amino acid requirements of infants over six months of age or with special needs, and of young children;
- mapping and monitoring (potential and actual) food shortages and undernutrition in developing countries and globally, including early warning systems (e.g. Food Insecurity and Vulnerability Information and Mapping Systems);
- research on relationships between excessive or deficient protein intakes and long-term health outcomes or the occurrence of diseases.

Ideally, a group set up to advise on requirements would include representatives of a wide range of disciplines, but this was not feasible either in 1985 or in 2002. The multidisciplinary perspective could be appropriately incorporated when food-based guidelines are being designed at a national level. As the 1985 report (*1*) shows, despite all the work that has been done, many difficult biological and statistical problems remain. The statistical and broader tasks identified by the 2002 Consultation are: estimation of the distribution of the requirements; and interpretation of the distribution of recommended requirements. As in previous reports, the primary task of this Consultation has been to provide the United Nations agencies with tools for addressing practical questions on such matters as the adequacy of food supplies, targets for food and nutrition policy, and labelling of protein quality, as well as for providing specific recommendations for infants, children and adults throughout the life-course. It is hoped that, like the guidance provided in past reports, the conclusions and recommendations in the present report will help guide the decisions of national committees in developing estimates of requirements appropriate to local conditions and applications.

International meetings of experts, conferences and other forums have been extremely productive in generating new ideas and stimulating new research. This is particularly apparent in relation to protein requirements; each successive meeting, building on the work of its predecessors, has identified gaps in current knowledge, which research workers in many countries have done their best to fill. There continues to be a need for more research results from developing countries. It is hoped that capacity-building activities of the United Nations agencies and others will help this. Identifying problems and stimulating further research continue to be extremely important functions of expert consultations.

This report is not an end-point, but an important step in the continuous quest for scientifically-based answers, and for understanding the implications of these answers in terms of improved nutrition and health. In this report of the 2002 Joint WHO/FAO/UNU Expert Consultation on Protein and Amino Acid Requirements in Human Nutrition, the primary objectives were as follows:

- to review, revise and update protein and amino acid requirements for all age groups (infants, children, adolescents, adults, elderly), and for women during pregnancy and lactation;
- to review and develop recommendations on protein requirements in health and disease, including their implications for developing countries;
- to develop recommendations on protein quality and labelling, with respect to new requirement levels, for use worldwide and in the Codex Alimentarius.

The conclusions of the 2002 Consultation are as well grounded as is possible, given the present state of knowledge. As recognized in the 1985 report (*1*), future experience will show how realistic the recommendations are. Participants in the 2002 Consultation nevertheless feel that these new recommendations, while often not very different from previous recommendations, represent a major step forward in addressing protein and amino acid requirements in human nutrition.

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2. Conceptual framework for estimating protein and amino acid requirements

Access to sufficient food of an adequate quality to maintain normal body composition and function throughout the life-cycle is fundamental to maintaining health. A source of protein is an essential element of a healthy diet, allowing both growth and maintenance of the 25 000 proteins encoded within the human genome, as well as other nitrogenous compounds, which together form the body's dynamic system of structural and functional elements that exchange nitrogen with the environment. The amount of protein that has to be consumed, as part of an otherwise nutritionally adequate diet, to achieve the desired structure and function is identified as the requirement.

2.1 Basic concepts

A generic model for the dietary protein requirement (as with any nutrient) defines the requirement in terms of the needs of the organism, i.e. *metabolic demands,* and the dietary amount which will satisfy those needs, i.e. *efficiency of utilization*, thus: dietary requirement = metabolic demand/efficiency of utilization.

For planning and public health purposes and to minimize risk of deficiency, requirements are expressed as dietary allowances, which take into account variation between individuals. This aspect is discussed in section 3.

Metabolic demand is determined by the nature and extent of those metabolic pathways that consume amino acids and are conventionally identified in most factorial models of requirements as maintenance and special needs. Special needs include growth, pregnancy and lactation. Maintenance comprises all those processes that consume amino acids and give rise to urinary, faecal and other losses, which include a small component of net protein synthesis in skin, hair and secretions.

Dietary requirement is the amount of protein or its constituent amino acids, or both, that must be supplied in the diet in order to satisfy the metabolic demand and achieve nitrogen equilibrium. The requirement will in most cases be greater than the metabolic demand because of those factors that influence the efficiency of protein use, i.e. net protein utilization. These are factors

associated with digestion and absorption, which influence the digestibility and consequent amount of dietary nitrogen lost in the faeces, and the cellular bioavailability of the absorbed amino acids in relation to needs, which influences the biological value (see section 6).

There is general agreement that when the dietary intake of nitrogen is zero, and energy and all other nutrients are consumed in adequate amounts, there is an ongoing loss of nitrogen from the body, identified as *obligatory nitrogen losses*. There is also general agreement that as the intake of protein, amino acids and nitrogen increases, there is a level of intake adequate to enable nitrogen balance to be achieved, which we can define as the *minimum protein requirement*. This is the lowest level of intake that has to be consumed in order to enable nitrogen equilibrium to be achieved in the short and long term, i.e. it will involve the highest efficiency of utilization. In practice, measurements of the minimum protein requirement have varied widely within and between individuals – and to a greater extent than observed with measurements of the obligatory nitrogen losses – for a variety of reasons, some of which are not entirely understood. For this reason, identification of the minimum protein requirement is inherently difficult. This is in sharp contrast with the basal metabolic rate, from which energy requirements can be calculated, after taking into account other components of energy expenditure, and which can be measured with relatively little variation, under carefully defined standardized conditions. What follows describes those factors that can influence the minimum protein requirement.

2.1.1 *Metabolic demand*

The metabolic demand for amino acids and protein is the flow of amino acids through those pathways that together maintain the structure and function of the body. This comprises conversion of some individual amino acids into important metabolites, which are further transformed into nitrogenous end-products, mainly urea and other compounds in urine, faeces or sweat, as well as net synthesis of proteins lost from the body as skin, hair and any other secretions. This demand is inherently variable between individuals and in the same individual at different times during the day and at different stages in life. A complete description of the metabolic demand would include the rates at which each individual amino acid flows through all metabolic pathways under all likely circumstances, as well as the interconversions of the different forms of nitrogen available to provide for the appropriate amino acid proportions. Measurement of such a demand cannot be made with any certainty or precision, but can be exemplified or characterized for different situations.

The basal demand for nitrogen is usually considered to equate to the obligatory nitrogen losses, the sum of all nitrogenous losses by all routes from the

body after stabilization on a nitrogen-free but otherwise nutritionally adequate diet. It is, however, recognized that the obligatory nitrogen losses represent the special circumstances where the metabolic demands are met by protein mobilized from body tissues. If the pattern of amino acids in body protein is not an exact match of the pattern of the metabolic demands, and there are reasons to believe that it is not, then the obligatory nitrogen losses will include nitrogen from amino acids which are surplus to demand, and to this extent will overestimate the magnitude of the metabolic demands, at least in terms of total amino acid equivalents.

The extent and amino acid pattern of demand will vary according to genotype and those factors that determine phenotype, i.e. programmed metabolic capacity, age, sex, diet, body composition, physiological state, pathological or environmental stressors, and lifestyle, especially physical activity, with all factors potentially acting separately and together. Where necessary, adaptive factors may be brought into play to enable the demand to be met, and these factors may or may not be either fully effective or costless. Current knowledge extends to only a limited understanding of this range of variation in the demand, namely the effect of other factors of importance in "model" or "reference" situations.

While it might reasonably be assumed that genotype, programmed metabolic capacity, sex, age, and body composition are all factors that might contribute to the variation in the basal demand, in practice the extent of such influences has yet to be quantified. Indeed it is not clear how far the observed variability in the basal protein requirement can be attributed to methodological considerations, or reflects inherent biological variability.

2.1.2 *Growth*

During growth in infancy and childhood, there are increases in length, mass, development and maturation of function. For pregnant and lactating women, there are the demands for net tissue deposit or milk formation. In each of these cases, the needs are for a pattern of amino acids that matches the material being deposited, including extracellular proteins, DNA, RNA, cell membranes, creatine, haem, etc. There is good evidence that the pattern of amino acids that is needed to meet these demands is different from that in the basal state.

During the rapid gain in weight associated with recovery from a pathological insult, there may be extensive deposit of lean tissue. This has been used as a suitable model with which to characterize the energy and nutrient needs for net tissue deposition. The information obtained from studies using this model is of value in extending our understanding, but the needs thus determined for tissue repletion of a deficit cannot be presumed to be the same as the needs during normal growth and development.

2.2 Dietary influences on demand

Efficient dietary provision of protein, amino acids and nitrogen to meet basal demands in an individual will occur only when demands for energy and all other nutrients for normal cellular and tissue function are met. There are complex responses of protein and amino acid metabolism to alterations in dietary intakes of other nutrients.

2.2.1 *Energy*

There are well-established responses to variation in dietary energy and protein intakes. At constant levels of energy expenditure, increased energy intake improves nitrogen balance independently of the nature of the excess energy (i.e. carbohydrate or fat). The basis for this is not entirely clear, although the hormonal responses to energy intake, especially insulin secretion, can reduce demands by minimizing net protein loss through the inhibition of both proteolysis and the oxidation of amino acids. In contrast to this, an excess of dietary energy also leads to the accumulation of excess adipose tissue, which results in an increase in lean body mass and an associated increase in demands over time.

Overall food consumption is, for most situations, determined by the level of energy expenditure, and the greatest variability reflects differences in levels of activity. A more active person expends greater amounts of energy, consumes greater amounts of food, and hence has a higher absolute level of protein consumption. Since, with increasing activity, the demand for amino acids and nitrogen increases to a much lesser extent (if at all) than energy demands, it becomes easier to satisfy nitrogen demands, and the amino acid pattern of the diet becomes of lesser importance. In contrast, as activity levels fall, food consumption falls and hence absolute protein intake falls, so any relative imbalance between the pattern of amino acids provided by the diet and the pattern required by the body will become more evident. Thus, at lower levels of food consumption, a diet that might have been adequate for protein at high levels of activity, may no longer be adequate at lower levels of activity.

2.2.2 *Micronutrients*

The pathways of amino acid metabolism and interchange are critically dependent upon an adequate micronutrient status, and hence upon the amount and quality of food consumed (*1*). Although to date this is a poorly understood area, inadequate amounts of B vitamins or zinc will influence dietary biological value. In addition, with either supplementation or food fortification, disposal of any excess consumption of micronutrients can impose a metabolic demand or stress on the body. For example, excessive dietary zinc induces the synthesis of metallothionein, which can increase demands for sulfur

amino acids, whereas the synthesis of ferritin in response to excessive iron can divert amino acids from other functions such as growth.

2.2.3 *Lifestyle and environmental influences*

The most important lifestyle influence that may modify demands for protein is the level of physical activity. Variation in levels of activity influences the patterns of food consumption, as well as influencing body composition and metabolic demands (see section 5). Indeed, activity itself can play an important part in the integration of intermediary metabolism, influencing amino acid interchange and the availability to the rest of the body of compounds containing nitrogen, for example the flow of nitrogen from branched-chain amino acids through glutamine to arginine or other compounds. While activity can increase the demand for protein, the extent of this may be minimized by training and by adequate and appropriate energy intake (*2*). There is evidence that the high protein intakes consumed by some athletes may increase the oxidation of amino acids during exercise and thus increase apparent demands.

Smoking (*3*) and alcohol consumption can each influence both intake and demand. Also detoxification and excretion of those chemical agents and xenobiotics consumed as a normal part of the diet can place unbalanced demands upon amino acid metabolism. Medications that consume amino acids during their detoxification include paracetamol (acetaminophen), which can account for a considerable demand for sulfur amino acids.

Exposure to environmental challenge imposes metabolic stress, which induces either nonspecific inflammatory responses, or more specific immune responses when infections occur. At their most severe, such exposures result in a complete re-ordering of metabolic priorities, unbalanced losses from the system, and a fundamental change in the requirements for protein, amino acids and nitrogen. Furthermore, recovery from such responses requires increased and altered metabolic demands to make good the specific losses.

2.3 Achieving nitrogen balance

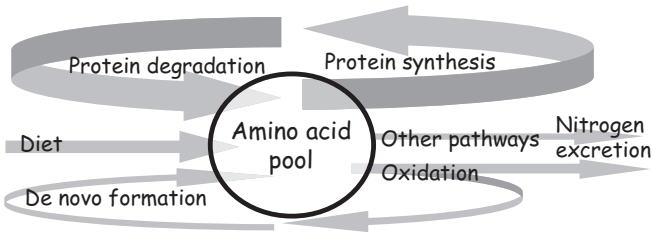
Adequate amounts of amino acids of a suitable pattern must be provided in the diet, either in a preformed state, or as appropriate precursors that can be used to generate a suitable mix of amino acids following endogenous transformations, in order to match the demand for protein synthesis and other metabolic pathways. Any demand that involves an irreversible net removal of part or all of indispensable amino acids from the system can only be satisfied from the diet.

The most general model that characterizes this movement of amino acids is shown in Figure 1. The demand within the system is the flow of amino acids to protein synthesis and other metabolic pathways, with any amino acids in excess of this demand flowing through oxidative pathways. This demand has to be satisfied with amino acids that derive from protein degradation, from the de novo synthesis of amino acids, or from dietary ingestion. To a substantial degree, the amino acids deriving from protein degradation will match the amount and pattern going to protein synthesis (apart from a minor fraction of amino acids which have undergone post-translational modifications such as methylations), so the demand will be dominated by the flow to other pathways.

One area of uncertainty relates to the usual assumption that dispensability and indispensability are absolute and mutually exclusive categorizations. This is almost certainly an oversimplification. For those amino acids identified as dispensable, there is in fact a variable extent to which endogenous formation might take place, with little reliable information about the upper limit of this capability, i.e. whether the endogenous capacity for their formation can always meet their demand. For the indispensable amino acids, this capacity is assumed to be zero, but in fact there is now evidence for some de novo synthesis of such amino acids following urea salvage in the lower gut. The extent of this and its nutritional significance remains uncertain, but has critical practical implications given the widespread use of stable isotope studies of amino acid oxidation as alternatives to nitrogen balance studies. Similarly, the extent to which de novo formation of dispensable amino acids might limit function in certain circumstances is important in terms of ensuring dietary adequacy during the formulation of special diets in clinical nutrition.

By its nature the system is more complex than we can characterize effectively at present (*4*) but there are elements which can be measured with variable degrees of reliability. Ultimately, the practical objective is to determine the extent and form of dietary nitrogen needed to enable a flow of amino acids sufficient to maintain health (body weight, nitrogen balance, and physiological, metabolic and psychological function).

Figure 1
General model for amino acid metabolism and interchange



2.3.1 *Maintenance amino acid catabolism and obligatory nitrogen losses*

As indicated above, the obligatory nitrogen losses on a protein-free but otherwise adequate diet have been assumed to indicate the magnitude of the maintenance metabolic demand (e.g. *5*). The obligatory nitrogen losses are assumed to reflect a demand for amino acid precursors for any net protein synthesis (mainly epidermal losses, plus menstrual blood in premenopausal women), for all non-protein products derived from amino acids that give rise to urinary nitrogen end-products, and any nitrogen lost in the large bowel. In reviewing the nature of the obligatory nitrogen losses, Reeds (*6*) emphasized that, with the exception of phenylalanine, tryptophan and methionine, current understanding indicates that the maintenance demand for amino acids is mainly for dispensable or conditionally dispensable amino acids. These obligatory nitrogen losses are assumed to be a fixed function of the lean body mass, although this is not known with any certainty. Factorial estimates of protein requirements calculate a dietary protein supply which provides for such losses, after adjustment for any inefficiency of utilization of dietary protein (e.g. *7, 8*). This assumes that on a protein-free diet the extent of amino acid catabolism indicates the usual metabolic demand, which is met from net tissue protein catabolism. Since the 1985 report (*9*) there has been an increasing effort to understand the nature of amino acid oxidation and the way nitrogen excretion is regulated.

It is agreed that there is a demand for amino acid precursors for a range of non-protein products deriving from either amino acid carbon skeletons or amino nitrogen, such as nucleic acids, diverse smaller molecules such as creatine, taurine, glutathione, catecholamines, thyroxine, serotonin, dopamine or nitric oxide, and some irreducible amino acid catabolism (e.g. of the branched-chain amino acids) which has not been identified as purposeful (see *6*). These various compounds are themselves catabolized, giving rise to various nitrogenous end-products. There is also catabolism during bacterial fermentation of carbon skeletons of amino acids that pass into the large bowel, with amino nitrogen reabsorbed as ammonia. In other words, the ileal digestibility of some indispensable amino acids is less than faecal digestibility. The overall pattern of these various pathways is unknown, but it has long been accepted that the amino acid pattern of the maintenance metabolic demand differs from that needed for tissue growth (see *5*). Also, there is evidence that addition of individual amino acids (sulfur amino acids and threonine) to protein-free diets can lower the nitrogen excretion, in the same way that diets from which deletions of single amino acids have been made will induce negative nitrogen balances that are not proportional to the tissue content of the deleted amino acids (e.g. *10, 11*).

There is little evidence to suggest that the magnitude of the obligatory nitrogen losses varies much between comparable age groups from different parts of the world (*9, 12, 13*). There may be differences in the distribution of these losses between faecal and urinary nitrogen, with higher faecal losses in subjects from developing countries, who maintain a higher faecal biomass resulting in higher overall losses. Thus in Indian and Nigerian men, faecal nitrogen = 40% (urinary nitrogen + faecal nitrogen), compared with 20% for Massachusetts Institute of Technology students, although it is not clear whether this reflects the higher non-protein nitrogen content of the experimental diets. Nevertheless, this higher faecal nitrogen seems to have metabolic implications, since it is associated with an inverse correlation between urinary and faecal nitrogen excretion (*14*, *15*) suggestive of a link between the size and nitrogen content of the faecal biomass and urea salvage, which might influence urinary urea excretion.

2.3.2 *Digestibility of dietary proteins*

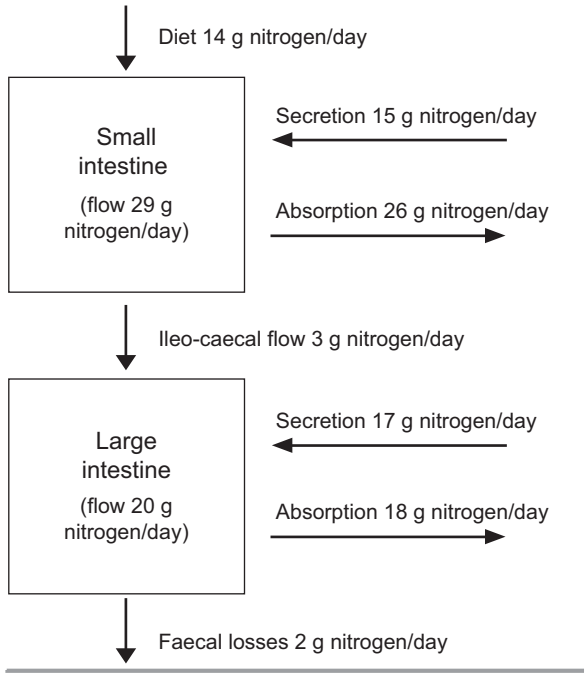
The concept of digestibility, usually defined in terms of the balance of amino acids across the small intestine (mouth to terminal ileum: ileal digestibility), or across the entire intestine (mouth to anus: faecal digestibility), is based on the principle that the difference between intake and losses provides a measure of the extent of digestion and absorption of food protein as amino acids by the gastrointestinal tract for use by the body. In fact, such net balance across the intestine involves considerable exchange of nitrogen in terms of protein, amino acids and urea between systemic pools and the gut lumen, as shown in Figure 2. Thus digestibility is more complex than usually assumed (*16, 17*).

The dietary supply of nitrogen-containing compounds into the system is predominantly protein, but also includes free amino acids, nucleotides and creatine, each of which may be important for health. The integrity of the gastrointestinal tract is maintained through net secretion of nitrogen-containing compounds such as mucins and antibodies, and the ongoing sloughing away of enterocytes. Further, there is significant secretion of proteins associated with the processes of digestion and absorption. Most proteins are digested and the resulting amino acids or peptides absorbed. The overall flow of endogenous nitrogen-containing compounds into the lumen of the small intestine is not known with reliability, but is estimated to be around 70 to 100 g protein each day. This mixes with amino acids derived from dietary consumption and, regardless of dietary consumption, the luminal concentration of amino acids appears indistinguishable by the mid-jejunum. Dietary protein and endogenous secretion are substantially absorbed by the time they reach the terminal ileum. Hence “ileal digestibility”, the difference between consumed amino acids and those appearing in the terminal ileum, is at best a very

crude approximation of the handling of nitrogen-containing materials in the small intestine. The use of labelled amino acids to trace the fate of dietary components reinforces the suggestion that most dietary protein is digested and absorbed with great efficiency.

The ileal effluent represents one aspect of the flow of nitrogen into the large bowel, and is only slightly greater (50%) than the nitrogen that is lost in the stool. This gives the impression in balance terms of little exchange of nitrogen compounds across the large intestine. However, tracer studies show that faecal nitrogen derives from a pool of nitrogen which includes not only ileal effluent and any residue from the dietary consumption, but also sloughed away cells and mucins derived within the colon, and nitrogen-containing compounds from the systemic circulation of the host. There is strong evidence for the movement into the gut of molecules such as urea, and reasonable evidence for the movement of molecules such as uric acid and creatine. These various forms of nitrogen are available as metabolic substrates for resident micoflora, but given the magnitude of faecal nitrogen loss it can be concluded that there is considerable reuptake of nitrogen from the colon. Furthermore, since according to circumstances the net effect of the amino acid metabolism associated with bacterial biomass can be to either remove

Figure 2
Exchange of nitrogen between the intestine and systemic circulation



[a]Adapted from reference 17 with permission from Vevey, Nestec Ltd/ S. Karger AG, Basel, Switzerland.

from or add to amino acids passing through the terminal ileum, ileal digestibility of individual amino acids is unlikely to be a more reliable measure of the systemic availability of dietary protein than faecal digestibility. The pattern of consumption of complex and non-digestible carbohydrates in the diet exerts an important influence on the metabolic behaviour of the colonic microflora because of their importance as an energy source. Faecal nitrogen is increased in individuals who consume diets containing large amounts of non-digestible carbohydrate, especially if this is susceptible to fermentation by the resident microflora, increasing the bacterial biomass and soluble nitrogen-containing compounds. With most diets this probably occurs to an extent that is largely independent of the protein associated with the non-digestible carbohydrate. Thus, when diets contain large amounts of non-digestible carbohydrate, faecal nitrogen cannot be used as a reliable measure of digestibility. Furthermore, an increase in faecal nitrogen can be matched by an equivalent decrease in urinary nitrogen, with the practical consequence that urinary nitrogen becomes a less reliable marker for nitrogen balance.

Thus, the concepts of both ileal digestibility and faecal digestibility are subject to important limitations under certain conditions. These conditions are most likely to apply where there is a need to determine the critical nutritional value of foods at the margins of satisfying dietary requirements, and therefore these methods cannot be used with any confidence in the development of policy options, unless the limitations of the underlying assumptions have been taken into account adequately.

2.3.3 *Protein quality: matching the supply to the demand*

The effectiveness with which nitrogen balance can be achieved for a given amount of absorbed dietary nitrogen is defined in terms of biological value, i.e. nitrogen utilized/digestible nitrogen intake. Biological value is most often discussed in terms of indispensable amino acid patterns relative to demand, permitting the identification of mixtures of dietary proteins that allow deficiencies of indispensable amino acid in one protein to be complemented with a relative excess in another protein, resulting in an appropriate overall dietary mixture. However, it needs to be recognized that biological value is in fact markedly influenced by the relative amounts of dispensable and indispensable amino acids and other nitrogen-containing compounds (*18, 19*).

Furthermore the definitions “dispensable” and “indispensable” for amino acids need to be interpreted with care. Work carried out during the 1960s to define minimal dietary amino acid and protein requirement levels, in which dietary sources were varied independently and together, showed that the “efficiency” of utilization of indispensable amino acids depends upon the total nitrogen and the form of nitrogen in the diet. The higher the total nitrogen

in the diet, the lower the consumption of indispensable amino acids to achieve nitrogen balance. A "good" mixture of dispensable amino acids is more effective than any other form of nitrogen, but even relatively poor sources of nitrogen, such as ammonia and urea, can exert a beneficial effect if the level of consumption is sufficiently high. Importantly, dispensable amino acids exert varying effects, with the provision of dietary glycine being especially effective in meeting the needs for indispensable nitrogen (*20*). In fact the minimum nitrogen intake for nitrogen balance is determined by the intake of indispensable amino acids. Thus, at a given level of nitrogen intake with animal proteins such as egg, milk or beef, the nitrogen balance improves when the protein is partially replaced by dispensable amino acids. Thus, the requirement for indispensable amino acids is not an absolute value, but can be expressed only in relation to the total nitrogen intake. The demonstration that the consumption of any form of dispensable nitrogen reduces the need for indispensable nitrogen implies that at lower levels of total nitrogen consumption, indispensable amino acids are being used inefficiently as a source of nitrogen for the formation of dispensable amino acids. The implications of this are that there is an absolute metabolic need for both indispensable and dispensable amino acids, and the rate of formation of dispensable amino acids in the body appears to be determined by the total intake of nitrogen, and at lower levels of total nitrogen consumption the formation of adequate amounts of non-essential amino acids is impaired.

Taken together this means that the concept of biological value, which is usually applied only in the context of matching individual indispensable amino acid intakes with the pattern of demand by the body, should also be extended to include dietary adequacy in terms of allowing for endogenous formation of the dispensable amino acids, and hence for total dietary nitrogen, to match the needs of the body. This becomes of practical importance in subjects fed diets based on amino acid mixtures. For example it may be that the ability of wheat-based diets to maintain long-term nitrogen balance (e.g. *21*), even though lysine intakes are much below apparent "requirement" intakes predicted in many of the more recent stable isotope studies, results from the use, in all of the tracer studies, of amino acid mixtures based on egg protein, with much less non-essential nitrogen than in diets based on cereal protein. Ultimately, in order to assess protein quality, direct study by measuring nitrogen balance along with body weight and body composition will be required to establish the utilization of proteins or diets with any certainty.

2.3.4 *Protein utilization and nitrogen balance*

Nitrogen balance measurements of the requirement usually involve subjects fed different levels of protein intake until they attain nitrogen equilibrium, i.e. when intake = loss, and balance = 0. Often a linear regression is used, so

that the intake for nitrogen equilibrium (the requirement) is defined by an intercept (the nitrogen loss at zero intake) and a slope. The intercept is an estimate of metabolic demands, i.e. the obligatory nitrogen losses. The slope indicates the efficiency of dietary protein utilization, which incorporates both digestibility and biological value: i.e. net protein utilization.

$$\text{Requirement} = \frac{\text{metabolic demand (intercept, obligatory nitrogen losses)}}{\text{net protein utilization (slope)}}$$

Balance studies in rapidly growing animals and young children show clear and predictable differences between protein sources in terms of digestibility, biological value and consequent net protein utilization, with values ranging from near-perfect utilization (net protein utilization = 1) for animal proteins, to much lower values for some plant-based diets. For human adults, however, it has long been known that the interpretation of balance studies poses some major difficulties. Thus, slopes and intercepts vary widely between studies with the same protein sources (*15*), and the outcome usually differs from the predictable value. This is clearly shown in a recent meta-analysis of all nitrogen balance studies reported to date (*13*). The median requirement (0.66 g/kg per day) was more than twice the obligatory nitrogen losses (≈0.3 g/kg per day) because the slope was <0.5. Furthermore, there was no significant influence of variation in the protein sources (animal, vegetable or mixed protein) on the slope and consequent requirement. This implies that for human adults, net protein utilization values for diets of most sources are similar, but much lower than would be predicted. Agreement has not yet been reached on an explanation for this, although one suggestion is that it is a consequence of incomplete adaptation (*22*). In any event, it is an indication of the importance of gaining a better understanding of how the organism adapts to variation in protein intake.

2.4 Response to variation in protein intake

The human organism can and does tolerate a wide range of dietary protein concentrations at no obvious cost. The difficulty for defining nutritional requirements for protein and amino acids lies in identifying the lower and upper limits of this intake range, beyond which any further adaptation may involve costs of one sort or another. At the outset, "tolerate at no obvious cost" needs to be defined. In the previous report, maintenance of nitrogen equilibrium in adults and achievement of satisfactory rates of growth in children were accepted as suitable end-points, but the way these might be achieved was discussed in terms of both changes in body nitrogen content and changes in protein and amino acid metabolism and turnover. Each of these two types of response requires discussion.

2.4.1 *Changes in body composition*

The growth potential of an individual in height and overall shape is likely to be achieved through the regulation of bone growth (*23*). This is genetically determined so that each individual follows a growth curve canalized in terms of both extent and time course if conditions are favourable (*24*), subject, of course, to optimal fetal programming to the extent that this influences postnatal height growth. Clearly, favourable conditions include adequate nutrition. Dietary protein plays a key role in this. It would appear that the growth of skeletal muscle, the largest component of the lean body mass, is also canalized, and arguments have been presented that for each genotype the muscle mass phenotype is characterized by a maximum size achievable with optimum nutrition and physical activity (*23*). In contrast, the sizes of many other organs, especially those of the splanchnic bed, are variable, responding to lifestyle factors that influence both energy expenditure and dietary composition, and that in turn regulate energy and protein intake. Thus, the protein content of the liver, gastrointestinal tract, kidney, etc. varies in response to functional demand and may increase with increasing dietary protein intakes associated with both high-protein diets and increased food intake in general associated with obesity, although there may be an upper limit (*25*). Muscle mass may also increase with obesity. Thus defining an optimal body protein content is difficult in relation to the entire lean body mass. Restricting such a definition to optimal muscle mass may simplify the situation. Organ size is now measurable, so that it should be possible to examine any change in organ size in relation to a change in protein intake.

2.4.2 *Labile protein reserves*

One feature of the response to changes in protein intake is gains and losses of body nitrogen, assumed to be protein and described as the labile protein reserves. Should the size of these be considered as an endpoint in nutritional evaluation? Although labile protein stores have been known and written about from the earliest times they remain largely unexplained. Garlick, McNurlan & Patlak (*26*), in reviewing a well-documented example of such losses of protein following changes in protein intake from 3 g to 1 g protein/kg per day (*27*), identified changes in the body urea and free amino acid pools in addition to any changes in tissue protein, which are probably too small to be detected. Since we know from animal studies that hepatic protein mass varies with protein intake, it may well be that labile protein reserves include in part changes in the size of those splanchnic organs which vary with functional demand. Another approach to understanding labile protein reserves is to identify them in metabolic terms, as changes in cellular protein which are a consequence of a delay in the mechanisms involved in the adaptive regulation of protein turnover, amino acid catabolism and nitrogen excretion to match

protein intake and achieve nitrogen equilibrium. Such equilibrium can clearly eventually be achieved over a wide range of intakes. Indeed many of the studies documenting labile protein reserves are conducted within "normal" ranges of protein intakes, when the "low" protein intakes are in fact above previously accepted safe allowances, e.g. 1 g egg protein/kg per day (*27*). Importantly this adaptation happens slowly in humans, requiring at least several weeks. In studies of the obligatory nitrogen loss, subjects fed a protein-free diet took between 10 and 17 days to achieve a constant low level of urea nitrogen excretion (*28*). In studies involving diets in which protein intakes were reduced from adequate to 0.35 g/kg per day, adult men took from 7 to 28 days to achieve nitrogen equilibrium (*29*). The subjects studied by Oddoye & Margen (*27*) took from 16 to >40 days to achieve balance after the reduction in intake from 3 to 1 g protein/kg per day.

It is unclear whether the protein gained during periods of increased protein intake is retained, or whether the protein lost during periods of low protein intake is regained if the treatments are continued, as there has been no systematic study of body composition of adults in relation to variation of protein intakes within the normal range in well-fed societies. However, attempts to increase muscle mass by increases in protein intake within the normal range have generally failed. Thus Lemon et al. (*30*) fed protein at 2.62 g/kg per day or 1.35 g/kg per day for 1 month during intensive weight training in a randomized double-blind cross-over study, and found no difference in measured strength (voluntary and electrically evoked) and muscle mass (density, creatinine excretion, muscle area by CAT scan, and biceps nitrogen content).

It follows that there is no convincing reason why any consideration should be given to a particular level of labile protein reserves in discussing body protein content in relation to the protein requirement.

2.4.3 *Protein turnover and amino acid recycling*

Discussion of metabolic responses to intake is complicated by the need to take account of the periodic nature of food intake and the consequent diurnal nature of overall daily balance. Because there is net protein catabolism with loss of tissue protein once the organism enters a post-absorptive state, during subsequent feeding net protein deposition will be required to replace post-absorptive losses, if overall balance is to be maintained. This periodic cycle of nitrogen gains and losses means that acute measurements of protein or amino acid metabolism relate in only an indirect way to the daily balance, unless measurements are made during both fed and post-absorptive states.

The magnitude of daily protein turnover, an amino acid flux several-fold greater than intake (*31*), requires reutilization of amino acids released by protein breakdown for protein synthesis. There has been considerable

research into the way that changes in protein turnover and consequent recycling of amino acids might influence dietary protein needs. However, there is little evidence for, or indeed reason why, dietary protein requirements should reflect protein turnover rates, since amino acids are recycled, except those with post-translational modifications (e.g. the 3-methylation of histidine). There is a general correlation between rates of protein turnover and endogenous nitrogen losses, with rates of both processes changing in relation to organism size and basal metabolic rate, most likely a reflection of the generally parallel metabolic changes in many cellular processes that make up the basal metabolic rate and contribute to both protein turnover and the obligatory nitrogen losses.

Very obvious responses of protein turnover to dietary inadequacies are seen in growing animals, especially in skeletal muscle (*31, 32*). However, in human adults there is not a simple relationship between protein intake and turnover that can be used as an indicator of dietary protein adequacy. Thus, the response of protein synthesis and especially proteolysis to feeding and fasting is sensitive to the level of protein intake (e.g. *33*), as it must be, given that the amount of post-absorptive protein loss and replacement with feeding varies with protein intake (*34*). However, overall daily rates of protein turnover change little with protein intake over a wide range (*26, 33*). Furthermore, with malnutrition, and in relation to ageing, changes in protein turnover are complicated by the changes in body composition. In malnourished adults, whole-body protein turnover rates appear to be increased when expressed per unit of lean body mass, probably because of the relatively greater losses of muscle compared with tissues with more rapid turnover. (*35*). Similarly, a lower proportion of skeletal muscle because of sarcopenia may explain why average daily rates of protein turnover change little with ageing (*36*), even though a fall in turnover of skeletal muscle protein with ageing has been reported (e.g. *37*). Thus, with protein turnover expressed per kg body weight, reflecting the relative size of the fat-free mass and its composition, the extent to which reduced turnover in non-muscle tissues occurs is difficult to identify (*36*).

Thus, whole-body measurements of protein synthesis have not proved to be a sensitive metabolic indicator of adequacy of protein intake, or a proxy that the requirement is being met. While the magnitude of net protein synthesis, i.e. the difference between protein synthesis and degradation, does constitute an important part of the metabolic demand for dietary protein, this appears to adapt in a complex way to meal feeding patterns and protein quality (*38*). Thus, the extent of net protein synthesis cannot be assumed to be a proxy for an adequate requirement.

2.5 Definition of requirement

On the basis that dietary protein requirements must provide for maintenance and any special needs of growth, reproduction and lactation, the protein requirement can be defined as: *the lowest level of dietary protein intake that will balance the losses of nitrogen from the body, and thus maintain the body protein mass, in persons at energy balance with modest levels of physical activity, plus, in children or in pregnant or lactating women, the needs associated with the deposition of tissues or the secretion of milk at rates consistent with good health.*

It is acknowledged that this definition of the requirement in terms of nitrogen balance does not necessarily identify the optimal intake for health, which is less quantifiable. It is assumed, however, that the body protein mass is maintained at a desirable level, as discussed in section 2.4.1 above. The impact on health of intakes higher than the requirement defined above is discussed in section 13.

2.6 Adaptive mechanisms

Adaptation to low intakes was briefly discussed in the 1985 FAO/WHO/UNU report and has been often revisited (*1, 17, 22, 39*). However, there are difficult issues involved and general agreement on the importance of adaptation has yet to be reached. The prevailing view of the 1985 consultation (*9*) was that for an individual adult, the requirement is genetically fixed over time, with inter-individual but no intra-individual variability. In that report, the previous safe allowance of 0.57 g/kg per day was increased to 0.75 g/kg per day, in part because of adverse responses to an intake of 0.57 g/kg per day in long-term nitrogen balance studies (*40–42*). An alternative view had been proposed (*43*), which allowed for intra-individual variability (i.e. adaptation), with a range of intakes within which protein homeostasis could be maintained, down to an intake equivalent to the obligatory nitrogen losses (i.e. 0.36 g/kg per day), based on a limited number of long-term nitrogen balance studies, some of which were considered in preparing the 1985 report (*29*). Although subjects in these studies ultimately achieved nitrogen balance, they lost weight, so the data are difficult to interpret, and the idea of intra-individual variability was not incorporated into the 1985 report. Indeed, since then, the derivation of dietary allowances and calculation of risk of deficiency have assumed no intra-individual variability (see section 3).

Since 1985, different views have emerged in relation to adaptation and these have become important in both the interpretation of nitrogen balance studies and in the design and interpretation of stable isotope studies. Some authors take no account of adaptation in their experimental design, arguing that prior adaptation is not needed (*44*). However, most authors accept that adaptation

occurs to some extent. Indeed, in the context of defining protein and amino acid requirements, Hegsted recently noted "healthy people must be considered to be 'adapted' to their current diet. If the requirement … is to be defined, the subjects must be allowed the time to adapt, otherwise one simply estimates the nutrient supply in the current diet, which has little nutritional significance" (*45*). Young has discussed the issue in relation to the extent and timescale, firmly rejecting the idea of long-term adaptation to intakes much below 0.6 g/kg per day, and proposing that "the limits of adaptation … are reached over a relatively short time frame" (*46*). This is consistent with the incorporation of a 7-day adaptation period into studies of amino acid requirements by Young and co-workers. Others have argued that an understanding of metabolic adaptation is central to both identification of requirements and their use in relation to risk assessment and management (*22, 47*).

2.6.1 *Adaptation of amino acid oxidation*

Millward & Rivers (*18*) introduced the concept of an adaptive component of pathways of amino acid oxidation, which has been verified by ^{13}C stable isotope studies of the way the metabolic demand varies with habitual protein intake (*38*), allowing an adaptive metabolic demands model of the protein requirement to be proposed (*22*). The key feature of this model is that loss of amino acids in other pathways (Figure 1) includes both an obligatory and an adaptive component, with the additional adaptive metabolic demand representing amino acid oxidation at a rate varying with the habitual protein intake. This adaptive metabolic demand is relatively insensitive to acute food or protein intake, changing only slowly (over many weeks or longer) with a sustained change in protein intake. The consequence of this for nitrogen homeostasis is a diurnal cycle of fasting losses and fed-state gains of increasing amplitude with increasing habitual intake. This has been demonstrated with both 12-hour nitrogen balances and short-term [1-^{13}C] leucine balances in subjects fed a wide range of protein intakes (*34, 48*). Thus, the marked losses of body nitrogen sustained during the transition from a high to a lower intake (e.g. *27*), previously identified as a loss of labile protein reserves, become a consequence of the time taken to reduce or adapt this aspect of amino acid oxidation rates. Because part of nitrogen excretion involves losses associated with this adaptive metabolic demand, the efficiency of protein utilization is higher than that indicated by the traditional model. This is evident when efficiency is measured as postprandial protein utilization, which takes the adaptive component of the metabolic demand into account (*49–51*).

The metabolic explanation of the adaptive metabolic demand is that, in order to be able to rapidly dispose of dietary protein in excess of minimal needs and maintain the very low tissue concentrations of the potentially toxic

branched-chain, aromatic and sulfur amino acids, the capacity of the pathways of oxidative catabolism of these particular amino acids adapts to match the habitual protein intakes. Although these pathways are to some extent regulated by feeding and fasting, this regulation is incomplete so that amino acid oxidation continues to occur after dietary protein is disposed of, continuing into the postabsorptive state.

To date there is limited experimental application of these principles involving either [1-^{13}C] leucine tracer balance studies with milk and wheat proteins in normal subjects and in the elderly (*22, 50, 51*), or some ^{15}N-studies with intrinsically ^{15}N-labelled proteins which involve somewhat different model assumptions (*52*).

2.6.2 *Adaptation of urea metabolism: nitrogen metabolism in the lower gut*

Jackson (see *1, 19, 53, 54*) has shown with tracer studies in children and adults, with ^{15}N-labelled urea, that an important aspect of adaptation to protein intake is a variable degree of urea salvage by bacterial hydrolysis in the gut, with recycling of nitrogen into the amino acid pool. The conventional view of urea salvage is that urea hydrolysis in the colon is minimally regulated or unregulated (e.g. *52*), and that nitrogen returns from the colon as ammonia. However, emerging evidence points to a more complex regulatory system in which urea can directly enter the colon, linking regulation of water balance and urea balance. While it had been thought that the colon is not equipped with sufficient amino acid transporters to allow the extensive retrieval of bacterial amino acids from the colon, this is now thought to be incorrect (*55, 56*).

The potential for the de novo synthesis of indispensable amino acids following urea salvage is a possibility identified many years ago (*57–59*), with the suggestion that the utilization of urea nitrogen in Papua New Guinea highlanders could improve their nitrogen balance on low- and poor-quality protein diets (*60, 61*). More recent work with pigs (*62*), human adults (*63, 64*) and malnourished infants (*65, 66*) has clearly confirmed that lysine and other amino acids derived from urea after intestinal microbial synthesis do appear in the circulating pool.

Thus it is clear that in humans, urea salvage does occur to a variable extent, with some of the salvaged nitrogen returned to the systemic pool as indispensable amino acids. Although the existing experimental database is small, the suggested magnitude of the process could be nutritionally significant. Furthermore, this has important consequences for studies of amino acid and protein requirements based on amino acid oxidation. Balance studies based on leucine oxidation, for example, should generally be more negative than expected, unless account is taken of de novo synthesis. It is clear, therefore, that

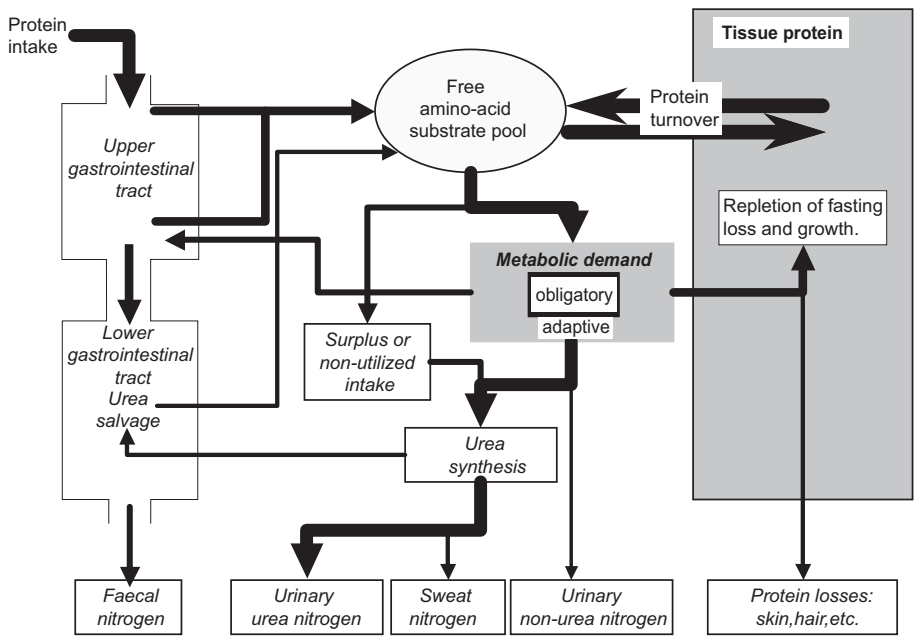
there is a need for a much better understanding of both the extent and regulation of urea synthesis, and of nitrogen metabolism in the lower gut, so that the quantitative importance of urea salvage can be incorporated into our overall understanding of amino acid homeostasis.

2.7 Summary metabolic model

Clearly, there remains some uncertainty about many individual aspects of amino acid, protein and nitrogen metabolism that together determine the metabolic demands for protein in the human diet. However, there is sufficient evidence to support the overall schematic representation of the metabolic demands shown in Figure 3.

The metabolic demand for amino acids is to maintain tissue protein at appropriate levels, to provide for all amino acid-derived metabolites, and any additional needs during growth, pregnancy and lactation. This demand is supplied from the free amino acid pool, the size of which, for most amino acids, is regulated within narrow limits. Regulation involves supply from three sources: dietary proteins after digestion and absorption from the upper gastrointestinal tract; tissue protein after proteolysis during protein turnover; and de novo formation, which may include amino acids and ammonia derived from urea salvage, after hydrolysis and bacterial metabolism in the lower gastrointestinal tract. Removal of free amino acids occurs by reactions in

Figure 3
Schematic representation of the metabolic demands for amino acids



which they act as substrates, and these reactions are shown as three pathways, one of which is the metabolic demand. This pathway involves a number of irreversible pathways, including net protein synthesis and other irreversible metabolic transformations of individual amino acids. The quantitatively largest pathway is the removal for protein synthesis during protein turnover. At nitrogen equilibrium, because turnover involves the reversible removal of amino acids with replacement through proteolysis, it does not exert a net metabolic demand (other than for those amino acids irreversibly modified during or subsequent to protein synthesis). Finally, amino acids may also be removed irreversibly by oxidation and nitrogen excretion provoked, for example, by the transient increases in some or all free amino acids after a protein meal. This would represent an inefficient utilization.

The metabolic demand for amino acids appears to involve obligatory and adaptive components. The obligatory component for subjects at equilibrium (i.e. maintenance) comprises conversion of some individual amino acids into important metabolites that are further transformed into nitrogenous end-products, mainly urea and other compounds in urine, faeces or sweat, as well as net synthesis of proteins lost from the body as skin, hair and any other secretions. The magnitude of the maintenance component is assumed empirically to be equal to the sum of all nitrogen losses from the body observed on a protein-free diet, after losses have stabilized at a low level, i.e. the obligatory nitrogen losses. Under these circumstances net tissue proteolysis is assumed to provide for the non-protein components of the obligatory demand, at a rate determined by the metabolic consumption of the rate-limiting amino acid (the amino acid with the highest ratio of molar proportion in the metabolic demand to molar proportion in protein). Because the obligatory metabolic demand is for a mixture of amino acids with a profile that is unlikely to match that of tissue protein, the actual nitrogen content of the metabolic demand is likely to be less than that in tissue protein mobilized to meet such demands, i.e. less than the obligatory nitrogen losses. This is because all amino acids mobilized to provide for the metabolic demand must be oxidized and will contribute to the nitrogen excretion, whereas only some of them will serve useful functions. The evidence for this is the lowering of the obligatory nitrogen losses in response to feeding selective amino acids such as methionine or threonine. Any net protein synthesis associated with growth, pregnancy and lactation is also included in the obligatory metabolic demand.

The adaptive component of the metabolic demand represents amino acid oxidation at a rate varying with the habitual protein intake, which occurs as a result of the increasing activities of the pathways of oxidation of amino acids that regulate free amino acid pool sizes. The reason for this is that humans grow very slowly, or maintain constant weight on diets that contain protein considerably in excess of minimum needs. Thus, in order to be able to rapidly dispose of excess protein and maintain the very low tissue

concentrations of those amino acids, such as the branched-chain, aromatic and sulfur amino acids, that may be toxic at higher concentrations, pathways of oxidative amino acid catabolism adapt (increase their *V*max) enabling them to operate at the appropriate rate set by habitual protein intakes. Importantly, the adapted rate, characteristic of habitual intake, changes only slowly in response either to a change in the level of dietary protein intake or to feeding and fasting. This has two main consequences. First, when intake falls below its habitual level, mobilization of tissue protein occurs, with a negative nitrogen balance for as long as it takes to adapt to the lower level of intake. This was previously identified as the labile protein reserve. The adaptive demand model of Millward & Rivers (*18*) assumes that for intakes greater than the minimum requirement, full adaptation to the new level will include not only a change in the adaptive metabolic demand to match intake, but also repletion of most tissue nitrogen lost during the adaptive transition. However, there is no experimental evidence to support this hypothesis, and failure to replete body protein must otherwise be regarded as disadvantageous. Second, because the adaptive rate of amino acid oxidation continues to some extent into the postabsorptive state, there are varying postabsorptive losses of tissue protein and nitrogen excretion with varying habitual intake. Because of this the adaptive metabolic demand model includes a component of net protein synthesis which replaces postabsorptive losses. The magnitude of this varies in a complex way with meal eating pattern, and with the amount and quality (amino acid score) of the habitual protein intake.

Although amino acid oxidation and urea synthesis are assumed to be irreversible, in fact this is not entirely true because of urea salvage. Thus the rate of urea synthesis is usually in excess of the rate of urea excretion, because some urea enters the lower gastrointestinal tract and is hydrolysed by bacteria. Most of this nitrogen is utilized by bacteria and, since little is lost as faecal nitrogen, it is eventually returned to the systemic pool as ammonia and amino acids, including indispensable amino acids. Although the extent and nature of this salvaged urea nitrogen is poorly understood, it may provide nutritionally important amounts of amino acids.

The dietary requirement for protein will be the minimum intake which satisfies metabolic demands and which maintains appropriate body composition and growth rates, after taking into account any inefficiency of digestion and of metabolic consumption. To satisfy the metabolic demand, the dietary protein must contain adequate and digestible amounts of nutritionally indispensable amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine), and amino acids that can become indispensable under specific physiological or pathological conditions (conditionally indispensable: e.g. cysteine, tyrosine, taurine, glycine, arginine, glutamine and proline), plus sufficient total amino acid nitrogen, which can be supplied from any of the above amino acids, from dispensable

amino acids (aspartic acid, asparagine, glutamic acid, alanine and serine) or from other sources of non-essential nitrogen. Minimum metabolic demands and consequent protein requirements will occur when the adaptive component has fallen to the lowest possible level. While it is not known with any certainty how long such adaptation would take, it may well be longer than the periods employed in short-term balance studies. This implies that short-term balance estimates of the minimum protein requirement may overestimate the value; and some of the variability in protein requirements between studies may reflect variable completeness of adaptation to the test diets.

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3. Statistical concepts and procedures involved in deriving recommendations for protein and amino acid requirements

The objective was to determine the dietary requirements for protein and amino acids for individuals and groups of individuals, or populations. The dietary requirements for protein and amino acids can then be expressed as reference intakes, which can be used as the basis for making dietary recommendations for groups of individuals or populations. The following points were considered to be fundamental.

- Dietary requirements for protein and amino acids are characteristics of an individual.
- Individual dietary requirements for protein and amino acids within a population can be characterized as a probability distribution.
- The status of a population in terms of dietary adequacy for protein and amino acids is estimated by comparing the distribution of its intakes with the distribution of its requirements.
- The distinction between an adequate or inadequate intake and the health or ill-health of a population and the societal problems that may follow from inadequacy is important in the application of reference requirements as the basis for recommendations. Using the terminology of Codex (*1*), inadequate intake is a hazard, while ill-health and societal problems are risks. Since the consequences of inadequacy of protein, and especially of amino acids, for ill-health are poorly understood, and not usually part of the methodology for assessing requirements, the risk resulting from the hazard of various degrees of inadequate intake is difficult to assess.

3.1 Overview

Estimating protein and amino acid requirements presents two major problems. One is that individuals vary in their demand for, and utilization of, these nutrients provided by foods. The other is that unequivocal indicators of the dietary inadequacy of protein and amino acids can rarely be identified, until gross dysfunction has developed. A large amount of data has been accumulated relating to various aspects of these phenomena. Since the 1985 report (*2*), additional and different forms of data have been gathered and a better

understanding of the fundamental phenomena has evolved. This has made possible a more unified and comprehensive examination of new and existing data, a more complete description of requirements, and a broader range of potential uses. Of necessity this whole procedure involves complex statistical methodologies that have been developed for exploring highly variable biological phenomena. This section discusses those statistical concepts and methods that are relevant to this report.

Estimating the protein and amino acid requirements for individuals ideally proceeds in four stages: gathering relevant data; estimating requirements of individuals; exploring whether individual requirements vary with anthropometric or demographic differences; and estimating and describing the distribution of these requirements. These steps are outlined below (section 3.2). After this, suitable intakes for individuals and populations need to be identified, so that dietary recommendations for individuals and populations can be developed. Because dietary recommendations can be used for different purposes, such as to estimate the adequacy of dietary patterns of populations, to plan feeding programmes or to develop food labelling, the last stage needs to be considered with particular care.

3.2 Phases of requirement estimation

3.2.1 *Gathering and screening the data*

Any statistical procedure requires that the data should accurately reflect the phenomena of interest. This dictates that the first, and perhaps most important, step in estimating nutrient requirements is preparation of the database: finding, collecting, standardizing, and screening those data that are relevant. Data on different aspects of dietary consumption of proteins and amino acids and their metabolism have been generated in different laboratories around the world at different times, using differing methods and for different purposes. In some cases, especially for amino acids, there are varying views about the strengths and weaknesses of particular approaches. However, for protein, there is an agreed method for determining the adult requirement, which is the short-term, multipoint nitrogen balance method. It is important that the assemblage of data, initially and throughout the process, be screened for inconsistencies and incompatibilities. This process of data review and selection, and the identification of data points that are outliers, is only partly a statistical task. This Consultation adopted a conservative approach, which accepted that data should be included unless there were compelling biological or experimental reasons for removing specific points from the database, and that inclusion or exclusion influenced the results. Thus, while specific statistical tests do exist for the detection of anomalous data (*3*), the general approach taken was to examine the data graphically and identify influential

points, and then both examine their source and run analyses with and without the putative influential points (*4*).

3.2.2 *Estimating the requirements of individuals*

As for any other nutrient, the requirement for protein is assumed to be an individual characteristic. The population distribution has been estimated directly from estimates of individual requirements, as far as is possible recognizing that somewhat arbitrary decisions need to be made about the nature of the variability associated with reported measurements. For the purpose of this report, the protein requirement of an individual is defined as the minimum intake that enables nitrogen equilibrium (zero nitrogen balance), so that the results analysed were based primarily on those studies that measured nitrogen balance in an individual at several different levels of protein intake. For each individual, the level of protein intake that would enable zero nitrogen balance was interpolated (using linear regression), and that level of intake was defined as the maintenance protein requirement for that person. For infants and children, and for pregnant and lactating women, the above procedures aimed at identifying a maintenance requirement were combined with further analyses of body composition changes and growth rates, and with estimates of the magnitude of the products of conception and lactation rates, to estimate the additional requirements for dietary protein that would enable an acceptable rate and pattern of net tissue deposition or milk production.

For the amino acids, the methodological diversity of the experimental data often precludes aggregation, making statistical analysis difficult. Furthermore, for studies in which nitrogen balance or stable isotope tracer balance has been determined, the results are generally not sufficient to estimate individual requirements, and an estimate of the average requirement for the group under study was obtained by various regression techniques.

Where possible, the estimates of requirements were compared with intakes which have been shown to maintain satisfactory growth in infants, e.g. breast milk, or weight maintenance in adults, as a proxy for validation. These specific procedures are detailed in the individual results sections.

3.2.3 *Examining influential factors*

The database of adult protein requirements was examined to determine whether individual requirements differed by age, sex, and diet, by analysing homogeneous subgroups of the data. The data could not be assumed to follow a normal distribution, nor be easily transformed to normality, and therefore non-parametric analogues of analysis of variance (ANOVA) were used for the statistical analyses. Typically these methods use only the relative rankings of the data and in general they are less powerful than parametric

methods, requiring more data to establish significance of differences, although for larger sample sizes they approach these methods in efficiency. The methods used included the Mann-Whitney (non-parametric two-sample t-test) and the Kruskal-Wallis test (the non-parametric one-way ANOVA) (see reference *5* and Annex for details of the development and application of these tests). As discussed in section 7, for some variables (e.g. age) the data were limited. In general, not enough data existed to explore the effects of these variables for amino acid requirements.

3.2.4 *Estimating the distribution of requirements within a population*

Given a set of data that estimate the requirements of a representative sample of the population of interest, the next step was to characterize that distribution (*6*) and estimate its parameters (*4*).

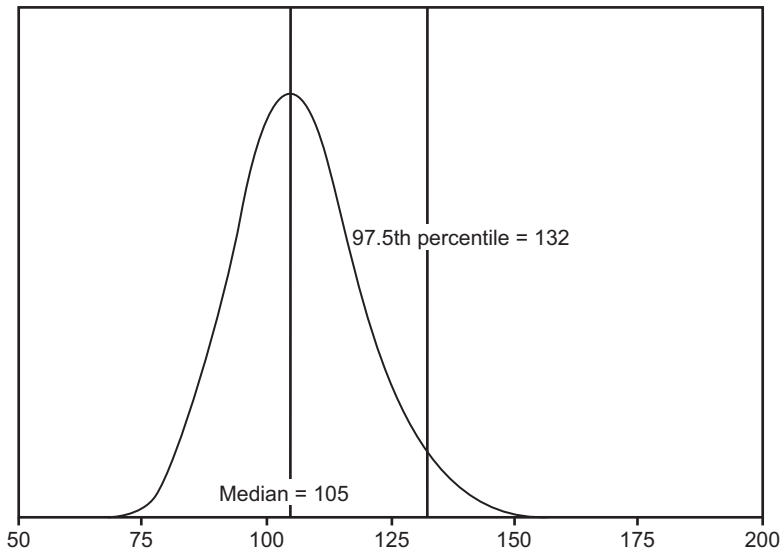
For the adult protein requirement, enough data were available to explore alternatives to the normal distribution. Graphical and analytical techniques were used to determine that protein requirements followed a log normal distribution, and thus the distribution could be summarized by a mean value of the logs of individual requirements and the variability between individuals. Estimation of the mean value is the straightforward average. However, the observed variability between individuals is inflated by the variability (lack of reproducibility) of the individual data. Analysis of variance was used to partition the observed variability and derive an estimate of between-individual variability (*7*). These two parameter estimates completely characterize the population distribution of protein requirements.

For amino acid requirements there are generally insufficient unequivocal data to identify the extent or nature of the population distribution of the requirement. Thus, judgments were made about "best estimate" values from reported mean values and no estimates of variability were derived (techniques that were used are detailed in references *4* and *6*).

Based upon the data available for normal adult humans, protein requirement has a log normal distribution (*7*), as shown in Figure 4. This distribution has a median of 4.654, equivalent to 105 mg nitrogen/kg per day or 0.656 g protein/kg per day. The 97.5th percentile was calculated as the log median plus 1.96 times the SD (standard deviation) of 0.12 in log units i.e. 4.8892. Exponentiation of this value gave 133 mg nitrogen/kg per day or 0.83 g protein/kg per day as the estimate of the 97.5th percentile.

$$\ln(\text{requirement}) \sim \text{normal}(\text{mean} = 4.654, \text{SD} = 0.12).$$

Figure 4
Distribution of the adult protein requirement



3.3 Reference requirements and intakes

As discussed in the 1985 report (*2*), the many applications of estimates of the average requirement value and its distribution within a population can be grouped into two main categories. In diagnostic applications, the estimates are used to judge the probable adequacy or inadequacy of observed intakes. In prescriptive applications, the estimates are used to suggest what intakes should be. How the requirement estimates are used within diagnostic or prescriptive applications may vary, especially when dealing with individuals as compared with populations, and in what follows these two circumstances are discussed separately.

3.3.1 *Reference intakes and risk of dietary inadequacy for individuals*

If information is available about an individual's usual intake of utilizable protein per kg of body weight, the interpretation and application of requirement estimates are relatively straightforward. The probability that consumption of protein at a specific level will meet the requirement of an individual can be identified simply as the area under the *requirement* distribution curve below a value equivalent to that level of consumption. This can be calculated using the standard formula for the cumulative unit normal distribution, i.e. $\Phi(z)$ = the area under the unit normal distribution to the left of z, where z is the number of standard deviations above (positive values) or below (negative

values) the mean. Φ can be calculated directly from z, either by the NORMDIST function in Microsoft Excel, or by NORMDIST (x, mean, standard deviation, 1), where x is the intake level (*8*).

Thus for an individual with intake level Q, the probability of dietary adequacy (i.e. positive nitrogen balance) = $\Phi(\ln Q - 4.654)/0.12$. For example, for an individual with an intake of 85 mg nitrogen/kg per day the probability that this protein intake is adequate is $\Phi(4.443 - 4.654)/0.12 = \Phi(1.76) = 0.039$ = approximately 4%.

Moreover, the level of intake necessary to ensure any specific probability of adequacy can be calculated by the reverse procedure. Thus the level of intake that would virtually ensure (with 99% probability) that an individual was receiving adequate protein nutrition is simply the requirement level that corresponds to the 99th percentile on the normal curve, which is 2.326 standard deviations above the mean: 99th percentile = 139 mg nitrogen/kg per day = exp(4.654 + 2.326*0.12). It should be noted, as discussed below, that the probability that a specific level of intake will meet the protein needs of an individual will be equivalent to the probability that it will meet the needs of a population *only* in the particular and unlikely circumstances that all within the population are provided with that level of intake.

By this method, Table 1 gives the certainty of adequacy associated with selected intake levels.

For the purpose of prescription, levels of intake that carry varying degrees of confidence that they are adequate for the random individual can be recommended. As with the previous report (*2*), the safe level of intake is defined

Table 1
Probability of adequacy for an individual consuming various protein intakes

Intake level (mg nitrogen/kg per day)	SD units from the mean	Probability (%)
72	3.21	0.1
79	2.42	1.0
83	2.00	2.5
86	1.70	5.0
90	1.31	10.0
97	0.67	25.0
105	0.00	50.0
114	0.70	75.0
122	1.28	90.0
128	1.68	95.0
133	2.01	97.5
139	2.39	99.0
152	3.15	99.9

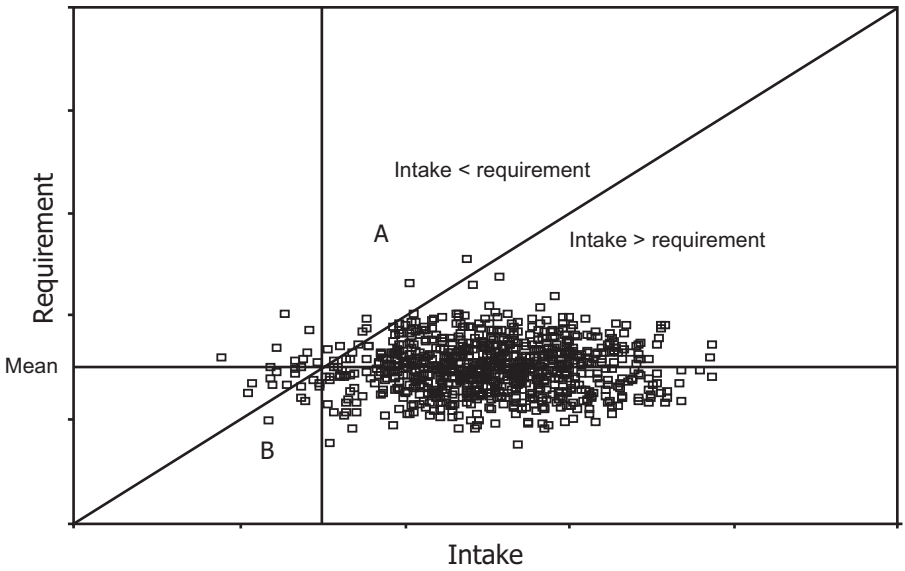
on the basis of a probability of adequacy of 0.975 (i.e. adequate for all but 2.5% of individuals). On this basis, for individuals the term *safe intake level* can be defined as: level of intake that is sufficient for 97.5% of the population = exp(4.654 + 0.12*1.96) = 133 mg nitrogen/kg per day (0.83 g protein/kg per day). Supplying this level to an individual will ensure an acceptably low level of risk (2.5%) that their needs will not be met, and conversely a high degree of probability that they will receive more than their requirement. However, the term *safe intake* also includes the concept that there is no risk to individuals from excess protein intakes up to levels considerably above the safe intake (see section 10). Indeed, many populations have habitual intakes of protein considerably in excess of this safe intake level. This is in contrast to considerations of energy requirements, where providing and consuming an excess of energy would be judged detrimental (see *9*).

3.3.2 *Reference intakes and risk of dietary inadequacy for a population*

Judging the adequacy of intakes and, especially, defining appropriate reference values are much more difficult when dealing with populations rather than individuals, for the simple reason that neither the intake nor the requirement is known for individuals within the population. Thus account must be taken of the distribution and variability of both the requirement and the intake. This has not been sufficiently understood in the past, and reference intake or safe intake levels defined as above for individuals have been incorrectly applied to populations. In fact, as discussed below, a safe population intake, which is appropriate for any population, can be defined only in terms of a relatively complex function of the characteristics of the distributions of requirements and intakes, and this is true even after taking into account difficulties related to identifying appropriate intakes (e.g. under-reporting, and ensuring that intakes are expressed in the same way in relation to bioavailability, as requirements), as discussed in the 1985 report (*2*). Furthermore, the practically useful measure is the average requirement, where "average" is used synonymously with median or mean, in the present case 0.66 g protein/kg per day (i.e. exp(4.654) = 105 mg nitrogen/kg per day). In fact, as discussed below, with certain assumptions, the percentage of a population that is consuming less than this intake level approximates to the percentage of the population with inadequate intakes.

Assessing the prevalence of nutrient inadequacy in a specific population requires a comparison of the intakes of the population with their requirements (*10–12*). This comparison is shown in Figure 5, a plot of a hypothetical population in terms of individual intakes (the *x*-axis) versus individual requirements (*y*-axis). Here it is assumed that there is no correlation between intake and requirement. The prevalence of individuals with intake less than their

Figure 5
Comparison of individual requirements in a hypothetical population[a]



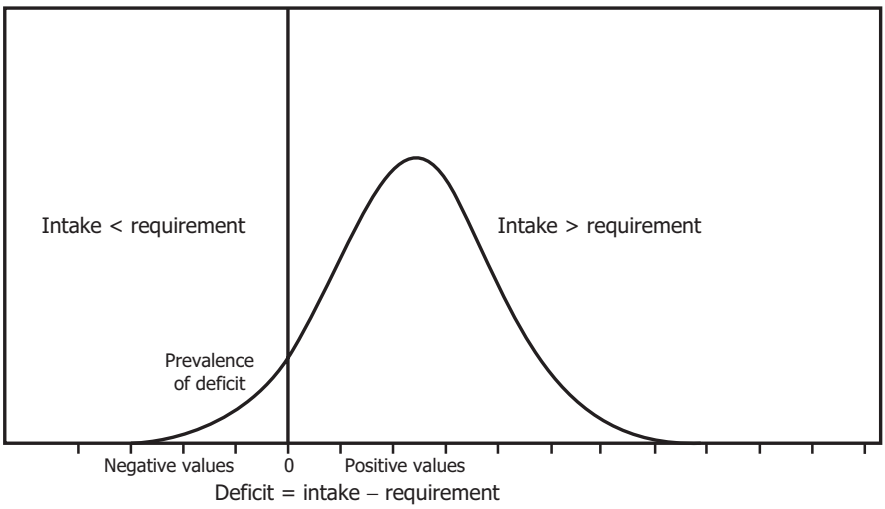
[a] The diagonal line is the line of equality dividing individuals in deficit (intake < requirement) from those in surfeit (intake > requirement). The number of individuals to the left of the vertical line (intake = mean requirement) approximates to the number above the diagonal to the extent that the number of individuals in the triangle identified as A equals those in the triangle identified as B.

requirement is just those who lie above the diagonal line of equal intake and requirement.

In practice, we cannot determine the intakes and requirements of the members of any specific population simultaneously. However, given the *distributions* of both requirements and intakes for the population of interest, we can derive the *distribution* of how much each individual is consuming above or below his or her requirement, i.e. the individual nutrient deficit (see Figure 6). The distribution of this deficit (intake less requirement) is the convolution of the two individual distributions, and this distribution can be estimated, either theoretically or through simulation.

In the case of the protein requirement, both of these distributions are log normal and their joint distribution is bivariate normal. In this case the distribution of protein deficit is especially simple, since the difference between two normally distributed variables is itself normally distributed (see Box 1). The fraction of individuals who are consuming below their requirement, i.e. those with negative deficits, can be easily calculated using the unit normal distribution $\Phi(z)$, as defined above.

Figure 6
Distribution of individual nutrient deficit for the hypothetical population illustrated in Figure 5[a]



[a] The proportion of the population in deficit (intake < requirement) is represented by the area under the curve to the left of the zero deficit vertical line.

In this case, we need to know the prevalence of individuals with protein intakes less than their requirement (i.e. with a deficit ≤ 0). The zero or negative deficit values can be expressed in terms of the number of SD values below the mean, i.e. $-M_D/S_D$, so that the prevalence of deficit becomes: $\Phi(-M_D/S_D) = \Phi(-(M_R-M_I)/S_D)$. The value of S_D is calculated from SD values for requirement and intakes, as shown in Box 1.

Inspection of the simulation in Figure 5 shows that the proportion of subjects in deficit (above the line of equality) may approximate to the proportion of subjects with an intake less than their *average requirement* (to the left of the vertical line). In other words, the number of subjects within the triangle A is similar to that in the triangle B. This similarity has been used in the past to calculate an approximate value of prevalence of deficit (the cut-point method). Thus, in this case deficit prevalence is the area under the intake curve that lies below an intake level equivalent to the average requirement, i.e.

$$\text{deficit prevalence} = \Phi(-(M_R-M_I)/S_I).$$

This approximation will converge with the correct value as S_D approaches S_I, or, in the absence of correlation, when S_I is much larger than S_R. In fact, at values of S_I that are $2.2 \times S_R$ or greater, S_I will be 90% or more of the value

of S_D. It should be noted that this formulation is appropriate only when the requirement and intake distributions are either normal or can be transformed to normality.

As an illustration of this procedure, consider estimating the prevalence of protein undernutrition in a well-fed population, for example one with a log normal intake distribution with mean intake equal to the estimated *safe level* and variability similar to that of requirement. Explicitly for the population, the log of intake has a normal distribution with mean $M_I = 4.894$ and standard deviation $S_I = 0.12$, to be compared with requirement, which has a log normal distribution with $M_R = 4.654$ and standard deviation $S_R = 0.12$. Using the above formulae (and assuming that intake and requirement, both expressed on a per kg body weight basis, are not correlated) the distribution of the protein deficit is log normally distributed with mean $M_D = M_I - M_R = 0.24$, and $S_D = \sqrt{(0.12^2 + 0.12^2)} = 0.17$. The prevalence of protein undernutrition is the area under this distribution for deficit less than zero, which is calculated as the cumulative unit normal less than M_D/S_D, $\Phi(-M_D/S_D) = \Phi(-1.414) = 7.9\%$. Table 2 shows the prevalence for several different hypothetical populations. As would be expected from the arguments above, the cut-point method gives prevalence estimates for protein undernutrition that are closest to the actual prevalence values when S_I is larger than S_R.

Box 1 Distribution of protein deficit

If log(requirement) is normally distributed
with mean M_R and standard deviation S_R
and log(intake) is normally distributed
with mean M_I and standard deviation S_I
with correlation = R,

then log(deficit) = log(intake) – log(requirement) is normally distributed
with mean: $M_D = M_I - M_R$ and
standard deviation: S_D = square root of $(S_I^2 + S_R^2 - 2\ R\ S_I S_R)$.

It is clear from the last column in Table 2 that for a population, an average intake equal to the *safe intake* as described above (mean requirement + 1.96SD) is associated with varying degrees of risk according to the relative variability of requirements and intakes. This is shown graphically in Figure 7, where actual safe population intakes are calculated in terms of numbers of SD values for both requirements and intakes above the mean requirement. It is evident that, in order for the safe *individual* intake to be also a safe *population* intake, the variability in intakes must be less than half

Table 2
Prevalence (percentage) of individuals estimated to be consuming protein below their requirement, for hypothetical populations with differing intake distributions

	Median intake (Cut-point estimates are shown in parentheses. Zero correlation between intake and requirement is assumed.)		
SD of intake (log units)	M_I = log(0.58 g protein/kg per day) (1 SD below requirement)	M_I = log(0.74 g protein/kg per day) (1 SD above requirement)	M_I = log(0.83 g protein/kg per day) (1.96 SD above requirement)
S_I = 0.06 i.e. half the SD of requirement	81.4% (98%)	18.6% (2.3%)	3.7% (<0.1%)
S_I = 0.12 i.e. equal to the SD of requirement	76.0% (84%)	24.0% (16%)	7.9% (2.3%)
S_I = 0.24 i.e. 1.96SD of requirement	67.5% (69.5%)	32.5% (30.5%)	18.2% (15.4%)

that of the requirement, a highly unlikely situation. Furthermore, in the more likely circumstances where the SD of the intake is greater than the SD of the requirement, the safe level will approximate to an intake that is somewhat greater than the requirement plus 2 SD of *intake.*

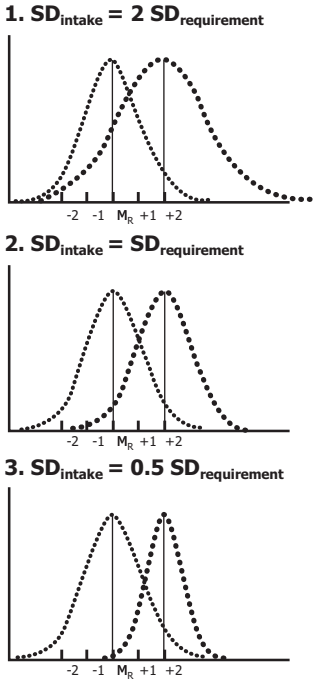
3.4 The cost of protein deficiency

The above discussion has focused on assessing the extent to which an individual or population is able to meet its requirements, i.e. estimating the fraction of a population that is consuming less than their requirement. Thus the examination of the distribution of protein inadequacy (deficit) permits the calculation of the prevalence of any specific level of inadequacy in a population for which the intake distribution is known. However, this approach does not differentiate the sequelae of different degrees of undernutrition, essentially equating all levels of inadequacy from mild to severe.

As recently pointed out by Rand (*12*), while the protein requirement is defined in terms of achieving and maintaining balance, the planner needs better definitions of the response in terms of health outcomes, so that deficits and surfeits of intake can be translated into well-defined health risks (or societal costs), with the deficit distribution – the amount of inadequacy of different levels of protein intake – linked to the clinical or societal cost associated with such inadequacy. This would require a dose–response function relating levels of

Figure 7

Prevalence of individuals estimated to be consuming protein below their requirement for populations with average intakes at the *safe individual* intake but with different intake variability[a]



	Prevalence of deficit %		Safe population intake ie. risk<2.5%	
	True	Cut-point	SD above mean requirement	
1. $SD_{intake} = 2\ SD_{requirement}$	19.1%	16.4%	5.0 $SD_{requirement}$	2.5 SD_{intake}
2. $SD_{intake} = SD_{requirement}$	8.3%	2.5%	2.86 $SD_{requirement}$	2.86 SD_{intake}
3. $SD_{intake} = 0.5\ SD_{requirement}$	4%	<0.1%	2.2 $SD_{requirement}$	4.4 SD_{intake}

[a] Distributions shown to be normal: i.e. values of ln protein requirements and intake.

deficit to its cost, either a continuous function of deficit or a step function, allowing the cost of mild or severe deficiency to be defined by different descriptors of severity. Clearly, such a cost function would be of great value, allowing the consequences of a specific degree of protein deficit for a population to be calculated in health terms: i.e. the average of deficit weighted by the cost function (the integral of the cost times the deficit over the whole range of intakes). However, cost functions for protein deficiency in the adult population have yet to be identified. In practice, this will be a most difficult task, if possible at all, given that many important nutrients are "fellow travellers" with protein in the diet.

Thus, protein-deficient diets are almost certain to be generally nutrient-poor diets, deficient to varying degrees in a range of other nutrients, and also often associated with other environmental factors that can adversely influence health. For the elderly, the population group with the highest protein:energy ratio of their requirement, and therefore most vulnerable to protein deficient diets (see sections 5 and 9.1), potentially adverse health outcomes of protein

deficiency (e.g. poor bone health, see section 11) are certainly multifactorial diseases. In the case of young children, the population group traditionally believed to be the most vulnerable, deficiency syndromes that have been associated in the past with protein deficiency, namely stunting and kwashiorkor, are now believed to reflect quite complex interactions between multiple nutritional deficiencies and other adverse environmental factors, including infection. For these reasons it is highly unlikely that cost functions unique to protein deficiency will be defined.

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4. General methods used for determining protein and amino acid requirements

The 1985 report (*1*) presented values for the requirements for protein and for all the indispensable amino acids, based on values published earlier (*2*). The important changes were that histidine was recognized as indispensable, and that new data had become available, allowing values for the requirements of preschool children to be presented as a separate group. As with the earlier report (*2*), the values for both protein and amino acid requirements were determined primarily from results of studies of nitrogen balance, and they agreed closely with those presented earlier. The derived values for protein requirement showed a rapid decrease with age in the very young, and a progressively slower fall with increasing age, as values approached those for adults. By contrast, values for amino acid requirements showed a fall by about 2-fold in young children compared with 3–4-month-old babies, but no further fall in older children. However, there was a further 3-fold decrease in requirement estimates of adults compared with older children, despite the very slow growth of the latter. In the years since 1985, this apparent discrepancy has been called into question by results of new measurements employing stable isotopic techniques. These have suggested that the original studies in adults, using nitrogen balance, might have seriously underestimated the requirements of this age group. The emphasis of this chapter is therefore to describe the methods used for determining protein and amino acid requirements, and to discuss the basis for preferring certain values for amino acid requirements over others obtained by a different technique.

4.1 Nitrogen balance

Nitrogen balance is the classic approach, which has been used for almost all determinations of protein requirement and a large number of studies of amino acid requirements since the pioneering work of Rose (*3*). The basic concept is that protein is by far the major nitrogen-containing substance in the body, so that gain or loss of nitrogen from the body can be regarded as synonymous with gain or loss of protein. Second, it is implicit in the method that in the healthy subject, body nitrogen will be constant (in the adult) or increasing maximally (in the growing child) if the dietary intake of the specific test nutrient, such as an indispensable amino acid, is adequate. It follows from this

that if body nitrogen is decreasing or is not increasing adequately, then the diet is deficient. These assumptions are less than secure, as there is no direct evidence that maintenance of the body protein mass is synonymous with health. However, as no more direct method for assessing adequacy in relation to health has been devised, nitrogen balance has remained an important, and until now the major, method for assessing protein and amino acid requirements. Moreover, there can be little doubt that maintenance or gain of body protein (nitrogen) in adults or children, respectively, is a prerequisite for health.

The main limitations of the nitrogen balance method have been well described (*4–6*). These result mainly from practical aspects related to the difficulties of making the appropriate measurements with sufficient accuracy, and to the interpretation of the results.

4.1.1 *Practical aspects*

The nitrogen balance technique requires accurate quantification of all routes of intake of nitrogen and all routes of loss. The former can be achieved by analysing duplicate portions of food and by very careful attention to collection of all food not consumed, such as spillage and residue on plates. However, although the errors in the former are likely to be random, and therefore not likely to influence the mean value, the error in the latter is always likely to underestimate the losses, thus overestimating intake (*4*) and leading to an erroneously positive nitrogen balance. The loss of nitrogen from the body occurs primarily via the urine, which can be measured accurately. Similarly the intestinal losses can be measured by collection of faeces. However, losses also occur through the skin, by sweat and desquamation, and through loss of hair, nails and various bodily secretions. These "miscellaneous losses" were neglected in many of the published studies using nitrogen balance, including those of Rose (*3*) and Leverton (*7*), which were used to derive the values for amino acid requirements in adults in the 1985 report. Very careful measurements have shown that miscellaneous losses vary slightly with the dietary nitrogen intake, amounting to about 5 mg/kg per day on an average diet, i.e. total integumental losses = $0.0043N_{intake}$(mg nitrogen/kg per day) + 3.6 (*8*) although it has also been shown that the exact amount is also influenced by heavy work (*9*). The 1985 report assumed somewhat higher values, of 8 mg/kg per day for adults and 12 mg/kg per day for children younger than 12 years, but acknowledged that no single figure will be applicable under all conditions (*1*). It has been suggested that overall losses are more likely to be underestimated than overestimated (*4, 10*). This would lead to an erroneously positive nitrogen balance, which would add to that resulting from an overestimate of intake described above. Thus it is likely that nitrogen balances will tend to err on the positive side, with the result that protein or amino acid requirements will tend to be underestimated.

After the diet has been changed, a period of time is usually allowed for adaptation to occur. This is important, as not only does metabolism take time to adapt to the new intake, but also the body urea pool must adjust to the change. The urea pool expands and contracts with increased or decreased protein intake, but with a half life of about 8–12 hours, in excess of 48 hours is required to reach its new size, during which time the urea nitrogen excretion is not indicative of the oxidation of amino acids.

The exact period required for adaptation has been the subject of debate. The 1985 report concluded that the major adjustment appears to be complete during the first 5–7 days in a range of age and sex categories (*1*). On this basis, studies of shorter duration than 1 week are unlikely to yield reliable data, and most studies have used diet periods of 1–3 weeks. However, Waterlow (*11*) has pointed out that there might be a further phase of "adaptation" involving a gradual loss of body protein, which might ultimately come back into equilibrium at a lower lean body mass. Indeed, there is evidence from a long-term study in men given a moderately low-protein diet, that the nitrogen excretion rate continued to fall for at least 90 days (*12*). Nevertheless, the practicality of studies in which the duration of each diet period is months rather than weeks limits their likelihood of being achieved. This point will be revisited later.

The other dietary constituents, especially energy, can also have an important influence on the values derived for requirements. The influence of energy intake on protein and amino acid requirements and their measurement is discussed in section 5.

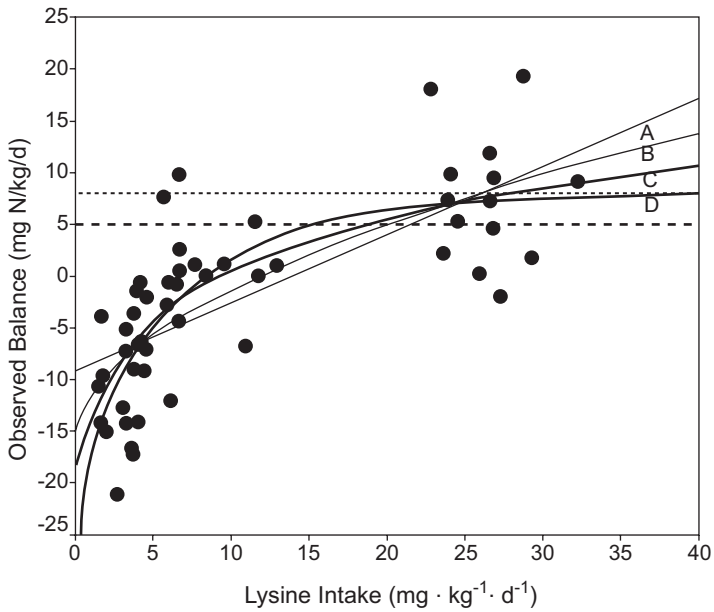
4.1.2 *Data interpretation*

The 1985 report (*1*) took the lowest dietary intake of the amino acid that gave positive nitrogen balance (in men) or 0 ± 5% of the intake (in women) as the average requirement. In general, however, nitrogen balance data have been analysed by plotting the data for balance against intake, drawing a straight line through the points, and either extrapolating or interpolating to determine the intake required for zero balance in adults or for a specific growth rate in children. This procedure makes two important assumptions: first, that the curve should be linear, and second, that the line will pass smoothly through the balance point, giving positive balances at high intakes. It is important, therefore, to examine in detail the validity of these assumptions.

The assumption of linearity is not in general valid. It has been shown that the gradient is higher at very low intakes, and declines appreciably as the balance point is approached (*12, 13*). This means that results calculated by plotting lines through data that include very low intakes are likely to lead to an underestimate of requirement. Therefore, more recent studies with nitrogen

balance have employed a range of intakes that encompasses the expected requirement, which enables the point of intersection with zero balance to be determined more accurately. Rand and colleagues (*14, 15*) have employed regression techniques to examine this issue in more detail. In particular, the individual data points from the study of lysine requirement in young women by Jones, Baumann & Reynolds (*16*) were fitted to each of four different shaped curves (Figure 8): linear (curve A), logarithmic (B), square root (C), and exponential asymptotic (D). Curves B, C and D have declining gradients as the balance point is approached, which is more consistent with the likely biochemical mechanisms involved in the control of protein balance. The conclusion was that exponential fitting was not satisfactory, as it was found to be too imprecise for routine use. However, the other three curves gave similar median values for the estimated requirement. The actual values for the median lysine requirement obtained by these three procedures were in the range 28.0 to 29.0 mg/kg per day when the miscellaneous losses were taken as 8 mg/kg day and 20.4 to 22.7 mg/kg per day when miscellaneous losses of 5 mg/kg per day were assumed. This illustrates the rather high sensitivity of the nitrogen balance method to the miscellaneous losses. An advantage of the regression approach is that it permits the variance of the estimated balance

Figure 8
Non-linear regression of nitrogen balance against lysine intake, using four different functions *(15)*[a]



[a]Reproduced from reference *15* with permission from *The Journal of Nutrition,* American Society for Nutrition.

point to be partitioned into within and between subjects, the latter value being required to derive the intake that will maintain balance in 97.5% of the population (*14*). This advantage is only obtained, however, if each subject's data are fitted to an individual equation, as opposed to treating each separate data point as if it were an individual subject (one-fit procedure), as was done in an earlier re-analysis of the same data (*17, 18*). Moreover, the one-fit procedure always gives lower estimates of the requirement than individual fitting (*15*). The values for the requirement derived by these procedures are 2-fold or 3-fold higher than those calculated from the same data by the original authors (*16*), because of the different data analysis and the inclusion of an estimate of miscellaneous losses, which were not measured in the original study.

The second assumption of the nitrogen balance method relates to the interpretation of the positive nitrogen balances frequently demonstrated in adults at high intakes. This is most apparent in subjects who are receiving very high total protein intake, when positive balances of 1 g nitrogen per day have been demonstrated over as long as 55 days under the most carefully controlled conditions (*19*). However, positive nitrogen balances are also seen in studies of amino acid requirements, when the total protein intake is modest (e.g. Figure 8). The question is, are subjects on modest intakes of protein increasing their body nitrogen content, and by inference their lean body mass, and if so, how should this information be interpreted? There are a number of reasons why balances might be artefactually positive. As discussed above, the errors inherent in the nitrogen balance technique are the tendency to overestimate intake, because of unmeasured wastage and spillage, and to underestimate nitrogen losses, because of failure in collection of excreta and the difficulty of accurately quantifying miscellaneous losses. All of these errors contribute to an apparent balance which is artefactually positive. In addition, the possibility that nitrogen can be lost from the body by other routes must be considered. Ammonia in the breath has been investigated, and shown to be a small component, as has nitrate excretion in urine and faeces which is not detected in the Kjeldahl assay (*8*). The possibility of conversion to nitrogen gas has also been investigated, but has not been confirmed (*20, 21*). Thus, although there is no known mechanism by which nitrogen balance might be artefactually positive, there remains the possibility that true protein balance can indeed be associated with apparently positive nitrogen balance. If this were to be true, then the analysis should take it into account, since the requirement calculated from zero apparent balance underestimates the true requirement. For example, a regression method could be employed, such as those proposed by Rand & Young (*15*), in which the criterion of adequacy is not zero balance, but the achievement of the maximum apparent positive balance, since this would equate with zero protein balance. This would result in higher estimates of requirement.

If it is accepted that the reported positive balances are genuine, then intakes higher than the requirement can result in accretion of protein in adults, in an analogous way to the loss of protein that occurs with lower intakes. The logical extension of this is that the intake corresponding to the zero nitrogen balance is a reflection of the habitual dietary intake of that subject. As the loss or gain is very slow, typically a few milligrams of nitrogen per kilogram per day, it could persist over an extended period of time and need not involve a large total gain or loss of protein. This corresponds to the "further phase of adaptation" described by Waterlow (*11*), which involves a gradual gain or loss of body protein, which might ultimately come back into equilibrium at a higher or lower lean body mass. Such a mechanism seems quite feasible when the dietary variable is total protein, as it need not involve very large changes in lean body mass. Nonetheless, this is a theoretical concept; there is no functional measurement to show that a slow chronic loss of body protein does not have an adverse effect on health and body functioning. Further, it appears to be a less plausible mechanism when the only dietary variable is a single indispensable amino acid and the total protein intake is similar to the subject's customary intake. Under these circumstances, it seems unlikely that, if the test amino acid were given in excess, an increase in lean body mass would ensue.

The weight of evidence in the above discussion leads to the conclusion that positive nitrogen balances in studies of amino acid requirements probably result from technical errors in the measurement of balance, or an inappropriate choice of value for the miscellaneous losses (when not measured directly).

4.1.3 *Other factors*

Although the indispensable amino acids cannot be synthesized in human tissues, the possibility that they can be made available to the body by the gut microflora has been much discussed (e.g. *22*). It has been shown in pigs given dietary [^{15}N]diammonium citrate that ^{15}N can appear in lysine and threonine, two amino acids that do not partake in transamination reactions, and that must therefore have become labelled by the colonic microflora (*23*). A study in adult humans ingesting $^{15}NH_4Cl$ and [$^{15}N_2$]urea has suggested that between 11 and 130 mg/kg per day of lysine is synthesized in the intestine and made available to the body (*24*), with similar findings reported in adult human ingesting [$^{15}N^{15}N$]urea (*25*). The nutritional significance of this finding, and in particular the extent to which this lysine can spare dietary lysine, is not yet clear. However, this does not directly invalidate the estimates of dietary lysine requirement by nitrogen balance, so long as it is appreciated that the dietary requirement is in addition to any lysine made in the intestine.

A factor that might influence the validity of nitrogen balance studies is the possibility that depletion of a specific amino acid might occur through loss of proteins that contain it in abundance. Thus, nitrogen balance might be maintained at the expense of a change in the concentration of specific proteins. One example of this is histidine restriction, which results in depletion of carnosine, a very abundant histidine-containing dipeptide, before there is any influence on total protein balance (*25*). Subsequently, body protein is maintained at the expense of haemoglobin, which is also rich in histidine (*26–28*). This might explain why the studies of Rose did not identify histidine as indispensable.

4.1.4 *General conclusion on nitrogen balance*

It is clear that the nitrogen balance technique has serious technical drawbacks that may result in requirement values that are too low. The analysis by appropriate linear and curvilinear regression techniques taking into account realistic estimates of unmeasured losses is a logical and important step forward, which enables more realistic estimates of requirements to be calculated from existing data.

4.2 Carbon balance

The carbon balance method relies on the assumption that the requirement of a specific indispensable amino acid in adults is the dietary intake of that amino acid which balances all routes of loss. For certain indispensable amino acids, for example leucine, by far the major route of loss is by oxidation, and this can be quantified by use of isotopic labelling in the carboxyl group (e.g. [1-^{13}C]leucine), which is released quantitatively into the bicarbonate pool during the first irreversible step in oxidation. The labelled amino acid is infused intravenously, generally preceded by a priming bolus, whereupon the isotopic enrichment (equivalent to specific radioactivity for radioactive labels) of the amino acid in the plasma rises to a plateau value (*29*). Simultaneously, measurements are made of the expiration rate of labelled carbon dioxide in the breath, which permits the rate of oxidation to be calculated from a simple precursor–product relationship (*30*). Measurements of amino acid oxidation are generally of between 4 and 24 hours' duration, so a study of the requirement of an amino acid involves a period of adaptation to the diet, the last day of which includes the labelled amino acid infusion.

4.2.1 *Practical aspects*

In this section, a number of practical problems that may influence the validity of carbon oxidation or balance studies are described. As these have a varying influence on the results, depending on the design of the specific measurement

protocols employed, their effect on the estimates of requirements will be discussed later in relation to specific protocols. In the following discussion, the labelled amino acid is assumed to be [1-^{13}C]leucine, unless specified otherwise.

In order that the rate of production of labelled CO_2 in the breath can be used to calculate the rate of leucine oxidation, the enrichment of leucine at the site of oxidation (the precursor enrichment) must be known accurately. Oxidation takes place intracellularly, primarily in skeletal muscle, where the enrichment of leucine is lower than that in the plasma. However, direct measurement of leucine enrichment in muscle can be avoided by measuring instead the enrichment of keto-isocaproic acid, which is produced by transamination of leucine and is subsequently decarboxylated to release CO_2. Analogous methods can be used for valine and isoleucine, but for other amino acids direct measurement of the precursor enrichment cannot be made, and the plasma amino acid must be used in its place. The consequences of this will be discussed later.

In principle, the constant infusion method requires that a metabolic steady state be maintained throughout. This requirement has an important effect on the way in which studies are performed. The subject is confined on a bed or chair, with limited activity, both because of the infusion lines and blood sampling, but also to prevent large changes in the rate of CO_2 production. Frequent or continuous monitoring of CO_2 production is necessary, usually by means of a ventilated hood system over the bed, which further restricts activity. It is unclear to what extent this influences the metabolism of the test amino acid on the day of the measurement. It has also been regarded as important to regulate the intake of food during the infusion. Food has therefore been given as small meals at regular intervals, for example hourly for 12 hours followed by 12 hours fasting, with the intention of mimicking the natural diurnal pattern of food intake, without unduly disturbing the steady state. However, the way in which the oxidation rates during the periods of feeding and fasting are integrated to derive the 24-hour rate of oxidation can have a substantial impact on the derived values for requirement, as will be discussed in relation to specific protocols.

A problem which is difficult to avoid when using stable isotopic labels is that the amino acid cannot be given as a true tracer. As pointed out previously (*22*), in one study with lysine the intake from the isotope infusion was three times the lowest dietary intake studied. The measurement of stable isotopes by mass spectrometry is insensitive compared with radioactivity counting, so the labelled amino acid must be given in an amount that is quantitatively significant compared with the dietary intake. Some of the earlier studies of requirements did not adequately take account of this. Some of the more recent

studies have avoided errors from this source, during the feeding period, by omitting from the diet on the day of the infusion an amount of the test amino acid equal to that infused as the "tracer". However, this still leaves considerable uncertainty over what to do about the isotope given during the fasting state, and whether this can be regarded as nutritionally available, in the absence of intake of the other indispensable amino acids (see specific protocols below).

There is some uncertainty in the measured rates of amino acid oxidation, because labelled CO_2 is sequestered in the body bicarbonate pool. Thus the rate of labelled CO_2 output in the breath is lower, by some 30%, than the production rate (summarized by Waterlow, *11*). This loss of label can be measured and corrected for by performing infusions of labelled bicarbonate in separate experiments, preferably in the same subjects on the same diet. However, it is not certain how well intravenously infused bicarbonate mimics the endogenous production of bicarbonate in the cells where keto-isocaproic acid is oxidized, and this introduces a degree of imprecision in the method. Additionally, the ^{13}C-enrichment of the natural CO_2 in the breath varies during the day, depending on the substrate being oxidized. This occurs because different dietary sources of carbon have different natural enrichments (e.g. cane sugar and maize products have a much higher enrichment than potato or wheat products). This problem can be minimized, first, by standardizing all diets with low enrichment ingredients, and second, by performing collections of respiratory CO_2 in the same subjects on the same diet, but without an infusion. The enrichments so obtained are then subtracted from those obtained during infusions of the labelled amino acid and bicarbonate. This procedure is necessary to avoid serious systematic errors in the determination of oxidation rates, but as with the labelled bicarbonate correction, it adds substantially to the overall variability of the method.

4.2.2 *Specific protocols*

Over the past 15 years, the protocols for assessing amino acid requirements by carbon balance have been refined and modified to take account of the need to know the total amount of the amino acid oxidized in a complete day, including both fed and fasting periods. Three basic procedures have been used. Initially, measurements were made in the fed state only (frequent small meals), employing a short tracer infusion of 3–6 hours' duration to study leucine (*31–33*), lysine (*34*), threonine (*35*), valine (*36*), and branched-chain amino acid interactions (*37*). After it became apparent that measurements were also needed in the fasting state, to account for the possibility that the fasting rate adapts to dietary intake, an 8-hour infusion protocol was used, including 3 hours of fasting followed by 5 hours of frequent small meals. Studies of phenylalanine/tyrosine (*38*), methionine/cysteine (*39–42*) and the

"Massachusetts Institute of Technology diet" versus the "Food and Agriculture Organization diet" (*43, 44*) were made by this approach. However, most of the more recent work has employed a more rigorous protocol in which measurements are made continuously over a 24-hour period, during which the first 12 hours are fasting (overnight) and the second 12 hours are fed (small meals at half-hourly intervals). Leucine (*45–49*), phenylalanine/tyrosine (*50–53*) and lysine (*54*) have been studied extensively by this protocol. In addition, as discussed below, measurements of [^{13}C]leucine balance over 24 hours have been used as a surrogate for nitrogen balance to study other amino acids (*55*). The 24-hour protocol is clearly the most sophisticated and accurate, as it takes account of a full 24-hour day. However, only leucine, phenylalanine/tyrosine, lysine and valine have so far been studied by this method. As threonine was studied only by the fed-state-only protocol, and methionine/cysteine only by the 8-hour fed/fast protocol, the advantages, drawbacks and likely accuracy of each of the three protocols are discussed, starting with the 24-hour protocol.

4.2.3 *The 24-hour protocol*

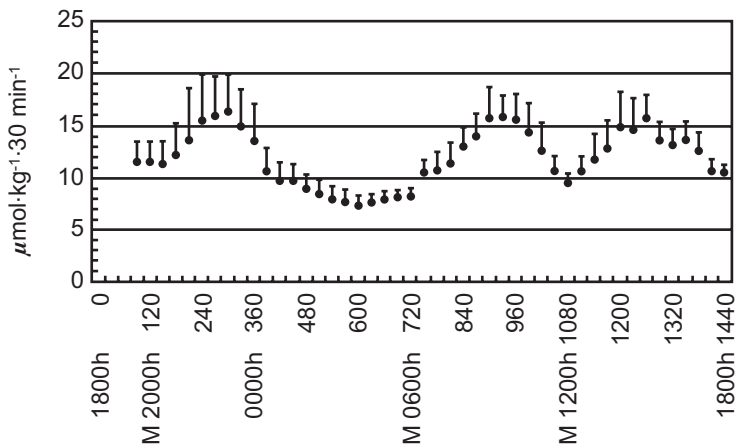
The main advantage of the 24-hour protocol is that the complete day is monitored, including periods of feeding and fasting, which makes it similar, but not identical, to the previous days of adaptation to the diet. It has been shown that the daily rate of protein oxidation, calculated from the leucine oxidation measured during this protocol, is closely similar to that derived from the measurement of nitrogen excretion in urine and faeces (*45*). Moreover, the subjects were close to leucine balance when consuming a diet that was adequate (1 g protein/kg per day).

There are, however, some drawbacks. The most serious relates to the question of whether the protocol involving small meals for 12 hours is representative of a "normal" day, involving larger and fewer meals. This was tested in a study which compared the protocol described above with an otherwise similar protocol in which three discrete meals were given (*47*). Despite receiving the same total dietary intake with the two protocols, the daily leucine balances were quite different. Whereas the half-hourly small meal protocol gave approximately zero balance, the discrete meal protocol gave a balance that was equivalent to 28 mg nitrogen/kg per day more positive. Moreover, similar differences between the two protocols were also noted in the rates of nitrogen excretion. The positive aspect of this is that the leucine oxidation and nitrogen excretion were consistent with each other. However, the apparent difference between the two protocols leads to concern over whether the less natural half-hourly small meals protocol is less conducive to protein retention and therefore might give rise to higher estimates of amino acid requirements. On the other hand, there is no obvious reason why healthy subjects on a

maintenance-energy, moderate-protein diet, taken as three normal-size meals, should gain nitrogen at a rate of 28 mg nitrogen/kg per day, about 12 g of protein or 70 g of lean tissue per day. It is probably the result of unidentified errors in the measurement of balance, as a more recent study of lysine requirement, using leucine as the tracer and the half-hourly small meals protocol, has also shown a similar positive leucine balance (*48*). This requires further investigation, as it is crucial to the determination of requirements by the carbon balance method.

There are two other potential problems with the 24-hour protocol. The first concerns the way in which the meals are given on the day that the infusion starts. During the adaptation period, the subjects take three equal meals each day, at 08:00 hours, 12:00 hours and 18:00 hours (*47*). However, as the infusion starts at 18:00 hours, the last meal for that day is given earlier, at 15:00 hours. This is done because the first 12 hours of the infusion, starting at 18:00 hours, is the fasting period. However, it means that 67% of the total day's intake is taken in the 6 hours prior to 18:00 hours, with the possibility that there is continued absorption of food well into the infusion period. This seems likely, as the effect of large meals in the protocol described above persisted for 6 hours after the meal was given (Figure 9). This carry-over would not be a problem if there were a similar carry-over from the fed to the fasting periods at the end of the infusion. However, the design of the small meals schedule makes this unlikely, as the meals are spread over 10 hours, leaving the last 2 hours of the infusion without food. The effect is apparent in the data (*45, 46*, and Figure 10). At the beginning of the infusion, the first values for oxidation are higher than those at the end. Moreover, although no

Figure 9
Leucine oxidation over 24 hours, with food given as three large meals[a]

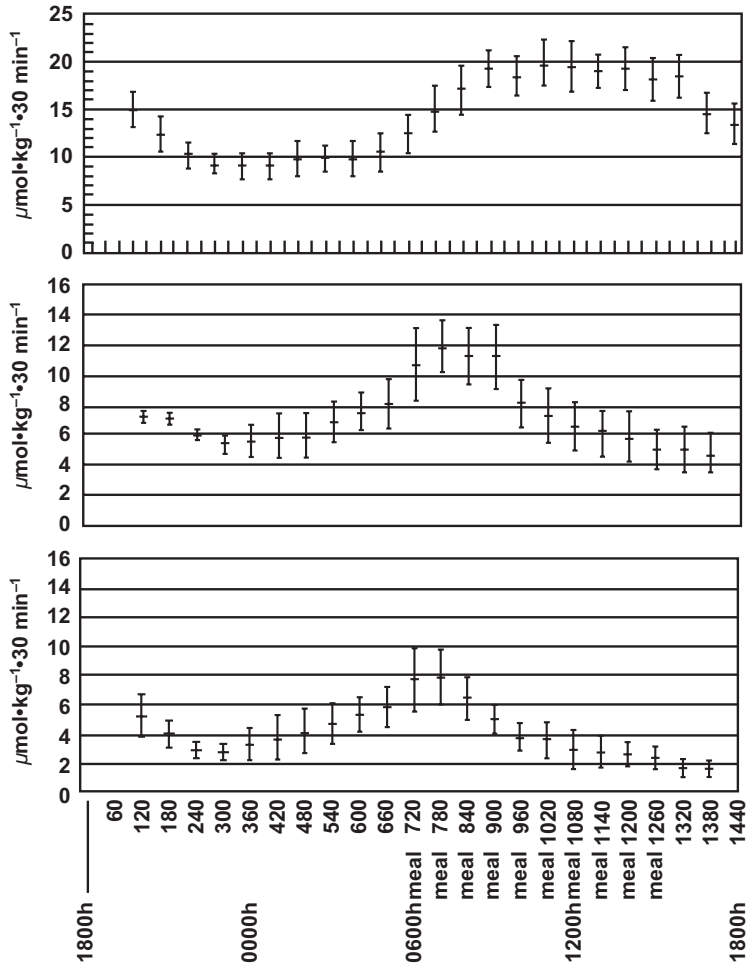


[a] Reproduced from reference *47* with permission from *The American Journal of Clinical Nutrition.*

values were recorded during the first 2 hours, it is clear from the shape of the curve that they would be higher still. The discrepancies were most apparent for the diets with lower leucine (*46*). The net result of this was an overestimate of oxidation, approximately 6 mg/kg for the high leucine diet and 2 mg/kg for the lower leucine diets.

A second potential problem is infusion of nutritionally significant amounts of labelled amino acid. In some of the studies employing the 24-hour protocol (*45, 47, 49, 50*), the total amount of leucine given on the day of the infusion was higher than on previous days, by the amount given as the infusion, usually by 9.6 mg/kg per day, but in one study with labelled phenylalanine and tyrosine by as much as 26 mg/kg per day (*50*). As all of these studies involved relatively high dietary intakes of the test amino acid, and the infused tracer was accounted for in the calculation of balance, it is unlikely that the additional amounts would have significantly altered the balance. In the other 24-hour studies, a different strategy was employed, and the interpretation of this is less straightforward. On the day of the infusion an amount of leucine equal to the amount infused was omitted from the diet, so that the total leucine given was the same as that on the previous days of adaptation. An equivalent strategy was used in studies employing labelled phenylalanine and tyrosine, when unlabelled phenylalanine and tyrosine equal in amount to what was infused were added to the diet on the adaptation days, but not on the infusion day (*51–53*). The problem is that only half of this infused amino acid is given during the fed phase, and the remainder is given during fasting. During feeding, the amino acid mixture given is therefore relatively depleted in the test amino acid in relation to the nominal intake. For example, at the lowest intake of leucine studied by El-Khoury et al. (*46*), the actual intake during feeding was only 60% of the nominal intake. The question is, can the amino acid given during fasting be utilized efficiently to support protein anabolism in the fed phase? The data of El-Khoury et al. (*46*), shown in Figure 10, show a gradual increase in oxidation during fasting, which suggests that this leucine is being oxidized, rather than retained for later use during feeding. The potential error involved is not insubstantial, amounting to an underestimate of about 5 mg/kg per day of "effective" leucine intake. This is similar in magnitude to the negative leucine balance of 6.2 mg/kg per day observed by El-Khoury et al. (*46*) on the diet containing a nominal 38.3 g leucine/kg per day. The possible error is greater with the studies of aromatic amino acids: about 12 mg/kg per day compared with an estimated requirement of 30–40 mg/kg per day (*53*). However, a more recent study has suggested that that this error might not be important, as infusions of three different levels of leucine during the fasting phase were shown to have no significant effect on leucine oxidation (*56*), suggesting that additional leucine would therefore be available for protein synthesis in the fasting state.

Figure 10
Rates of leucine oxidation over 24-hour periods[a]
Note: Diets contained 90 (top), 38 (middle), or 14 (bottom) mg/kg per day leucine.



[a] Reproduced from references *45* and *46* with permission from *The American Journal of Clinical Nutrition.*

4.2.4 *The fed-only protocol*

With the fed-only protocol, the isotope infusion was of short duration, 3–5 hours, and small meals were given during, and also for several hours before, the infusion. The main shortcoming of this procedure is that measurements are made only after a few hours of feeding, so it is difficult to extrapolate findings to a normal day with any level of confidence. A value for the oxidation rate during fasting has to be derived, and Meguid and others (*31, 36*) used the values obtained at the lowest dietary intakes studied (4 mg/kg per

day for leucine and valine). Similar assumptions were made for lysine (*34*) and threonine (*35*).

These fed-only studies also took insufficient account of the amino acid given as the infusion. First, the isotope was given in addition to the amino acids in the meals on the day of infusion. It is difficult to be certain how this would affect the measured amino acid balance, which would probably reflect an intake somewhere between that occurring on the previous days of adaptation and that appropriate to the diet plus the additional amino acid from the infusion. Second, the method for calculating the balance underestimated the contribution of the amino acid infused as the label. In this calculation, the measured hourly oxidation rate was multiplied by 12 to obtain the value for the 12-hour fed period. Similarly, in calculating the total intake, the hourly intake from food was multiplied by 12, but to account for the isotope, only the amount actually infused during the 3–5-hour infusion period was added. In fact, the hourly amount of isotope infused should have been added to that from food, and the sum multiplied by 12 to obtain the total fed value. This miscalculation gave rise to underestimates in balance for leucine of 9 and 13 mg/kg per day (*31, 36*), lysine of 8 mg/kg per day (*34*) and threonine of 5 mg/kg day (*35*), and hence to overestimates of requirements.

4.2.5 *Short-term fasting/feeding protocol*

To account for the changes in amino acid oxidation resulting from food intake, a short, 8-hour infusion protocol, in which no food is given for the first 3 hours and small meals are given during the remaining 5 hours, has been employed. This circumvents many of the problems associated with the fed-only procedure, as separate measurements are made for the fed and fasting states. The infusion is started at 08:00 hours, with the fasting measurement, and this has been criticized by Millward & Rivers (*57*), because at the time that the measurements are made the subjects are either 12–15 hours fasted or 3–5 hours fed, which might not be representative of an average day. Comparison with data from the last hour of fasting and the 5th hour of feeding with the 24-hour protocol shows that results of the two procedures agree quite well for an adequate leucine intake, but with lower leucine intakes larger differences are apparent (*46*).

In all of the studies employing the fasting/feeding protocol, the isotope infusion was given in addition to dietary amino acid, thus increasing the total intake. Hence the actual intake was greater than the nominal intake, which would lead to a small underestimate of requirement.

4.2.6 *Data interpretation*

There are several factors that influence the interpretation of data from amino acid oxidation and metabolism studies, regardless of the exact protocol employed. Potentially the most significant is the measurement of the enrichment of the amino acid at the site of oxidation or metabolism. As pointed out above, leucine is oxidized intracellularly, especially in skeletal muscle, where the enrichment is about 20% lower than that in the plasma. Error from this can be avoided, however, by using instead the enrichment of the transamination product, α keto-isocaproate, which is produced from leucine in the tissue and transported out into the plasma. However, this course of action is available only for branched-chain amino acids. A study of lysine oxidation suggested that the enrichment of neither the plasma lysine nor the urinary aminoadipic acid adequately assessed the enrichment at the site of lysine oxidation in the liver (*54*). Whether plasma lysine or urinary aminoadipate was used as precursor, or the label given intravenously or orally, similar rates of lysine oxidation were obtained in the fasting state. However, in the fed state, the values from urinary aminoadipate were about 50% higher than those calculated from the plasma lysine enrichment. Moreover, the daily lysine balances calculated by the different methods showed considerable variation, which would seem to preclude studies of lysine requirements by the carbon balance method.

The identification of the appropriate precursor enrichment is more complex in studies of phenylalanine conversion to tyrosine and oxidation. Both processes take place largely in the liver, which is not readily accessible to direct measurement, so instead, the plasma enrichments of phenylalanine and tyrosine have been used (*38, 50–53, 58*). The formula used to calculate the rate of hydroxylation of phenylalanine to tyrosine includes the ratio of phenylalanine to tyrosine enrichment. However, although the enrichment of phenylalanine in liver is lower than in the plasma, the enrichment of tyrosine is higher at its site of synthesis in the liver than in the plasma. Consequently, the ratio of enrichments in the plasma is appreciably lower than that in the liver, leading to a considerable underestimate of the rate of tyrosine synthesis.

Although oxidation or conversion to other amino acids is by far the major route of disposal of indispensable amino acids, the other pathways might not be insignificant when considering small changes in the balance. The routes of amino acid loss are listed in Box 2. All amino acids are lost via the urine, skin and intestine. Intestinal losses have been estimated by collecting ileostomy fluid from subjects given a protein-free diet (*60*), suggesting that the intestine is potentially a major route of amino acid loss. The unanswered question is how much of the ileal amino acids is subsequently reabsorbed in the colon. Whereas it has been demonstrated that amino acids can be absorbed

Box 2
Major routes of obligatory amino acid loss[a]

Oxidation

Intestinal losses

Skin (sweat and skin cells)

Urinary excretion

Synthesis of other amino acids

Irreversible modification

Synthesis of non-protein substances

[a] Modified from reference *59*.

from the colon, it is not known whether this is complete. It has been assumed that faecal nitrogen is mostly contained in the microflora, but it is not known whether loss of nitrogen in the faeces also results in loss of indispensable amino acids that would otherwise have been available to the body.

In addition, some of the indispensable amino acids are important precursors of other compounds in the body (e.g. tryptophan is the precursor of neurotransmitters), and there is a continuous loss of lysine and histidine by their irreversible methylation. The conclusion of this discussion is that oxidation is not the only route of loss of indispensable amino acids. Neutral balance of an indispensable amino acid calculated only from dietary intake minus oxidation occurs when the "true" balance is still negative as a result of the other losses. For this reason, the carbon balance method will tend to underestimate requirements.

Two other potential problems of interpretation were discussed under nitrogen balance, but have a different effect on carbon balance. The first is the possibility that amino acids synthesized in the intestine are made available to the body. In this case, the increased intake would lead to higher oxidation. The apparent negative amino acid balance would give rise to an overestimate of the amount of dietary amino acid required to maintain balance. The second potential problem is the possibility that depletion of a single amino acid, such as histidine, could take place without a significant overall loss of body protein. If in this case the tracer is the amino acid that is depleted, the depletion would be detected by the carbon balance method.

4.2.7 *General conclusion on carbon balance method*

The protocol for assessing carbon balance has been refined to such a degree that few serious potential sources of error remain. The problem of precursor compartmentation can be avoided for the branched-chain amino acids but for other amino acids the balance is likely to be in error because the precursor enrichment cannot be directly measured. Data from short-term fed and fasted protocols that make measurements lasting only a few hours can be used to predict the 24-hour balance, but this yields less reliable estimates of requirement. The 24-hour protocol represents the current state of the art, but problems still remain, including allowing for the mass of isotope infused and the extent to which the experimental feeding protocol, e.g. frequent small meals, is representative of a normal day. Finally, the logistics of performing the studies means that it is difficult to ensure that sufficient different intake levels are fed around the requirement level, with most studies published to date being underpowered in this respect.

4.3 Indicator amino acid method

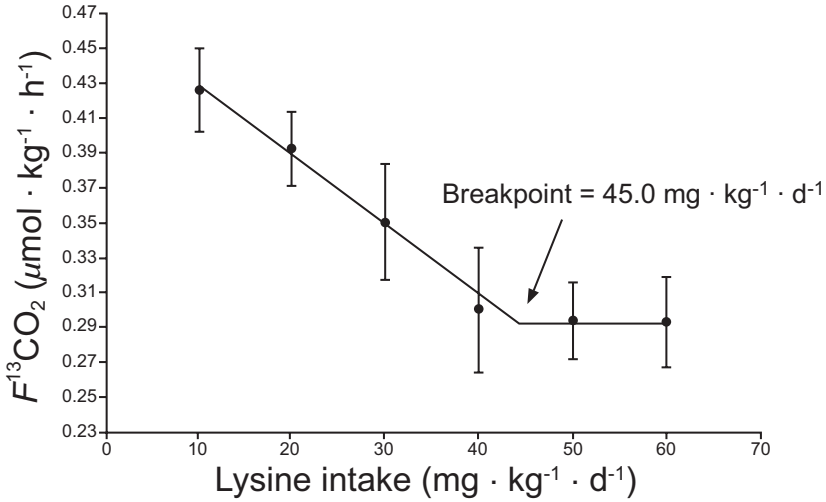
4.3.1 *General approach*

The indicator amino acid method also relies on stable isotopes to measure amino acid oxidation, but it differs from the carbon balance approach in that the oxidation of an amino acid other than the test amino acid is measured. The theory behind the method is that if one amino acid in the diet is below requirement (i.e. is limiting), then all other indispensable amino acids cannot be fully utilized for protein synthesis and the excess is therefore oxidized. As the amount of the limiting amino acid is increased, the others will be progressively better utilized and their oxidation rates will progressively fall to a lower limit at the point where the requirement of the test amino acid is reached. Intakes above this should no longer influence the oxidation of the test amino acid, which should remain low and constant. The aim is to detect a "breakpoint" in the curve for oxidation of the "indicator" amino acid against the intake of the test amino acid. An example is shown in Figure 11.

The indicator method was originally used to study amino acid requirements in pigs (*62, 63*), but is now extensively used for studies in humans (e.g. *64*). The general procedure differs in a number of important respects from that traditionally used to determine requirements, so it deserves a detailed discussion.

With this approach, subjects are given infusions of the indicator amino acid, generally [1-^{13}C]phenylalanine, at 2-day intervals, while on a diet with a low natural carbon-13 content (to stabilize the enrichment of breath CO_2), adequate phenylalanine (above requirement), and generous tyrosine to ensure

Figure 11
Breakpoint analysis of $^{13}CO_2$ production against dietary lysine intake during infusion of [1-^{13}C]phenylalanine[a]



[a] Reproduced from reference *61* with permission from *The American Journal of Physiology,* The American Physiological Society.

that excess phenylalanine converted to tyrosine is then oxidized to CO_2 (*64*). In some cases the pre-infusion diet also includes generous amounts of the amino acid under investigation, e.g. lysine (*64*). On each infusion day, subjects consume equal small meals containing the test level of the amino acid given at hourly intervals, beginning 3 hours before the isotope infusion. Blood and breath samples are taken in order to determine the oxidation rate of phenylalanine. Each subject repeats this procedure 6 or 7 times with varying test amino acid intakes. This is possible in short time periods because no period of adaptation to each intake is employed (*64*). The rate of oxidation of the indicator amino acid is then determined and plotted against the intake of the test amino acid. The position of the breakpoint for each individual is then ascertained by a specific regression procedure (two-phase linear crossover; *65*).

This technique has several theoretical and practical advantages over carbon balance approaches although, as discussed below, such advantages need to be weighed against concerns that have been raised about certain aspects of this approach.

First, unlike the carbon balance method, the test amino acid and the tracer are separate. Hence there is no problem of giving nutritionally significant quantities of the tracer, because it is separate from the test amino acid. Moreover, in the protocol described above, there is no measurement during

fasting, which is the time when the problem of the amount of infused amino acid is greatest. In addition, since the intake of the indicator amino acid is kept constant, there will be smaller changes in its concentration than for the test amino acid. Changes in concentration of an amino acid alter the rates of transport into cells, and hence the intracellular to plasma enrichment ratio (*66*). For amino acids such as phenylalanine and lysine for which there is no surrogate measure of the intracellular enrichment, such as the keto-isocaproate for leucine, the change in this ratio might confound the results. Above all, the requirement of any amino acid can be determined with a single indicator. This is especially important since, as pointed out above, only the branched-chain amino acids are ideally suited to measurements of carbon balance. Because in this approach balance is not measured, the choice of indicator amino acid can be based on other considerations of what is theoretically and practically the most sound and convenient. In practice, [1-^{13}C] phenylalanine has been used most frequently. Because of the low concentration of phenylalanine in blood and tissues, it was suggested that its metabolism would be rapidly sensitive to changes in protein balance (*61*). Although phenylalanine decarboxylation takes place after conversion to tyrosine, with $^{13}CO_2$ release occurring from tyrosine oxidation, the loss of the ^{13}C into the protein-bound tyrosine pool or tyrosine metabolites has been minimized by giving a high-tyrosine diet before the study (*61, 67*).

A second apparent advantage of this approach is its practical simplicity resulting from the lack of need to make balance measurements or for prior dietary adaptation to each of the varying intake levels. Thus, there is no need to have sophisticated facilities and staff to run accurate balance studies, and the subjects do not have to spend a week or more in a metabolic facility prior to each measurement. In addition, the measurements are made during a short period of feeding only, which reduces the overall complexity still further. Not only can many such measurements be made in a relatively short time, but each subject can conveniently be studied over a complete range of intakes (6 or 7). Hence, the oxidation results from each subject can be plotted separately and an individual breakpoint determined. This is valuable, because it enables the authors to report both a mean requirement and its inter-individual variance.

A third advantage is that the method is not reliant on high levels of precision or accuracy in the measurement of amino acid oxidation. The breakpoint is an operational indicator of the adequacy of intake of the test amino acid that does not depend on whether the indicator oxidation rate is measured accurately. The reason is that there is no source of error that varies systematically with the intake of the test amino acid, which most believe to be a reasonable assumption. Therefore, there is no need to measure the CO_2 recovery. Indeed, the same breakpoint can be obtained by plotting rates of

labelled CO_2 production rather than phenylalanine oxidation (*64*). In addition, the problem of measuring the intracellular amino acid enrichment does not invalidate the result. Indeed, in most reports, no influence of amino acid intake on any measure of tracer turnover is detectable. It does not matter whether amino acids are lost via routes other than oxidation, and if there were to be absorption of indispensable amino acids synthesized by the intestinal microflora, the breakpoint would indicate the requirement for additional dietary amino acids.

Because of these advantages, the method has been extensively deployed, resulting in a considerable amount of new information about the requirements of most of the indispensable amino acids (see section 8). However, the theoretical basis of the approach differs from other methods, and what is measured bears a different and quite complex relationship to the amino acid requirement measured by the traditional balance approaches.

As mentioned above, the indicator method was originally used to study amino acid requirements in pigs (*62, 63*), prior to its more recent use in human for studies (e.g. *61, 64*). It was developed to assess the amino acid requirements for growing animals, which are almost entirely those for growth. It therefore aims to assess the amino acid pattern for net protein deposition and the breakpoint will be most apparent when there is a clear change from no growth (high rates of indicator oxidation) to rapid growth (low rates of oxidation).

In the human adult in overall balance, this approach can be deployed in practice only to assess the requirement pattern for postprandial net protein synthesis, and this raises two problems. First, because this net protein synthesis may be less intense than in a growing animal, the demonstration of a breakpoint is often much less convincing. Certainly the statistical analysis to yield the average breakpoint and consequent requirement value is by no means transparent. This is often the case with inspection of the oxidation rate data points from individual subjects where identification of a breakpoint is difficult to justify. Thus, individual breakpoints indicated values ranging from 10 to 35 mg/kg per day for threonine requirements (*68*) and 2 to 5 mg/kg per day for tryptophan (*69*). Second, our current model for amino acid requirements (see section 2) assumes that the amino acid pattern of the adult requirement is for maintenance, which may differ from that for growth, i.e. net protein deposition. Thus, this method is not a direct measure of the maintenance requirement in the same way that the 24-hour carbon balance method is, but is mainly a measure of the intake of the test amino acid as a proportion of its content in the amino acid mixture required for postprandial protein deposition.

Within the diurnal cycle of tissue gains and losses, the extent of protein deposition after meals does relate to the overall requirement and must balance

fasting losses for balance to be achieved, but this relationship is complex. The rate of total protein intake (less the test amino acid) during feeding determines the maximum rate of protein retention that can be achieved when the test amino acid intake meets the requirement. This means that the protocol design in terms of the rate of protein intake per meal determines the maximum rate of protein anabolism achievable, which in turn determines the apparent amino acid requirement. Giving the daily intake in 8 rather than 12 hourly meals would mean a 50% higher hourly intake and potential net protein deposition rate, and a similar increase in the apparent requirement. In real life, meal sizes and timing vary, and only after a full 24 hours or longer can the body balance intake and output. This will involve regulation of both the gains and losses of body protein, and the rates of amino acid oxidation throughout the 24-hour cycle.

This different approach to measuring amino acid requirements raises the practical and theoretical issue of the need for a period of adaptation to the varying intakes prior to each infusion. Some period of adaptation is a usual feature of multilevel nitrogen or carbon balance studies, but this is a complicated issue in studies with mixtures of amino acids. The experimental design can involve variation not only in the intake levels of the test amino acid but also in the overall nitrogen intake, and there is evidence that adaptive responses occur in each case. Thus El Khoury et al. (*45, 46*) showed an adaptation of leucine oxidation in the fasting state over a 2-fold range after 6 days of leucine intakes at 89, 38 and 14 mg/kg per day at a constant nitrogen intake. These are similar to changes in fasting and fed nitrogen losses or leucine oxidation shown to occur in response to 2-week periods of widely varying protein intakes (*17*). Thus, adaptation should influence the overall need for amino acids, even when measurements are limited to the fed state.

The advocates of the indicator oxidation method cite two sets of experimental data to support their view that prior adaptation is unnecessary. They argue from the data of Zello, Pencharz & Ball (*67*) that adaptation to either 4.2 or 14.0 mg/kg per day phenylalanine for periods of 3, 6 or 9 days did not influence the result. They also point to two separate studies of the lysine requirement at total protein intakes of 0.8 (*61*) or 1.0 g/kg per day (*70*) which yielded very similar breakpoints. It is unlikely, however, that any adaptive response to such a small difference in protein intake could be detected. Indeed close inspection of the actual design shows that rates of intake of protein during the measurement were almost identical (0.067 versus 0.071 g/kg per day). This is because the two intake levels were given as half of the day's intake divided into 7 portions for the 1.0 g/kg per day intake or 6 portions for the 0.8 g/kg per day. Thus, questions remain as to whether measurements in the fed state only, with no prior periods of adaptation to experimental diets, can accurately reflect the dietary requirement.

More recently, the idea of indicator oxidation and breakpoint analysis has been extended to 24-hour oxidation studies. In this case, 24-hour [1-^{13}C] leucine oxidation and balance is the indicator of the requirement intake of another amino acid such as lysine (*55, 71*). Following the initial validation of the 24-hour [1-^{13}C]leucine oxidation and balance approach (*45*), 24-hour [1-^{13}C]leucine oxidation studies at varying lysine intakes showed a negative leucine balance with 12 mg/kg per day lysine and a positive leucine balance with 28 mg/kg per day lysine, indicating a requirement close to 28 mg/kg per day (*71*). However, in a second study (*55*), with four different levels of lysine, 12, 20, 28 and 36 mg/kg per day, positive leucine balance was observed at all lysine intakes, so that it was not possible to calculate the requirement level from the leucine balance alone. However, on the assumption that the positive balances were artefactual (although not explained), it was possible to determine a breakpoint in the dose–response curves for 12-hour fed-state oxidation, 24-hour oxidation or leucine balance and in each case it was close to 29 mg/kg per day. In other words, two-phase linear regression was used in this case as a specific curve-fitting procedure for the balance or oxidation data, although whether the number of measurements (only four points) is sufficient for this is debatable. As indicated above, 24-hour ^{13}C balance measurements at five or six different levels of intake for each individual, with a design including adaptation for 1–2-week periods, is prohibitively costly. For this reason, in practice each subject has been studied at only two or three levels of intake, with intakes randomized within and between individuals to span a total of four or six intake levels. Thus a separate breakpoint cannot be derived for each individual, and exact details of detection of the breakpoint have not been given. The best account of the two-phase linear crossover method in humans is given by Zello, Pencharz & Ball (*67*).

4.3.2 *Summary of indicator amino acid method*

The indicator amino acid approach when performed over a 24-hour period is probably the most satisfactory method on theoretical grounds, representing the current state of the art. However, the logistics and cost of the work have meant that only one group has to date been able to perform such studies, reporting results based on [1-^{13}C]leucine oxidation and balance for lysine (*55, 71*), threonine (*72*) and methionine (*73*). To date a comparison of estimates of the requirement for these three amino acids by the 24-hour method with the fed-state indicator approach of Ball & Pencharz (see section 8) indicates no evidence of a systematic difference, i.e. 30 mg/kg per day and 37 mg/kg per day, respectively, for lysine, 15 mg/kg per day and 19 mg/kg per day for threonine, and 16 mg/kg per day and 13 mg/kg per day for methionine (see section 8). Clearly this is an area where more work is required.

One question regarding all studies involving purified amino acids, whether by nitrogen or stable isotope oxidation, is how well the results relate to the amino acid requirements of subjects consuming protein within mixed diets. Amino acid mixtures will be absorbed very rapidly, and limited data on the influence of digestion and absorption rates on protein utilization point to a lower efficiency with "fast" proteins such as whey compared with slow proteins such as casein (*74*).

4.4 Predictions from the obligatory nitrogen loss

The theoretical prediction of the amino acid requirement pattern from the magnitude of the obligatory nitrogen loss and the pattern of tissue protein (*6, 75*) is a controversial issue (*17, 22, 76*). If valid, this would enable values to be derived for amino acids for which there is a lack of experimental data. It is assumed, quite reasonably, that the individual amino acids contribute to the obligatory nitrogen loss in subjects on a protein-free diet in proportion to their representation in whole-body protein. However, it is then assumed that these calculated obligatory oxidative losses (obligatory nitrogen losses as mg nitrogen/kg per day × amino acid mg/g tissue nitrogen) reflect the basal demands for each indispensable amino acid, from which the individual amino acid requirements are calculated after adjusting for the efficiency of utilization (e.g. 47% in adults; see section 7). It is possible, however, that the requirement of only one amino acid can be identified in this way, i.e. the limiting amino acid, with the highest ratio of metabolic demand for maintenance to tissue protein content (*57*). In other words, the consumption of this one amino acid in effect "drives" the obligatory nitrogen losses, and this is believed to be methionine. On this basis, this approach has been used in this report to estimate the sulfur amino acids.

4.5 Indirect estimates through measurement of protein utilization

One source of indirect evidence about the requirement values for individual amino acids is the direct evaluation of protein quality with nitrogen balance studies of dietary protein sources known to be limited in terms of specific amino acids. However, this has not proved to be generally very useful because of lack of reproducibility between studies (*65*). More recently, short-term feeding and fasting studies of [1-^{13}C]leucine oxidation and balance, which have been used to explore the adaptive metabolic demands model of protein requirements (*77*), have also been applied to measurements of utilization of milk and wheat proteins in terms of the relative efficiency of postprandial protein utilization. In this case [1-^{13}C]leucine balance is used as a surrogate for acute measurement of nitrogen balance (*78*). From these acute measurements of [1-^{13}C]leucine balance, nitrogen balance is predicted, allowing estimation of the metabolic demand for protein (from postabsorptive nitrogen

balance), the efficiency of postprandial protein utilization (from the slope of the nitrogen-intake nitrogen-balance regression), and the requirements for wheat protein and lysine, assuming that wheat protein is lysine-limited (*79, 80*). The values reported by this approach are dependent on various theoretical assumptions within the metabolic demands model, and studies where postprandial protein utilization is measured at two levels of protein intake (*80*) within a single three-phase 9-hour infusion proocol (fasting, fed low protein, fed high protein) provide a more satisfactory measure than a single meal, fast-fed protocol (*80*). The estimates of the lysine requirement at the level of adaptation obtaining in the subjects at the time of study are an indirect measure, and have generally indicated somewhat lower values than those indicated by the methods described above.

4.6 Conclusions

While the estimation of human requirements for protein and amino acids remains an inherently difficult problem, the Consultation was guided by the following considerations in selecting appropriate values for this report:

- The only available method for estimating the requirement for total protein (nitrogen) is by nitrogen balance. The large body of data available from adults is the subject of a recent meta-analysis (*81*), from which the nitrogen intake corresponding to zero nitrogen balance has been estimated by regression techniques, and this forms the basis of the adult requirement in this report (section 8). Similarly, there have been a number of studies of nitrogen balance at varying intakes in children, and these have been used in combination with the factorial approach to form the basis of the estimates of the children's protein requirements (section 9).

- At present, no method is entirely reliable for determining the dietary requirement for indispensable amino acids. The available nitrogen balance data have been shown to yield greatly differing estimates according to the assumptions about unmeasured nitrogen losses and the statistical method employed to analyse the balance data. The carbon balance method using carbon-13 labelled amino acids is subject to considerable uncertainty, with reasonably reliable estimates currently available only for amino acids that lose the label quantitatively and irreversibly when oxidized, and for which reliable estimates of precursor enrichments can be obtained. This limits the approach to the branched-chain amino acids (in practice, leucine). Although on theoretical grounds the most reliable approaches are the 24-hour indicator/carbon balance approaches, which take account of most of the potential theoretical and practical problems that have been expressed, these have been applied only to lysine, threonine and methionine. In making recommendations for the other amino acids, the data from various methods that have been employed were taken into consideration (section 8).

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5. Protein–energy interactions

In the 1985 report (*1*), discussion of protein–energy interactions was limited to the well known influence of changes in food energy intake below or above energy needs on nitrogen balance, and the potential problem posed by inadequate energy density of diets, especially weaning foods, for ensuring that protein and other nutrient needs are met. The potential use of the protein:energy ratio as a measure of dietary quality was discussed at somewhat greater length. In fact there are important practical and conceptual issues that need to be recognized. These relate both to the design and interpretation of nitrogen balance studies of protein and amino acid requirements (*2*) and to the relationship between energy and protein requirements and the likelihood of risk of deficiency (*3*).

Both units of energy measurement have been used in this report: SI conversion factors are: 1 kJ = 0.239 kcal; and 1 kcal = 4.184 kJ.

5.1 Energy intakes and protein requirements

5.1.1 *Nitrogen retention and variable energy balance*

Protein utilization and deposition are energy-dependent at all stages of amino acid transport and interconversion, protein synthesis and proteolysis. In addition, amino acids are a potential cellular fuel, especially for hepatic and renal metabolism, but also within skeletal muscle. Thus adequate non-protein energy from carbohydrate or fat is indispensable to ensure that sufficient dietary amino acids remain available as substrates to satisfy the amino acid demand and to fuel associated energy demands.

Studies by Calloway & Spector (*4*) and Inoue, Fujita & Niiyama (*5*) suggested a variable sensitivity of nitrogen balance to energy intake over the protein intake range from very low to excess. The 1985 report (*1*), drawing mainly on the work of Calloway (*4, 6*), estimated the magnitude of the impact of energy intake on nitrogen balance both below and above nitrogen equilibrium to be 1–2 mg of retained nitrogen/kcal. More recently Pellett & Young (*7*) evaluated all published nitrogen balance studies in adults where both protein and energy intakes were varied (n = 361 balances) by multiple

linear regression. This showed that increases in both energy intake and nitrogen intake were separately effective in improving nitrogen balance, with 53% of the variation in nitrogen balance explained by nitrogen intake and energy intake in combination, i.e.

$$\text{nitrogen balance} = 0.17\ \text{nitrogen intake} + 1.0\ \text{energy intake} - 69$$

The slope implies an energy intake, nitrogen balance equivalence of 1 mg nitrogen/kg per day gain per extra 1 kcal/kg per day intake. This allows an estimate to be made of the likely extent to which errors in ensuring adequate energy intakes will influence estimates of the protein requirement. For example, for a moderately active young adult male, with an estimated energy requirement of 45 kcal/kg per day (i.e. 1.8 times the predicted basal metabolic rate), an error of ±10% in estimating basal metabolic rate and consequent energy needs, i.e. 4.5 kcal/kg per day, would account for a variability in nitrogen balance of ±4.5 mg nitrogen/kg per day (at 1 mg nitrogen balance per 1 kcal energy intake). This is equivalent to a variability in requirement of ±10 mg nitrogen/kg per day (given the slope of the nitrogen intake versus nitrogen balance of about 0.5). According to the recent meta-analysis of nitrogen balance estimations of the protein requirements (*8*), this error in protein requirement attributable to the likely error in establishing energy balance is substantial, at about one-third of the total between-individual variance (SD = 31.9 mg nitrogen/kg per day), or ~85% of the estimated true between-individual variance. In practical terms, in multilevel nitrogen balance studies aimed at measuring protein or amino acid requirements, actual rates of energy expenditure and consequent energy requirements are seldom measured. Instead, body weight monitoring is the usual measure of energy sufficiency or deficiency during the study. In this case, how well energy balance was maintained would depend on the vigilance of body weight monitoring in what are usually short-term studies. An overestimate of 4.5 kcal/kg per day could result in 0.5–1 g of tissue gain/kg per day, equivalent to 0.25–0.5 kg of weight gain per week for a 70-kg adult. Clearly this would be substantial weight gain, if maintained, but might be considered within the normal range in a short-term study. More often an underestimation of energy requirements has been made, as in the only multilevel study of protein requirements in elderly people reported to date (*9*), in which energy intakes were low at only 1.33 times basal metabolic rate (see *10*). This could have resulted in an overestimation of the protein requirement by 0.125 g protein/kg per day.

5.1.2 *Nitrogen retention and variable energy turnover*

The effect of variation in energy turnover through variable physical activity at energy balance on nitrogen balance is an important, but much less understood, issue. In the developing world, where rates of energy expenditure may

be higher than those associated with the more sedentary lifestyles in the developed world, the question of how changes in energy flux through increased activity influence nitrogen balance may be of great practical importance. However, this important question is poorly understood. On the one hand it has been reported that increasing physical activity can optimize dietary protein utilization in normal adults (*11*). On the other, work related to examination of the effect of exercise on protein requirements generally indicates that exercise increases amino acid oxidation and nitrogen losses, although in the appropriately trained individual with adequate energy supply, such nitrogen losses may be minimal or even less than in sedentary individuals (*12*). Clearly this is an area where more work is needed.

5.1.3 *Energy intakes and the interpretation of requirement studies*

The interdependence of energy and protein requirements makes it difficult to interpret the results of nitrogen balance trials, and these difficulties have not been resolved. Studies performed since the 1973 report (*13*), have generally aimed at providing minimum energy intakes to avoid weight gain. This has meant that during a long-term nitrogen balance trial at an intake of 0.6 g high-quality protein/kg per day, "increased" energy intake was needed to maintain nitrogen balance, implying to the authors that this level of protein intake was inadequate (*14*). An alternative view would be that chosen energy intakes had been too low at this lower end of the adaptive range for protein. Another important controversial study is the long-term test of the adequacy of wheat-based diets (*15*), in which weight, fitness and positive nitrogen balance were maintained for 50 days on a wheat-protein-based diet providing only 18 mg lysine/kg per day. One view is that "the high dietary energy intakes (1.7–2.2*BMR) provided by the experimental diet confound interpretation of the N-balance data" (*15*). The other is that in the absence of weight gain, energy intakes were consistent with demands of high levels of physical activity, and that the study is a valid test of the adequacy of an intake of 18 mg lysine/kg per day (*2*). Studies at realistic levels of physical activity are arguably most relevant to subjects in developing countries.

5.1.4 *Protein–energy interactions in children*

In children, given their capacity for lean tissue growth, protein–energy interactions are quite complex. First, any endogenously driven protein deposition and growth, e.g. as a consequence of height growth (see *16*), can enable positive nitrogen balance which is to some extent independent of energy intake, and which can occur in negative energy balance, with energy demands for growth met by mobilizing body fat. Second, there is a wide range of observed variability in the composition of weight gain. This has very marked effects on the potential relationships between energy and protein intakes and

weight gain, given a 5-fold difference in the required energy intake to support adipose, as opposed to lean, tissue (*17*). Thus changes in the composition of deposited tissues can dramatically influence growth rates, as observed during the marked increase in growth rate with no change in energy intake in children supplemented with zinc, which had become limiting for lean tissue growth (*18*). While some attempts have been made to formalize the prediction of dietary protein–energy interactions in relation to rates of weight gain and the composition of deposited tissue during catch-up growth (*19*), the various assumptions that have to be made limit the predictive value and practical applicability of such equations, especially during rapid weight gain. Hence, values must be assumed for the energy cost of fat and lean tissue deposition, the digestibility and metabolizability of dietary energy and the efficiency of protein utilization. Thus, during rapid weight gain of infants fed two levels of protein intake, who consumed slightly different energy intakes, whereas the predicted weight gain was consistent with the observed outcome in terms of energy intakes, variations in the efficiency of protein utilization meant that weight gain was not predicted by protein intakes.

5.1.5 *Protein-sparing effect of non-protein energy substrates*

At very low levels of either protein or energy intakes, the protein-sparing effect of carbohydrates is greater than that of lipids (*20*), and it has been proposed that this is also true as energy intakes increase towards maintenance (*21*). However, more recent work suggests that lipids become as effective as or even more effective than carbohydrates in the maintenance range (*22, 23*). In the clinical environment, most studies show an equivalent nitrogen-sparing effect of glucose or lipids during total parenteral nutrition in adults and children, with a lipid–glucose regimen sparing more nitrogen than glucose alone, and with medium-chain triglycerides being particularly effective in promoting nitrogen balance. Furthermore, high-fat regimes have been developed to ensure rapid weight gain and very efficient nitrogen utilization in children given an energy-dense, high-fat diet with a fat:carbohydrate ratio of up to 4:1 (*22, 23*).

The protein-sparing effect of dietary carbohydrates is mediated in part by increased insulin secretion, which inhibits proteolysis, hepatic gluconeogenesis and renal ammoniagenesis. Dietary lipids are also insulinogenic, through the enteroinsular axis and incretins (e.g. glucose-dependent insulinotropic polypeptide (GIP), which is released in response to dietary glucose or fat), and through an influence of circulating non-esterified fatty acid on insulin secretion (*24, 25*). Also, a protein-sparing effect of dietary lipid may be attributable to a reduction of amino acid oxidation, through an effect of free-fatty acid oxidation in the liver, whereby the increase in the NADH/NAD ratio inhibits branched-chained keto-acid dehydrogenase.

5.2 The protein:energy ratio

In the 1985 report (*1*), the protein:energy ratio was reviewed as a measure of dietary quality, and in relation to definition of reference values for requirements with which the adequacy of diets could be evaluated for individuals and different population groups. While the use of protein:energy ratios can be of great value, the issues involved are complicated, so that care is required in both calculating and using such ratios.

5.2.1 *Protein:energy ratios as a measure of dietary protein quality*

Food consumption is primarily determined by energy expenditure, a function of basal metabolic rate and physical activity level. However, basal metabolic rate varies with age, sex and body weight, and physical activity varies with lifestyle and patterns of behaviour, so that consumption of food and consequently protein is also determined by age, sex, body weight, occupation, lifestyle and patterns of behaviour. Thus, when enough food is eaten to satisfy energy needs, the needs for protein (or any other nutrient) will also be satisfied if the ratio of protein (or other nutrient) to energy is appropriate. Thus the protein:energy ratio becomes a measure of dietary quality, a concept first developed by Platt, Miller & Payne (*26*). The utilizable or net protein (i.e. total protein corrected for digestibility and biological value) is converted to metabolizable energy (17 kJ or 4 kcal/g protein) and then expressed as a percentage of total dietary metabolizable energy (i.e. net dietary protein calories %, or NDPcal%). While this was most useful in describing the quality of diets to support growth in experimental animals, its use in human nutrition needs to be considered with care, and there are some quite difficult problems which need to be recognized if the adequacy of diets to provide for protein requirements is assessed in these terms.

"Utilizable protein" or the net utilization of a protein is calculated from digestibility and biological value, the latter being a function of amino acid score, which is its amino acid pattern in relation to the amino acid pattern of the requirements (see section 6). Because of the higher amino acid requirements in infants and children than in adults, an age-specific amino acid scoring pattern is required to assess the biological value of any diet. Assuming that digestibility is not age-dependent, the net protein utilization of any diet or protein will vary with age, according to the variation in biological value. Thus for diets limited by their amino acid content (biological value), net protein utilization will be lower for infants and children than for adults.

A further complication in calculating the protein:energy ratio of the diet relates to the actual available energy content of foods, i.e. taking into account true digestibility and metabolizability of the dietary energy. The 1985 report suggested that the influence of dietary fibre on energy digestibility reduced

available energy by 2–3% at moderate levels and by an additional 2–3% at the levels found in vegetarian diets. Thus a correction for a 5% energy loss was suggested for diets with "moderately large" amounts of fibre from fruit, vegetables and wholemeal bread. It was further suggested that this "…may not be enough for some populations in developing countries". Since then, the issue has been evaluated by FAO in its recent report on food energy, methods of analysis and conversion factors (*27*). Energy losses attributable to fermentation of resistant starches in the lower gut can be accounted for by calculating metabolizable energy from general Atwater factors applied to values for available carbohydrate by difference, or by using the specific Atwater factors (*28*). These different approaches result in slightly different values (up to 3% difference in overall metabolizable energy for some diets). In addition, calculations of net metabolizable energy take into account not only fermentation losses, but also losses attributable to the thermic effect of protein. However, having reviewed these issues in judging the energy content of food in relation to energy requirements, FAO concluded that energy should continue to be calculated from general Atwater factors for protein, fat, available carbohydrate and fibre, and that the likely differences between these values and best estimates of net metabolizable energy will be small (*27*). Thus in most cases for regular diets not containing excessive protein, corrections may not be necessary, i.e. the correction will usually be less than 2.5% and can be ignored (*29*). Clearly this is an area that requires more work, especially in relation to diets which may contain anti-nutritional factors, such as those found in legumes, or where assessment of available carbohydrate and fibre is difficult (e.g. *30, 31*).

Most difficulties relate to calculation of the protein:energy ratio of the requirement, especially the derivation of requirement values that can be compared with protein:energy ratios of diets, in order to assess the risk of deficiency.

5.2.2 *Protein:energy ratio of requirements and dietary assessment*

Energy requirements change not only with age, sex and size, but also with the physical activity associated with lifestyle. In contrast, in this report – as in the past – we define protein requirements as independent of size, sex (in adult life) and adult age. Thus "situation-specific" values of the protein:energy ratio of the requirement need to be calculated based on weight, age, sex and lifestyle (physical activity).

Beaton has discussed the procedures involved in the assessment of risk of dietary protein deficiency within a population group and derivation of reference protein:energy ratios (*32, 33*). His ideas have been incorporated into the report *Dietary reference intakes: application in dietary assessment* (*34*).

These ideas have been reviewed recently (*3*). For a complete assessment, information is required about appropriate values for requirements and intakes of protein and energy, and the nature and extent of both the within-individual and the between-individual variation in these values (see Tables 3 and 4). Information is also required about the extent of any correlations between (a) intakes and requirements for energy, (b) intakes and requirements for protein, and (c) energy and protein requirements. When such data are available to enable the generation of representative distributions for both intake and requirements, it is possible to use a probability approach to calculate from the distribution curves the proportion of the population that is deficient. However, representative data on intakes and requirements are generally not available within the same population groups, and therefore it is necessary to develop approaches within which reasonable assumptions can be made about the variables of importance, and which can be used to identify safe or reference protein:energy ratios for individuals and for population groups.

5.2.3 *Derivation of reference protein:energy ratios*

At the outset, as discussed in section 3, a distinction needs to be made between assessment of risk of deficiency for an individual as opposed to a population, the latter being much more difficult than the former. Most of the following discussion relates to assessment of risk of deficiency for an individual. As with calculation of safe or reference protein requirement values, the identification of a safe or reference protein:energy ratio, which describes an intake associated with a low risk of inadequacy for an individual, involves the assumption that there is no correlation between protein intake and requirements, i.e. that adaptation of metabolic demands to dietary protein intake does not occur or that appetite is not driven by the need for protein. Furthermore, because an individual's risk of inadequacy for any dietary protein:energy ratio depends on the variability of both his or her protein and energy requirements, the calculation of a "safe" protein:energy ratio is complex. Since there has been no clear agreement on the most appropriate way, or even the feasibility, of deriving a reference protein:energy ratio for a population group, a pragmatic approach can be adopted. In Appendix 9A of the 1985 report (*1*), a method for calculating a reference protein:energy ratio was described that would ensure that, at a specific probability (e.g. 97.5%), a diet with the calculated protein:energy ratio would meet or exceed the actual protein requirements of a randomly selected individual. When the energy and protein requirements have similar variability, and are not correlated, the calculated value for adults approximately corresponds to:

$$\text{reference protein:energy ratio} = \frac{\text{mean protein requirement} + 3\text{SD (as energy)}}{\text{energy requirement}}$$

For infants and children, the calculated value corresponds to a protein value in the numerator of between 3SD and 4SD above the mean.

Calculation of a reference protein:energy ratio to be used to judge the adequacy for a population of their intake is especially complex. As discussed in section 3, any formula will be a function not only of means and variances of the protein and energy requirements, but also of means and variances of their intakes. Arguments can be developed (e.g. *3*) for defining values in which the protein requirement term in the numerator of the ratio ranges from average requirement +3SD to much higher values up to average requirement +8SD. However, use of the latter value results in reference protein:energy ratios which are unachievable in terms of intakes for many populations. Although the former value, similar to the reference protein:energy ratio of intake for an individual, is likely to be lower than the true safe intake appropriate for a population, any deficiency identified with such a value will be less than the actual risk, so that diets judged inadequate with these values can be confidently assumed to be inadequate. Clearly, use of these values also means that diets of populations judged adequate could still be inadequate, so a cautious approach needs to be adopted when judging the risk of deficiency.

In Annex 1 to the 1985 report (*1*), an approximate formula was given for calculating reference protein:energy values. Examples of the mean and reference protein:energy ratios of energy and protein requirements are shown in Table 4, calculated as indicated in the 1985 report (*1*), for males and females of two adult body weights and at three levels of physical activity, using the protein and energy requirements shown in Table 3.

As would be predicted from the way energy requirements change with basal metabolic rate, which is assumed to be lower (per kg) for women than men, to fall with age in adults, and to be lower in heavier than lighter adults of any age, the mean and reference protein:energy ratios increase with age, are higher for females than males, higher for small compared with large adults at any age, and of course are higher in sedentary than in active individuals. Thus the protein:energy ratio is highest when energy requirements are lowest, i.e. in sedentary elderly large women. Conversely, the high energy requirements of infants and children, which are much higher relative to adult values compared with protein requirements, mean that the protein:energy ratios of requirements are lowest in infants and young children. Thus, assuming the reference protein:energy ratio represents a safe or "desirable" protein:energy ratio that has to be provided to an individual by the diet, a sedentary elderly woman who weighed 70 kg would require food with more than twice the protein concentration relative to energy compared with that needed by very young children. It follows that a diet that can meet both the energy and protein needs of the infant, such as breast milk (i.e. a low-protein food consumed in

Table 3
Age-related changes in the protein:energy ratio

Age (years)	Mean protein:energy ratio[a]						Reference protein:energy ratio[b]					
	Males			Females			Males			Females		
	Light physical activity level	Moderate physical activity level	Heavy physical activity level	Light physical activity level	Moderate physical activity level	Heavy physical activity level	Light physical activity level	Moderate physical activity level	Heavy physical activity level	Light physical activity level	Moderate physical activity level	Heavy physical activity level
	1.55	1.75	2.2	1.55	1.75	2.2	1.55	1.75	2.2	1.55	1.75	2.2
0.5		0.056			0.056			0.078			0.076	
2.5		0.036			0.039			0.050			0.053	
5.0		0.036			0.039			0.050			0.052	
10	0.054	0.046	0.040	0.059	0.050	0.043	0.074	0.062	0.054	0.081	0.068	0.059
15	0.061	0.052	0.045	0.068	0.060	0.050	0.084	0.071	0.062	0.093	0.082	0.069
Adults at 70 kg body weight												
18–29	0.068	0.060	0.048	0.068	0.069	0.055	0.094	0.083	0.067	0.108	0.096	0.076
30–59	0.071	0.063	0.050	0.071	0.074	0.059	0.098	0.087	0.069	0.117	0.103	0.082
>60	0.085	0.075	0.060	0.085	0.082	0.065	0.117	0.104	0.083	0.128	0.113	0.090
Adults at 50 kg body weight												
18–29	0.059	0.052	0.041	0.059	0.061	0.049	0.081	0.072	0.057	0.096	0.085	0.068
30–59	0.059	0.052	0.041	0.059	0.060	0.048	0.081	0.072	0.057	0.094	0.083	0.066
>60	0.073	0.064	0.051	0.073	0.068	0.054	0.100	0.089	0.071	0.106	0.094	0.075

[a] Calculated from the values for protein and energy requirements in Table 4 as (protein (g/kg) × 16.7) /energy (kJ/kg).

[b] Safe protein:energy ratio for an individual calculated from the values for protein and energy requirements in Table 4 according to the formula in the Annex, with the SD for the energy requirement calculated assuming a coefficient of variation of 12%. The correlation between energy and protein requirements is 0.1, and the safe protein requirement is mean +2SD. These values are generally similar to calculations assuming a reference protein requirement +3SD for adults and +3SD to +4SD for infants and children.

Table 4
Age-related changes in protein and energy requirements[a]

Age (years)	Protein requirements (g/kg per day)				Energy requirements (kJ/kg)					
	Males		Females		Males			Females		
	Mean	SD	Mean	SD	Light physical activity level	Moderate physical activity level	Heavy physical activity level	Light physical activity level	Moderate physical activity level	Heavy physical activity level
					1.55	1.75	2.2	1.55	1.75	2.2
0.5	1.12	0.10	1.12	0.10		335			340	
2.5	0.75	0.09	0.76	0.09		348			334	
5.0	0.69	0.08	0.71	0.09		315			305	
10	0.75	0.08	0.74	0.09	233	275	315	210	248	285
15	0.71	0.08	0.69	0.08	195	230	265	170	193	230
Adults at 70 kg body weight										
18–29	0.66	0.09	0.66	0.09	162	183	230	141	159	200
30–59	0.66	0.09	0.66	0.09	155	175	220	131	148	186
>60	0.66	0.09	0.66	0.09	130	147	185	120	135	170
Adults at 50 kg body weight										
18–29	0.66	0.09	0.66	0.09	187	212	266	159	180	226
30–59	0.66	0.09	0.66	0.09	188	212	266	162	183	230
>60	0.66	0.09	0.66	0.09	152	172	216	144	163	205

[a] Energy requirements from reference *34*.

large quantities), may satisfy the energy needs of older children or adults, but may fail to meet their needs for protein at the level of consumption required to meet their needs for energy. These concepts are explored further in section 9.2 in relation to dietary intakes in developed and developing countries.

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6. Protein quality evaluation

Protein quality evaluation aims to determine the capacity of food protein sources and diets to satisfy the metabolic demand for amino acids and nitrogen. Thus any measure of the overall quality of dietary protein, if correctly determined, should predict the overall efficiency of protein utilization. Safe or recommended intakes can then be adjusted according to the quality measure, so that demands can be met.

As discussed in section 2, protein utilization is generally discussed in terms of *digestibility,* a measure of the dietary intake which is made available to the organism after digestion and absorption, and *biological value,* a measure of how well the absorbed amino acid profile matches that of the requirement. Overall protein utilization, i.e. *net protein utilization* (NPU), will therefore reflect both digestibility and biological value. Although net protein utilization has been most widely used in animal growth studies of protein utilization, where terms such as $NPU_{standardized}$ and $NPU_{operative}$ distinguish between studies at fixed or varying dietary protein concentrations, it is also used in human studies to describe the slope of the relationship between nitrogen balance and nitrogen intake.

Protein quality is of course a measure of protein bioavailability, the generic term for the proportion of any nutrient that can be absorbed from the diet and utilized. Bioavailability comprises digestibility, chemical integrity, and freedom from interference in metabolism, highlighting those aspects of amino acid utilization that may be important with specific foods and food processing methods. Assessment of bioavailability can be made – with varying degrees of difficulty – in humans directly, but clearly the use of model animals is attractive in terms of cost and time (*1*).

From the outset it must be recognized that there are a number of difficulties relating to protein quality evaluation that have not been fully resolved. These are both conceptual and technical, and for this reason, in this report, protein quality evaluation is addressed only in general terms, highlighting areas of current concern and identifying likely circumstances where poor protein quality may compromise nutritional status.

An important conceptual difficulty is that of establishing values for the quality of individual proteins and dietary protein mixtures which enables their utilization in human nutrition to be accurately predicted in absolute terms. This is because, in most circumstances in human nutrition, protein utilization appears inefficient, independently of the dietary protein source. This is evident from the fact that the adult requirement value for good-quality protein determined in nitrogen balance studies appears to be about twice the value of the obligatory nitrogen loss (see section 7), implying a net protein utilization of only about 50%. Only in particular circumstances, such as the rapid growth observed during catch-up from malnutrition, does the apparent net protein utilization of dietary protein approach the values achieved in animal growth trials. Although the reason for this is poorly understood, and may reflect the adaptive nature of human protein utilization (*2*), the low overall efficiency of protein utilization in human nutrition has long been recognized. Thus in practice, protein quality evaluation has aimed to predict relative utilization of different protein sources rather than absolute values.

6.1 Prediction of protein quality using the protein digestibility corrected amino acid score (PDCAAS) approach

The 1985 report (*3*) suggested the combined use of age-related scoring patterns and measures of digestibility to calculate either the safe level of intake of a diet as consumed, or the effective intake as compared with a reference protein. Scoring patterns were calculated as age-related amino acid requirement levels divided by the safe level of protein intake. This approach was formalized by a joint FAO/WHO Expert Consultation on protein quality evaluation (*4*), which was convened to review methods that could replace the rat growth assay of the protein efficiency ratio. This ratio assessed rat growth on the test protein or proteins as a function of protein intake, and for a variety of reasons it had been concluded to be unsatisfactory. Thus an assay based on measures of digestibility and amino acid composition was suggested, namely the protein digestibility corrected amino acid score (PDCAAS). This was proposed as a means of assessing the protein quality of both dietary mixtures (e.g. wheat, chickpea and milk) and individual protein food sources.

6.1.1 *Protein digestibility*

Digestibility, the proportion of food protein which is absorbed, is defined from measurements of the nitrogen content of foods and faeces, with "true" digestibility taking into account the extent to which faecal nitrogen is "endogenous", which in turn is measured as faecal nitrogen loss on a protein-free diet, i.e.

$$\text{apparent protein (N) digestibility (\%)} = \frac{I-F \times 100}{I}$$

$$\text{true protein (N) digestibility (\%)} = \frac{I-(F-F_k) \times 100}{I}$$

where I = nitrogen intake, F = faecal nitrogen loss on the test diet, and F_k = faecal nitrogen loss on a protein-free diet.

Some values for the percentage digestibility of proteins in humans are shown in Table 5. As discussed below, the relative biological significance and practical importance of faecal as opposed to ileal digestibility have become a major issue.

6.1.2 *Biological value*

The amino acid profile is assumed to determine the effectiveness with which absorbed dietary nitrogen can be utilized, which is usually defined in terms of *biological value*, i.e.

$$\text{apparent protein (N) biological value (\%)} = \frac{(I-F-U) \times 100}{I-F}$$

$$\text{true protein (N) biological value (\%)} = \frac{I-(F-F_k)-(U-U_k) \times 100}{I-(F-F_k)}$$

where U = urinary nitrogen loss on the test diet, and U_k = urinary nitrogen loss on a protein-free diet

6.1.3 *Amino acid score*

The amino acid score determines the effectiveness with which absorbed dietary nitrogen can meet the indispensable amino acid requirement at the safe level of protein intake. This is achieved by a comparison of the content of the limiting amino acid in the protein or diet with its content in the requirement pattern:

$$\text{amino acid score} = \frac{\text{mg of amino acid in 1 g test protein}}{\text{mg of amino acid in requirement pattern}}$$

then PDCAAS = digestibility × amino acid score.

If biological value is determined solely by the amino acid profile, then PDCAAS should predict biological value.

Table 5.
Values for the digestibility of protein in humans (*4*)

Protein source	True digestibility (%)	Protein source	True digestibility (%)
American mixed diet	96	Oatmeal	86
Beans	78	Oats, cereal	72
Brazilian mixed diet	78	Peanut butter	95
Chinese mixed diet	96	Peanuts	94
Corn, cereal	70	Peas, mature	88
Corn, whole	87	Rice, cereal	75
Cottonseed	90	Rice, polished	88
Egg	97	Soy flour	86
Farina	99	Soy protein isolate	95
Filipino mixed diet	88	Sunflower seed flour	90
Indian rice + beans diet	78	Triticale	90
Indian rice diet	77	Wheat flour, white	96
Indian rice diet + milk	87	Wheat gluten	99
Maize	85	Wheat, cereal	77
Maize + beans	78	Wheat, refined	96
Maize + beans + milk	84	Wheat, whole	86
Meat, fish	94		
Milk, cheese	95		
Millet	79		

6.2 Current concerns about the protein digestibility corrected amino acid score (PDCAAS) approach

Since the 1991 report (*4*) a number of important technical issues have arisen relating to the PDCAAS approach. First, as with the 1973 report (*5*), the 1985 (*3*) and 1991 (*4*) reports calculated scoring patterns from amino acid requirement values divided by the safe level of protein intake. This was done because the 1973 report related "estimates of the upper range of amino acid requirements to estimates of the upper range of protein requirements (the safe level)". However, scoring patterns that have been suggested since then have been based on amino acid requirement values, which generally reflect best estimates of average requirements (e.g. *6, 7*), as were the values derived by Hegsted (*8)* from his regression analysis of nitrogen balance data. The values assembled in the present report have also been chosen as best estimates of the average values. For this reason scoring patterns should be based on amino acid requirement values divided by the mean protein requirement.

A second concern relates to the assumed value for digestibility, especially the extent to which correction for faecal as opposed to ileal digestibility should be used. The use of ileal digestibility was discussed in the preparation of the

1991 report (*4*) but was not adopted: instead faecal digestibility determined in a rat assay was suggested as the appropriate method. Since that time there has been increasing support for the use of true ileal amino acid digestibility coefficients (*9–11*). The data available for humans are limited at present, in some cases showing quite small differences, or even no differences, between ileal and faecal digestibility of lysine (*9*). However, in a recent comprehensive review, Moughan (*11*) concludes that experimental observations in humans to date are consistent with findings in other monogastrics, such as the pig, where ileo-faecal differences which are of practical importance have been described. Thus, while faecal digestibility is likely to remain the appropriate measure of overall nitrogen digestibility, it is unlikely to be a true measure of amino acid digestibility, with measurements at the ileal level a better measure of amino acid digestibility and losses of both dietary and endogenous origin. A complementary and still unresolved aspect is to take into account the recycling of intestinal nitrogen and bacterial amino acids to the body. These areas require further research (*1*).

A third concern is that of accounting for reduced bioavailability of some amino acid residues in proteins which have been chemically transformed during manufacturing of processed foods. Lysine is an important example, and Moughan has described a specific assay for "reactive" lysine in foods which would distinguish it from biologically unavailable lysine that has undergone Maillard reactions (*11*).

A fourth important and controversial matter relates to truncation of the amino acid score and consequent PDCAAS value, i.e. expressing the maximum value for individual proteins as no greater than 1.0 or 100%, when actual calculated values for the amino acid score are higher than this because all indispensable amino acids are present at higher concentrations than in the reference scoring pattern. Thus it has been argued that truncation removes any differences between two proteins such as milk and soya protein, even though actual concentrations of important indispensable amino acids, which may be limiting in some diets, are higher in milk than in soya protein (*12*). Thus the ability of milk protein to improve the amino acid profile of a dietary mixture will be greater than that of soya. It is argued that this could be recognized by giving individual proteins an amino acid score of greater than unity or >100% as a "protein source quality index".

In the 1991 report (*4*), truncation was not used for the amino acid score, but was applied to the PDCAAS value (see Table 11 of reference *4*). In fact there is considerable confusion about this issue, which was not adequately discussed or resolved in the 1991 report.

The PDCAAS value should predict the overall efficiency of protein utilization in terms of its two components, digestibility and biological value, where

biological value is utilized nitrogen divided by digestible nitrogen, a function of its amino acid score. The principle behind this is that utilization of any protein will be first limited by digestibility, which determines the overall available amino acid nitrogen from food, and biological value describes the competence of the absorbed amino acids to meet the metabolic demand. Biological value can never exceed 1, since for any quantity of absorbed nitrogen the best that can be achieved is that the amino acid pattern is an exact match of requirements, so that all of the amino acids are utilized. In this context the PDCAAS value would be used to adjust dietary protein intakes to meet requirements, i.e. for any diet, recommended intake = safe level of protein/ PDCAAS value of diet. Thus a PDCAAS value >1 would never be used, since this would mean that for "high quality" diets, adjusted intake would be less than the safe level.

In fact, while score is determined only from indispensable amino acid content (and usually only for those few amino acids that limit protein quality in practice), the metabolic demand is for both indispensable amino acids and nonessential nitrogen. Because of this, when any or all indispensable amino acids are present in excess of the demand, the absorbed mixture is unbalanced and limited by dispensable amino acids. It is assumed that these will be supplied from oxidation of surplus indispensable amino acids. If such conversion of indispensable to dispensable amino acids occurs, then all of the absorbed nitrogen will be utilized in the same way as that of an absorbed mixture which exactly matches the demand (the reference pattern). On this basis it might be concluded that there can be no benefit from an amino acid score >1 with the theoretical possibility of a disadvantage if interconversion were incomplete.

These arguments need to be carefully considered in the context of calculating amino acid scores for PDCAAS >1 as a quality index for food protein sources and diets. The argument for defining a quality index for food protein sources based on non-truncated values of the amino acid score is built on the advantage of identifying proteins as rich sources of indispensable amino acids that can be used to complement other sources that are deficient in indispensable amino acids. Clearly, since the debate has focused entirely on amino acid composition, if such a quality index were to be advocated it could be done only in relation to amino acid score. This was not made clear in the 1991 report, which incorrectly listed several proteins with PDCAAS values greater than their digestibility when the amino acid score was >1. Thus, for soybean concentrate, with values for digestibility and amino acid score, respectively, of 95% and 1.04, the PDCAAS value was quoted as 0.99. This implies that its slight excess of indispensable amino acids could make up for the loss of 5% of total nitrogen during digestion and absorption, and this is arguably incorrect. Thus logically, on the basis that digestibility is first limiting, the PDCAAS value should be calculated from a truncated amino acid score value,

indicating that the PDCAAS value for soybean concentrate is 0.95, the same as its digestibility.

Another misunderstanding relates to the calculation of the amino acid score for a dietary protein mixture, especially where the digestibility of individual constituents varies. In this case, amino acid score is calculated for the diet from the overall amino acid profile of the dietary amino acid mixture without identifying the score of component proteins. However, on the principle that digestibility is first limiting, the composition and amino acid score of the absorbed available amino acids will reflect the relative digestibility of the individual food protein constituents. Thus the amino acid score for food mixtures should be calculated from the weighted average *digestible* amino acid content. This is in contrast to the 1991 report (Table 10 of reference *4*), which gave an example of the calculation of the PDCAAS value for a mixture of wheat, chickpea and milk powder, for which the score was calculated from the weighted amino acid content per gram of dietary protein before digestibility was calculated. While the error resulting from this miscalculation is only small in this particular case, if the digestibility of individual dietary constituents varies markedly, the error could be significant. An example of a correct calculation for a mixture of wheat, chickpea and milk is shown in Table 6, using the new reference scoring patterns derived in sections 7 and 8. Since in practice dietary proteins are likely to be limited only by lysine (most cereal proteins), the sulfur amino acids (legume proteins), tryptophan (some cereals such as maize) or threonine (some cereals), in calculating scores it is usually only necessary to use a pattern based on these four amino acids. As discussed in section 9, given the minor changes in the scoring patterns for schoolchildren and adolescents, just one scoring pattern is adopted for these age groups, based on that derived for 4–10-year-olds.

6.3 Conclusions

It is clear that there are several aspects of protein quality evaluation that require further consideration. Thus a complete listing of the digestibility and amino acid scores of food proteins based on updated data on amino acid composition, and on the new scoring patterns quoted in this report, is beyond the scope of this report and will be the subject of a new technical report. In the meantime, however, the principles discussed here and illustrated in Table 6 can be applied. Thus, protein quality evaluated in terms of PDCAAS value is calculated from the best estimate of digestibility and the amino acid score based on a comparison of the amino acid composition of digestible protein with the scoring pattern appropriate for age. It is clear that when such PDCAAS values are used to adjust the intakes of the dietary mixtures to meet the safe level, the score of the mixture should not exceed 1. Whether there is

Table 6
Calculation of PDCAAS value for a mixture of wheat, chickpea and milk powder[a]

	Analytical data							Digestible quantities in mixture[b]				
	Weight	Protein	Lysine	Sulfur amino acids	Threonine	Tryptophan	Digestibility	Protein	Lysine	Sulfur amino acids	Threonine	Tryptophan
	(g)	(g/100 g)		(mg/g protein)				(g)		(mg)		
	A	B	C	D	E	F	G	A×B×G/ 100 =P	P×C	P×D	P×E	P×F
Wheat	400	13	25	35	30	11	0.85	44	1105	1547	1326	486
Chickpea	100	22	70	25	42	13	0.8	18	1232	440	739	229
Milk powder	35	34	80	30	37	12	0.95	11	904	339	418	136
Totals								73	3241	2326	2483	851
Amino acids: mg/g protein (total for each amino acid/total protein)									44	32	34	12
Weighted average digestibility: sum of digestible protein/ total protein							0.85					

Age group (years)	Reference patterns: mg/g protein[c]				Amino acid score for mixture: amino acids/g protein per reference pattern				PDCAAS value: lowest score × digestibility
	Lysine	Sulfur amino acids	Threonine	Tryptophan	Lysine	Sulfur amino acids	Threonine	Tryptophan	
Infants (0–5 years)	57	28	31	8.5	0.78	1.14	1.10	1.38	0.67
Preschool children (1–2 years)	52	26	27	7.4	0.85	1.22	1.26	1.58	0.72
Older children and adolescents (4–18 years)[d]	48	23	25	6.5	0.93	1.38	1.36	1.80	0.79
Adults (>18 years)	45	22	23	6.0	0.99	1.45	1.48	1.94	0.84

[a] Values for protein, amino acid content and digestibility from reference *4*.
[b] Protein and amino acids calculated as digestible amounts.
[c] Reference patterns from section 9.
[d] Based on the scoring pattern derived for the 2–10-year age group.

a case for defining an index of protein quality in terms of non-truncated amino acid scores >1 for individual proteins will require further evaluation.

Finally, significant advances have been made in amino acid analysis methods and amino acid digestibility techniques since the 1991 report (*4*), which allow an expansion of the database presented in that report. The recent technical report on food energy conversion factors (*13*) includes recommendations on methods of analysis and expression of protein and amino acid content of foods, especially that protein should be measured as the sum of individual amino acid residues (the molecular weight of each amino acid less the molecular weight of water) plus free amino acids, whenever possible, recognizing that there is no official Association of Analytical Communities (AOAC) method for amino acid determination in foods. Collaborative research and scientific consensus would be needed in order to bring this about.

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7. Protein requirements of adults, including older people, and women during pregnancy and lactation

The historical development of ideas and approaches to measurement of protein requirements in adults has been reviewed in detail by Carpenter (*1–5*), by Rand, Pellett & Young (6) in their recent meta-analysis of nitrogen balance studies for estimating protein requirements in healthy adults, and by Millward (*7*).

As indicated in section 2, the protein requirement of adults can be defined as the minimum intake that will allow nitrogen equilibrium (zero nitrogen balance), at an appropriate body composition during energy balance and at moderate physical activity. In practice, the nitrogen balance studies that formed the basis of the previous (*8*) and present reports involve studies on healthy adults assumed to be in energy balance, usually on the basis of weight maintenance and of an "appropriate" body composition, but without specific measurement to ensure that this was the case. Also, the majority of reported studies were conducted after a relatively brief (usually two-week) period of adjustment to a change in test protein intake. Although the nitrogen balance technique has serious shortcomings, as discussed in the 1985 report (*8*) and by others (*9–14*), this method remains the primary approach for determining protein requirement in adults, in large part because there is no validated or accepted alternative.

The meta-analysis described by Rand, Pellett & Young (*6*) forms the basis of the current report and will be referred to below as "the meta-analysis". For the present analysis, only studies which presented data on nitrogen balance as a function of nitrogen intake among healthy people were included. The resultant papers were examined on the basis of the primary intent of the study and their inclusion of individual data. They were divided into three major types of studies ("estimation", "test" and "obligatory") and analysed separately. Estimation studies were explicitly designed to estimate the protein requirement by studying many different nitrogen intakes near purported requirements (27 studies, involving 411 subjects). These were subdivided into those studies that presented individual balance data for subjects studied at >3 intakes, (primary estimation studies, 19 studies involving 235 individuals) and those that presented either grouped data only or data from different

individuals at different intakes (secondary estimation studies). Test studies were those designed to measure the nitrogen balance at 1 or 2 specific nitrogen intakes, which were analysed independently from the estimation studies. Obligatory studies were those that reported on endogenous or obligatory nitrogen losses after providing subjects with very low amounts of dietary protein.

7.1 Evaluation of published nitrogen balance studies

7.1.1 *Adjustment for dermal and miscellaneous nitrogen losses*

The majority of nitrogen balance studies report measurements of urinary and faecal losses without measurements of dermal or miscellaneous nitrogen losses, so adjustments were made in the meta-analysis to take such losses into account. In the previous report (*8*) an allowance of 8 mg nitrogen/kg per day was chosen for dermal and miscellaneous losses for adults, without detailed justification, although it has been argued that a value of 5 mg nitrogen/kg per day, or less, would be more appropriate (*12*). Studies in infants (*15*) and pre-adolescent children (*16, 17*) suggest that dermal or sweat nitrogen losses are approximately 10 mg/kg per day or more and that they vary with the nitrogen intake level (*18*). Because body surface area and the consequent likely rate of loss of nitrogen via the skin will vary significantly with body size, measurements on adults are best used for estimating a suitable allowance for dermal and miscellaneous losses. Meticulous studies of dermal (mainly sweat) and miscellaneous nitrogen losses in healthy adults (*19*) indicated that dermal nitrogen loss varied with nitrogen intake and that additional miscellaneous nitrogen losses (via nails, hair, tooth brushing, etc.) were reasonably constant at about 115 mg nitrogen per day or 1.8 mg nitrogen/kg per day.

The meta-analysis examined published information on dermal and miscellaneous losses (see Table 7). It is apparent from studies conducted in different countries at different times of the year that dermal nitrogen losses, reflecting mainly urea, were consistently higher for those studies conducted in the tropics or during the hot season compared with those conducted in temperate regions or cold weather. Also there were significant linear relationships between nitrogen intake and dermal nitrogen loss for the temperate region studies but not for studies in the tropics. Notwithstanding the limited number of studies available, there was a consistency of the results in the requirement range of nitrogen intake, so that dermal plus miscellaneous losses were assumed to amount to a constant 11 and 4.8 mg nitrogen/kg per day for the tropical and temperate region studies, respectively, and in the meta-analysis these values were used to adjust the reported nitrogen balance studies of the requirement.

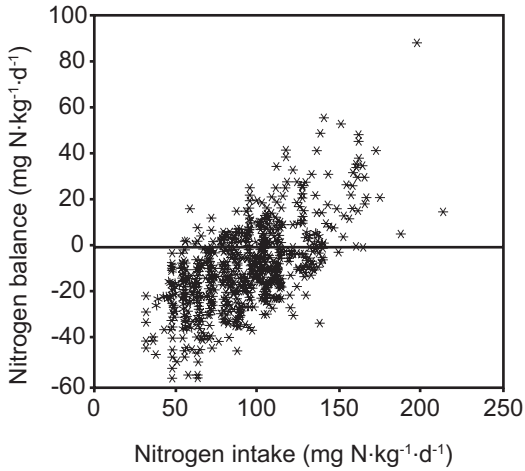
Table 7
Integumental and other miscellaneous nitrogen losses in healthy adults

Integumental and other miscellaneous losses: mg nitrogen/kg per day		Reference
Tropical	**Temperate**	
9.2		*20*
12.2		*21*
	4.1	*19*
8.1		*22*
11.5		*23*
	3.8	*24*
8.3		*25* egg diet
10.3		mixed diet
	5.6	*26* cool temperature
13.9		high temperature
14.5		27 winter
	5.4	summer
	5.2	*28*
	6.5	*29*
	4.4	*30*
11.0	5.00	Average
2.4	0.9	Standard deviation

7.1.2 *Statistical analysis of nitrogen balance data*

The studies included in the primary analysis are listed in Table 8, and the individual nitrogen balance points are shown in Figure 12. It is well known that positive nitrogen balances are almost invariably reported in non-growing adults at generous nitrogen intakes, even after correcting for dermal and other miscellaneous nitrogen losses. Such positive nitrogen balances, at intakes often similar to habitual intakes, are assumed to reflect one or more as yet unidentified technical problems resulting in an overestimation of nitrogen intake or an underestimation of nitrogen losses. Were this not the case they would result in continuing increases in tissue nitrogen concentration or continuing growth of the lean body mass. Given such overestimation of balance at generous intakes, it might be suggested that there should be some specific positive nitrogen balance value used to identify the requirement intake. However, with the lack of any obvious plateau value in the nitrogen balance data (see Figure 12), and a consequent lack of any objective method of identifying a suitable positive value, there is currently no justifiable alternative to choosing zero nitrogen balance as the measure of the requirement intake.

Figure 12
Nitrogen balance versus nitrogen intake[a]



[a]Reproduced from reference *6* with permission from *The American Journal of Clinical Nutrition.*

Rand, Pellett & Young (*6*) identified 19 studies that were designed to estimate individual protein requirements (Table 8). The studies involved 235 individuals in total, and the authors selected only those individuals who were studied at three or more different intake levels, with individual balance values at each intake. The entire data set of individual nitrogen balance points is shown in Figure 12. It is clear that there is a high level of variability in the balance responses, with some individuals in positive balance with protein intakes approaching a low level of 50 mg nitrogen/kg per day and others in negative balance at intakes of 150 mg nitrogen/kg per day.

As far as the requirement values for each individual studied are concerned, since balance studies rarely provide an intake level that results in exactly zero balance, the estimation of the individual requirement intakes involves interpolation between the intake levels studied. On a population basis, the results in Figure 12 suggest that balance varies linearly with intake. As discussed by Rand, Pellett & Young (*6*), while it is highly unlikely that the biological relationship between nitrogen intake and retention will be linear over the entire range of possible intakes, and while several different models might be used to describe the intake–balance response relationship, given the few data points for each individual (i.e. 3–6) it is difficult to justify any analysis other than linear regression. Thus linear regression of the balance data for each individual was used to estimate the slope, intercept and consequent intake for zero balance as an estimate of requirement (*48*). Also, given the small number of data points for each individual, no analysis was made of the goodness of fit.

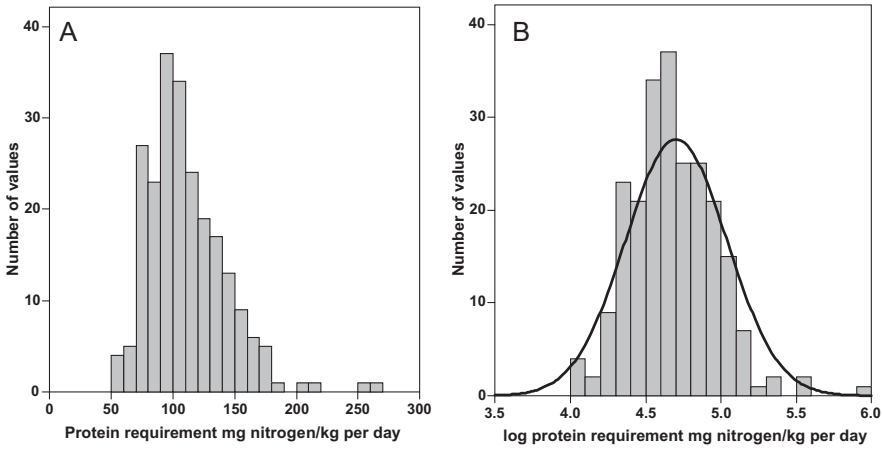
Table 8
Nitrogen balance studies used to estimate the protein requirement of healthy adults

Diet (A, animal, Mx, mixed, V, vegetable) and source	Number of individuals studied	Sex (M, F)	Climate	Age in years (Y, young; O, old)	Reference
V: rice, wheat	11	M + F	Tropical	Y: 25–39	*31*
Mx: beef, rice	15	M	Tropical	Y: 19–21	*32*
A: milk + V: corn, beans	11	M	Temperate	Y: 20s	*33*
Mx: milk, wheat, rice	6	M + F	Temperate	Y: 22–26	*34*
V: rice, beans	9	M	Tropical	Y: 18–28	*35*
A: egg + V: lupin	14	M	Tropical	Y: 18–31	*36*
Mx: rice, wheat, beef	12	F	Tropical	Y: 21–32	*23*
Mx: meat, wheat, potatoes + V: rice, beans, potatoes	14	M + F	Tropical	Y: 21–26	*37*
Mx: mixed	8	F	Tropical	Y: 18–27	*38*
A: fish + V: soy + Mx: fish, soy	20	M	Temperate	Y: 19–28	*39*
V: soy	8	M	Temperate	Y: 18–26	*40*
Mx: mixed	12	F	Temperate	Y: 18–24	*28*
M: wheat, yogurt	11	M	Temperate	Y: 19–26	*41*
A: milk + V: soy	22	M	Temperate	Y: 18–23	*42*
V: cottonseed	7	F	Temperate	Y: 18–23	*43*
A: egg	13	M	Tropical	Y: 19–27	*44*
A: egg	14	M + F	Temperate	O: 68–84	*45*
Mx: wheat, milk + A: egg	15	M	Temperate	Y: 20–31	*46*
A: egg + V: soy	15	M	Temperate	Y: 20s	*47*

7.2 Population distribution of protein requirement and determination of the median

Visual examination of the requirement levels of the 235 individual subjects indicated a skewed distribution with several probable outliers present, whereas detailed analysis indicated a log-normal distribution of requirements (see Figure 13). Two median requirement values were calculated: 105 mg nitrogen/kg per day (95% CI =101, 110) was the requirement for the population for the entire sample of 235 individuals weighting all individuals equally; and 102 mg nitrogen/kg per day (95% CI = 96, 112) was the median requirement of each of 32 distinct sub-studies identified in terms of diet, sex, or age, from the original 19 studies (i.e. each sub-study was weighted equally). Since these two procedures gave such similar results, the one based

Figure 13
Distribution of protein requirement as mg nitrogen/kg per day (A) or as log mg nitrogen/kg per day (B)



on the individual values was used. It should be noted that, since individual requirements follow a log-normal distribution, the log of the median of the requirement distribution is an estimate of the mean of the distribution of the log values.

7.3 Estimation of the variability and population reference intakes

The estimation of the true between-individual variability of the requirement, the nitrogen intake for zero balance, is an essential part of deriving population reference intakes. Whereas calculation of the median requirement value as described above is relatively straightforward, calculation of its variability is much more difficult, both in practice and conceptually. As discussed below, there are uncertainties about the extent to which energy balance is achieved in nitrogen balance studies and about whether complete adaptation to the test intakes has been achieved. Such uncertainties can markedly influence the outcome. However, in the absence of information relevant to these issues, a pragmatic approach to estimation of variability of the requirement must be adopted.

The available nitrogen-balance data include variability arising through experimental and methodological error within and between studies, through day-to-day variability of individuals, and because of true between-individual variation, the ultimate variability estimate of interest. In practice, neither the distribution (normality or otherwise) nor the magnitude of the between-individual variability of the true requirement can be identified with any certainty from the available information. In the meta-analysis (*6*) a statistical approach was adopted, after first trimming 5% for probable outliers, i.e.

removing the 2.5% highest and lowest values. This left 225 individual requirements. The between-study and within-study variability of the log-transformed data was then identified by partition of the variance by analysis of variance. This indicated the variance to be distributed as 40% between and 60% within studies. Identification of within-individual variation requires replicate estimates of requirement measured in the same individuals, and within the data set this was limited to 20 individuals in four studies, who were investigated twice. Analysis of variance (ANOVA) of these data indicated variance to be distributed about two-thirds as within-individual variance (temporal or experimental error), with the remaining one-third representing true between-individual variance. Thus, about 20% of the total variance represented true between-individual variance, yielding a standard deviation of 0.12 for the (log) requirement (ln 4.65 =105 mg/kg per day). On this basis the 97.5th percentile of the population distribution of requirement, an estimate of the safe intake level log median + (1.96 x 0.12), gave a value after exponentiation of 133 mg nitrogen/kg per day. Whereas a meaningful standard deviation cannot be calculated for the requirement itself because of its skewness, half the difference between the estimated 16th and 84th percentiles (which would contain those individuals within one standard deviation of the mean for a normal distribution) was calculated as 12.5 (log), yielding a coefficient of variation of about 12% for the purposes of calculating the safe level.

7.4 Basal metabolic demands: the obligatory nitrogen loss

As discussed in section 2, current models of protein requirements assume that the basal metabolic demand for amino acid nitrogen is equivalent to the obligatory nitrogen loss, the loss of nitrogen in urine and faeces by subjects fed adequate amounts of energy and nutrients but with very low or no protein intake. The meta-analysis estimated the magnitude of this in two ways. First, an analysis of published investigations designed to specifically estimate the obligatory nitrogen loss (Table 9), including separate studies in young and older adult men and women, indicated a mean nitrogen balance at near zero nitrogen intake of −47 mg nitrogen/kg per day. Second, the mean intercept corresponding to zero nitrogen intake was then calculated from the regressions of the balance studies at variable nitrogen intakes that were used to estimate the requirement value. This gave a very similar value, −48 mg nitrogen/kg per day. The data in Table 9 indicated no variation with age but a lower value for women than men (−48.8 and −35.4 mg nitrogen/kg per day, respectively, $P = 0.005$), although there were far fewer studies in women.

The similarity of these two estimates of the obligatory nitrogen loss give confidence in the value, demonstrating that basal demands for dietary protein are quite low, equivalent to 0.3 g protein/kg per day. Furthermore this

Table 9
Summary of data for obligatory nitrogen losses in healthy adults

Sex	Age	Number of subjects	Intake	Urine	Faecal	Mean balance	SD balance	Reference
			mg nitrogen/kg per day					
M	Y	15	14.8	44.8	20.2	-59.43	5.7	*20*
F	Y	11	1.8	30.7	7.7	-41.4	6.1	*49*
M	Y	13	1.8	30.9	8.8	-42.7	6.8	*49*
F	Y	25	3	25.2	8.7	-35.7	4.1	*50*
M	Y	13	0	38	14	-55.59	7.6	*51*
M	Y	50	5	33.4	13.1	-52.5	5.3	*52*
M	Y	9	2	33.3	12.7	-52	3.7	*53*
M	Y	9	14.7	34	23	-53.3	6.4	*54*
M	Y	83	11	37.2	8.8	-39.8	6	*55*
F	O	11	10	24.4	9.8	-29	6.3	*56*
M	Y	4	0	34.9	12.6	-58.5	4.2	*57*
M	O	8	0	34.5	12.2	-51.5	11.2	*58*
M	Y	8	6.7	36.2	16.1	-50.4	9.9	*59*
M	Y	8	6	36.6	9	-44.4	3.2	*60*
M	O	6	0.9	27.3	9.5	-40.32	3.4	*30*
Male 12 studies						-50.0	6.6	
Female 3 studies		Sex comparison $P = 0.005$				-35.4	5.6	
Young 12 studies						-48.8	6.0	
Old 3 studies		Age comparison $P = 0.14$				-40.3	7.7	
All 15 studies						-47.1	6.4	

suggests that the relationship between nitrogen balance and nitrogen intake in the submaintenance-to-maintenance range of protein intake is not significantly non-linear, and gives confidence in the analytical model of linear regression.

7.5 Potentially important influential factors identified in the meta-analysis

The overall studies identified by Rand, Pellett & Young (*6*) involved a number of subpopulations which varied in terms of climate, sex, age, and protein source, allowing investigation of the extent of any variability because of these factors. The small size of the consistent subsets, however, prevented an examination of the interactions between these factors. Indeed, most studies included only a single level of the factors evaluated (a single sex or age group, for example) so that "paired" analyses that would control for these factors could not be conducted. Instead, both parametric and non-parametric statistical tests were applied to comparisons of (i) all individual data relevant

to each subpopulation and (ii) the medians of the sub-studies (as defined above). Differences were recognized only where these two approaches produced similar results. This is likely to result in a conservative interpretation of the data. The results are shown in Table 10.

7.5.1 *Dietary source of protein*

Within the primary studies analysed, dietary protein intakes derived from animal (n=64), vegetable (n=77) or mixed (n=94) protein sources. Several of

Table 10
Estimation of nitrogen requirement in healthy adults

Source of data	Factor	Number of points	Median slope	Median intercept[a]	Median requirement[a]
Individuals	All	235	0.47	−48.1	104.6
		95% CI	(0.44, 0.50)	(−51,−45)	(101, 110)
Climate	Temperate	154	0.45	−45.3	102.8
	Tropical	81	0.50	−51.9	113.3
	***P*-value**		**0.20**	**0.011**	**0.047**
Age	Young	221	0.48	−49.4	103.9
	Old	14	0.31	−36.7	130.5
	***P*-value**		**0.003**	**0.025**	**0.401**
Sex	Male	181	0.46	−49.4	109.3
	Female	54	0.47	−43.1	91.4
	***P*-value**		**0.47**	**0.20**	**<0.001**
Diet	Animal	64	.46	−48.8	104.0
	Vegetable	77	.47	−49.4	106.7
	Mixed	94	.48	−46.6	104.2
	***P*-value**		**0.62**	**0.81**	**0.62**
Primary sub-studies	All	32	0.49	−47.1	101.5
		95% CI	(0.42, 0.53)	(−64, −53)	(96,112)
Climate	Temperate	22	0.45	−43.0	100.8
	Tropical	10	0.52	−54.8	111.3
	***P*-value**		**0.10**	**0.020**	**0.27**
Age	Young	30	0.50	−48.9	101.5
	Old	2	0.31	−36.7	110.9
	***P*-value**		**0.12**	**0.23**	**0.97**
Sex	Male	24	0.50	−48.9	101.5
	Female	8	0.46	−42.0	101.8
	***P*-value**		**0.45**	**0.27**	**0.62**
Diet	Animal	9	0.50	−48.1	100.5
	Vegetable	11	0.50	−45.9	103.5
	Mixed	12	0.48	−49.7	101.5
	***P*-value**		**0.88**	**0.83**	**0.72**

[a] Values in mg nitrogen/kg per day.

the experimental diets characterized as vegetable included complementary mixtures of vegetable proteins, such as corn and beans (*33*), and rice and beans (*35*), or good-quality soy protein (*39, 40, 42, 47*). Analysis of these three dietary protein groups indicated no differences in intercept, slope or the requirement in either the individual analyses or the sub-study medians.

7.5.2 *Climate*

A third of reported studies were conducted in the tropics, and the requirement was higher for these studies. However, whereas the difference was marginally significant ($P < 0.047$) for requirements derived from individual data, the analysis of sub-studies did not show significance.

7.5.3 *Age*

A very small subsample (one report on 7 men and 7 women) involved elderly subjects (*45*). For the 14 subjects, both the slope (efficiency of nitrogen utilization) and intercept (basal demands) were significantly lower than for young adults, but the higher median requirement of 131 mg nitrogen/kg per day compared with 104 mg nitrogen/kg per day for young adults was not statistically different. However, on the basis of a comparison of the sub-study medians (n=2), there were no significant differences in either efficiency of nitrogen retention or requirement in older versus younger subjects. Furthermore, in this single report on elderly men and women, energy intakes were very low (1.33 times basal metabolic rate).

7.5.4 *Sex*

Comparison of the 54 females with the 181 males indicated a lower basal demand for females, a similar slope (efficiency of utilization) and a highly significant lower requirement: i.e. 91 mg nitrogen/kg per day compared with 109 mg nitrogen/kg per day for males. However, the closeness of the 95% CIs for these estimates, (84.7, 103.5 for females and 103.6, 113.5 for males), suggests that caution is needed in interpreting the results, especially as analysis of the 8 female and 24 male sub-study medians indicates near-identical values for the requirement.

7.6 Comparison with previous reports and other information

The previous report (*8*) relied on a limited number of short-term and longer-term nitrogen balance studies to derive the protein requirement of adults. Some of these were designed to identify a requirement and others to test the safe level (0.58 g protein/kg per day) identified in the 1973 report. Taken together they were interpreted as indicating a mean requirement of 0.6 g protein/kg per day, with a coefficient of variation estimated to be 12.5%, after

examining the total variation of the available short-term balances. This resulted in a safe protein intake of 0.75 g/kg per day, i.e. a value at 2SD above the average requirement (0.6 g protein/kg per day) which would provide for the needs of nearly all individuals (97.5%) within a target population.

7.6.1 *Dietary source of protein*

The 1985 report based its estimates of the adult requirement value on only those available balance studies conducted with high-quality protein, since few of the studies with plant proteins included in the recent meta-analysis had been published. Furthermore, in discussing likely influences on the protein requirement, although digestibility was identified as an important factor that might increase the protein requirement with some plant-based diets, biological value was discussed mainly in terms of amino acid scoring. Thus, given the marked fall with age in amino acid requirements assumed in that report, the values identified for the adult amino acid requirements resulted in an adult scoring pattern which identified the dietary amino acid pattern of all likely diets to be adequate. The revised, somewhat higher, amino acid requirement values for adults discussed in section 8, and the suggested scoring pattern, mean that this conclusion needs to be re-evaluated, although on the basis of the new scoring pattern, mixtures of cereal proteins with relatively modest amounts of legumes or oil seeds, or animal proteins are unlikely to be limited through their amino acid content.

Although a lower efficiency of utilization of individual plant compared with animal protein sources has been reported in individual nitrogen or [^{13}C]leucine balance comparisons (e.g. for wheat gluten compared with beef (*61*), egg (*62*) or milk (*63, 64*), or for rice compared with egg (*53*), or for lupin compared with egg proteins (*36*)), the differences are often less than the differences seen between studies with the same protein (see *65*). Indeed, in contrast to the ease with which differences in the biological value of proteins in relation to the amino acid content and chemical score can be demonstrated in laboratory animals, in humans it is extraordinarily difficult. This is because of a lack of reproducibility between studies with the same protein, and high inter-individual variability of biological value within individual studies, which can be up to 50% (*47*). Thus analysis of the variability observed in the balance trials done at one experienced centre (*66*) showed that the biological value would have to differ by more than 50% before significant differences could be demonstrated with realistic numbers of subjects. In the meta-analysis, this large between-study variability was apparent in the "test" studies. This indicates that protein utilization in humans may be dependent on complex extrinsic factors that influence the behaviour of the organism but that have not been captured in the short-term nitrogen balance

studies, as well as by the intrinsic properties of the protein, such as amino acid content.

7.6.2 *Protein requirement of elderly people*

A frequently discussed area of concern in relation to protein nutrition in elderly people is sarcopenia, the age-related loss of skeletal muscle mass and consequent fall in muscle strength (see *67*). In fact, the main determinant of sarcopenia appears to be the decline in resistance-type physical activities, with no evidence yet identified for any nutritional component. Furthermore, recent detailed balance and body composition studies have shown that with a suitable programme of resistance exercise sarcopenia can be reversed and muscle strength increased on a protein intake of 0.8 g/kg per day (*68*). This intake is similar to the 1985 safe allowance and lower than usual intakes in this population.

Whether variation in protein intake towards marginal levels is detrimental in the elderly population consuming self-selected diets was examined in two important studies (*69, 70*), neither of which could identify any such relationship (see *12*). This suggests that free-living elderly individuals are able to adapt to protein intakes over a wide range, with no benefit from increased intakes in terms of either biochemical indicators of protein sufficiency or measured balance.

In the previous report (*8*), four nitrogen balance studies in elderly people were reviewed: the one such study included in the meta-analysis, and three others. Taken together these studies were inconsistent and the report concluded that the safe intake of protein should not be lower than 0.75 g/kg per day for older adults and the elderly. Two studies have specifically addressed the question of the extent of any age-related changes in protein requirements, with studies on both younger and older subjects (*71, 72*). No differences were identified and the study design does not allow a requirement value to be predicted with any confidence. A 30-day balance study aimed to test the adequacy of the safe allowance (0.8 g/kg, *73*) showed zero nitrogen balances for the group as a whole, even though the study involved energy intakes that may well have been inadequate. Thus none of these published studies provides convincing evidence that protein requirement of elderly people differs from the protein requirement for younger adults.

The interpretation of nitrogen balance data is especially difficult in the case of elderly people, not least because there are more constraints on experimental design than with studies on younger subjects. Furthermore, some have re-analysed or aggregated the available nitrogen balance data in such a way that protein requirements would appear to be higher in elderly people (*74, 75*). However, on the basis of a rigorous reassessment of all available data, it was

concluded that there was no change with age in the protein requirement per kg body weight and that no studies unequivocally demonstrate that the protein requirement would be higher than the safe allowance defined in the previous report (*8*).

In the previous report it was stated that "it is an accepted fact that protein utilization is less efficient in the elderly". The evidence for this is hard to identify. Some authors have suggested an apparently higher first-pass splanchnic extraction of dietary leucine (*76*) and phenylalanine (*77*) in healthy elderly subjects, as compared with younger adults, although in one of these studies (*76*) this was observed only in subjects with a markedly higher body mass index, suggesting an influence of body composition on tracer kinetics. Other [1-^{13}C]leucine balance studies reached different conclusions. Thus measurements of metabolic demand, efficiency of utilization, and apparent protein requirements (metabolic demand/efficiency of utilization) in a group of elderly and young men and women, showed that, whereas the demand was lower in both elderly men and women, efficiency of utilization was unchanged (*67, 78*). The apparent requirement was lower in the elderly people on a body weight and fat-free mass basis, with a significant inverse correlation between age and apparent protein requirements. While such studies indicate metabolic responses to protein intake in energy balance only in a clinical setting, i.e. fed at subjects' habitual protein intake with a specific feeding mode of frequent small meals of highly digestible protein (milk-based), any physiological changes with age in the metabolic demand or in the efficiency of protein utilization under laboratory conditions should be revealed. Thus, these data lend confidence to the overall conclusion drawn from the nitrogen balance data that the physiological protein requirement does not increase with age.

Nevertheless, it should be noted that for any diet in which protein intake is likely to be limiting, sedentary elderly people are the population group most at risk from protein deficiency (see section 5). Thus assuming that protein requirements per kg do not change with age during adult life, the low energy requirement of sedentary elderly people means that the protein:energy ratio of their requirement is higher than for younger age groups. Since this will also be true for their requirement for many other nutrients, it is clear that the most appropriate response to this is to encourage increased activity, energy expenditure and consequent increased food intake. In this way, the needs for protein and other nutrients are more likely to be supplied by any given diet. Clearly, factors such as infection, trauma and disease, which tend to reduce food intake and which are more common in elderly than in young people, also require particular vigilance.

7.6.3 *Protein requirement of women*

The 1981 Consultation (*8*) concluded from studies of obligatory nitrogen losses and short-term nitrogen balance studies that there was no firm evidence to suggest that a distinction could be made between adult males and females when setting the safe protein intake, which was thus set at 0.75 g/kg per day for both sexes. Clearly, the sex differences in body composition, generally higher fat and lower lean content of women compared with men, might be expected to result in a lower requirement per kg in women in line with their lower basal metabolic rate. The Schofield equations predict that in adult women the basal metabolic rate is 7–15% lower than in men according to age and weight, and this is in line with the lower protein requirement indicated by the meta-analysis of individuals. However, given the overall variability of the database, the small difference likely to occur and the lack of any adverse effect of any overestimation, in practical terms it is probably better to maintain similar values for adult men and women. It should be recognized, though, that maintaining a requirements model in which there are no sex differences for protein, but lower requirements for energy in women than in men, means that the protein:energy ratio of the requirement for women is higher than for men. Thus for the population group most vulnerable to marginal protein intakes, elderly people, women will appear to be more vulnerable than men. However, such higher vulnerability may be more apparent than real.

7.7 Protein requirements during pregnancy

Adaptations in protein metabolism appear to occur in anticipation of maternal and fetal needs (*79*). A decrease in total plasma α-amino nitrogen, a lower rate of urea synthesis, a lower rate of branched-chained amino acid transamination, and constant rate of weight-specific protein turnover are seen during pregnancy. These adaptive changes are aimed at conservation of nitrogen and increased protein synthesis. Protein synthesis estimated using [^{13}C]leucine or [^{15}N]glycine demonstrated 1%, 15% and 25% increases in protein synthesis (g nitrogen/day) in the first, second and third trimesters, respectively (*80*). Relative to body weight, there was no progressive change in protein synthesis, but relative to lean body mass there was a significant increase as pregnancy advanced (*81*). Kinetic studies of leucine metabolism showed no significant change in leucine carbon turnover, but lower rates of leucine nitrogen turnover, suggesting lower rates of leucine transamination (*79*). A significant reduction in urea synthesis has been shown to occur in the first trimester and to be sustained throughout pregnancy, promoting nitrogen retention (*82*). Based on [$^{15}N_2$]urea tracer-dilution method, a 30% decrease in urea synthesis was observed in healthy and diabetic women during the third trimester of pregnancy (*83*). The decreased concentration of plasma α-amino

nitrogen in blood seen in pregnant women has been attributed to the lower rate of urea synthesis. During the second half of pregnancy, insulin secretion increases and insulin resistance develops, at a time when fetal protein synthesis increases markedly. The relative insulin resistance is associated with higher levels of circulating glucose that potentially can spare amino acids for protein synthesis in the fetal compartment.

The 1985 Consultation (*8*) assessed protein needs on a calculated increment of 925 g protein, i.e. the average gain, plus 30% (2SD of birth weight), to cover the protein gains during pregnancy of nearly all normal women, after adjusting for an efficiency of 70% for the conversion of dietary protein to fetal, placental, and maternal tissues. This gave safe levels of additional protein of 1.2, 6.1 and 10.7 g/day in the first, second and third trimesters, respectively, but an average of 6 g/day to be added to the non-pregnant allowance throughout pregnancy was selected on the basis that more protein may be deposited early and somewhat less very late in pregnancy.

For the present report two approaches have been taken to estimate the protein requirements of pregnant women. First, as for the 1985 report (*8*), a factorial approach sums the components of protein (nitrogen) gain during pregnancy, and then the amount of dietary protein needed to meet the incremental need is estimated. Second, the dietary protein requirement can be estimated from nitrogen balance studies.

7.7.1 *Factorial approach*

The deposition of protein in the fetus and maternal tissues was first determined on a theoretical basis. Protein is deposited predominantly in the fetus (42%), but also in the uterus (17%), blood (14%), placenta (10%), and breasts (8%) (*84, 85*). Protein is deposited unequally across pregnancy, predominantly in late pregnancy. Hytten & Chamberlain (*85*) estimated that 925 g protein are deposited in association with a 12.5 kg gestational weight gain (GWG) (Table 11). Protein deposition was estimated to be 36 g, 165 g and 498 g for first, second and third trimesters, respectively.

Second, protein deposition has been estimated indirectly from measurements of total body potassium accretion, measured by whole body counting in a number of studies of pregnant women (Table 12). The study design (cross-sectional or longitudinal), stage of pregnancy and type of whole-body counter differed across studies. MacGillivray & Buchanan (*86*) studied eight women in early pregnancy and another 16 in late pregnancy: since the same women were not studied repeatedly, the increase in total body potassium is questionable. The results of Emerson, Poindexter & Kothari (*87*), based on a sample size of five women, are questionable: the potassium per kilogram gained was high, and total body potassium did not decline in the postpartum period

in three of the subjects. King, Calloway & Margen (*88*) observed a rate of 24 meq/week between 26 and 40 weeks of gestation. Pipe et al. (*89*) found a 312 meq increase in potassium. Lower increments, of 110 and 187 meq at 36 weeks, were found over pre-pregnancy values (*90, 91*). Based on a

Table 11
Deposition of protein in the fetus and maternal tissues during pregnancy

Protein deposition (g)	Weeks of pregnancy			
	10	20	30	40
Fetus	0.3	27	160	440
Placenta	2	16	60	100
Amniotic fluid	0	0.5	2	3
Uterus	24	55	102	166
Breasts	9	36	72	81
Blood	0	30	102	135
Total[a]	35	165	498	925

[a]Values rounded up.

Table 12
Increment in total body protein estimated from changes in total body potassium (TBK) of well-nourished women during pregnancy

Number of individuals studied	Study interval (weeks of pregnancy)	TBK measurement	Increase in TBK (meq)	TBK (meq/day)	TBK (meq/kg gained)	Increment in protein (g)	Reference
8	11.2–37.3	1952 meq	589	3.22	42.1	1712	*86*
16	cross-sectional	2541 meq					
10	26–40	24 meq/week	336	3.41	44.3	977	*87*
	longitudinal						
5	20, 24, 28, 32, 35	2712 meq	480	3.43	86.5	1395	*88*
	longitudinal	3192 meq					
27	10–14, 24–28, 36–38	2442 meq 2754 meq	312	1.78	30.0	907	*89*
	longitudinal						
22	0–36	2397 meq	110	0.44	9.4	320	*90*
	longitudinal	2507 meq					
34	0–36	2604 meq	187	0.79	12.8	544	*91*
	longitudinal	2770 meq					

TBK, total body potassium.

potassium:nitrogen ratio in fetal tissues of 2.15 meq/g nitrogen, total protein deposition estimated from four longitudinal studies (*87, 89–91*) was 686 g. However, protein is not deposited equally throughout pregnancy. In well-nourished women with a mean gestational weight gain of 13.8 kg, total protein deposition (686 g) was distributed as 1.9 g/day in the second trimester and 7.4 g/day in the third trimester, based on the distribution observed in women studied before, during and after pregnancy (*91*). Interestingly, total body potassium and total body nitrogen measured by prompt-gamma neutron activation did not differ significantly before and after pregnancy, indicating no net accretion of protein during pregnancy. The mean gestational weight gain found in a WHO collaborative study on maternal anthropometry and pregnancy outcomes was 12.0 kg (*92*). If protein deposition were proportional to gestational weight gain, total protein deposition would be 597 g, distributed as 1.6 g/day and 6.5 g/day in the second and third trimesters, respectively.

7.7.2 *Nitrogen balance*

Nitrogen balance studies during pregnancy, in which protein intake was not the intentional variable in the study design, were compiled by Calloway (*93*). A total of 273 metabolic balances were determined the majority of which were from women at or beyond 20 weeks of gestation. The average nitrogen retention was 1.8 g/day from 20 weeks onward, and 1.3 g/day before 20 weeks. Miscellaneous nitrogen losses were unaccounted for in these studies, but were estimated to be 0.5 g/day. With or without correction for miscellaneous nitrogen losses, the nitrogen retention calculated from the balance studies was appreciably greater than the theoretical nitrogen deposition (Table 13). As a result, the efficiency of nitrogen utilization estimated from these studies was very low. For balances beyond 20 weeks of gestation, the value was 26%, compared with 47% in non-pregnant women (section 7.6).

Table 13
Nitrogen deposition during pregnancy[a]

	Theoretical nitrogen deposition (g/day)	Observed nitrogen retention (g/day)	Observed nitrogen retention, corrected for miscellaneous nitrogen losses (g/day)
0–10 weeks	0.10		
10–20 weeks	0.29	1.20 (*n*=39)	0.70
20–30 weeks	0.77	1.75 (*n*=119)	1.25
30–40 weeks	0.98	1.85 (*n*=184)	1.35
Mean	0.53	1.60	1.10

[a]From references *85, 94*.

A somewhat higher efficiency value of 42% (after omitting the two subjects who gave negative gradients) was obtained in nitrogen balance studies performed in 10 primiparous teenagers during the last 100 days of pregnancy (*87*). This is similar to the value derived from non-pregnant adults (47%; see section 7.6). Hence 42% has been employed in the factorial calculation of the protein requirement during pregnancy.

7.7.3 *Recommendations for protein intake during pregnancy*

The additional protein intake needed during pregnancy was derived from the newly deposited protein and the maintenance costs associated with increased body weight (Table 14). Mean protein deposition has been estimated from total body potassium (TBK) accretion in normal healthy pregnant women, gaining 13.8 kg. The efficiency of protein utilization was taken to be 42%. The maintenance costs were based upon the mid-trimester increase in maternal body weight and the adult maintenance value of 0.66 g/kg per day. The safe level was derived from the average requirement, assuming a coefficient of variation of 12%.

- Based on an efficiency of protein utilization of 42%, an additional 1, 9 and 31 g/day protein in the first, second and third trimesters, respectively, are required to support 13.8 kg gestational weight gain.
- In view of the literature indicating a controversial increase in neonatal death with supplements that are very high in protein (34% protein:energy; see below), it is recommended that the higher intake during pregnancy should consist of normal food, rather than commercially prepared high-protein supplements

Table 14
Recommended additional protein intake during pregnancy

Trimester	Mid-trimester weight gain (kg)	Additional protein maintenance (g/day)[a]	Protein deposition (g/day)	Protein deposition, adjusted efficiency (g/day)[b]	Additional protein requirement (g/day)	Additional safe intake (g/day)[c]
1	0.8	0.5	0.0	0.0	0.5	0.7
2	4.8	3.2	1.9	4.5	7.7	9.6
3	11.0	7.3	7.4	17.7	24.9	31.2

[a] Mid-trimester increase in weight x estimated average requirement (EAR) for maintenance of protein for adults 0.66 g/kg per day.
[b]Protein deposition adjusted for the efficiency of protein utilization during pregnancy: 42%.
[c]Safe intake, calculated as the average requirement plus allowance for estimated coefficient of variation of 12%.

7.7.4 *Diet interventions during pregnancy*

According to the 2001 Cochrane Library's Pregnancy and Childbirth Database, balanced protein and energy maternal supplements were the only intervention that improved birth weight (*94*). The balanced supplements provided less than 25% of their energy as protein (no minimum was defined). The 14 supplementation trials included in the Cochrane review demonstrated a modest increase in maternal weight gain, a small but significant increase in birth weight, and a nonsignificant increase in birth length and head circumference. Overall there was a 32% reduction in risk of the fetus being small for gestational age, a 21 g/gestational week increase in maternal weight, and a 32 g increase in birth weight. In a recent trial in the Gambia, biscuits providing 1017 kcal and 22 g protein were given to pregnant women starting at 20–24 weeks of gestation (*95*). Remarkable improvements in birth weight were seen in both the dry season (+136 g birth weight and a 39% reduction in low body weight) and the rainy season (+201 g birth weight and a 42% reduction in low body weight). These benefits of supplementation are consistent with dietary assessments in unsupplemented mothers, showing that those who had the highest protein intakes (around 100 g protein/day) had the best pregnancy outcome (*96*). However, as reviewed recently (*97*), untoward effects on pregnancy outcome were reported with high-protein supplements (providing >34% of energy) in studies conducted in New York (*98*) and India (*99*). The high-protein supplement (470 kcal and 40 g protein/day) in the New York trial was associated with a small, non-significantly higher weight gain, and a higher, non-significant increase in neonatal death, and no difference in fetal growth.

7.7.5 *Twin pregnancy*

It is reasonable to suppose that women supporting the growth of two fetuses (twins) have higher protein needs than women having singleton births. Indeed, there are data from the Montreal Diet Dispensary (*100*) showing that application of the Dispensary's assessment and treatment programme to women with twins improves pregnancy outcome (*101*), decreasing low birth weight rate by 25% ($P<0.05$) and very low birth weight by 50%. The nutritional intervention also reduced preterm delivery by 30%. Target protein intakes were 50 g extra from the 20th week and 1000 additional kcal, double the pregnancy allowance for women with singleton pregnancies. Unfortunately, this study did not measure how much protein (or energy) that a woman bearing twins actually took, but these women did gain 2 kg more than the controls.

7.7.6 *Adolescent pregnancy*

It is well established that both the mother's pre-pregnant weight and her weight gain during pregnancy are correlated with birth weight of the infant

(*101*, *102*). The problem with adolescent pregnancy is compounded by whether the mother has completed her growth or not (*103*, *104*). In those who have not completed their growth, it appears that there is competition between maternal and fetal growth (*103*, *105*, *106*).

The Montreal Diet Dispensary has also studied the effect of its programme on 1203 pregnant adolescents compared with 1203 controls. The programme significantly increased mean birth weight and reduced low birth weight rate by 39% ($P < 0.001$). Overall protein intake in adolescent females taking part in the programme was 96 g/day (*107*).

Nitrogen balance data on 15–19-year-old pregnant adolescents (*87*) indicated that their pregnancy protein requirement was 1.5 g/kg per day pregnant weight. A pre-pregnant weight of 55 kg and an average weight gain of 12.5 kg would indicate a protein intake at term of 101 g/day, which is very similar to the estimate based on the Montreal Diet Dispensary data (*107*).

7.8 Protein requirements during lactation

A factorial approach was taken to derive the protein requirements during lactation. Mean production rates of milk produced by well-nourished women exclusively breastfeeding their infants during the first 6 months postpartum and partially breastfeeding in the second 6 months postpartum (*108*) were used together with the mean concentrations of protein and non-protein nitrogen in human milk (*109*) to calculate mean equivalent milk protein output. Human milk contains a relatively high concentration of non-protein nitrogen, of the order of 20–27% of total milk nitrogen, much of this being in urea. Whether this merely reflects a diversion of urea excretion from urine to milk or deliberate secretion is not known, but diversion from the urine seems more likely, as urea is an abundant end-product of metabolism. Thus, for the calculations below it was assumed that the increased nitrogen needs of the lactating woman should cover protein nitrogen, but not the non-protein nitrogen. The factor 6.25 was used to convert protein nitrogen to protein equivalents.

The efficiency of protein utilization for milk protein production is unknown. The efficiency associated with the production of milk protein was taken to be the same as for protein deposition in the non-lactating adult (47%). Thus, the additional dietary protein requirement during lactation will be an amount of digestible protein equal to milk protein, divided by an efficiency of 0.47. The safe protein intake was calculated as mean + 1.96SD with 1SD calculated on the basis of a coefficient of variation of 12%. As shown in Table 15, the additional safe protein intakes during the first 6 months of lactation ranged from 19 to 20 g protein/day with an average value of 19 g protein/day, falling to 12.5 g protein/day after 6 months.

7.9 Areas of uncertainty

There are several difficulties associated with identification of population average protein requirements and reference intakes which result from the analysis of the available information, all of which raise questions of the usefulness of the traditional approach to estimating protein requirements.

One important issue relates to the large variability in protein requirements, as derived from nitrogen balance studies. This variability arises both within and between studies. Thus, the variance identified in the meta-analysis (*6*) as true between-individual variance, equivalent to an SD of about 12%, was only about 20% of the overall variance, with the upper limits of the overall range of published requirement values equivalent to a value of about 1.1 g protein/kg per day. While the methods available to disentangle the different sources of variability were limited given the available information, an understanding of the nature of this variation is clearly important in the derivation of population reference requirement values.

Achievement of nitrogen equilibrium is dependent on several factors that may be difficult to control, of which energy balance is particularly influential. Thus, if energy requirements are underestimated, protein requirements will be overestimated, and vice versa (see section 5). An analysis of most published data of nitrogen balance studies in adults where protein and energy intakes were varied indicated a relationship between energy intake and nitrogen balance in which there was a gain of about 1 mg nitrogen/kg per day for an extra 1 kcal/kg per day of intake (*110*). Thus for a moderately active young adult male with an estimated energy requirement of 45 kcal/kg per day (i.e. 1.8 × predicted basal metabolic rate), the likely error of ±10% in estimating basal metabolic rate and consequent energy needs, i.e. 4.5 kcal/kg per day, would account for a variability in nitrogen balance of ±4.5 mg nitrogen/kg per day, equivalent to a variability in requirement of 9 mg/kg per day (with a nitrogen balance versus nitrogen intake slope of about 0.5). With the total variability reported in the meta-analysis of nitrogen balance estimations of the protein requirement equivalent to a standard deviation of 27.5 mg nitrogen/kg per day, or the estimated true between-individual variance equivalent to a standard deviation of about 12 mg nitrogen/kg per day, then >80% of between-individual variability could reflect a lack of energy balance. While an overestimate of 4.5 kcal/kg per day could result in 0.25–0.5 kg of weight gain per week for a 70-kg adult, this might be considered within the normal range in a short-term study.

In addition to the extent of energy balance per se, there may be an effect of energy turnover in relation to physical activity levels on protein metabolism (*111–113*) and, therefore, on dietary nitrogen utilization and body nitrogen balance. This possible effect may depend on the physical condition and state

Table 15
Additional protein requirements during lactation

Months postpartum	Milk intake (g/day)[a]	Milk intake (g/day)[b]	Protein concentration (g/litre)[c]	Non-protein nitrogen (protein equivalent) (g/litre)[c]	True protein secreted (g/day)	Non-protein nitrogen (protein equivalent) (g/day)	Requirement: (efficiency of milk protein synthesis applied to true protein[d]) (g/day)	Safe level[e] (g/day)
1	699	734	10.4	3.13	7.60	2.30	16.2	20.2
2	731	768	9.6	2.83	7.33	2.17	15.6	19.5
3	751	789	8.8	2.38	6.97	1.88	14.8	18.5
4	780	819	8.2	2.21	6.73	1.81	14.3	17.9
5	796	836	8.1	2.11	6.79	1.76	14.4	18.1
6	854	897	8.1	2.11	7.29	1.89	15.5	19.4
6–12	550	578	8.1	2.11	4.69	1.22	10.0	12.5

[a] From reference *109*.
[b] Milk intake corrected for insensible water losses during test-weighing measurement (5%).
[c] Protein = nitrogen × 6.25.
[d] Efficiency of milk protein synthesis = 47%.
[e] Coefficient of variation of 12%.

of training of the individual (*114*) and this could influence the status of nitrogen balance in any given experiment as well as the variability within and among experiments which were not controlled for these effects.

A third potential source of variability relates to the short-term (<2 weeks) duration of most of the nitrogen balance studies included in the meta-analysis, and the potential for incomplete adaptation to the sub-maintenance intake levels fed in the studies. Rand, Scrimshaw & Young argued against this possibility on the basis of their own analysis of the results of long-term balance studies (*115*, *116*), which contrasted with long-term balances at low protein intakes, discussed in the 1985 report on the basis of evidence of adaptive mechanisms previously proposed by Sukhatme & Margen (*117*). However, incomplete adaptation could account for the low and very variable apparent efficiency of utilization observed in the published balance studies. As indicated here, the average efficiency of nitrogen utilization for retention is slightly less than 50% in healthy adults, with no differences in relation to diet. Clearly this is inconsistent with a model of protein utilization in which only digestibility and biological value influence protein utilization. Rand, Pellett & Young (*6*) point out that the choice of linear regression used to interpolate for individual nitrogen equilibrium and consequent requirement, while of practical necessity given the usually limited number of individual data points, may not be the most biologically realistic actual response. It has been suggested that the response curve might be artefactually low, indicating a low apparent efficiency of protein utilization, as a consequence of incomplete adaptation (*118*). Thus, if part of nitrogen excretion involves losses associated with an adaptive component of the metabolic demand, this would imply a higher efficiency of protein utilization than that calculated assuming demand to be basal (i.e. the intercept) at all intakes, as in the traditional model. This is evident when efficiency is measured as postprandial utilization (*119*), which takes the adaptive component into account, indicating near-perfect utilization for milk proteins (*63*, *64*, *67*). In contrast, as pointed out by Baker (*120*), studies in growing animals have shown linear responses of retention of single indispensable amino acids in carcass protein, over a wide range of intakes from near zero to 80–90% of requirement, although the efficiency of utilization is low (38–75%) and different for each amino acid. Equivalent studies have not been performed in humans.

7.10 Summary of protein requirement values for adults, including women during pregnancy and lactation

The requirement indicated by the meta-analysis (*6*) (a median requirement of 105 mg nitrogen/kg per day or 0.66 g/kg per day of protein) can be accepted as the best estimate of a population *average requirement* for healthy adults. Although there is considerable uncertainty about the true between-individual

variability, the *safe level* was identified as the 97.5th percentile of the population distribution of requirement, i.e. 133 mg nitrogen/kg per day, or 0.83 g/kg per day protein. Thus 0.83 g/kg per day protein would be expected to meet the requirements of most (97.5%) of the healthy adult population. Because the distribution of requirements was log-normal, and thus skewed, direct calculation of a standard deviation was not possible. However, an approximate value was derived as half the difference between the estimated 16th and 84th percentiles (which would contain those individuals within one standard deviation of the mean for a normal distribution), yielding an apparent coefficient of variation of about 12%. This value was employed in the calculations of safe levels for protein and amino acids of children and adults when direct experimental evidence for their values was not available (sections 9 and 10).

These values for average and safe intakes are about 10% higher than the value of 0.6 g proposed in the 1985 FAO/WHO/UNU report (*8*). While there are important questions about food intakes for older individuals, there is at present no firm evidence warranting different values for this population group. Similarly, there is as yet no justification for any differentiation between males and females. However, additional protein is recommended for pregnant women of 1, 9 and 31 g protein/day in the first, second and third trimesters, respectively, or additional food energy with a protein:energy ratio of 0.03, 0.12 and 0.23. For lactating women, an average of 19 g protein/day is required, falling to 12.5 g protein/day after 6 months.

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8. Amino acid requirements of adults

The requirements estimates in the 1985 FAO/WHO/UNU (*1*) report were taken directly from the 1973 FAO/WHO report (*2*). These were based on the nitrogen balance studies by Rose (*3*) in men, on similar studies by various investigators in women as summarized by Irwin & Hegsted (*4*), and on both sets of data having been re-analysed by regression of $\log_e$ intakes on balance by Hegsted (*5*). As discussed in section 4, since 1985 concerns have been expressed about the derived values, and all now agree that they were certainly too low. While the chosen requirement estimates in the 1973/1985 reports were conservative, being based on the highest values available (in each case, the values from Rose, *3*), all reviewers of these studies (e.g. *6*–*8*) have concluded that they are unsatisfactory for a variety of reasons, especially in the studies reported by Rose. The most serious problem in all studies was that no allowance was made for miscellaneous losses, so the values selected in the 1973/1985 reports were certainly too low. A set of higher values calculated with a realistic value for the miscellaneous losses have been reported by Fuller & Garlick (*7*) and by Millward (*8*). Also Rand & Young (*9*) have re-analysed one set of values relating to the lysine requirements. These recalculated higher values represent the best estimates from nitrogen balance studies.

These nitrogen balance estimates are, however, in many cases lower than newer estimates made in stable isotope studies. As argued in section 4, these stable isotope methods involve a number of assumptions in their interpretation and there is, as yet, no complete consensus as to their relative merits. Those studies which involve prior dietary adaptation are logistically difficult and laborious, so only a limited number of intakes can be studied. Thus the estimates obtained are approximate and probably represent conservative overestimates, rather than underestimates, of the true values. Those stable isotope approaches that have not involved prior dietary adaptation have covered a wide range of intakes, but concern has been expressed about the interpretation of the results of such studies. In some cases, stable isotope approaches have been used to estimate the lysine requirement from measurements of the relative utilization of wheat compared with milk protein. The values obtained in these studies nevertheless depend on several assumptions and require cautious interpretation.

Among the stable isotope studies, the Consultation agreed that the most reliable approach involved measurements over an entire 24-hour period representative of a normal day with ^{13}C tracers which can be reliably interpreted in terms of calculation of oxidation rates after some adaptation to the intakes. Recognizing that this limits the database to [^{13}C]leucine studies of lysine, leucine and threonine, all other published studies were considered as sources of variable quality which need to be interpreted cautiously, and judgments were made on final values for recommendations. All of the methods are based on a physiological response to graded intakes of the test amino acid. To best define the requirement level of the test amino acid, a range of intakes must be used, but the logistics of conducting 24-hour tracer balance studies means that in many cases fewer intakes were tested than would have been desirable.

Theoretical predictions of the requirement pattern have also been published based on the obligatory oxidative losses, i.e. the pattern of tissue protein and the magnitude of the obligatory nitrogen losses, although as discussed in section 4, the theoretical basis of such predictions has been questioned, and this approach has not received general support. All agree, however, that these values are a likely guide to the magnitude of one rate-limiting amino acid which determines the magnitude of the obligatory oxidative losses – probably methionine.

Finally, it must be recognized that these new values have not been validated in any entirely satisfactory way, i.e. in long-term studies at the requirement intakes with measurement of body weight, body composition and well-being. Indeed such studies, based on nutritionally complete real food containing the requirement pattern and adequate dispensable nitrogen, would be difficult to design. Only in the case of lysine have attempts been made with studies of the nutritional adequacy of wheat-based diets, which are generally agreed to be lysine-limited. However, while these studies provide useful information on the adequacy of one intake level, they do not enable a requirement intake to be defined.

8.1 Requirements for indispensable amino acids

The indispensable amino acids are leucine, isoleucine, valine, lysine, threonine, tryptophan, methionine, phenylalanine and histidine. Histidine is considered to be an indispensable amino acid because of the detrimental effects on haemoglobin concentrations that have been observed (*10*) when individuals are fed histidine-free diets.

8.1.1 *Lysine*

The requirement for lysine has received most attention given its nutritional importance as the likely limiting amino acid in cereals, especially wheat.

Reported values are shown in Table 16. The Consultation's estimate of the requirement for lysine (30 mg/kg per day) is derived from tracer studies using the 24-hour indicator amino acid method (*11, 12*), which are considered to provide the best stable isotope data currently available. However, even with these studies the possibility of a value intermediate between 22 mg and 30 mg cannot be ruled out, in line with the most recent of these studies. Intakes of either 30 mg/kg per day or 45 mg/kg per day maintained similar, slightly positive [^{13}C]leucine balances in Indian subjects with low body mass index fed their habitual diet after treatment for intestinal parasites (*13*). Studies with the indicator amino acid oxidation method, with [^{13}C]phenylalanine in the fed state only and without prior adaptation to the diet (*10, 14–16*), have generally indicated higher values (35–45 mg/kg per day) for reasons that are not understood. Two of these latter studies (*10, 16*) allow a comparison between females and males using the same tracer model. Also, [^{13}C]lysine oxidation studies in the fed state only or over 24 hours have yielded values of 20–30 mg/kg per day (*17–19*).

The 1973/1985 FAO/WHO values for lysine derived from a value of 800 mg/day (*3*) expressed as 12 mg/kg per day, the highest value of a number of studies on men and women (*20–22*). The most extensive single nitrogen balance study is that on young women (*21*), although even in this study intakes are either below (<13 mg/kg per day) or above (>23 mg/kg per day) the predicted requirement value. Recalculation after curve fitting indicates values ranging from 13 mg/kg per day to 36 mg/kg per day according to the fitting model and value chosen for miscellaneous nitrogen loss (*5, 7–8*). The best estimate from these re-analyses of this nitrogen balance study is about 17 mg/kg per day (95% CI =14–27 mg/kg per day) based on the current estimate of miscellaneous loss, 5 mg/kg per day (*23*).

Indirect estimates of the lysine requirement have been reported from [^{13}C] leucine balance studies in normal adults, which measure leucine retention from wheat and the efficiency of wheat protein utilization, i.e. postprandial protein utilization. The most secure value, i.e. 23 mg/kg per day, derives from assessment during a small repeated meal protocol at two levels of protein intake, where postprandial protein utilization should reflect only protein utilization (*24*). A slightly lower value, 18 mg/kg per day, has been reported from calculations of postprandial protein utilization using a large single meal protocol, where the model assumptions are different. This is less secure, since postprandial protein utilization is a measure of the utilization of the whole meal, with leucine balance influenced by energy intake as well as protein (*25*).

Finally, two long-term nitrogen-balance studies in male college students fed wheat-based diets provide support for a value between 17 mg/kg per day and

Table 16
Lysine requirement measured using different approaches

Type of study	Lysine requirement (mg/kg per day)	Reference
Nitrogen balance	12	*1–3*
	17	*5, 7–9*
[^{13}C]leucine oxidation studies		
24-hour multi-level lysine intakes	29	*11*
24-hour multi-level lysine intakes 21-day adaptation	31	*12*
[^{13}C]lysine oxidation studies		
Fed state only, multi-level lysine intakes	>20 <30	*17*
24-hour multi-level lysine intakes	30	*18, 19*
[^{13}C]phenylalanine oxidation studies		
Fed state only, multi-level lysine intake	37 45 37 35	*14, 15, 10, 16*
[^{13}C]leucine oxidation studies		
Leucine retention from wheat (postprandial protein utilization)	18–23	*24, 25*
Recommendation	**30**	

30 mg/kg per day. In these studies, diets providing either 0.94 g protein/kg per day (*26*) or 0.51 to 0.73 g protein/kg per day (*27*) showed maintenance of nitrogen balance, body weight and fitness with lysine intakes over 2 months of 18 mg/kg per day, or 20–30 mg lysine/kg per day. Although the authors of each of these reports state that the subjects were in energy balance, concern has been expressed over whether the high energy intakes associated with high levels of physical activity could have influenced the outcome (*9*).

8.1.2 *Leucine*

Leucine is the most abundant amino acid in tissue and food proteins but specific demands for non-protein functions have not been identified. Reported requirements are shown in Table 17. The Consultation's estimate of the requirement for leucine (39 mg/kg per day) derives from 24-hour [^{13}C]leucine balance studies which indicated leucine requirements of 37 mg/kg per day and 40 mg/kg per day (*28, 29*). A limitation of these studies is that the highest test dose of leucine fed was 40 mg/kg per day, and thus did not exclude the possibility that the requirement was higher than 40 mg/kg per day. Conversely, others have argued that these values may be overestimates because of the way the tracer and meal leucine intakes were distributed between the fed and fasted state, although a study to investigate this possibility did not

Table 17
Leucine requirement measured using different approaches

Type of study	Leucine requirement (mg/kg per day)	Reference
Nitrogen balance	14	*1, 2, 3*
Re-evaluation of nitrogen balance	26	*5, 7, 8*
[^{13}C]leucine oxidation studies		
24-hour multi-level leucine intakes	40	*31*
	37.3	*28*
	39.6	*29*
Fed state only, multi-level leucine intakes	20<40	*33*
Recommendation	**39**	

identify any design problem (*30*) and previous 24-hour [^{13}C]leucine balance studies indicated similar values (≈40 mg/kg per day; *31, 32)*. Earlier fed-state-only [^{13}C]leucine oxidation studies indicated 20–40 mg/kg per day (*33*). Indicator amino acid oxidation studies using [^{13}C]phenylalanine in the fed state (*34*) derived a leucine requirement from the total branched-chain amino acid requirement of between 47 mg/kg per day and 55 mg/kg per day. Although these values could have been overestimated by about 10% (*35*), they are still higher than the values given by the 24-hour studies. The 1973/1985 FAO/WHO values for leucine derived from a value of 1100 mg/day (*3*) expressed as 14 mg/kg per day. Recalculation on the basis of Hegsted's regression values indicates a value of about 26 mg/kg per day with 5 mg/kg per day miscellaneous loss (*5–7*). Similar values have also been inferred from analysis of plasma amino acid responses (*33, 36*).

8.1.3 *Isoleucine and valine*

Reliable direct tracer experimental data from which the requirements of isoleucine and valine could be calculated are not available, although one multi-level tracer study (direct amino acid oxidation) of valine balance in the fed state only suggested a valine requirement >16 mg/kg per day (*37*). A 24-hour indicator oxidation and balance study on the valine requirement of well-nourished Indian subjects has also suggested values between 17 mg/kg per day and 20 mg/kg per day (*38*). Reported values are shown in Table 18. Indicator amino acid studies with [^{13}C]phenylalanine in the fed state (*34–35*) derived total branched-chain amino acid requirements ranging from 110 mg/kg per day to 134 mg/kg per day depending on outcome used and taking into account an initial 10% overestimate.

Table 18
Isoleucine and valine requirement measured using different approaches

Type of study	Isoleucine or valine requirement (mg/kg per day)	Reference
Nitrogen balance		
Isoleucine	10	*1, 2, 3*
Valine	10	*1, 2, 3*
Re-evaluation of nitrogen balance		
Isoleucine	18	*5, 7, 8*
Valine	14	*5, 7, 8*
[^{13}C]valine oxidation studies		
Fed state only, multi-level intakes	>16	*37*
24-hour multi-level intakes	17–20	*38*
Calculation from leucine (see text)		
Isoleucine	20	
Valine	26	
Recommendation		
Isoleucine	**20**	
Valine	**26**	

The 1973 and 1985 FAO/WHO values for isoleucine and valine derived from values of 700 mg/day and 800 mg/day (*3*) expressed as 10 mg/kg per day in each case. Recalculation on the basis of Hegsted's regression values indicates a value of about 18 mg/kg per day for isoleucine and 14 mg/kg per day for valine with 5 mg/kg per day miscellaneous loss (*5, 7, 8*).

Since the three branched-chain amino acids share a common catabolic pathway for their oxidation, and because their maintenance requirements reflect mainly their basal rates of catabolism, the Consultation estimated isoleucine and valine requirements from an assumed proportionality with leucine, based on the amino acid composition of body protein (*39*). Assuming a value of 39 mg/kg per day for the leucine requirement, this procedure yields values of 26 mg/kg per day for valine and 20 mg/kg per day for isoleucine.

8.1.4 *Threonine*

The threonine requirement is particularly nutritionally important, since it has been suggested that, after the sulfur amino acids, it is the second rate-limiting amino acid in the maintenance requirement (*40–42*), probably because it accounts for the largest single component of the ileal loss into the large bowel (*43–45*). It is also present at low concentrations in cereal proteins. The Consultation's estimate of the requirement for threonine derives from two 24-hour [^{13}C]leucine balance studies which each indicated a value of 15 mg/kg per day (*46, 47*) (Table 19).

Table 19

Threonine requirement measured using different approaches

Type of study	Threonine requirement (mg/kg per day)	Reference
Nitrogen balance	7	*1, 2, 3*
Re-evaluation of nitrogen balance	16	*5, 7, 8*
[^{13}C]leucine oxidation studies		
24-hour multi-level intakes	15	*46*
	15	*47*
[^{13}C]threonine oxidation studies		
Fed state only, multi-level intakes	10–20	*49*
[^{13}C]phenylalanine oxidation		
Fed state only, multi-level intakes	19	*48*
Recommendation	**15**	

Studies in the fed state with the indicator amino acid oxidation method with [^{13}C]phenylalanine suggest a threonine requirement of 10–20 mg/kg per day (*48*). Other fed-state direct amino acid oxidation tracer studies also suggested requirement values between 10 mg/kg per day and 20 mg/kg per day (*49*).

The 1973 and 1985 FAO/WHO values for threonine derived from values of 500 mg/day (*3*) expressed as 7 mg/kg per day. Recalculation on the basis of Hegsted's regression values indicates a value of about 16 mg/kg per day.

8.1.5 *Aromatic amino acids*

Of the aromatic amino acids, phenylalanine and tyrosine, the former is nutritionally indispensable while the latter, as a metabolic product of phenylalanine catabolism, is dependent on there being sufficient phenylalanine to supply the needs for both amino acids. Thus studies have either measured the total requirement for phenylalanine plus tyrosine, by giving diets lacking, or very low in, tyrosine, or examined the ability of tyrosine intake to lower the apparent requirement for phenylalanine. The total aromatic amino acid requirement is set at 25 mg/kg per day, which is close to the midpoint of a range of requirement estimates all of which have some considerable uncertainty (see Table 20).

The 1973 and 1985 FAO/WHO values for phenylalanine plus tyrosine derived from a value of 1100 mg/day (*3*) expressed as 14 mg/kg per day, higher than the value of 7 mg/kg per day deriving from a re-evaluation of the existing data using Hegsted's regression equation (*5*) after adjusting for 5 mg nitrogen/kg per day miscellaneous loss (*7*). Reported values are shown in Table 20. However, it has been suggested (*8*) that the most satisfactory nitrogen balance

study is that of Tolbert & Watts (*50*), which included suitable additions for miscellaneous losses. They reported the value to range between 13 mg/kg per day and 25 mg/kg per day.

The interpretation of the tracer estimates of the needs for the aromatic amino acids is particularly problematic because of tracer compartmentalization problems; no completely reliable methods are available. Some of these tracer studies have suggested higher values, of 22–39 mg/kg per day by 24-hour phenylalanine oxidation and balance using [^{13}C]phenylalanine (*51, 52*), and 19–36 mg/kg per day by 24-hour measurement of tyrosine balance using [^{13}C]tyrosine (*53*).

Regarding the sparing effect of tyrosine on the phenylalanine requirement, studies that have assessed this include the early nitrogen balance studies of Leverton et al. (*54*), which showed a low requirement of 3.7 mg/kg per day for phenylalanine when the diet contained generous amounts of tyrosine. A re-evaluation of these data by Hegsted (*5*) suggested a value nearer to 9 mg/kg per day. These and other data (*50, 55*) suggest that appreciable sparing of phenylalanine requirement by tyrosine intake occurs.

Similarly, the value of 9 mg/kg per day for the phenylalanine requirement in the presence of generous tyrosine, derived by break-point analysis of amino acid oxidation measured with [^{13}C]phenylalanine (*56*), suggests sparing by tyrosine. A direct measurement of the magnitude of sparing of phenylalanine by tyrosine was made by the indicator amino acid oxidation method using [^{13}C]lysine in the fed state only (*57*). At an intake of phenylalanine of 9 mg/kg per day, a break-point in the lysine oxidation curve was demonstrated at a tyrosine intake of 6 mg/kg per day, and this was taken to imply a combined aromatic amino acid requirement of 15 mg/kg per day. However, the phenylalanine intake of 9 mg/kg per day used in this study was based on the estimated phenylalanine requirement in the presence of generous tyrosine derived from the earlier study (*56*), which, in the context of more recent isotopic measurements yielding values for the combined requirement in the range 19–39 mg/kg per day (*51–53, 58, 59*), might have been an underestimate. If so, it is possible that the phenylalanine intake in the study of Roberts et al. (*57*) would have been suboptimal, and would therefore have restricted the utilization of tyrosine and lowered the apparent combined requirement.

There is thus considerable uncertainty, but taking all the above into account the recommended best estimate of total aromatic amino acid requirement is set at 25 mg/kg per day. It is not possible at present to set a specific value for the ability of tyrosine to spare phenylalanine intake.

Table 20
Aromatic amino acid requirement (phenylalanine and tyrosine) measured using different approaches

Type of study	Phenylalanine or tyrosine (mg/kg per day)	Reference
Nitrogen balance: no tyrosine	14	*1, 2, 3*
Re-evaluation of nitrogen balance	9.1	*7*
Nitrogen balance: no tyrosine	25	*50*
[^{13}C] phenylalanine oxidation studies		
24-hour single intake (no tyrosine)	< 39	*51*
24-hour single intake (no tyrosine)	>22	*52*
24-hour multi-level intake (no tyrosine)	< 36 >18.5	*53*
Fed-state multi-level intakes (high tyrosine)	9.1	*56*
Fed-state multi-level intakes: 6 mg tyrosine	15	*57*
Recommendation	**25**	

8.1.6 *Tryptophan*

Whereas the occurrence of tryptophan in proteins is generally less than many other amino acids, it is nutritionally important since it is a precursor for important metabolites such as serotonin and nicotinamide, in the latter case giving it vitamin-like properties through its ability to replace dietary niacin. Its content is low in cereals, especially maize, where it may be the nutritionally limiting amino acid in some varieties. The value of tryptophan requirement is set at 4 mg/kg per day, based on an average of values derived from a variety of approaches, each yielding results close to this value. Values examined are included in Table 21.

The 1973 and 1985 FAO/WHO values for tryptophan derived from a value of 250 mg/day (*3*) expressed as 3.5 mg/kg per day. This was increased only slightly to 3.7 mg/kg per day (*5, 7, 8*) after adjusting for 5 mg nitrogen/kg per day miscellaneous loss, because the regression equation reported by Hegsted (*6*) exhibited a steep slope. Tracer studies are limited but [^{13}C]phenylalanine indicator amino acid oxidation studies in the fed state indicate a tryptophan requirement of 4.0 g/kg per day (*59*). Additional evidence for this figure comes from the examination of the plasma amino acid response curve, which suggested a requirement value between 3 mg/kg per day and 5 mg/kg per day (*60*).

Table 21
Tryptophan requirement measured using different approaches

Type of study	Tryptophan (mg/kg per day)	Reference
Nitrogen balance	3.5	*1, 2, 3*
Re-evaluation of nitrogen balance	3.7	*5, 7, 8*
[^{13}C]phenylalanine oxidation studies		
Fed state, multi-level intakes	4	*59*
Recommendation	**4**	

8.1.7 *Sulfur amino acids*

Of the total sulfur amino acids, methionine and cysteine, the former is nutritionally indispensable while the latter, as a metabolic product of methionine catabolism, is dependent on there being sufficient methionine to supply the needs for both amino acids. They are important nutritionally since their concentrations are marginal in legume proteins, although they are equally abundant in cereal and animal proteins. Although their occurrence in proteins is less abundant than other amino acids, they are important metabolically to the extent that their relative requirement for maintenance is probably higher than that for human growth. For this reason it is believed that the obligatory nitrogen losses occur at a rate determined by the need to mobilize tissue protein to supply the sulfur amino acid requirements (*61*). This makes it possible to predict their requirement from the magnitude of their obligatory oxidative losses, as discussed below.

Values examined are included in Table 22. The 1973 and 1985 FAO/WHO values for methionine and cysteine, derived from nitrogen balance studies with methionine but without dietary cysteine (*3*), were 1100 mg/day expressed as 13 mg/kg per day. However, the balance studies were generally unsatisfactory and Hegsted's re-analysis identified a very shallow slope of the intake–balance regression, so any additions for miscellaneous loss dramatically increased the requirement (*5*), e.g. to 30 mg/kg per day at 5 mg nitrogen/kg per day (*7*).

Tracer studies are not entirely satisfactory, but a requirement for total sulfur amino acids close to 13 mg/kg per day has been suggested by a short-term direct amino acid balance technique (*62*), a short-term indicator amino acid oxidation technique (*63*), and the 24-hour indicator amino acid balance technique (*64, 65*). This value is similar to the estimate of 13 mg/kg per day derived from the obligatory oxidative losses (*61*, *66)*.

Meakins, Persaud & Jackson (*67*) showed that young women given diets that provided methionine and cysteine at, respectively, 6 mg/kg per day and 4.8 mg/kg per day for 1 week could be brought into balance with additional non-essential nitrogen, but not by an isonitrogenous amount of methionine (10 mg/kg per day), indicating that methionine at these levels of consumption was not limiting, but the availability of non-essential nitrogen was limiting.

The calculations of obligatory oxidative losses and the consequences for total sulfur amino acids requirement were re-evaluated in the light of current estimates of the magnitude of the obligatory nitrogen loss, i.e. 47.7 mg/kg per day (*23*), and the sulfur amino acid composition of the tissue protein-bound amino acids mobilized to meet this nitrogen loss. It is assumed that if the metabolic demand for methionine does determine the rate of net tissue proteolysis to supply the obligatory nitrogen loss on a protein-free diet, then the methionine content of an amount of tissue protein equivalent to the obligatory nitrogen loss may indicate the upper limit of the metabolic demand for methionine.

The first step is to define the total sulfur amino acid content of tissue protein. This was taken as the mean of the total sulfur amino acid content of liver (*68*) and of muscle protein. The latter value was taken as the methionine plus cysteine content of the weighted average mix of the principal human muscle myofibrillar proteins as indicated by the genome database (*69*). On this basis, an obligatory nitrogen loss of 47.7 mg/kg per day is equivalent to obligatory oxidative losses of methionine and cysteine of 10.4 mg/kg per day and 4.1 mg/kg per day, respectively.

The second step is to identify an appropriate value for the efficiency of dietary utilization to provide for the metabolic demand. The dietary requirement for methionine will be the obligatory oxidative loss corrected for any dietary inefficiency of utilization, only if the obligatory oxidative loss is equal to the metabolic demand: i.e. when mobilization of tissue protein which generates the obligatory nitrogen loss is 100% efficiently linked to providing the demand for methionine. Thus the obligatory oxidative loss is an upper limit of the demand. However, given that high-quality proteins are utilized with a near 100% efficiency when measured in an appropriate way (*70*), and given that the obligatory oxidative loss for methionine may in fact be greater than its metabolic demand as argued above, there seems little justification for adjusting the obligatory oxidative loss value upwards to account for dietary inefficiency.

The third step is to decide whether the demand for cysteine can be entirely supplied by methionine catabolism. Although this is not known with certainty, the onward metabolism of methionine to cysteine and to taurine is regulated, appears functionally important, and is limited or compromised in

several circumstances, such as poor B-vitamin status, infancy and alcohol consumption (*71*). Furthermore, there are conflicting studies on the ability of cysteine intake to reduce the requirement for methionine. Sparing was demonstrated in a study using the indicator amino acid approach, and with a diet containing sulfur amino acids in a ratio of 5:1 cysteine to methionine (*72*). However, two other studies employing the ^{13}C direct amino acid oxidation method suggested that sparing might not occur at intake ratios nearer to unity (*73, 74*). This is the ratio that would be more likely to occur with normal diets. Finally in an indicator amino acid balance study with graded levels of cysteine intake (0, 5 and 12 mg cysteine/kg per day) there seemed to be a sparing effect of cysteine on the methionine requirement. However, the effect could not be quantified with any certainty (*75*). For this reason it was decided to assume that cysteine intake could not entirely reduce the requirement for methionine, and that there should be separate recommendations for methionine and cysteine. Given these arguments, values for the total sulfur amino acid requirement are calculated on the assumption that methionine acts as a continuous source of sulfur for cysteine formation under steady-state conditions, but cannot supply the entire demand for cysteine. Thus the dietary requirement for methionine plus cysteine will be the obligatory oxidative losses (methionine plus cysteine), i.e. 10.4 mg/kg per day methionine and 4.1 mg/kg per day cysteine =14.5 mg/kg per day total sulfur amino acids rounded to 15 mg/kg per day. This value is similar to total sulfur amino acid requirement values obtained by the indicator amino acid balance method, and is therefore suggested for the total sulfur amino acid requirement.

It is clear that considerable uncertainty exists about the sparing effect of cysteine on the methionine requirement, and it is suggested that this should be a subject of future research.

8.1.8 *Histidine*

The minimum physiological requirement for histidine in adults is based on the investigations by Kopple & Swendseid (*76, 77*). Histidine was accepted in the 1985 report (*1*) as an indispensable amino acid in human adults, despite controversy regarding its essentiality. Whereas short-term (6–8 days) studies by Rose and others (*78, 79*) indicated no histidine requirement for nitrogen equilibrium, longer-term studies (~30 days) were equivocal (*76*, *80, 81*). Kopple & Swendseid (*76*) found that nitrogen balance was negative in healthy adults and uraemic patients when diets were devoid of histidine for 25–36 days; but in the study by Wixom et al. (*80*) nitrogen balance remained positive in one subject receiving parenteral nutrition without histidine for 27 days. In the study by Cho et al. (*81*), nitrogen balance was approximately zero or positive in subjects consuming a histidine-deficient diet for 56 days. However, upon reintroduction of histidine into the diet, nitrogen balance rapidly

Table 22
Sulfur amino acids requirement measured using different approaches

Type of study	Methionine or cysteine requirement (mg/kg per day)	Reference
Nitrogen balance: methionine only	13	*1, 2, 3*
Re-evaluation of nitrogen balance: methionine only	30	*5, 7*
[^{13}C]methionine oxidation studies		
Fed state, single intake, no cysteine	13	*62*
Fed state, single intake, variable cysteine	6–13	*73*
Fed state, variable methionine and cysteine	6–13	*74*
[^{13}C]phenylalanine oxidation studies		
Fed state, multi-level intakes, no dietary cysteine	13	*63*
[^{13}C]leucine oxidation studies		
24-hour multi-level intakes	15	*64*
24-hour multi-level intakes	16	*65*
Obligatory amino acid losses	13	*8, 61, 66*
Recommendation (see assumptions in text)		
Methionine	**10.4**	
Cysteine	**4.1**	
Total sulfur amino acids	**15**	

became more positive. To date the indispensability of histidine in healthy adults remains unresolved.

The difficulty of establishing conclusive indispensability of histidine may be attributable to the maintenance of large histidine pools in haemoglobin and carnosine. When a histidine-deficient diet was consumed for a prolonged period, a decrease in haemoglobin, in conjunction with a rise in serum iron, was observed (*76, 81*). These data suggest that during limited dietary supply, histidine pools may be maintained through the release of histidine from the degradation of haemoglobin (*75*), or through the reduction in haemoglobin synthesis (*82*). Histidine may also be released from carnosine (alanyl-L-histidine), a dipeptide present in large quantities in skeletal muscle (*83*). Carnosine concentrations in muscle or olfactory bulb were reduced in rats (*84–87*), chickens (*88*), cockerels (*89*) and dogs (*90*) receiving a histidine-deficient diet. Free carnosine concentration in muscle tissue in normal adults is about 20–180 mg/100 g (*91*). Thus, during consumption of a histidine-deficient diet, the amount of carnosine in muscle tissue is theoretically sufficient to provide enough histidine to maintain nitrogen balance in older children and adults for several weeks (*83*), but not in infants, because of the

limited amount of the enzyme carnosinase (*92, 93*). Decreased oxidation and degradation of histidine (*84*, *94, 95*) is another possible adaptive response in the body to maintain histidine adequacy during consumption of a histidine-deficient diet. A recent study (*96*) confirmed many of these findings and showed that histidine depletion over 48 days resulted in a fall in albumin and transferrin, as well as a 24–28% decline in whole-body protein turnover. Because of the extended period of time (>56 days) that is required to deplete body histidine pools in adults, it has not been possible to determine histidine requirement experimentally. On this basis the consultation endorsed the conclusion of the previous report (*1*), i.e. that histidine requirements "may be" between 8 mg/kg per day and 12 mg/kg per day (*76*), and identified a requirement of 10 mg/kg per day.

8.2 Dispensable amino acids

As discussed in section 2 in relation to matching the supply to the demand, effective utilization of digestible dietary protein requires an appropriate balance among the indispensable amino acids, those which are dispensable, and other nitrogen-containing compounds. Clearly protein synthesis requires the appropriate mix of all amino acids, but at maintenance, with protein synthesis supplied largely through amino acids recycled from proteolysis, metabolic consumption in other pathways drives most of the amino acid requirement, so that much of this demand is for dispensable amino acids. Thus the diet must provide dispensable amino acids or a utilizable source of "nonspecific" nitrogen (*1*) to enable their synthesis, as well as the synthesis of other physiologically important nitrogen-containing compounds, such as purines and pyrimidines, glutathione and creatine. Indeed effective utilization of intakes of indispensable amino acids at the lower end of their requirement range can occur only with adequate amounts of dispensable amino acids or non-essential nitrogen. Furthermore, the consumption of indispensable amino acids at intakes in excess of their requirements will in turn consume dispensable amino acids in detoxifying them, and thus increase overall requirements for dietary nitrogen. For example, increasing the dietary intake of methionine increases 5-oxoprolinuria, presumably because the effective metabolic disposal of methionine places a competitive demand on the availability of glycine (*67*). The rate of formation of dispensable amino acids in the body appears to be determined by the total intake of nitrogen. At lower levels of total nitrogen consumption, the formation of adequate amounts of dispensable amino acids is impaired, and the critical limitation may be the ability to provide adequate amounts of glycine and glutamine (*97*). The ability to generate adequate dispensable amino acids might also be dependent upon effective salvage of adequate amounts of urea nitrogen.

The endogenous synthesis rate of glycine could be rate-limiting (*98*), especially in rapidly growing babies (*99*) or where the metabolic demand for

glycine is increased (*67, 100–102)*. Glycine synthesis is reduced when nitrogen intake and dispensable amino acids are low (*102*), and even at an adequate dietary nitrogen level, a lower glycine intake results in an increased excretion of urinary L-oxoproline, which is an index of glycine adequacy (*103*).

Based on the foregoing, it is clear that humans require a preformed source of α-amino nitrogen in addition to that supplied by the indispensable amino acids. Under usual dietary conditions, this would be met by the dispensable or conditionally indispensable amino acids liberated via digestion of food proteins. However, in experimental circumstances it seems possible that glutamate alone or glutamate plus glycine would serve as an efficient source of α-amino nitrogen. The relative efficacy of these two sources in comparison with other simple nitrogen-containing mixtures to meet the demand for the formation of dispensable amino acids in adequate amounts and appropriate proportions will be determined by the degree of metabolic interconversion required, and a rank order for the different sources of dispensable amino acids, including diammonium citrate and other forms of nitrogen, is provided below (*104*):

glutamate and glycine
diammonium citrate and glycine
diammonium citrate and glutamate
glycine and urea
diammonium citrate
urea.

8.3 Summary of amino acid requirements in adults

Whereas some uncertainty remains over the adult indispensable amino acid requirements, the best current estimates are shown in Table 23, as mg/kg per day and as mg/g protein (i.e. the requirement pattern, calculated as the individual amino acid requirement divided by the total protein requirement). With the exception of histidine, the sulfur amino acids and tryptophan, all values are about twice as high as the values in the previous report. The total daily nitrogen requirement is effectively met through the provision of an appropriate intake level and balance of indispensable amino acids together with sufficient dispensable amino acids providing α-amino nitrogen. While there is a need to determine the magnitude of the demand for dispensable amino acids, glutamate and glycine appear to be the most effective form of this component.

Based on the mean requirement estimates for the indispensable amino acids identified above and assuming a mean total protein requirement of 0.66 g/kg per day, intakes of about 0.18 g/kg per day and 0.48 g/kg per day of indispensable and dispensable amino acids, respectively, or preformed

Table 23
Summary of the adult indispensable amino acid requirements

Amino acid protein[b]	Present estimates		1985 FAO/WHO/UNU[a]	
	mg/kg per day	mg/g protein[b]	mg/kg per day	mg/g protein[b]
Histidine	10	15	8–12	15
Isoleucine	20	30	10	15
Leucine	39	59	14	21
Lysine	30	45	12	18
Methionine + cysteine	15	22	13	20
Methionine	*10*	*16*	–	–
Cysteine	*4*	*6*	–	–
Phenylalanine + tyrosine	25	38	14	21
Threonine	15	23	7	11
Tryptophan	4	6	3.5	5
Valine	26	39	10	15
Total indispensable amino acids	**184**	**277**	**93.5**	**141**

[a] From reference *1*.
[b] Mean nitrogen requirement of 105 mg nitrogen/kg per day (0.66 g protein/kg per day).

α-amino nitrogen (28 mg nitrogen/kg per day and 78 mg nitrogen/kg per day, respectively), should be sufficient to maintain body nitrogen homeostasis in healthy adults.

8.4 Safe intakes of indispensable amino acids

There is no information on the variability of requirements for individual amino acids. Therefore, approximate values were calculated on the assumption that the inter-individual coefficient of variation of the requirements for amino acids is the same as that for total protein, i.e. 12%. On this basis, the safe levels of intake for the indispensable amino acids are 24% higher than the values for average requirement shown in the first column of Table 23.

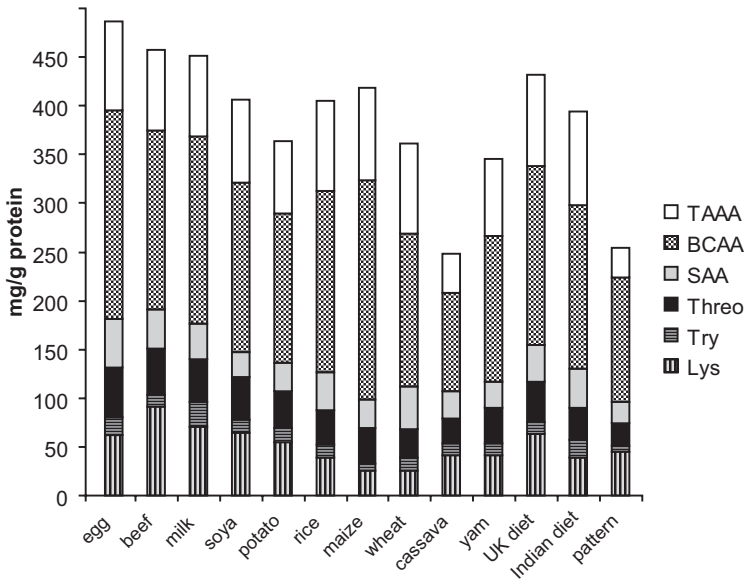
8.5 Indispensable amino acid requirements in elderly people

The data based on the currently acceptable methodologies described above are inadequate to make a separate recommendation for indispensable amino acid requirements in elderly people. Where available, the results of the nitrogen balance and tracer-based approaches are fragmentary and conflicting. Therefore, it is recommended that the indispensable amino acid pattern of requirement for elderly people is the same as that for adults in general.

8.6 Requirement values compared with the amino acid content of food proteins and diets

The nutritional implications of these values are discussed in full in section 10, but Figure 14 shows the amino acid content of the major food proteins compared with the proposed requirement pattern, and Table 24 shows the same values expressed as a percentage of the requirement pattern values. Clearly the content of indispensable amino acids in these examples of sources of animal proteins, legumes, root crops and cereals is considerably greater than the proposed requirement values overall, and for each individual amino acid, with two exceptions. First, lysine is present in cassava and yam at just over 90% and in cereals at between 57% and 86% of requirement levels. For this reason, lysine is below the requirement level (87%) in the Indian diet (mean value for 17 states). Given the uncertainty which exists in identifying a secure value for the lysine requirement, it is clear that more work is required in this area. Second most limiting are the branched-chain amino acids, which are limiting in cassava. However, their requirement value is based on that for

Figure 14
Indispensable amino acids in food proteins and diets compared with the requirement pattern



TAAA: Total aromatic amino acids
BCAA Branched-chain amino acids
SAA Sulfur amino acids
Threo: Threonine
Try: Tryptophan
Lys: Lysine

Table 24
Distribution of amino acids in food proteins and diets

	Percentage of requirement pattern[a]											
	Egg	**Beef**	**Milk**	**Soya**	**Potato**	**Rice**	**Maize**	**Wheat**	**Cassava**	**Yam**	**UK diet[b]**	**Indian diet[c]**
Lys	139	203	158	144	121	**86**	**58**	**57**	**92**	**91**	140	**87**
Tryp	293	213	417	217	240	224	117	217	192	213	211	293
Threo	223	202	191	191	167	153	157	127	115	157	177	143
SAA	225	182	164	114	131	176	132	203	124	125	174	182
BCAA	168	144	151	136	120	146	177	122	**79**	116	143	132
TAAA	301	275	271	281	243	305	314	306	135	265	311	317

Lys, lysine; Tryp, tryptophan; Threo, threonine; SAA, sulfur amino acids; BCAA, branched-chain amino acids; TAAA, total aromatic amino acids.

[a] See reference *105* for food amino acid contents.
[b] Reference *106*.
[c] Reference *107*.

leucine, for which the new requirement, which derives from the stable isotope studies, is much higher than that for the recalculated nitrogen balance studies. Thus more work is also required in this area.

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9. Protein and amino acid requirements of infants and children

As indicated in section 2, the protein requirement of infants and children can be defined as the minimum intake that will allow nitrogen equilibrium at an appropriate body composition during energy balance at moderate physical activity, plus the needs associated with the deposition of tissues consistent with good health. In practice, all previous reports have focused on a detailed consideration of the breastfed infant and the adult, with interpolations for intermediate ages. This was the case for the 1985 report (*1*), which adopted a modified factorial approach derived after consideration of intakes of breast milk. The assumption is made that for the first 6 months of life human milk from a healthy well-nourished mother can be regarded as providing an optimal intake of protein for the infant (*2*). Short-term nitrogen balance data were used to select a maintenance value (120 mg nitrogen/kg), rounded up from the highest of a range of values (80–118 mg nitrogen/kg per day). A growth requirement, calculated as mean nitrogen increment plus 50% to account for day-to-day variation in growth, scaled up assuming a 70% efficiency of utilization, was added to this maintenance value to give the average requirement. A safe level was calculated as average plus 2SD, assuming a coefficient of variation derived from the coefficients of variation for growth and maintenance, which fell from 16% at 6 months to 12% at 2 years of age.

The justification for this factorial method was derived from a comparison of the requirement values with protein intakes of breastfed infants of healthy mothers, which showed that the derived *average* protein requirement for the 3–4-month-old infant (1.47 g protein/kg per day) was very similar to average milk protein intake values (1.49 g protein/kg per day) for this age group. With protein intakes of breastfed infants of healthy mothers assumed to provide adequately for the infants' protein needs, the similarity of the derived value for the average requirement with the average intake was taken as support for the validity of the assumptions within the factorial calculations.

It has since been argued by members of the Consultation that produced the 1985 report that this was an error (*3–5*) and that the 1985 report considerably overestimated the protein requirement. As discussed in section 3, on the basis of a model in which protein requirements are not correlated with protein intakes, in a population consuming an average intake which is the same as the

average requirement, half the population will be consuming an intake less than their requirement. Thus if the average protein requirement of breastfed infants is similar to their average protein intake, this implies that 50% of breastfed infants are in deficit, with intake less than requirement. In fact if, as is normally assumed, nearly all breastfed infants meet their protein requirements, then their average intake should equate to a requirement value at the upper limits of the overall range, i.e. somewhat above the *safe level* for protein intake, i.e. >2SD higher than the mean requirement.

All of the issues relating to protein requirements of infants were comprehensively reviewed by Dewey et al. (*2*) in a report commissioned by the International Dietary Energy Consultancy Group to review the 1985 infant and childhood protein requirements. Dewey et al. re-examined the assumptions and evidence for the derivation of factorial estimates of protein requirements for the breastfed infant from birth to 6 months, and suggested that the requirement values for breastfed infants should be 10–25% lower than those in the 1985 report. This was achieved by adopting a lower maintenance value (90 mg nitrogen/kg per day) and replacing the 50% increase in the protein allowance for growth to cover day-to-day variation with an increase in the coefficient of variation for growth. The growth rates assumed were derived from the WHO 1994 breastfed pooled dataset (*6*) and the efficiency of utilization of dietary protein for growth was again assumed to be 70%.

Dewey and Beaton examined these new estimates using the "probability approach" to assessment of observed intakes developed by Beaton (see *2*). Thus the individual components of the factorial requirement were modelled in the light of intake data (total nitrogen) for a cohort of healthy United States breastfed infants at 3 months of age, all assumed to be adequately nourished. The predicted prevalence of "inadequacy" associated with various estimates of the requirement was estimated. The factorial model adopted in fact predicted somewhat higher than expected prevalence rates of inadequacy (8.1%). This suggested that the modelled estimates remained somewhat high, either because the value for maintenance was slightly overestimated, or the efficiency of dietary protein utilization was slightly underestimated, or a combination of both, for the breastfed infant. The value adopted for the intake of nitrogen from breast milk for the modelling was total nitrogen, which contains about 25% non-protein nitrogen. Any assumption that not all non-protein nitrogen is utilizable would have resulted in lower effective milk nitrogen intake, and consequently even higher prevalence rates of inadequacy on the basis of the revised factorial requirement values. Nevertheless, rather than revise the factorial values downwards, the suggested requirement values were assumed to be reasonable, on the basis that they were derived for a range of ages and feeding modes and were based on relatively conservative estimates for maintenance requirement and for efficiency of utilization of dietary nitrogen.

In this report, estimates of the protein requirements in early infancy and from 6 months to adulthood derive from a factorial model similar in principle to that reported by Dewey et al. (*2*). Thus, values for growth, from recently reported rates of protein deposition, adjusted to account for the estimated efficiency of dietary utilization of protein to provide for that growth, have been added to values for maintenance derived on the basis of the discussion below, to give the average requirement. This is then adjusted according to the expected variability of maintenance and growth to give a value equivalent to the 97.5th percentile of the distribution, as a measure of the recommended intake. For estimates of the requirements in the first 6 months, the model assumptions have been tested by comparison with breast-milk intakes. As discussed below, in selecting values for maintenance and growth efficiency for ages greater than 6 months, the likelihood that mixed diets consumed after weaning are utilized less efficiently is taken into account, as is the maintenance value for adults identified in section 8.

9.1 Maintenance requirement for protein

9.1.1 *Interpretation of experimental information*

Two types of studies have been reported which help identify a maintenance requirement value. Overall, these studies were performed in approximately equal numbers of males and females, and involved European, African, Central American, and Chinese infants and children. Three studies involving 57 subjects were designed to measure nitrogen losses at very low or zero protein intakes, a measure of the basal demand (Table 25). These studies indicated a mean value of 62 ± 12 mg nitrogen/kg per day. The other 10 studies were designed to examine the relationship between protein intake and nitrogen balance, both above and below maintenance (Table 26). All these multipoint nitrogen balance studies of children fed different levels of protein (nitrogen)

Table 25
Basal nitrogen loss of infants and children as estimated from studies with very low or zero protein intake

Study	Number of subjects	Nitrogen intake (mg nitrogen/kg per day)	Basal loss (from nitrogen balance) (mg nitrogen/kg per day)	Reference
1.	11	20	67 ± 7	*7*
2.	6	0	58 ± 4	*8*
3.	34	10	63 ± 15	*9*
Total	51			
Mean			63 ± 12	

Table 26

Regression analysis of nitrogen balance studies on children from 6 months to 12 years of age to determine the maintenance protein requirement and efficiency of utilization

Study	Diet	Age	Number of subjects	Basal loss[a,b,c] (mg nitrogen/kg per day)	Slope[b,c]	Maintenance[a,b,c] (mg nitrogen/kg per day)	Reference
1.	Milk	34–62 months	6	35.4	0.52	76	*10*
2.	Soy	34–62 months	7	58.2	0.51	127	*10*
3.	Mixed	8–10 years	8	67.3	0.54	126	*11*
4.	Mixed	12–14 years	8 (pooled)[d]	61.43	0.573	107	*12*
5.	Milk	9–17 months	31 (points)[d]	77.53	0.693	112	*7*
6.	Egg	9–17 months	30 (points)[d]	81.63	0.713	116	*7*
7.	Rice and fish	18–26 months	7	53.6	0.52	102	*13*
8.	Rice and beans	22–29 months	5	98.1	0.68	149	*14*
9.	Milk	17–31 months	10	52.0	0.70	66	*15*
10.	Soy	17–31 months	10	55.5	0.55	90	*15*
All individual estimates (7 studies – excluding 4–6)			53	57.5	0.56	108	
All studies (10 studies)				57.4	0.58	110	
All milk and egg studies (4 studies)				61.6	0.66	93	

[a] A correction factor of 6.5 mg nitrogen/kg per day was applied for miscellaneous losses of nitrogen.
[b] Multiple data on individual subjects not available.
[c] Values are for the median.
[d] Regression estimate of study requirement.

shown in Table 26 were analysed following a linear regression approach, as described for adults (see section 3). The individual data were fitted to the linear model:

$$\text{nitrogen balance} = A + B \times \text{nitrogen intake},$$

where A is the extrapolated nitrogen loss at zero intake and B (the slope) represents the efficiency of utilization. The individual maintenance requirement is estimated as the intake that provides zero nitrogen balance.

It is clear that there is a very limited database of nitrogen balance studies of infants and children, with only one multipoint balance study that involved infants (9–17 months of age studied by Huang, Lin & Hsu (*7*)). Furthermore, as pointed out by Dewey et al. (*2*) and as reported by the WHO Working Group on Infant Growth (*6*), the infants' energy intake in this study (77 kcal/kg per day) was below the then recommended level (>100 kcal/kg per day) for this age range. However, it is within the range of the recent lower estimate (82.4 kcal/kg per day for 1–2-year-old boys) made by FAO/WHO/UNU (*16*). The values for the four milk and egg studies were used for the factorial estimate of protein requirements in the first 6 months of life. The data from all 10 studies were used for the factorial estimation of the protein requirements of older infants, children and adolescents (6 months to 18 years).

Average values for basal loss were similar in the two groups of studies, at 62 mg nitrogen/kg per day and 57 mg nitrogen/kg per day (Tables 25 and 26). Maintenance values range from 66 mg nitrogen to 149 mg nitrogen, equivalent to 0.42 g protein/kg per day to 0.93 g protein/kg per day. The values for children who were fed animal protein (egg and milk) indicate a lower maintenance requirement (93 mg nitrogen/kg per day or 0.58 g protein/kg per day) compared with values obtained with mixed diets or plant protein sources, as might be expected given the lower quality, especially digestibility, of these protein sources. Taken together, these experimental values do support the argument that the maintenance value for infants and young children is lower than the 120 mg nitrogen/kg per day assumed in the 1985 report (*1*).

In selecting an appropriate value for maintenance for infants and young children, the question can be posed as to whether their maintenance requirement differs from that of adults. The adult value, i.e. 105 mg nitrogen/kg per day (see section 5), derived from a data set which involved an overall range for the mean values within each reported study of 67–148 mg nitrogen/kg per day for the primary studies (19 studies) and 73–153 mg nitrogen/kg per day for the secondary studies (8 studies). Thus the overall range for maintenance values for infants and children shown in Table 26 (66–149 mg nitrogen/kg per day) is similar to the adult range, although the values from studies with animal protein lie at the lower end of this range. The values for basal loss are

somewhat higher than such losses observed in adults (47 mg nitrogen/kg per day), although the studies with infants or children reported in Table 25 involved somewhat shorter periods on low-protein diets than in most adult studies. On this basis it is difficult to determine with any certainty whether maintenance values for infants differ from those for adults.

One difficulty in interpreting nitrogen balance data in terms of identifying the efficiency of protein utilization in the infant during the first 6 months of life is the extent to which faecal nitrogen excretion varies and represents endogenous rather than dietary nitrogen. Foman, DeMaeyer & Owen (*9*) reported nitrogen balance data on infants aged 4–6 months, implying values of 0.49 when faecal nitrogen losses, which increased with nitrogen intake, were included, with much higher efficiency (0.91) indicated on the basis of changes in urinary nitrogen. In most studies, faecal nitrogen varies little with intake and accounts for on average 15% of intake (*17, 18*). In one study of neonates (*19*), urinary nitrogen did not change with intakes ranging from 0.8 g protein/kg per day to 3 g protein/kg per day, indicating an apparent biological value of breast milk approaching 100%, as reported by Waterlow & Wills some years ago in infants recovering from malnutrition (*20*). Although faecal nitrogen was not measured in the latter studies, the authors (*20*) assumed that about 23% of the intake was lost via the faecal route, reporting an overall efficiency of 0.77. In Table 26, the four studies with animal protein indicate a slope of 0.66.

The experimental evidence in Tables 25 and 26 was reviewed extensively by Dewey et al. (*2*), and as these data are very limited in terms of the range of ages, study techniques and diets, the choice of a maintenance value is by no means clear-cut. Dewey et al. (*2*) chose a value of 90 mg nitrogen/kg per day (0.56 g protein/kg per day). A similar value of 91.2 mg nitrogen/kg per day, i.e. 0.57 g protein/kg per day, would be indicated by a basal demand of 60.2 mg nitrogen/kg per day, the mean of the values shown in Tables 25 (63 mg) and 26 (57.4 mg – mean of all studies), and an efficiency of utilization of 0.66, the slope indicated by the four studies with animal protein. The value indicated in Table 26 for the four studies with milk and egg is 93 mg nitrogen/kg per day (0.58 g protein/kg per day), and this value was selected for infants up to 6 months. The value indicated for all 10 studies, 110 mg nitrogen/kg per day (0.69 g protein/kg per day), is close to the adult maintenance value of 105 mg nitrogen/kg per day (0.66 g protein/kg per day), so this latter value was selected for the maintenance value for ages greater than 6 months. While there is no a priori reason why maintenance should change with age, it is possible that during the rapid growth of infancy, dietary utilization to meet basal demands might be more efficient. In the past, requirement values for intermediate ages between infancy and adulthood have been interpolated. For this report, the change in the requirement model occurs at 6 months, consistent

with the major change in feeding mode associated with the adoption of the mixed diet at weaning.

9.1.2 *Variability of maintenance*

Given the paucity of the information and complete absence of any replicate studies on the same individual, no judgements can be made about the variability of maintenance requirement of children or whether it differs from that of adults. In the latter case a coefficient of variation of 0.12 has been derived (see section 7). This value was assumed therefore to apply to infants and children.

9.2 Protein deposition

The availability of new estimates of rates of protein deposition for infants and children from 6 months to 18 years, together with an expansion of information on the amino acid composition of whole-body protein, allows an improvement in the factorial estimates for overall protein and indispensable amino acid requirements during growth. Thus, average daily rates of protein deposition can be derived from the measurements of whole-body potassium reported by Butte et al. (*21*) and Ellis et al. (*22*).

9.2.1 *Infants and young children, age 0–2 years*

In a longitudinal study, Butte et al. (*21*) followed 76 individuals from birth to 2 years with measurements at 0.5, 3, 6, 9, 12, 18 and 24 months. The total protein data for each of the 71 individuals who had at least 5 data points, including one at 18 or 24 months, were fitted to individual quadratic equations:

$$\text{total protein} = A + B\ \text{age} + C\ \text{age}^2 \quad (\text{average } R^2 = 0.99, \text{ no } R^2 \text{ below } 0.94).$$

The derivatives of these equations describe the protein deposition per day for each child:

$$\text{protein deposition} = B + 2C\ \text{age}.$$

Individual weight data were fitted to power curves

$$\ln(\text{weight}) = A + B \ln(\text{age}) \quad (\text{average } R^2 = 0.99, \text{ no } R^2 \text{ below } 0.95).$$

For each individual, the ratio of these two equations estimated protein deposition per day per kilogram body weight. Monthly values for each individual are calculated and summarized by their mean and standard deviation in Table 27.

Table 27
Protein deposition in infants[a]

Age (months)	Total protein (g)		Total protein deposition (g/day)		Weight (kg)		Protein deposition per kg weight (g/kg per day)	
	Average	SD	Average	SD	Average	SD	Average	SD
1	486.35	60.51	2.44	0.48	4.49	0.46	0.548	0.113
2	559.49	63.22	2.37	0.45	5.55	0.49	0.428	0.078
3	630.33	68.43	2.29	0.42	6.28	0.53	0.366	0.061
4	698.90	75.12	2.22	0.39	6.86	0.56	0.323	0.050
5	765.18	82.50	2.14	0.36	7.34	0.60	0.292	0.041
6	829.18	90.06	2.07	0.33	7.76	0.63	0.266	0.035
7	890.90	97.48	1.99	0.31	8.14	0.67	0.245	0.029
8	950.33	104.59	1.92	0.28	8.48	0.70	0.226	0.025
9	1007.47	111.27	1.84	0.26	8.79	0.73	0.209	0.022
10	1062.34	117.47	1.77	0.25	9.08	0.76	0.194	0.020
11	1114.92	123.18	1.69	0.24	9.35	0.79	0.181	0.019
12	1165.21	128.41	1.62	0.24	9.60	0.82	0.168	0.019
13	1213.23	133.21	1.54	0.24	9.84	0.85	0.157	0.020
14	1258.96	137.63	1.47	0.25	10.07	0.88	0.146	0.022
15	1302.40	141.75	1.39	0.26	10.28	0.91	0.135	0.023
16	1343.56	145.66	1.32	0.28	10.49	0.93	0.126	0.025
17	1382.44	149.47	1.24	0.30	10.69	0.96	0.116	0.027
18	1419.03	153.32	1.17	0.33	10.88	0.99	0.108	0.030
19	1453.34	157.32	1.09	0.36	11.06	1.01	0.099	0.032
20	1485.37	161.65	1.02	0.38	11.24	1.04	0.091	0.034
21	1515.11	166.45	0.94	0.42	11.41	1.06	0.083	0.037
22	1542.57	171.89	0.87	0.45	11.57	1.09	0.075	0.039
23	1567.75	178.11	0.79	0.48	11.73	1.11	0.068	0.041
24	1590.64	185.28	0.72	0.51	11.89	1.14	0.061	0.044

[a] Reproduced from reference *21* with the permission of the publisher.

9.2.2 *Children, age 4–18 years*

The protein deposition needs of children (4–18 years), given in Table 28, were based on the study by Ellis et al. (*22*). Since these data are cross-sectional, single models were fitted to the entire data set for each sex. The fitting procedure used mid-year for ages, and excluded the data for years 10 and 13 for the females. The protein data (yearly cohort averages) were fitted to a single cubic curve for each sex:

males: protein (kg) = $5.46 - 1.285\ \text{age} + 0.166\ \text{age}^2 - 0.00433\ \text{age}^3$ ($R^2 = 0.992$),
females: protein (kg) = $3.91 - 0.925\ \text{age} + 0.139\ \text{age}^2 - 0.00428\ \text{age}^3$ ($R^2 = 0.993$).

These curves were differentiated to give protein deposition rate estimates:

males: protein deposition (kg/year) = – 1.285 + 0.332 age – 0.0130 age^2,
females: protein deposition (kg/year) = – 0.925 + 0.279 age – 0.0128 age^2.

The weight data were fitted to a quadratic curve for each sex:

males: weight (kg) = 54.2 – 15.0 age + 1.89 age^2 – 0.0557 age^3 (R^2 = 0.991),
females: weight (kg) = 25.3 – 4.47 age + 0.730 age^2 – 0.0198 age^3 (R^2 = 0.972) .

The ratio of these two functions (adjusted to give daily values) estimates protein deposition per kilogram, as shown in Table 28.

In order to unify the two data sets, some interpolation was required for those ages between the data sets. This was necessary because any fitting procedure is less certain at the ends of its range, especially when, as here, this corresponds to the decline of infant growth velocity prior to any subsequent growth spurt. Also the two data sets involved different approaches, i.e. longitudinal

Table 28
Protein deposition in children[a]

Age (years)	Male			Female		
	Weight (kg)	Total protein (kg)	Protein deposition (g / kg per day)	Weight (kg)	Total protein (kg)	Protein deposition (g /kg per day)
4	20.9	2.7	0	17.8	2.2	0
5	19.5	2.6	0.007	18.7	2.2	0.022
6	20.2	2.8	0.032	20.5	2.5	0.039
7	22.7	3.1	0.048	23.0	2.8	0.048
8	26.6	3.6	0.055	26.1	3.3	0.051
9	31.6	4.2	0.056	29.7	3.8	0.050
10	37.4	4.9	0.054	33.8	4.3	0.047
11	43.6	5.6	0.050	38.1	4.9	0.043
12	49.9	6.4	0.045	42.5	5.5	0.037
13	56.0	7.3	0.041	47.0	6.1	0.031
14	61.5	8.1	0.036	51.4	6.6	0.025
15	66.1	8.9	0.032	55.6	7.0	0.018
16	69.4	9.6	0.027	59.5	7.3	0.012
17	71.2	10.3	0.023	63.0	7.5	0.005
18	71.1	10.8	0.018	65.8	7.5	0

[a] Reproduced from reference *22* with the permission of the publisher.

(*21*) or cross-sectional (*22*) studies. A quadratic model was used to span this gap, using data for the ages of 18 months, and 2, 4, 5 and 6 years to estimate deposition needs for the ages 2, 3, 4 and 5 years. The final estimates for protein deposition over the age range 6 months to 18 years are shown in Table 29, which shows small sex differences in deposition. As far as the significance of this sex difference is concerned, although there was a steady trend from males having higher deposition at 1 month to females having higher deposition at 24 months (crossing at about a year), which may be a significant trend, testing for sex differences in terms of the monthly data revealed no differences. Thus, an average value was also defined for each age.

9.2.3 *Variability of protein deposition*

Dewey et al. (*2*) reviewed available evidence for inter-individual and intra-individual variability in infant growth when examining the pooled data set for growth of breastfed infants. Coefficients of variation for one-month growth increments ranged from 26% at 1–2 months to 93% at 11–12 months with coefficients of variation for 3-month weight increments varying from 24% to 46% over the first year. Because the data set suggested that inter-individual variability in growth was more accurately determined over longer than shorter periods, it was argued that the 3-month values were better estimates. In the cohort of 104 breastfed infants reported by Dewey et al. (*2*) that was used to test various assumptions within a factorial model of the requirements, the variability of weight gain at 3 months over a 2-month interval was reported as 24.7%. In the 1985 report (*1*), a coefficient of variation of 37% was derived from Foman's monthly measurements in boys aged 3.5–6.5 months.

The approach adopted here for estimating the variability of protein deposition rates in infants is to derive values from the data of Butte et al. (*21*) for infants and young children. These data were gathered longitudinally, enabling the standard deviation of protein deposition at specific ages to be calculated (Table 27). These rates of protein deposition in Table 27 represent the best estimates currently available. The coefficients of variation of protein growth are on average 24%, and are highest as the growth rate slows.

For older children, in the absence of suitable information from the cross-sectional data of Ellis et al. (*22*), longitudinal data analysed by Tanner (*23*) were used to estimate the standard deviation of velocity of total weight increase for specific ages. This involved conversion of standard deviations of weight growth velocity (*23*) according to the fraction of weight as protein (*22*). Whereas this approach has limitations, representing measurements on different populations at different times, as well as a mix of longitudinal and cross-sectional measurements, the growth velocity values from the two data

Table 29
Protein deposition for infants and children [a]

Age (years)	Protein deposition (g/kg per day)			
	Females	Males	Both sexes	Standard deviation
0.50	0.266	0.266	0.266	0.035
1.00	0.168	0.168	0.168	0.031
1.50	0.108	0.108	0.108	0.029
2.00	0.076	0.073	0.075	0.026
3.00	0.044	0.034	0.039	0.022
4.00	0.026	0.013	0.020	0.019
5.00	0.022	0.009	0.016	0.017
6.00	0.038	0.032	0.035	0.016
7.00	0.048	0.048	0.048	0.016
8.00	0.051	0.055	0.053	0.016
9.00	0.050	0.056	0.053	0.017
10.00	0.047	0.054	0.051	0.017
11.00	0.043	0.050	0.047	0.018
12.00	0.037	0.045	0.041	0.018
13.00	0.031	0.041	0.036	0.018
14.00	0.025	0.036	0.031	0.017
15.00	0.018	0.032	0.025	0.015
16.00	0.012	0.027	0.020	0.012
17.00	0.005	0.023	0.014	0.008
18.00	0.000	0.018	0.009	0.005

[a] Source: references *21* and *22*.

sets were similar, thus validating the approach. Table 29 shows the rates of protein deposition of boys and girls calculated from the combined data from the two studies, with mean values and standard deviations as discussed above.

9.2.4 *Growth rates compared with previous estimates*

Table 30 shows the new values for growth during the first year of life compared with previous values reported by Fomon (*24*) and Dewey et al. *(2)*.

It is apparent that the new estimates are slightly lower up to 3 months of age and slightly higher after this.

9.3 Factorial estimates of protein requirements

As indicated above, the factorial method involves calculation of a mean value for the requirement, as maintenance plus the dietary requirement for growth (deposition/efficiency of utilization), and in addition, the safe level, which is

Table 30
Protein deposition during the first year of life: new values compared with previous estimates

Age (months)	Body protein gain (g/kg per day)		
	Dewey et al. 1996 *(2)*	Fomon's estimate *(24)*	Current[a] estimate
0–1	1.00	0.93	
1–2	0.69	0.70	0.486
2–3	0.44	0.50	0.399
3–4	0.35	0.34	0.348
4–5	0.20	0.27	0.311
5–6	0.25	0.26	0.283
6–9	0.20	0.23	0.237
9–12	0.15	0.18	0.188

[a] Midpoint (mean) of values shown in Table 27.

calculated as the mean requirement plus 1.96 times the SD (with 1SD calculated as the root mean square of the SDs for maintenance and growth). The experimental evidence summarized in section 9.1 above suggests maintenance requirements and the efficiency of dietary utilization for growth for infants <6 months of age to be 93 mg nitrogen/kg per day (0.58 g protein/kg per day) and 0.66 (66%), the observed intercept/slope and slope values for the nitrogen balance studies with animal protein shown in Table 26. Deposition rates are indicated in Table 27, and the factorial calculation of the average requirements and safe levels is shown in Table 31.

9.3.1 *Comparison with protein intakes of the breastfed infant*

On the basis of the arguments presented by Beaton & Chery (*3*) and discussed extensively by Dewey et al. (*2*), if the factorial model is correct it should predict values for breastfed infants at <6 months which are consistent with protein intakes from breast milk, i.e. the safe level of intake should be slightly lower than observed average intakes, and calculations of prevalence of inadequacy for breastfed infants should indicate low levels (2.5% or less).

However, estimating true protein intakes from breast milk is difficult because of the non-protein nitrogen fraction. Total nitrogen in human milk represents both protein, about 75%, and non-protein nitrogen. The latter is made up of urea, which accounts for up to 50% of non-protein nitrogen, amino acids and other nitrogenous compounds. The proportion of non-protein nitrogen in human milk is large compared with milk of most other mammals, and since the extent of its utilization is not entirely understood, in any comparison of

Table 31
Safe level of protein intake for infants less than 6 months of age

Age (Months)	Maintenance[a] requirement	Growth[b] requirement	Average requirement	Safe level[c] (+1.96 SD)	1985 Report *(1)*
		(g protein/kg body weight per day)			
1	0.58	0.83	1.41	1.77	2.25
2	0.58	0.65	1.23	1.50	1.82
3	0.58	0.55	1.13	1.36	1.47
4	0.58	0.49	1.07	1.24	1.34
6	0.58	0.40	0.98	1.14	1.30

[a] Calculated from maintenance value of 0.58 (balance results with egg and milk: Table 26), plus growth.
[b] Dietary growth requirements calculated from deposition rate in Table 27, adjusted for 66% efficiency of utilization (balance results with egg and milk: Table 26).
[c] Calculated from mean values plus 1.96 times the root mean square of the SD values for the growth requirement (Table 27, adjusted for 66% efficiency of utilization) and maintenance (12% of 0.58).

predicted requirements with breast-milk intakes, judgements must be made as to the amount of available nitrogen consumed as protein in breast milk. Thus utilized nitrogen is likely to include that in the form of free amino acids or small peptides as well as a proportion of urea nitrogen. Reported values vary from 10% to 43% (*25-28)*. In Table 32, values for protein intakes of breastfed infants are shown with the protein calculated as either crude protein (nitrogen times 6.38, the appropriate conversion factor for milk) or true protein estimated as 75% of crude protein. Thus utilizable intakes should comprise values intermediate between crude and true protein intakes. The mean and safe requirement values calculated by the factorial model for infants <6 months (discussed above) is also shown, which can be compared with utilizable intakes. The final column in Table 32 shows how much of the non-protein nitrogen fraction would need to be utilized for the safe requirement to exactly match the intake.

The factorial model (Table 32) predicts a safe protein requirement value which is similar to, but lower than, the "crude" breast-milk protein intake at all ages. In fact, on the basis of the calculation of the prevalence of inadequacy as outlined in section 3,[1] the requirement values at 3, 4 and 6 months indicate a prevalence of inadequacy of 13–15%. In terms of the Beaton & Chery reasoning (*3*) this would imply that the values for the safe level in Table 32 are somewhat too high. However, if protein intake and requirements are to some

[1] Prevalence of intakes < requirement is calculated from mean values of intakes and requirements from the expression $\Phi(-(M_R-M_I)/SD)$, where Φ is the unit normal distribution and $SD = \sqrt{(S_I^2 + S_R^2)}$.

Table 32
Factorial values for infant protein requirements and implications for breastfed infants at different stages of lactation [a]

Note: Table 32 - Expert Consultation calculations (data and methods described in Tables 26 and 27).

Age	Weight	Total nitrogen intake	Crude protein intake (nitrogen × 6.38)	Milk protein intake[b]	Requirement safe leave	Utilization of non-protein nitrogen[d]
(months)	(kg)	(mg/day)	(g/kg per day)		(g/kg per day)	%
1	4.76	1723	2.31	1.73	1.77	9
2	5.62	1486	1.69	1.27	1.50	56
3	6.29	1444	1.46	1.10	1.36	73
4	6.78	1408	1.32	0.99	1.24	75
6	7.54	1486	1.26	0.94	1.14	64

[a] Data modified from reference *2*, Tables 1 and 8.
[b] Milk protein intake = 75% crude protein.
[c] From Table 31.
[d] Calculated from: (safe level minus milk protein intake) ÷ (crude protein intake minus milk protein intake).

extent correlated, the calculated risk of deficiency will be less than the values indicated above. The protein requirements for infants up to 6 months of age as calculated by the factorial model shown in Table 32 are compatible with the assumption that human milk from a healthy well-nourished mother can support the protein requirements for infants for the first 6 months of life.

9.3.2 *Implications of the estimated protein requirements for formula-fed infants*

The estimated protein requirement values are likely to guide formulations of infant formula. However, differences in dietary protein digestibility, bioavailability and efficiency of utilization between human milk and infant formulas must be recognized. The 1998 Life Sciences Research Office (LSRO) Expert Panel on the assessment of nutrient requirements for infant formulas recommended a minimum of 1.7 g protein/100 kcal (*29*). Although the LSRO Expert Panel considered the minimum intake of 1.7 g/100 kcal of true protein from milk-based formula to be adequate, it recommended that new formulations providing protein levels at or near the minimum level should be clinically tested to demonstrate efficacy. In fact, a number of recent infant feeding trials tested lower than usual protein concentrations in infant formulas. Experimental formula with protein levels of 1.9–2.2 g/100 kcal resulted in similar indices of protein metabolism compared with breastfed and mixed-fed

(breast milk and formula) infants at 6 months (*30*). Raiha et al. (*31*) recently tested 1.8 g/100 kcal from a whey-modified infant formula from birth to 4 months, reporting similar weight gains and length gains or body mass indices compared with either breastfeeding or a standard formula with a protein:energy ratio of 2.2 g/100 kcal. Fomon et al. (*32*) studied infants fed a formula with 1.56 g/100 kcal from ages 8 to 27 days, gradually decreasing to 1.25 g/100 kcal from ages 84 to 111 days. Weight gain and serum albumin were not significantly different from a reference group fed 2.1 g/100 kcal, but length gain was significantly less. Protein intakes decreased from 1.85 to 1.18 g/kg per day and were judged inadequate. In another study, Foman et al. (*33*) tested a formulation with 1.7 g/100 kcal and concluded that protein intake was adequate in terms of gain in length, although they were puzzled by energy intakes and consequently weight gains that were higher than the reference group fed 2.2–2.5g/100 kcal. An inadequate protein:energy ratio could have stimulated milk intake; however, the authors stated that unknown factors unrelated to the protein:energy ratio may have been responsible for the higher energy intakes. In general, milk-based formulas with a protein:energy ratio of 1.7–1.8 g/100 kcal appear adequate and safe for term infants. The observed protein intakes exceed the estimated protein requirements shown in Table 31. For instance, in the 3-month-old infants in these studies, the observed protein intake was 1.76 g/kg per day on 92 kcal/kg per day (*31*) and 1.65 g/kg per day on 99 kcal/kg per day (*30*), respectively. Because the protein content of formula is constant, unlike human milk, and must be formulated to meet the protein needs of the infants at all times, including the first month when protein needs are highest, observed protein intakes will exceed protein needs by an increasing margin after the first month. Nevertheless, formulation of infant formula must compensate for differences in dietary protein digestibility, bioavailability and efficiency of utilization between human milk and formula to meet the protein requirements of formula-fed infants.

9.3.3 *Average protein requirements and safe levels for infants and children from 6 months to 18 years*

The similarity of growth rates of boys and girls prior to adolescence is such that requirement estimates can be calculated for both sexes combined, although during adolescence it is perhaps more appropriate that separate estimates are calculated for boys and girls. In the analysis presented in Table 26, for all subjects from 6 months to 18 years a maintenance value of 109.8 mg nitrogen/kg per day (0.69 g protein/kg per day) was derived. This is very similar to the maintenance value of 0.66 g protein/kg per day derived from the much larger data set from studies in adults (section 7). Hence, the value of 0.66 was also used for maintenance for the present calculations for children aged 6 months to 18 years.

From the deposition needs (Table 29) and an efficiency of utilization for growth of 0.58 (see Table 26), the average protein requirements for 6 months to 18 years are estimated as:

average protein requirement = maintenance + deposition / efficiency.

The assumption is made that requirement follows a normal distribution, and thus a safe level (exceeding the requirement of 97.5% of the population) is estimated as the average level plus 1.96 standard deviations. There are no data on the variance of maintenance in children, and therefore the Consultation chose to use the adult value of 12% (section 7). The rates of protein deposition at different ages and their standard deviations are shown in Table 29. The standard deviation for growth, like the average deposition (growth data), was adjusted for efficiency of utilization (0.58, as above).

Values for protein requirements for infants and children are shown in Table 33(a) for both sexes combined, and protein requirements for adolescent males and females are shown separately in Table 33(b). In each case, safe levels from the 1985 report (*1*) are also shown, so that the differences can be

Table 33a
Safe level of protein intake for weaned infants and children up to 10 years of age (sexes combined)

Age (years)	Maintenance[a] requirement	Growth[b] requirement	Average requirement	Safe level[c] (+1.96SD)	1985 report
		(g protein/kg body weight per day)			
0.5	0.66	0.46	1.12	1.31	1.75
1	0.66	0.29	0.95	1.14	1.57
1.5	0.66	0.19	0.85	1.03	1.26
2	0.66	0.13	0.79	0.97	1.17
3	0.66	0.07	0.73	0.90	1.13
4	0.66	0.03	0.69	0.86	1.09
5	0.66	0.06	0.69	0.85	1.06
6	0.66	0.04	0.72	0.89	1.02
7	0.66	0.08	0.74	0.91	1.01
8	0.66	0.09	0.75	0.92	1.01
9	0.66	0.09	0.75	0.92	1.01
10	0.66	0.09	0.75	0.91	0.99

[a] Derived from the regression of nitrogen balance against intake shown in Table 26.
[b] From Table 29, adjusted for 58% efficiency of utilization, derived from the regression analysis in Table 26.
[c] SD for maintenance based on a coefficient of variation of 12%. SD for growth calculated from SD of deposition in Table 29/0.58 (efficiency of utilization). SD for maintenance and for growth are calculated as described in the text.

Table 33b
Safe level of protein intake for adolescent boys and girls[a]

Age (years)	Maintenance[a] requirement	Growth[b] requirement	Average requirement	Safe level[c] (+1.96SD)	1985 report
	(g protein/kg body weight per day)				
Girls					
11	0.66	0.07	0.73	0.90	1.00
12	0.66	0.06	0.72	1.89	0.98
13	0.66	0.05	0.71	1.88	0.98
14	0.66	0.04	0.70	0.87	0.94
15	0.66	0.03.	0.69	0.85	0.90
16	0.66	0.02.	0.68	0.84	0.87
17	0.66	0.01	0.67	0.83	0.83
18	0.66	0.00	0.66	0.82	1.80
Boys					
11	0.66	0.09	0.75	0.91	0.99
12	0.66	0.08	0.74	0.90	0.98
13	0.66	0.07	0.73	0.90	1.00
14	0.66	0.06	0.72	0.89	0.97
15	0.66	0.06	0.72	0.88	0.96
16	0.66	0.05	0.71	0.87	0.92
17	0.66	0.04	0.70	0.86	0.90
18	0.66	0.03	0.69	0.85	0.86

[a]Calculations and notes as in Table 33a.

clearly identified. The new values are comparable with, but uniformly lower than, the earlier estimates for most ages, especially in the first 2 years of life. It is notable that although the requirement falls very rapidly up to 2 years of age, thereafter the decrease towards the adult value is very slow.

9.4 Amino acid requirements from infancy to 18 years

9.4.1 *Infants up to 6 months*

In the 1985 report (*1*) the amino acid requirements of infants were evaluated in terms of a comparison of their content in both formula and breast milk, plus available experimental evidence from Holt & Snyderman (*34*) and Fomon & Filer (*35*). This resulted in the choice of breast milk content as an appropriate requirement estimate. Dewey et al. (*2*) reconsidered the infant values on the basis of factorial calculations of maintenance and growth, based on values that were conservative (i.e. would overestimate rather than underestimate). This showed that breast milk (at intakes of 800 ml) provided on average a 45% excess of indispensable amino acids at 0–1 month and a 61%

excess at 1–3 months. Because of this, and given that intakes of breast milk from a healthy well-nourished mother are considered to satisfy protein requirements for the first 6 months of life, the Consultation endorsed the 1985 report in recommending the breast milk content of amino acids as the best estimate of amino acid requirements for this age group. However, it must be recognized that such intakes may be generous compared with actual demands. The amino acid composition of mixed human-milk proteins is shown in Table 34, and the amino acid intakes of breastfed infants calculated from these composition values are shown in Table 35. These values are calculated from the amino acid content of proteins in breast milk, with protein calculated as 75% of total nitrogen (as in Table 32). Since the non-protein nitrogen component of milk includes some free amino acids, the intakes shown in Table 35 will be slight underestimates of overall intakes.

Table 34
Amino acid composition of mixed human milk proteins[a]

Amino acid	Mean (mg amino acid/g total proteins)	Standard deviation
Lysine	69	9
Threonine	44	6
Methionine	16	3
Leucine	96	12
Isoleucine	55	8
Valine	55	8
Phenylalanine	42	14
Tryptophan	17	3
Histidine	21	5
Tyrosine	52	8
Arginine	23	3
Proline	80	11
Cysteine	17	3
Glycine	23	3
Glutamate + glutamine	178	19
Aspartate + asparagine	90	11
Alanine	38	5
Serine	50	7

[a]Average and standard deviation derived from references *36–38*.

9.4.2 *Older infants and children*

For older infants and children, the 1985 report (*1*) identified values for the amino acid requirements of preschool children and schoolchildren, but commented on the limited and unsatisfactory nature of the information available.

Table 35
Indispensable amino acid intakes of exclusively breastfed infants

Average amino acid content of breast-milk protein (mg/g)[a, b]									
	His	Ile	Leu	Lys	SAA	AAA	Thr	Trp	Val
	21	55	96	69	33	94	44	17	55
Age (months)	Amino acid intakes (mg/kg per day)[c]								
1	36	95	165	119	57	162	76	29	95
2	26	69	121	87	42	118	55	21	69
3	23	60	105	75	36	102	48	19	60
4	21	54	95	68	33	93	44	17	54
6	20	52	90	65	31	88	41	16	52

[a] Values from Table 34.
[b] His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; SAA, sulfur amino acids; AA, aromatic amino acids; Thr, threonine; Trp, tryptophan; Val, valine. Values derived from the protein content values in Table 32 (75% crude protein) multiplied by the average amino acid content as mg/g protein.

Further consideration of these values was given in a subsequent report on protein quality evaluation by FAO/WHO (*39*). Whereas the values for schoolchildren (*40–44*) were considered flawed (see *45*), the values reported for preschool children (*46*) were adopted as the basis of a scoring pattern within the protein digestibility-corrected amino acid score methodology for all ages, as an interim measure until more satisfactory values could be defined. However, these values for preschool children are difficult to interpret. They have not been peer reviewed and derive from a report that gives incomplete information about their origin. In particular, the limited details that are given, e.g. for lysine, suggest nitrogen accretion rates that are several-fold greater than expected for children of this age (see *45*), with values overall corresponding more closely to the needs of the 3–6-month-old infant than to those of a preschool child.

In the absence of secure values, a factorial approach was adopted, as suggested by Dewey et al. (*2*). Further support for adopting this factorial approach is derived from studies with stable isotopes of total branched-chain amino acid requirements, performed with the same indicator amino acid oxidation technique (see section 7) in adults and in children (*47, 48*). Briefly, the estimate obtained for total branched-chain amino acids in adults was 144 mg/kg per day, which represents the maintenance value. The growth component in the 6–10-year-old children studied by Mager et al. (*48*) was calculated to be approximately 10 mg/kg per day, and hence the factorial estimate of total branched-chain amino acid requirements in children was 144 + 10 = 154 mg/kg per day, which was not significantly different from the experimentally determined estimate for total branched-chain amino acids of 147 mg/kg per day.

The factorial approach, based on the maintenance and growth components of the protein requirement, was used to estimate amino acid requirements, on the assumption that (a) the pattern of maintenance amino acid requirements for infants and children is the same as that for adults, and (b) the pattern of amino acid requirements for growth is given by the amino acid composition of the whole body. On this basis, amino acid requirements are calculated in Table 36 as the sum of amino acids needed for maintenance, i.e. the maintenance protein requirement (g/kg per day) times the adult scoring pattern (mg/g protein), plus growth, (i.e. the tissue deposition rates in g/kg per day), adjusted for an efficiency of deposition of 0.58 (from Table 26) times the assumed human tissue pattern (mg/g protein). Scoring patterns are then shown as the requirement for each indispenable amino acid divided by the protein requirement for the selected age groups.

Table 36
Amino acid requirements of infants, children and adolescents (males and females combined)

			His	Ile	Leu	Lys	SAA	AAA	Thr	Trp	Val
Tissue amino acid pattern[a]			27	35	75	73	35	73	42	12	49
Maintenance amino acid pattern[b]			15	30	59	45	22	38	23	6	39
Protein requirements (g/kg per day) for			Amino acid requirements (mg/kg per day)[d]								
Age (years)	Maintenance	Growth[c]									
0.5	0.66	0.46	22	36	73	64	31	59	34	9.5	49
1–2	0.66	0.20	15	27	54	45	22	40	23	6.4	36
3–10	0.66	0.07	12	23	44	35	18	30	18	4.8	29
11–14	0.66	0.07	12	22	44	35	17	30	18	4.8	29
15–18	0.66	0.04	11	21	42	33	16	28	17	4.5	28
>18	0.66	0	10	20	39	30	15	25	15	4.0	26
			Scoring pattern (mg/g protein requirement)[e]								
0.5			20	32	66	57	28	52	31	8.5	43
1–2			18	31	63	52	26	46	27	7.4	42
3–10			16	31	61	48	24	41	25	6.6	40
11–14			16	30	60	48	23	41	25	6.5	40
15–18			16	30	60	47	23	40	24	6.3	40
>18			15	30	59	45	22	38	23	6.0	39

His, histidine; Ile, isoleucine; Leu, leucine; SAA, sulfur amino acids; AAA, aromatic amino acids, Thr, threonine, Trp, tryptophan; Val, valine.

[a] Amino acid composition of whole-body protein (*37*).
[b] Adult maintenance pattern (see section 8).
[c] From Tables 32 and 33, calculated as average values for the age range growth adjusted for protein utilization of 58%.
[d] Sum of amino acids contained in the dietary requirement for maintenance (maintenance protein x the adult scoring pattern) and growth (tissue deposition adjusted for a 58% dietary efficiency of utilization x the tissue pattern).
[e] Amino acid requirements/protein requirements for the selected age groups.

A comparison of the new factorial values for preschool children (aged 1–2 years) with previous values for this age group is shown in Table 37. The values for preschool children were adopted as a scoring pattern for all ages in the 1991 report on protein quality evaluation (*39*) because at that time no satisfactory adult values could be identified. The comparison shows that the new values represent on average 73% of the values reported for this age group in the 1985 report (70% for lysine). However, the new scoring pattern for children at this age is closer to the 1985 scoring pattern, being on average 94% of the previous values (91% for lysine). This is because these new values employ a scoring pattern based on the amino acid requirements divided by the average protein requirement, rather than the safe requirement employed in the 1985 report. Furthermore the new protein requirement for this age group is similar to that in the 1985 report (*1*).

After the age of 2 years there is very little further change in requirement or pattern until adulthood is reached. Thus for children aged over 2 years and adolescents, given the minor contribution that growth makes to the requirement for these age groups, the scoring pattern differs from that of adults to only a minor extent. For this reason, when judging protein quality for schoolchildren and adolescents, it is probably more practical to use just one pattern, i.e. that derived for the age group 3–10 years. The implications of these new scoring patterns in relation to dietary protein quality evaluation are discussed in section 6.

Table 37
Comparison of factorial requirements for preschool children with previous values

	Ile	Leu	Lys	SAA	AAA	Thr	Trp	Val
Amino acid requirements (mg/kg per day)								
1985 report *(1)*, preschool children (2 years)	31	73	64	27	69	37	13	38
New values, 1–2 years old	27	54	45	22	40	23	6.4	36
New/old	0.87	0.74	0.70	0.81	0.58	0.62	0.49	0.95
Requirements pattern (mg/g protein)								
1985 report (1), preschool children	28	66	58	25	63	34	11	35
New values,1–2 years old	31	63	52	26	46	27	7.4	42
New/old	1.11	0.95	0.90	1.04	0.73	0.79	0.67	1.20

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10. Catch-up growth

Weight deficits in children can be categorized in terms of thinness (weight-for height) and shortness (height-for-age) with wasting and stunting defining severe thinness and shortness, i.e. more than 2SD below the appropriate reference value (*1–4*). While these two kinds of deficit may often be found together in the same child, they represent separate biological processes and are statistically independent. Acute severe malnutrition that results in wasting makes a large contribution to infant mortality (*5*, *6*), with a peak prevalence in the second year of life. This coincides with the introduction of weaning foods and a high incidence of diarrhoeal disease (*7*). The natural history of stunting is different, with slowing in linear growth typically beginning within 3 months of birth and continuing for 2 or 3 years (*1*). Up to 60% of preschool children in developing countries are stunted in relation to the United States National Center for Health Statistics standards (*8*). Prevalence is highest in the 2-year-old and is associated with increased morbidity and mortality (*9*). As stated in the previous report (*10*), both wasting and stunting are principally environmental in origin, reflecting the combined effect of infections and poor nutrition. The removal of these adverse influences allows catch-up growth in both weight-for-height and height-for-age. However, it has not been entirely resolved whether complete catch-up growth in height of stunted children is achievable regardless of age (*1*, *11–13*), or why differences exist between countries in the extent to which restoration of height deficits appear to occur prior to adulthood (*1*, *3*).

In children with a weight deficit, once the factors responsible for growth inhibition have been removed, the rate and extent of catch-up growth will vary according to the nature of the deficit and the energy density, protein and other nutrient content of the food provided or available (*14–16*). As discussed in the 1985 report (*10*), the energy and protein requirements for catch-up growth have been evaluated in some detail (*17–19*), and reviewed further by Dewey et al. (*20*). In the wasted child in a clinical setting, after initial management with a low-protein maintenance regime and associated treatment (*21*, *22*), catch-up in weight can be extremely rapid and appears to be limited only by the amount of nutrient substrates that the infant can consume, with energy density most likely to be rate-limiting in otherwise nutritionally

complete formulae. Thus severely malnourished wasted infants become voraciously hungry and can achieve very high rates of weight gain, up to 20 times the normal rate, with energy-dense formulae provided on demand. This enables rapid restoration of appropriate weight-for-height (*1*, *16*, *23*). However, such rates of catch-up growth may be less easy to achieve with dietary interventions in community settings (*24*), although rates of catch-up growth at up to eight times the average daily growth rate have been described in the Gambia (*25*).

10.1 Protein requirements for rapid weight gain in the wasted child

While factorial calculations of the requirements for weight gain are relatively straightforward, they do require assumptions to be made about the composition of the weight gain (lean or adipose tissue), the magnitude of the maintenance values for protein and energy, the efficiency of both dietary protein and energy utilization, and the efficiency of net protein and fat deposition. Golden (*26*) has attempted to formalize such predictions of rates of weight gain in terms of its composition during catch-up growth, deriving equations for energy, protein and the protein:energy ratio of requirements as a function of weight gain and its composition. In fact, while such predictive tools are useful, especially in terms of predicting the energy requirements for weight gain, their predictive value for protein can be quite poor, especially during rapid weight gain. This is because the prediction equations depend on a single fixed value for the efficiency of protein utilization (e.g. 70%), as opposed to the wide range of actual efficiency values that can and do occur in practice. During rapid weight gain in children, there is ample evidence for a wide variation in the rate and the composition of the weight gained, suggesting marked variability in nitrogen utilization which is not a function of biological value or digestibility. Some of this variability relates to nutrient limitation for lean tissue growth (e.g. by zinc), but other factors are involved which are less well understood. Whatever the reasons, observed growth rates cannot be assumed to conform to predicted outcomes. In a study of rapid weight gain (*27*), previously malnourished infants were fed two levels of protein intake (3.1 g/kg per day and 4.6 g/kg per day) and slightly different energy intakes (165 kcal/kg per day and 154 kcal/kg per day). Growth rates were 16 g/kg per day and 12 g/kg per day, respectively: i.e. the observed weight gain was similar to that predicted in terms of energy intakes, but was not consistent with that predicted from protein intakes or dietary protein:energy ratio. In the latter case, the higher protein intake would have been predicted to result in a higher rate of weight gain. Thus the efficiency of protein utilization did not conform with the assumed values, decreasing with the higher protein: energy ratio. In careful studies of energy balance during rapid weight gain in malnourished children fed to satiety with a single energy-enriched

formula, Spady et al. (*17*) reported rates of weight gain that varied by up to more than 20 g/kg per day. The average value for energy deposition (intake less expenditure) was 3.3 kcal/g weight gain, equivalent to 14% protein (at 5.67 kcal/g gross energy) and 27% fat (at 9.36 kcal/g gross energy), with individual values ranging from 1.2 kcal/g to 5.7 kcal/g. This implies that lean tissue varied between about 100% and 50% of weight gain. Direct studies of muscle mass during rehabilitation of similar children at the same centre confirmed a wide variation (10–82%) in its contribution to weight gain.(*18*).

Table 38 shows examples of rates of weight gain in malnourished infants during catch-up as a function of protein and energy intakes, based on observed responses in malnourished wasted infants (*17*). The calculations assume a

Table 38
Protein and energy needs for catch-up growth at different rates of weight gain

	Typical composition of weight gain[a]			**High rate of fat deposition[b]**		
Net growth costs (kcal/g)[c]	**3.29**			**5.12**		
Gross growth costs (kcal/g)[d]	**4.10**			**5.99**		
	Dietary requirements					
Rate of gain (g/kg per day)	Protein[e] (g/kg/day)	Energy[f] (kcal/kg/day)	Protein/ energy (%)	Protein[g] (g/kg/day)	Energy[h] (kcal/kg/day)	Protein/ energy (%)
1	1.02	89	4.6	1.0	91	4.2
2	1.22	93	5.2	1.1	97	4.5
5	1.82	105	6.9	1.5	115	5.2
10	2.82	126	8.9	2.2	145	6.0
20	4.82	167	11.5	3.6	205	6.9

[a] 73:27 lean:fat equivalent to 14% protein and 27% fat.
[b] 50:50 lean:fat equivalent to 9.6% protein and 50% fat.
[c] Based on 5.65 kcal/g protein and 9.25 kcal/g fat.
[d] Net costs adjusted for a 90% and 73% metabolic efficiency of fat and protein deposition respectively (*28, 29*), plus metabolizable energy of additional non-utilized protein.
[e] 14% deposited tissue adjusted for a 70% efficiency of utilization plus the safe level of maintenance at 1.24×0.66 g/kg per day = 0.82 (see section 11).
[f] Maintenance energy at 85 kcal/g (which includes maintenance protein energy) + gross energy costs at 4.10 kcal/g weight gain.
[g] 9.7% deposited tissue adjusted for a 70% efficiency of utilization plus the safe level of maintenance at 1.24×0.66 g/kg per day = 0.82 g/kg per day; 1.27×0.58 g/kg per day = 0.737 (see section 11).
[h] As in footnote "f" except that gross energy costs are 5.99 kcal/g weight gain.

maintenance (zero growth) energy requirement of 85 kcal/g, derived from the intercept value of the regression of metabolizable energy intake on growth rates.

Two values for tissue composition are shown, equivalent to 3.3 kcal/g and 5.1 kcal/g net growth costs, i.e. the average value equivalent to 14% protein, 27% fat, and a high-fat value of 9.6% protein, 50% fat. The average value contains slightly more fat than that of normal tissue composition, i.e. 18% protein and 20% fat, as reported for normal infants between 9 months and 2 years (*30*). Thus total (gross) energy requirements for growth with the two outcomes shown, based on maintenance, deposited energy, the metabolic costs of protein and fat deposition, and the metabolizable energy of excess dietary protein not deposited, are 4.1 kcal/g and 6.0 kcal/g weight gain, so rates of weight gain would be slower in the latter case for a given energy intake. The factorial model for protein requirements is that used for normal children, discussed in section 9. The safe level for maintenance protein requirement is calculated from the value for maintenance identified in section 9 for infants and children >6 months (0.66 g protein/kg/per day) adjusted to a safe intake according to a coefficient of variation of 12% (i.e. 0.82 g/kg per day), and with the efficiency of dietary protein utilization for deposition assumed to be 70%, assuming that dietary protein utilization for growth is high during catch-up. This may underestimate actual rates of protein deposition if dietary protein utilization is more efficient, but in any case there is considerable uncertainty about the actual efficiency of both protein utilization and the metabolic costs of growth (*28*), especially in malnourished infants (*19*).

Several points are noteworthy with regard to the estimates in Table 38. The composition of the deposited tissue influences its energy density, the dietary energy requirement for growth and the consequent weight gain achievable for a given amount of food energy. The two examples equate to energy density values of 4.1 kcal and 5.99 kcal dietary energy per g of weight gain, which can be compared with the rounded value of 5 kcal/g quoted in the 1985 report. The highest rates of growth in the examples require 88–125% additional energy compared with a normal child, according to the level of fat repletion. Thus, with standard feeds (usually about 12% protein:energy), although rapid catch-up can be achieved, e.g. 12 g/kg per day (*27*), intakes are likely to be limited by bulk unless their energy density is increased by additional oil or sugar (*21*, *23*, *27*). High intakes of standard high-protein feeds are also likely to be limited by a blunting of appetite by dietary protein in excess of that needed for growth (*19*, *31*). However, with energy-dense feeds, although intakes will usually be self-limited once appropriate weight-for-height is restored, there is considerable potential for excess energy intakes and consequent development of excess adiposity.

For these children during rapid recovery, a high value of 70% for the efficiency of protein utilization was assumed, compared with the value assumed for normal children of 58%. The protein content of the required intake to achieve these high rates of weight gain is generally considerably less than that of standard formulae, except at very high rates of net deposition of relatively lean tissue.

In practice, the relative deposition of fat and lean is not uniform during recovery, so that weight gain on a given intake may be faster than expected initially, because of higher lean tissue deposition, and slower subsequently as a greater proportion of fat deposition occurs. Thus, as reported (*27*), malnourished infants fed a high-protein standard formula (protein:energy ratio = 0.12) gained weight initially at 7 g/kg per day on energy-limited intakes of 99 kcal/kg per day and 3.0 g/kg per day protein, and at 12 g/kg per day on intakes of 155 kcal/kg per day and 4.6 g/kg per day protein. This implies a predominance of lean tissue gain, with an energy equivalent of weight gain in the initial phase of about 2 kcal/g, increasing to 5.9 kcal/g subsequently as relative deposition of fat increases.

The protein:energy ratios shown in Table 38 relate to the specific examples of growth rates and tissue composition shown, and will differ from safe or reference protein:energy ratios derived to take account of inter-individual variability in protein and energy requirements (see section 5).

10.2 Catch-up in height in stunted children

In contrast to the potentially rapid catch-up of weight-for-height of wasted infants, rates of catch-up height deficits associated with low height-for-age will be much slower, occurring over a much longer time span, as the height deficit is restored (*1–3*). Indeed, when wasting and stunting coexist, whereas rapid catch-up in weight-for-height occurs immediately with appropriate feeding, the peak velocity for height growth does not commence until after restoration of weight-for-height. There are two important issues relating to the protein requirements for catch-up in height.

The first is the dietary provision for the additional tissue deposition in excess of normal growth. Requirements for this slow phase of catch-up will clearly depend on the rate at which such catch-up in height-for-age occurs, but in any case needs are likely to be modest, involving amounts associated with the lower rates of weight gain shown in Table 38. Worked examples reported in the 1985 report (*10*) and by Dewey et al. (*20*) for a stunted 2-year-old recovering over 6 months indicate growth rates of no more than 2 g/kg per day, which would require an extra 8–10 kcal/kg of a relatively low-protein feed providing less than 5% protein calories. Such amounts of energy are probably within the normal variability of intakes associated with variable physical

activity and energy expenditure, whereas diets that are nutritionally adequate in terms of micronutrients and minerals will provide protein intakes considerably in excess of such needs.

The second and probably more important issue in terms of the protein requirement relates to the dietary protein concentration necessary to provide the optimal regulatory stimulus or anabolic drive for linear growth. In fact, the nutritional regulation of and requirements for normal height growth remain poorly understood, as does the role of specific nutrient deficiencies in stunting. One reason for this is that specific deficiencies of those nutrients likely to be involved, the Type 2 nutrients defined by Golden (*32*), which include protein, zinc, potassium, etc., are all difficult to identify. Nevertheless, there is now clear experimental evidence from animal studies (*33*, *34*) that dietary protein provides an important anabolic drive for linear bone growth. The importance of this for human linear growth is suggested by the several intervention studies with additional protein-rich foods, which have shown increased linear growth (*35–38*). Although specificity for protein has yet to be shown conclusively in studies of linear growth in humans, these dietary supplementation studies suggest a similar specific stimulatory effect of protein on linear bone growth, as observed in animal studies at intakes in excess of basal needs (*33*), mediated through IGF-1(insulin-like growth factor) (*35*, *36*, *39*). Unfortunately there is very little evidence that allows the dietary protein effect to be quantified. While Kabir et al. (*35*, *36*) increased linear growth in 2–4-year-old children by increasing the protein:energy ratio of a standard diet from 7.5% to 15%, Fjeld, Schoeller & Brown (*23*) reported faster linear growth with 11% compared with 8% protein calories. Malcolm (*38*) markedly stimulated height growth by increasing the protein:energy ratio of children's diets from 0.043 to 0.084, with evidence that such a diet was energy- rather than protein-limited, judging by the fall in adiposity that accompanied the accelerated linear growth.

Taken together these studies strongly suggest a dietary protein requirement to enable satisfactory catch-up, or even normal linear growth, which is greater than the requirements for maintenance and tissue deposition developed by the factorial model discussed in section 8 and Table 38. However, without dose–response and intervention studies that are controlled for other nutrients, it is not possible to identify the optimal dietary level of protein. Nor is it possible to determine whether the level is higher than intakes likely to be achieved by mixed diets providing appropriate levels of other nutrients, or by a meat-free western vegetarian diet containing about 10% digestible protein calories (*40*, *41*).

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11. Influence of infection on protein and amino acid requirements

Nutrition and infection interact with each other synergistically (*1*). Recurrent infections lead to a loss of body nitrogen and worsen nutritional status; the resulting malnutrition, in its turn, produces a greater susceptibility to infection. In children, linear growth and weight gain, which are important indices of child health, are lower in underprivileged communities (*2–5*). This reduced growth rate, which leads to stunting in later life, is associated with long-term effects, including decreased productivity and functional deficits (*6*). The cause of this reduction in growth rate is likely to be multifactorial, reflecting the interactions of a poor diet and a poor environment (*7*), and particularly the consequences of bacterial infections and parasitic infestations (*8*).

Infections usually cause a set pattern of metabolic and clinical changes in individuals. The metabolic pattern, which includes hypermetabolism, a negative nitrogen balance, increased gluconeogenesis and an increased fat oxidation, is modulated by hormones, cytokines and other pro-inflammatory mediators (*9*), and is usually compounded by a reduced food intake. The clinical features include fever and, depending on the site of infection, such symptoms as cough or diarrhoea, compounded by nutritional changes and symptoms. In terms of endogenous protein availability for the metabolic processes, the major reservoir in the lean body mass is represented by muscle, as visceral mass is usually preserved in infection (*10*). The total body skeletal muscle mass varies in different populations, based on ethnicity or prior nutritional status (*11, 12*), and the negative nitrogen balance response after injury tends to be higher in muscular well-nourished individuals than in malnourished individuals (*13–15*). Significant reductions in lean tissue or, by extension, the muscle mass will attenuate the immunological response, as well as reduce physical activity. The provision of protein in the diet would meet some of the requirements for amino acids for the immune and restorative response, and this section explores the influence of infection on daily protein and amino acid requirements. The influence of infection on protein nutritional status has been extensively reviewed by Scrimshaw et al. (*14*), for different pathogens and nutrients.

11.1 Pattern of the protein and amino acid response to infection

Injuries or infection lead to an increased nitrogen loss from the body. The specific response to bacterial or viral infections, in terms of the nitrogen balance, has been reviewed by Powanda & Beisel (*16*). The catabolic response of adults to infections with different organisms such as bacteria, rickettsiae and viruses was prospectively evaluated by metabolic balance studies, during exposure as well as during overt infection. The infections were treated by antibiotics in the more severe cases. There was a loss of nitrogen in all cases, and the loss of nitrogen was proportional to the number of days the subjects had fever (*16*, Table 39), and was most serious with bacterial infections such as typhoid fever. Although the intake of the subjects fell with the illness, pair-fed healthy controls showed that only about a third of the infection-induced nitrogen loss could be attributed to dietary restriction (*17*). This therefore suggests that dietary supplementation could directly reduce part of the negative nitrogen balance observed. The striking feature of these data is the dependence of the negative nitrogen balance on the duration of fever, and negative nitrogen balance was not observed until the febrile response began. The negative nitrogen balance nevertheless persisted for days after the fever had subsided. In the mildest viral fever studied (sandfly fever, in which the febrile response was mild at 100 °C, and lasted for only 3 days), subjects took 10–11 days to recover from negative nitrogen balance when offered normal amounts of dietary protein and energy (*16*). In general, there is a catabolic response with increased nitrogen losses from the body, and Wilmore (*9*) has summarized the pattern of the catabolic response to infection with the following observations:

- The increased nitrogen loss occurs via the urine, mainly as urea, although it is possible that with fever and sweating, there may be significant losses through sweat (*14*).
- There is a dose response in that the greater the infection (in terms of fever), the more extensive the nitrogen loss.
- More nitrogen is lost from a well-nourished individual than a depleted patient following a comparable insult.
- The response is not constant, and follows a time course, increasing to a peak and then gradually returning to equilibrium.

Other responses also occur in the infected individual. The catabolic response is characterized by nitrogen loss and the flux of amino acid nitrogen from muscle. The principal amino acids released from muscle are alanine and glutamine (*18, 19*), although the amount released is greater than the amount contained in muscle protein. Therefore, there is de novo synthesis of these amino acids in muscle, using nitrogen from essential amino acids, with

Table 39

Severity of infection and nitrogen loss[a]

Infection	Nitrogen loss (g)	Average fever duration (day)
Severe typhoid	186	25
Moderate typhoid	87	15
Tularemia	52	6
Q fever	40	5
Sandfly fever	16	3

[a]Adapted from reference *16*.

an increased loss of skeletal muscle protein. This is evident in severe or prolonged infection as wasting of muscle. The export of amino acid nitrogen from muscle meets the needs of gluconeogenesis in the liver; the conversion of alanine to glucose results in nitrogen residues which are then excreted. Glutamine is a preferred fuel for enterocytes and immune cells such as lymphocytes and macrophages. In the kidney, glutamine supplies ammonia which combines with filtered hydrogen ions to form ammonium ions which are then excreted.

There is also a reduction in amino acids in the plasma and in skeletal muscle. In general, the majority of plasma amino acids show a decline early in the infection (*20, 21*), preceding the onset of fever (*22*). A decline in plasma glutamine is also seen (*23*). In critical illness, plasma levels of leucine, lysine, threonine, histidine and glutamine have been observed to fall (*24*). In contrast, plasma phenylalanine and tryptophan levels have been found to rise (*21, 24–26*). Acute-phase proteins, which are produced by the liver in response to infection, have a high phenylalanine and tryptophan concentration, and it may be that the increased plasma phenylalanine and hepatic uptake will fuel this process. These changes in plasma amino acid levels tend to persist until the infection subsides, and are associated with an anabolic response through cytokine-stimulated increase in the synthesis of positive acute-phase proteins by the liver (*27, 28*). Changes are also evident in muscle concentrations of proteins and amino acids. Total free amino acids and glutamine concentrations have been observed to fall by more than half during critical illness associated with bacteraemia. In contrast, branched-chain amino acid and aromatic amino acid concentrations increased with critical illness (*25*).

Tuberculosis is now a major cause of infection worldwide, particularly in underprivileged populations. It is estimated that 3−4 million people die of tuberculosis every year (*29*). The protein kinetic response in tuberculosis mimics, in general, the response to other infective insults; however, in a study of patients with tuberculosis (just diagnosed, without treatment), it was also

found that there was a reduced rate of muscle synthesis in response to feeding in the patients when compared with body mass index-matched controls (*30*). This anabolic block in response to feeding may be one of the reasons for the accelerated weight loss seen in these patients. The excessive production of sputum and expectoration may also be an additional source of protein loss (*31*). In patients with melioidosis, another chronic disease associated with wasting, protein turnover was increased by nearly 40% compared with uninfected subjects; however, in contrast to tuberculosis, the net catabolic rate was not significantly changed, nor was an anabolic block in response to feeding observed (*32*).

11.2 Implications of HIV/AIDS

The wasting that occurs in human immunodeficiency virus (HIV) infection and acquired immunodeficiency syndrome (AIDS) results in the loss of fat and lean tissue, and is attributable to a number of factors. The weight change is usually more evident with the onset of secondary infection, but may also precede it (*33*). This suggests that weight loss is attributable to the primary HIV infection as well as to the opportunistic infections that occur. HIV-induced weight loss therefore has to be evaluated in symptom-free HIV-positive patients, since the effects of opportunistic infections will synergistically alter protein kinetics. The primary pathology in HIV infection is a decrease in CD4 T lymphocytes, leading to an immunodeficiency that results in varied clinical presentations. The loss of lean tissue is related to survival time, as was shown by studies on total body potassium in these patients (*34*). The cause of the weight loss is related to a negative energy balance, linked more to the decrease in energy intake than an increase in energy expenditure (*35, 36*). The decrease in energy intake is probably associated with poor nutrient absorption. Another reason for the weight loss, and specifically the loss of lean tissue, is the net protein catabolic response, which is observed even when adequate amounts of protein are fed. Among HIV-positive subjects, those known to be at a higher risk of poor outcome were likely to have poor dietary intakes (*37*).

The weight loss in HIV patients tends to be episodic (*36*), with periods of stability between periods of weight loss associated with secondary infections. Approximately 60% of the weight lost is lean tissue (*38*). Since this loss of lean tissue is associated with survival, the aim of nutritional therapy should be to restore the lean mass. Simply providing protein does not seem to help in this process, and one study has shown that the benefit of resistance exercise in this regard outweighs the benefit of protein (whey) alone, in terms of increasing muscle mass, strength and quality of life indices (*39*).

Glutathione, which is present in high concentrations in mammalian tissues, has many diverse functions related to the protection of cells (*40, 41*). With regard to HIV, glutathione levels are low in plasma and other body fluids (*42, 43*), and this is clinically significant, since there is a strong association between survival and glutathione deficiency in CD4 cells in HIV patients (*44*). The deficiency of glutathione in HIV patients is in part attributable to a decreased synthesis rate (measured by erythrocyte glutathione synthesis), and this reduced rate of synthesis can be increased by about 25% with the supplementation of cysteine (*45*). Other approaches to measuring the adequacy of glutathione synthesis have also shown that symptom-free HIV patients are unable to increase their glutathione synthesis rates when given low doses of acetaminophen (*46*). The low glutathione levels in HIV-positive patients could be the result of an increased demand for the anti-oxidative property of this molecule; however, it does not seem that glutathione removal is increased in these patients (*42*). Rather, it is the decreased synthetic ability, possibly related to the inadequate supply of precursors for synthesis, that is responsible. Low intracellular concentrations of cysteine have been reported in HIV infection (*47, 48*), and erythrocyte glutathione synthesis rates increased with cysteine supplementation (*45*), suggesting that it is a cysteine deficiency, even with an adequate dietary protein intake, that is primarily responsible for the glutathione deficiency.

The rate-limiting amino acids in HIV infection can be reasonably identified as those that would have the lowest plasma concentration when compared with controls, after an amino acid infusion (*49*). Based on this paradigm, it was found that the plasma levels of threonine, valine, lysine and methionine remained low after a short amino acid infusion in HIV-infected individuals. However, the pattern of amino acid response would at least depend on the amount of amino acids infused relative to body size (in this case a fixed amount was infused), the route of infusion, the prior diet of the individuals, the proportions of amino acids in the mixture, and the hormonal milieu in the subjects. The insulin levels, for instance, increased more in the HIV-positive subjects (*49*). Nevertheless, this study remains the only attempt to identify the limiting amino acids in the requirement for HIV-infected patients.

11.3 Recommendations for a protein allowance in infection

Meeting the increased requirement for nutrients and protein in infection does not imply a complete therapeutic strategy. In general, infections need to be treated appropriately, with nutrition as an adjunct to the treatment. Severe infections need appropriate antibiotic treatment, and the accompanying fever, which increases the demands for nutrients, has to be treated.

In general, the increased allowance in acute infection aims to cover the losses sustained, and extends into the entire convalescence. The anorexia that accompanies illness prevents additional protein intake, and aggressive feeding of protein during the illness is counterproductive. In chronic infections, for instance tuberculosis, the aim is to replace the additional daily loss that prolongs the course of the illness and its treatment.

A greater concern at the population level is the situation of individuals in environments in which persistent immune activation, along with a decline in intestinal absorptive capacity, may be present, which is not manifested as an overt clinical syndrome but which will still increase the demand for protein (*50*). There is also a need to consider the allowance in chronic infections such as HIV and tuberculosis, which are widely prevalent in the world today.

From the viewpoint of chronic infections, an analysis of the nitrogen loss in these conditions based on kinetic data is presented in Table 40. Three conditions, tuberculosis, HIV and melioidosis (*30, 32, 35*), in three different countries, studied by the same technique using leucine kinetics as the marker for protein kinetics, have been compared. Since two of the studies presented oxidation data normalized to the lean body mass, these data were recalculated for body weight, so that all studies were comparable. Although these were short-term studies, measured over 4 hours in the fasted and fed state, leucine balance values for the fasted and fed state could be calculated as the difference between intake and oxidation for the fasted and fed phase, and extrapolated to 12 hours in each case. The 12-hour fasted and fed balance were added to get a 24-hour value; the 24-hour leucine balance was converted into a nitrogen value (based on a leucine content of diet and body protein of approximately

Table 40

Requirements for protein in different acute and chronic bacterial infections

Condition	Allowance % protein requirement
Untreated tuberculosis	25
Partly treated melioidosis	15
HIV (symptom-free)	50
Mixed intestinal parasites	10
Acute bacterial infection (convalescence)	20
Acute diarrhoea (convalescence)	30
Sepsis	30

Data for tuberculosis, melioidosis, HIV and intestinal parasites (*30, 32, 35, 53*) based on calculations in text. Symptom-free HIV patients were in neutral nitrogen balance at a nitrogen intake about 50% above the safe level of intake. Data for acute bacterial and diarrhoeal infection from Scrimshaw et al. (*14*). The requirement is calculated based on replacing the extra losses throughout convalescence. Data for sepsis from Ishibashi et al (*51*).

8%), and the nitrogen balance so obtained represented the increased requirement. Based on this recalculation, patients with chronic bacterial disease did have an increased nitrogen loss, while those with HIV, who were symptom-free, did not. In the tuberculosis patients, the balance was about 45 mg nitrogen/kg per day more negative than the controls for that study. To use these data in coming to a recommendation, the assumption was made that the same systematic error in extrapolation was made in both control and infected groups. Then, the difference in nitrogen balance between the control and infected groups represents the extra nitrogen loss in each disease, and this could be expressed as a percentage of the nitrogen intake in each study, to arrive at an allowance for meeting the increased requirement level for nitrogen. With this method, the nitrogen requirement in tuberculosis was about 25% higher than in the controls when the nitrogen loss was assessed against the nitrogen intake (Table 40). The HIV patients were in nitrogen balance and similar to their controls, which might mean that there was effectively no need for a higher protein intake, according to the present analysis. However, both groups received a generous energy intake of 156 kJ/kg per day along with a nitrogen intake of 200 mg/kg per day, which was about 50% higher than the nitrogen equivalent of the safe protein intake of 0.83 g protein/kg per day. An important difference between the tuberculosis and HIV group was that the tuberculosis group were all clinically ill, many with fever and cough, at the time of the study. Additionally, most of the tuberculosis patients had not yet begun treatment, as they had just been diagnosed. In the other chronic bacterial disease of melioidosis, there was an increase in nitrogen loss of about 15% by the same method of calculation as detailed above. The lower loss in this group could be a result of their relative heterogeneity in pathology, as well as their having received antibiotics for up to 2 weeks before the study, although they were mildly febrile on the day of the study. They also had a higher energy intake than the tuberculosis patients (Table 40).

Taken together, these data would suggest that in chronic bacterial infections, the allowance for protein is of the order of about 25%, while in symptom-free HIV, nitrogen balance can be achieved with an increased nitrogen intake of about 50% above the safe level of 0.83 g protein/kg per day, with adequate energy intake. The replacement of protein in wasting conditions may be most effective when physical activity is encouraged, and when adequate amounts of energy are also given.

Another approach to approximating the extra protein allowance is to measure, by nitrogen balance, the nitrogen loss during the course of the infection. This approach has been used in relatively acute and limited bacterial conditions (*31, 50*). If the mean additional loss of protein is 0.6 g/kg per day, and if it takes 2–3 times the duration of depletion to replace this protein in an individual, then assuming the additional protein in the diet was available during

convalescence, it would require 0.2–0.3 g protein/kg to meet the increased demand for protein. This is about 20–30% of the normal requirement, depending on the age of the individual, since protein requirements vary with childhood and growth, as well as in the elderly. If the protein losses are higher, as they are in diarrhoea and dysentery, or typhoid fever, it would take even more protein to cover the losses. Thus if the losses are assumed to be 0.9 g protein/kg per day in diarrhoea or dysentery, then the allowance, assuming a 2–3 week convalescence, would be 0.2–0.3 g protein/kg per day (*31, 50*). However, in chronic disease, where the course of the illness is in months or longer, it is important to provide for the nitrogen loss completely on a daily basis. There will continue to be an increased requirement in convalescence as well as resulting from the repletion process, and it is quite possible that alterations in the efficiency of utilization will persist in the recovery period.

The protein loss experienced in more severe critical illness such as sepsis and trauma can be more dramatic, associated as it is with the altered metabolic profile of these patients. A careful study on critically ill patients who were haemodynamically stable found that when their protein intake was raised by 30% to 1.2 g/ kg per day, with the same energy intake, the protein loss or negative protein balance was reduced by half, to about 1 g/kg per day (*51*).

There has only been one set of studies that specifically set out to identify the lysine requirement in undernourished men who had intestinal parasites (*12, 52, 53*). In these studies, the lysine requirement was found to be about 50% higher in chronically undernourished men living in slums when compared with well-nourished controls coming from a high socioeconomic stratum with clean environments. Most of this increased requirement could be attributed to the presence of intestinal parasites, since following successful treatment, the subjects were in amino acid equilibrium at the normal level of lysine intake.

Overall, there are insufficient data to propose quantitative allowances for amino acids during infection. Several studies based on clinical outcome show that clinical benefit may accrue from supplementing the diet with specific amino acids, but unless dose–response studies are available, these remain of only qualitative use. It is important to recognize that the over-supplementation of amino acids (with or without whole-protein supplementation) also has its problems. In rats, the over-supplementation of amino acids (particularly threonine, methionine and branched-chain amino acids) leads to an increased severity of malaria infection (*54*), and certainly the supplementation of methionine in the diet will increase the need for glycine, as dietary supplementation studies with methionine in normal women receiving low-protein diets have shown an increased 5-L-oxoproline excretion (*55*).

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12. Implications of the protein and amino acid requirements for populations in developed and developing countries

The recommendations for both protein and amino acid requirements in this report are significantly changed as compared with the previous report. For protein, while the values for adults differ only slightly (i.e. 10% higher), the recommended values for infants and children are appreciably lower, i.e. a reduction from 1.57 to 1.14 g/kg per day for children at 1 year of age and from 0.99 to 0.91 g/kg per day for 10-year-olds. These changes reflect a re-assessment of the implications of breast-milk intakes for the factorial model of the requirements of infants and young children. Thus the model has predicted values for protein intakes which meet demands for maintenance and current best estimates of protein deposition, and which are close to but still higher than requirement values that would be predicted from protein intakes from breast milk, assuming that such intakes are associated with minimal risk of deficiency

For amino acids, the major change relates to the higher values recommended for adults and the use of a factorial model to calculate requirements of infants and children. This model involves requirement patterns derived by combining values for maintenance, assumed to be similar to the adult pattern, with the current best estimate of the amino acid composition of deposited tissue. In the 1985 report there was a marked fall in requirement values with age from preschool children to adults. However, with the factorial model for calculating requirements of infants and children, this age-related change is much less marked. Thus, values for preschool children are 40% to 60% of previous values for lysine, threonine and tryptophan, but values are much higher for adults – especially for lysine and threonine, which are 2.7 times higher. However, the scoring pattern for preschool children is closer to the previous values, 63% to 85% of previous values for lysine, threonine and tryptophan, because of the use of the mean rather than safe protein requirement value in the calculation. Taken together these changes mean that for adults, diets which might previously be judged as adequate could now possibly be judged as limiting in terms of their biological value: this would be the case for the lysine limitation of cereal-based diets. For infants and children, the new values mean that diets that might previously have been judged marginally inadequate in terms of protein may now be judged adequate.

The use of breast-milk intakes to help identify appropriate values for infants, with the implicit assumption that breast-milk protein intakes from healthy mothers represent the ideal intake for infants, provides some confidence in the new values. Furthermore, as extensively reviewed by Dewey et al. (*1*), there is experimental evidence to support the assumed adequacy of breast-milk protein intakes. From a prescriptive perspective, the fact that the final model chosen predicts a requirement somewhat higher than breast-milk intakes would suggest that there is a margin of safety in the new values. However, for infants and children after weaning, while there is no a priori reason why demands for maintenance should be higher, and while demands for growth will generally be less, it is likely that the efficiency of dietary utilization to meet demands will be lower with mixed diets.

As discussed in section 6, both digestibility and biological value in terms of amino acid score need to be taken into account when matching requirement values with intakes of specific foods; so for most foods and diets, intakes will usually need to be higher than the proposed requirement values. Furthermore, specific account will need to be taken of any additional needs associated with illness or catch-up after periods of growth failure, as discussed in section 10. The factorial model adopted in this report does not include any additional component for such eventualities, or for any extra allowance to take into account day-to-day variability in growth, which was a feature of the model adopted in the 1985 report. Against this background, it is worth examining the new values in the context of what is known about actual intakes and growth rates in children.

12.1 Protein intakes of infants associated with adequate growth rates

Prior to the 1985 consultation, because of major concerns that recommendations in the previous 1973 report were too low, a major international research programme was commissioned to investigate growth in relation to protein intakes of infants and children fed traditional diets. This information was mainly published in two *Food and Nutrition Bulletin* supplements (*2, 3*) and is reviewed in the 1985 report (*4*). The limited long-term balance data available at the time of the 1985 report was reviewed (see Table 41). These were studies at a fixed level of intake with foods commonly eaten by poor people in the countries represented. The studies in preschool children are shown in Table 41 in terms of the digestible protein intakes, compared with the new safe intake levels. All these children were poor, more often than not with a history of malnutrition and stunting. Furthermore, many had minor febrile and afebrile illnesses during the studies, which may have accounted for some children not gaining weight at the expected rates during the studies. The digestibility of these diets was below that recorded for diets based on milk and eggs. As argued in the 1985 report, interpretation of these long-term balance

figures is difficult, not least because they represent different age ranges. Nevertheless weight gain was in general satisfactory, with nitrogen accretion usually quite large compared with what would be expected. Given that these are representative diets, they could be viewed as a measure of the likely intakes.

It is clear from Table 41 that in all cases these intakes are higher than the safe intakes of high-quality protein derived by the factorial model in section 9. Thus it is reassuring that these diets provide a margin of safety compared with the safe intake, at least to the extent that they are representative of diets likely to be consumed at home.

Another important study is a 90-day growth trial designed to assess the nutritional adequacy of a hybrid maize variety (su_2:o_2) as the sole protein source in recovering malnourished children (*9*). This is a variety with a markedly improved amino acid profile, i.e. 50% higher lysine and 78% higher tryptophan compared with normal maize. In these 13–29-month-old stunted children, growth rates in height and weight were compared with similar children fed a modified cow-milk formula. Energy intakes were 110 kcal/kg, with the maize providing 100% of their protein and fat intakes (and 90% of their energy) over the 90-day trial. Since the trial focused just on the adequacy of the maize as a protein source, a complete supplement of vitamins and minerals was provided. Linear growth, gains in height-for-age, weight gain, and final sums of fat folds were not different between the two diets. After correcting for the digestibility of energy (85%) and protein (80%), the utilizable protein:energy ratio was 0.080, supplying 1.88 g/kg per day digestible pro-

Table 41
Results of long-term nitrogen balance studies in preschool children[a]

Age	Protein source	Digestible protein intake[b] (g/kg)	Requirement[c] (g/kg)
8–12 months[d]	Rice:fish 70:30	1.35	1.20
22–40 months[e]	Beans + corn:other veg 95:5	1.07	0.93
29–46 months[f]	Beans + corn + other veg: animal 82:18	1.46	0.89
2–5 years[g]	Wheat or rice + veg	1.39	0.89

[a] From FAO/WHO/UNU Table 35 (*4*).
[b] Corrected to digestibility of cow's milk.
[c] Safe level (approximate values interpolated from Table 33a).
[d] Tontisirin, Amanwra & Valyasevi (*5*).
[e] Torun & Viteri (*6*).
[f] Torun & Viteri (*7*).
[g] Begum et al. (*8*).

tein, with lysine and tryptophan at 43 mg/g protein and 9.1 mg/g protein compared with the new scoring pattern of 52 mg/g protein and 7.4 mg/g protein. Thus, on the basis that lysine was limiting, the protein digestibility-corrected amino acid score value of this diet would be about 0.66 (0.80×43/52). Given a safe protein requirement for a normal 1–2-year-old of 1.1 g/kg per day, the safe protein intake for this unsupplemented maize diet would be 1.5 g/kg per day (1.1/0.7). i.e. 80% of the actual intake. Clearly this diet was atypical; in practice, appropriate micronutrients would have been included by complementing the diet with legumes and other vegetables, which would also have improved the amino acid profile. But the study does show that a cereal-based diet can supply adequate protein for normal growth with a margin of safety compared with the estimated average requirement.

Whereas the above studies indicate that likely intakes that allow adequate growth may be greater than the new requirement values, this does not mean that lower intakes closer to the requirement would be adequate. In fact, there are no long-term balance studies at lower intakes similar to the requirement values. However, more information on the actual adequacy of these diets in relation to the requirement is provided by a further detailed examination of one study shown in Table 42 (*5*).

This is a 4-month study on the adequacy of usual Thai weaning food, with 8–12-month-old previously malnourished Thai infants fed diets based on rice, fish and vegetables with fat provided at 10% energy intake. The diets provided 99 kcal/kg initially, falling to 93 kcal/kg after 4 months. The nitrogen balance data, indicated digestibility of about 75%, so the protein intakes of 1.6–1.8 g/kg per day provided digestible protein intakes 20–30% higher than the predicted requirement values (safe levels). This allowed growth at rates somewhat greater than those of the reference cohort studied by Butte et al. (*10*) with initial rates of protein deposition, as judged from the nitrogen balance data, that were considerably higher than reference rates for infants at this age. The data in Table 42 suggest that these infants exhibited some catch-up growth or body composition changes initially, as judged by the marked fall over the period in protein deposition to values approaching the reference rates. However, it is clear that the biological value (calculated from the nitrogen balance data as utilized nitrogen/absorbed nitrogen) fell from 0.77 initially to 0.35 after 4 months because of increasing urinary nitrogen excretion with no decrease in growth rate. This suggests that the digestible protein intake at the end of this study was still in excess of the requirement.

Table 42

Growth and protein intakes of Thai children fed a traditional Thai weaning food compared with reference data and new requirement values

Age (months)	Observed data					Reference/ predicted data[a]		
	Weight (kg)	Weight gain (g/kg per day)	Digestible protein intake[b]	Protein gain[c]	Biological value	Body weight (kg)	Protein deposition	Protein requirement[d]
			(g protein/kg per day)				(g protein/kg per day)	
10–11	8.29	1.5	1.33	0.58	0.77	9.22	0.188	1.14
11–12	8.61	1.2	1.29	0.48	0.51	9.48	0.175	1.13
12–13	8.68	1.0	1.27	0.39	0.45	9.72	0.163	1.11
13–14	8.98	1.1	1.19	0.24	0.35	9.96	0.152	1.09

[a] See section 11.
[b] True digestibility = 0.74.
[c] Nitrogen balance × 6.25.
[d] Safe level.

12.2 Population intakes and the new requirement values

As indicated above, for adult populations the revised adult scoring pattern, with a higher value for lysine, means that cereal-based diets that are lysine limited, which might previously have been be judged to be adequate, could now possibly be judged as inadequate in terms of their biological value. This was the conclusion reached by Young & Pellett (*11*) on the basis of their analysis of amino acid intakes compared with a scoring pattern with a similar lysine level to the one proposed in this report. However, judging the adequacy of protein and amino acid intakes of adults requires an understanding not only of the protein concentration and amino acid pattern of the diet, but also of the overall intake level. Clearly, food intakes must meet energy needs before an evaluation of protein intake and quality becomes relevant, but there can be considerable variability of intake levels between population groups in energy balance, because of variation in lifestyles, physical activity and consequent energy needs. Thus, a dietary pattern which supplies adequate protein and amino acids to physically active individuals may become limiting for protein at lower intakes associated with a more sedentary lifestyle. Recognizing this principle, one approach to assessing the implications of these new requirement values is to compare estimates of a reference protein:energy ratio of the requirements with protein quality-adjusted protein:energy ratios of intakes, as recently reported (*12*). In this way, at least in theory, account can be taken of the influence of the varying energy needs and consequent food intakes of various groups on their protein intakes, assuming that they are meeting their energy needs from different diets.

In practice, as discussed in section 3 for the protein requirement, whereas a safe intake for an individual within a population can be defined as the 97.5th percentile of the requirement distribution (i.e. average +1.96SD), a single reference value that can be considered "safe" for a population cannot be calculated, since it will vary as a function of the relative SD values for the requirements and intakes. Indeed, when the variability within populations is larger than that of the requirement, the population "safe" intake may be equivalent to the mean requirement plus 3–4 SD values. Within the United Kingdom National Dietary and Nutrition Survey of the elderly, the SD of protein intakes/kg body weight in subjects with measured energy intakes greater than 1.3 × basal metabolic rate was about 22% (Millward, unpublished information), i.e. nearly twice that of the assumed SD of 12% for the protein requirement. This would imply a "safe" population protein intake of about the mean requirement plus 4 SD values.

Calculation of a reference protein:energy value involves added complexity because of the need also to consider the variability of the energy requirement. As discussed in section 5, the reference protein:energy ratio of intake for an

individual which is associated with a prevalence of deficit of <2.5% approximates to a protein:energy ratio based on an average protein requirement plus 3SD. Furthermore, a safe reference protein:energy ratio of intake for a *population* is greater than this. As recently argued (*12*), use of the safe *individual* protein:energy ratio to identify prevalence of deficiency within a population will underestimate deficiency, so diets judged inadequate with these values will certainly be inadequate. Use of these values also means that diets of populations judged marginally adequate could still be inadequate. Thus, a cautious approach needs to be adopted when judging risk of deficiency.

It is apparent from the comparisons of age-related changes in protein and energy requirements and calculation of the protein:energy ratios of the requirements (see section 5) that the reference protein:energy ratio increases with age, is higher for females than males, is higher for small than large adults at any age, and decreases with physical activity. This means that a sedentary elderly woman who weighs 70 kg would require food with more than twice the protein concentration relative to energy than that needed by very young children. It follows that a diet that can meet both the energy and protein needs of the infant may satisfy the energy needs of older children or adults, but may fail to meet their needs for protein at the level of consumption required to meet the needs for energy.

While a detailed analysis of the implications of these requirement values in relation to diets of developing countries is beyond the scope of this report, for the present purposes a guide can be obtained by evaluating the three diets shown in Table 43. These are the UK omnivore and vegetarian diets (*13*), and a value representing the average diet for India (*14*), which is typical of a cereal-based low-meat diet in a developing country. These are shown in terms of their available protein, adjusted by protein digestibility-corrected amino acid score values according to the age-related lysine scoring pattern and assumed values for digestibility.

Two comparisons have been made. First, in Figure 15, reference protein:energy values for an individual of a selected population group are shown, together with the protein quality-adjusted protein:energy values of the three representative diets. Second, on the basis of the average protein:energy ratios of the requirements (see section 5), estimates of the prevalence of population deficit (intake < requirement) by sex, age and activity level, for populations consuming each of these three diets, is calculated in Table 44 according to the principles discussed in section 3. The assumption has been made that the diets are normally distributed with coefficients of variation of 16% while the coefficient of variation of the protein:energy ratio of the requirement is 12%.

Visual inspection of Figure 15 and comparison with actual prevalence of deficit in Table 44 shows that none of the three diets appears adequate for

all groups. Protein deficiency (i.e. a protein:energy ratio of the diet that is less than the reference protein:energy ratio) is most likely in an elderly sedentary woman and least likely in a moderately active young child. This is the opposite of what is usually assumed, even though the protein:energy ratio of breast milk at 0.06, which can be assumed to be a close match to the desirable protein:energy ratio of infants, is half that of the adult diet. However, for infants and very young children the assumption has to be made that the diet is sufficiently energy-dense that the bulk of the diet does not limit consumption so that it fails to satisfy energy requirements. This is probably correct for the 5-year-old, but may not be the case at 6 months. Among children and

Table 43
Available protein for various age groups from diets representative of developed and developing countries

		Diets		
		Indian average	**UK vegetarian**	**UK omnivore**
Protein:energy ratio[a]		**0.111**	**0.127**	**0.142**
Digestibility[b]		**0.80**	**0.81**	**0.89**
Lysine content (mg/g protein)[c]		**39**	**53.8**	**62.9**
Age (years)	**Lysine scoring pattern[d] (mg/g protein)**	**Available protein[e]**		
0.5	57	0.061	0.096	0.126
1–2	52	0.066	0.102	0.126
3–10	48	0.072	0.102	0.126
11–14	48	0.072	0.102	0.126
15–18	47	0.073	0.102	0.126
19–29	45	0.077	0.102	0.126
30–59	45	0.077	0.102	0.126
>60	45	0.077	0.102	0.126

[a] Indian diet from FAO food balance sheets 1961–1992, as reported in reference *14,* and UK diets from reference *13.*

[b] For UK diets the overall nitrogen digestibility is assumed to be the weighted mean of 95% and 80% for animal and plant protein sources respectively (see *15*) with the Indian diet assumed to derive from plant protein sources.

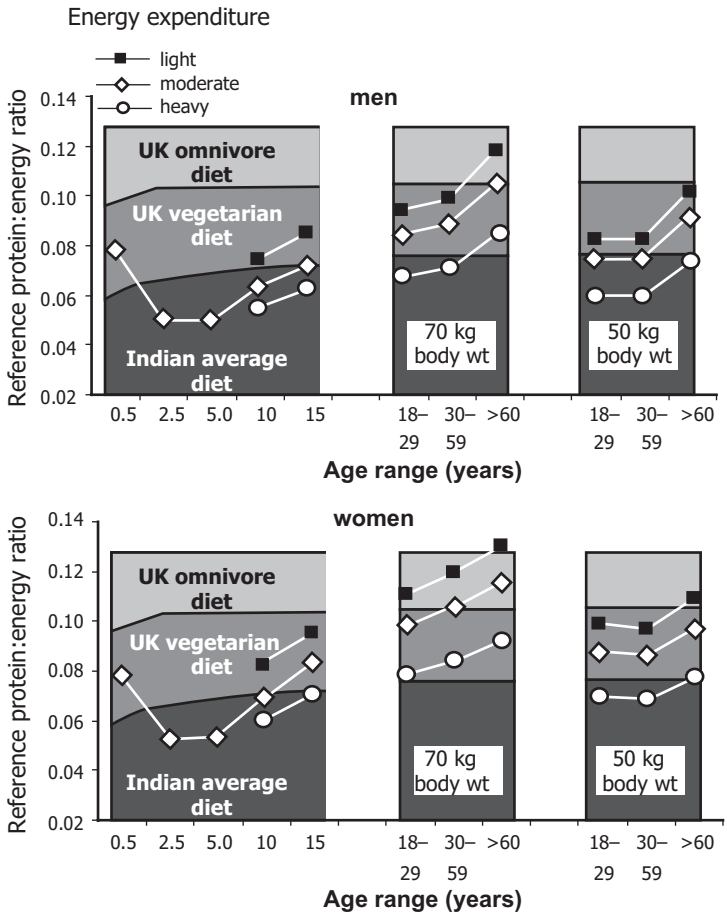
[c] From reference *15.*

[d] See section 9.

[e] Protein:energy ratio times digestibility times lysine score (protein digestibility-corrected amino acid score adjusted protein:energy ratio).

Figure 15

Reference protein:energy ratios for women and men compared with the available (protein digestibility-corrected, amino acid score-adjusted) protein:energy ratios of representative diets from developed and developing countries.



The shaded areas are the protein quality, age-adjusted protein:energy ratios of the three diets shown in Table 43.

Reference protein:energy ratio values at National Center for Health Statistics/WHO median weights for age for children 0.5–15 years are calculated as discussed in section 5, taking into account energy requirements calculated from Schofield equations adjusted for physical activity level values of 1.55 (light), 1.75 (moderate) and 2.2 (heavy) for both males and females, and protein requirements derived from sections 7 and 9.

adolescents, the most vulnerable group is the adolescent female at 15 years, an age when pregnancies may begin. Clearly, smaller body size reduces risk by increasing energy intake per kg, assuming that the prediction equations for body mass ratio accurately account for body weight variation. Thus with the UK omnivore diet, available protein intake is marginally insufficient for

Table 44
Prevalence of deficit for various age groups and activity levels with representative diets from developed and developing countries.[a]

			Indian average diet			
	Males			**Females**		
Age (years)	**Light activity**	**Moderate activity**	**Heavy activity**	**Light activity**	**Moderate activity**	**Heavy activity**
0.5		0.34			0.32	
2.5		0			0	
5.0		0			0.01	
10.0	0.09	0.02	0.01	0.19	0.05	0.01
15.0	0.19	0.05	0.01	0.41	0.17	0.04
			Adults at 70 kg body weight			
18–29	0.28	0.13	0.02	0.54	0.38	0.06
30–59	0.35	0.17	0.03	0.68	0.44	0.11
>60	0.69	0.45	0.11	0.82	0.62	0.21
			Adults at 50 kg body weight			
18–29	0.10	0.04	0.00	0.31	0.14	0.02
30–59	0.10	0.04	0.00	0.27	0.12	0.02
>60	0.39	0.19	0.03	0.49	0.27	0.05
			UK vegetarian diet			
		Males			Females	
Age (years)	Light activity	Moderate activity	Heavy activity	Light activity	Moderate activity	Heavy activity
0.5		0.01			0.01	
2.5		0			0	
5		0			0	
10	0	0	0	0.01	0	0
15	0.01	0	0	0.04	0.01	0
			Adults at 70 kg body weight			
18–29	0.03	0.01	0	0.10	0.04	0
30–59	0.04	0.01	0	0.17	0.07	0.01

>60	0.18	0.07	0.01	0.30	0.14	0.02
Adults at 50 kg body weight						
18–29	0.01	0	0	0.04	0.01	0
30–59	0.01	0	0	0.03	0.01	0
>60	0.05	0.02	0	0.08	0.03	0
UK omnivore diet						
	Males			Females		
Age (years)	Light activity	Moderate activity	Heavy activity	Light activity	Moderate activity	Heavy activity
0.5		0			0	
2.5		0			0	
5		0			0	
10	0	0	0	0	0	0
15	0	0	0	0	0	0
Adults at 70 kg body weight						
18–29	0	0	0	0.02	0	0
30–59	0	0	0	0.03	0.01	0
>60	0.01	0.01	0	0.07	0.02	0
Adults at 50 kg body weight						
18–29	0	0	0	0	0	0
30–59	0	0	0	0	0	0
>60	0	0	0	0.01	0	0

[a] Calculated as described in section 3 from $\Phi(-(MR-MI)/SD)$ where Φ is the unit normal distribution, MR and MI are mean requirements and intakes shown in Tables 42 and 43, and SD = (SI2 + SR2) assuming that intakes and requirements are not correlated.

a large elderly sedentary woman. With the UK vegetarian diet, available protein intake is insufficient for large elderly men with intakes appropriate for light or moderate physical activity, for women of all adult ages who are large, sedentary or moderately active, or small, elderly and sedentary.

It is clear that the average Indian diet would be associated with high prevalence rates of deficiency for the infant and the majority of adolescent and adult groups. Only those with small body size and high levels of physical activity could consume enough protein to meet requirements.

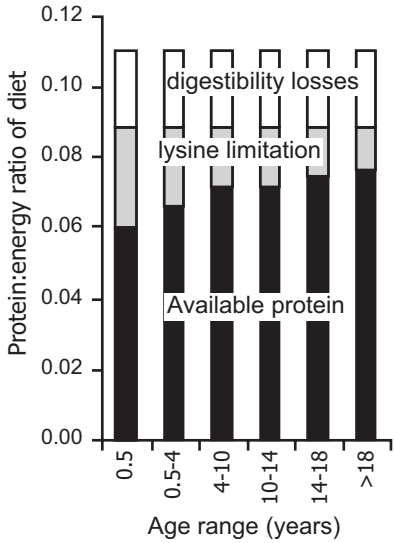
12.3 Implications of the apparent inadequacy of the diets in developing countries

It is clear that, on the basis of the definitions and data analysis associated with the derivation of the requirements for protein and amino acids adopted in this report, this analysis of risk of deficiency has identified a significant problem. Furthermore, while the problem is most marked for the diet typical of a developing country, it is also identified within some groups consuming vegetarian diets typical of developed countries, and even to a limited extent a typical omnivore diet. For these groups, in developed and especially in developing countries, there is inadequate protein consumption, in terms of both quantity and quality (digestibility and lysine requirement). As discussed elsewhere, these calculations are based on a number of assumptions that could influence the detail of the outcome (*12*). Certainly the calculations of risk prevalence in Table 44 are based on very limited data on dietary intakes, making assumptions about the distributions and variances of these intakes. However, on the basis that these estimations of risk are reasonable and that a high risk of protein deficiency is identified for many in the developed world, there are two alternative approaches that can be made.

One approach is to pursue risk management by considering the need to increase the availability of adequate supplies of high-quality protein such as legumes to those populations at risk of deficiency. A strategy along these lines has already been suggested by Young, Scrimshaw & Pellet (*16*), based on their opinion that the requirement for lysine has been underestimated and that lysine intakes are inadequate. The impact of the new lysine requirement on the calculation of the available protein in the Indian diet is to reduce it by 13% in adults (see Figure 16).

As discussed in section 7, the new value for the lysine requirement, like that of all amino acids, is associated with uncertainty, with reported values that are both higher and lower than the selected value of 30 mg/kg. Also the meta-analysis of nitrogen balance studies failed to identify any difference in utilization between animal and plant protein sources (see section 7). Furthermore, two long-term nitrogen balance studies based on wheat protein diets indicated body weight and fitness maintenance at lysine intakes of 17 mg/kg

Figure 16
Influence of lysine limitation and digestibility on the available protein from the Indian diet adjusted for age



per day (*17*) or between 20 mg/kg per day and 30 mg/kg per day (*18*). Clearly this is an important area for further research. However, calculation of deficit prevalence for the Indian diet on the basis of an adult protein:energy ratio of 0.88, i.e. assuming only a correction for digestibility, does reduce deficit prevalence, as shown in Table 45 for adult women, although considerable deficit remains for all but the most active age groups. This is especially true, given that the digestibility value chosen at 80% may well be an overestimate, with values of 50–80% reported for mixed diets in developing countries. Also, as discussed in sections 2 and 6, there are important considerations that raise concern about our current understanding of digestibility. In particular, we have insufficient understanding of the extent to which there is significant metabolism of amino acids and nitrogen in the lower gut, and how this might limit our ability to measure true digestibility. More work is needed in this area.

An important and contentious issue relates to the assumptions used in the calculation of reference protein requirements and risk of inadequacy. In deriving the protein requirement and reference protein:energy ratio, the assumption is made that protein intakes and requirements are independent. This is a basic and fundamental assumption for all the traditional approaches to the determination of safe levels of dietary protein. As discussed in section 2, the extent to which any form of adaptation might operate is unknown, although a role for adaptation has been proposed (*19, 20*) and its implications discussed (see section 7).

Table 45
Prevalence of deficit in adult females with the Indian diet assuming lysine is not limited[a]

Age (years)	Light activity	Moderate activity	Heavy activity
	Adult females at 70 kg		
18–29	0.28	0.13	0.02
30–59	0.41	0.21	0.03
>60	0.59	0.35	0.07
	Adult females at 50 kg		
18–29	0.12	0.05	0.01
30–59	0.11	0.04	0.00
>60	0.24	0.11	0.01

[a]Calculations as in Table 44.

The implications of adaptation include the possibility that a part of what is viewed as between-individual variability in the protein requirement results from incomplete adaptation to the sub-maintenance intakes in short-term balance studies and that the true requirement lies towards the lower end of the reported range. This would also explain the apparent inefficient utilization of protein, regardless of its source (see section 7).

Clearly, the incorporation of adaptation into the protein requirements model used in this report would pose difficult questions in terms of risk management and the development of public health nutrition policy. In the context of providing advice on safe diets, there is little merit in departing from the current approach. Certainly caution should be exercised in any recommendation which proposes that lower intakes of foods containing protein be considered safe, especially since many key micronutrients and minerals accompany dietary protein, and there may be benefit for general health and for reducing the risk of chronic disease, from protein intakes higher than the minimum for the achievement and maintenance of nitrogen balance (see section 13).

In the context of diagnostic use of requirement values, i.e. risk assessment aimed at identifying prevalence of deficit, it is nevertheless important that any analysis aspires to an acceptable balance between the numbers of false positives and false negatives. The analysis above shows very substantial risk; if the analysis is correct, the risk carries extremely serious implications. Without wanting to dismiss the possibility that there is a genuine problem, it has been suggested that the assessment of the extent of this risk is likely to be overestimated, given that the approach even indicates significant risk in populations in the United Kingdom that are generally considered to be well nourished (*12*). However, an adaptive model is relevant to discussion of deficiency only in terms of being unable to maintain nitrogen balance and an

appropriate lean body mass, after full adaptation to an otherwise nutritionally adequate diet which satisfies the demands for energy. Whether or not populations in this state enjoy optimal protein-related health in terms of immune function, bone health, growth in height or any other function are separate issues that are important and need to be addressed in their own right.

Another assumption of particular importance is that appetite and food intake over any extended period of time are determined by levels of energy expenditure, which influence energy consumption to maintain energy balance. The possibility has, however, to be considered that when the diet is marginally limiting in protein, there is a drive for protein consumption in its own right, similar to the increased appetite observed during catch-up growth. If meeting the needs for protein were to drive consumption, then there are important implications which need to be considered and addressed. If, for example in older people who lead a relatively sedentary lifestyle, or other population groups operating at the margin, protein consumption were consistently below requirements, then any drive to increase protein consumption would be associated with an intake of energy in excess of metabolic demands, predisposing people to positive energy balance and excess adiposity with its attendant risks.

Given the considerable importance which the underlying assumptions carry for policy formulation, there is a clear and important need for continuing research into processes and mechanisms which enable health to be achieved on protein intakes as habitually consumed. While maintenance of nitrogen balance or an appropriate lean body mass must remain the major outcome measure of protein-related health, it would appear that assessment of dietary adequacy in these terms is unlikely to be possible without a much better understanding of adaptive mechanisms.

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13. Protein intake and health

Nutritional assessment of diets and populations is most often concerned with dietary adequacy and the potential adverse effects of low or inadequate nutrient intakes. However, for a proportion of the population in the developed countries, many nutrients are not only abundant in the usual diet, but also may be taken as dietary supplements, so that overall intakes may be far in excess of requirements and recommended intakes. This is especially true for protein and amino acids. Average protein intakes of populations consuming the mixed diets of developed countries will usually be considerably in excess of recommended intakes, especially for meat-eaters. In addition to this, protein and amino acid supplements are readily available to the general public through pharmacies, grocery stores, and Internet vendors of nutritional products. Protein supplements are the most widely consumed ergogenic aid, whereas single amino acids are consumed for a wide variety of reasons, most of which have little or no secure scientific foundation. There are several issues which arise from the potential for protein intakes to be in excess of the recommended intake.

One issue is the potential for harm. The possibility of toxicity resulting from consuming very large amounts of individual amino acids is outside the scope of this report, but has been examined in various publications (*1–6*). While the previous report did not review the issue of protein intakes in excess of requirements, concern has been expressed in several national reports. In the United Kingdom, in the context of guidance on high intakes, several potential adverse effects were identified and it was concluded that it was prudent for adults to avoid protein intakes of more than twice the reference dietary amount (i.e. 1.5 g protein/kg) (*7*). It has since been pointed out that such intakes are easily exceeded by physically active individuals on normal diets, and that much higher levels of protein are consumed through the protein-enriched diets typical of men involved in body-building (*8*), as discussed below. Because of this, it is particularly important that potential adverse effects are appropriately identified.

A second issue is the possibility that protein intakes in excess of recommended intakes may confer health benefits, i.e. it may be that optimum protein

intakes are greater than a recommended intake derived as in this report. This section therefore examines the relationship between protein intakes and long-term health in relation to a number of specific disease states, and also whether it is possible to identify a maximum level of protein that can be consumed without adverse effects.

13.1 Renal function

There is clear evidence that high intakes of protein by patients with renal disease contribute to the deterioration of kidney function (*8–12*). However, the suggestion that the decline of glomerular filtration rate that occurs with advancing age in healthy subjects (*13*) can be attenuated by reducing the protein in the diet appears to have no foundation. This concept arose from studies in rats, in which low-protein diets were shown to delay the development of chronic renal failure (*9, 10*). However, it seems unlikely that this mechanism would operate in humans, in whom the decline in kidney function occurs through a fall in filtration by non-sclerotic nephrons, rather than by glomerular sclerosis as occurs in the rat (*14*). In a group of subjects covering a wide range of dietary protein intakes, the glomerular filtration rate was related to the protein intake, but albumin excretion, an indication of renal disease, was not (*15*). This indicates that chronic protein intake is a determinant of glomerular filtration rate, but does not suggest a role for protein intake in the deterioration of kidney function. It has been argued by Walser (*14*) that symptomatic renal failure does not result from the physiological decrease in glomerular filtration rate that occurs with age, because symptoms do not occur until the glomerular filtration rate has decreased much more than occurs with ageing. Moreover, protein restriction lowers glomerular filtration rate, suggesting that the decline of glomerular filtration rate with age is a natural consequence of the decline in protein intake as age progresses, and is unrelated to deterioration of renal function (*16*). As concluded by Walser (*14*), protein restriction on the grounds of renal function is justifiable and prudent only in subjects who are likely to develop kidney failure owing to diabetes, hypertension, or polycystic kidney disease.

13.2 Bone health

The relationship between protein intake and bone health appears to be more complex than was previously believed. Thus the potential negative effect of protein on calcium balance is a function of the overall dietary acid–base balance. In addition, protein seems to have direct anabolic effects on the bone matrix. It is well documented that diets containing high protein can result in an increase in urinary calcium excretion (*17–20*), amounting to a 50% increase in urinary calcium for a doubling of protein intake (*19*). Bone mineral balance is very sensitive to acid–base balance, and calcium can be mobilized

from bone in response to the need to buffer the acid load produced by oxidation of the sulfur-containing amino acids, methionine and cysteine (*21*). Accordingly, increased resorption of bone has been shown to occur as a consequence of increased protein intake (*20, 21*).

This raises the controversial issue of whether this process might lead to a decrease in bone calcium (*19, 22, 23*). Heaney (*24, 25*) has suggested that this is unlikely, as low protein intake itself leads to bone loss, and higher protein intake generally leads to a higher calcium intake. However, it is now clear that net renal acid excretion predicts calcium excretion, and that this can be predicted from the ratio of dietary protein to potassium, since the dietary intake of potassium occurs mainly as salts of weak organic acids and therefore has an alkalizing effect (*26*). Thus in women, lower intakes of endogenous noncarbonic acid (i.e. a lower protein intake but a higher potassium intake) were related to better measures of bone health (*27, 28*). This probably explains the beneficial influence of fruit and vegetables, the major dietary source of potassium, on bone health (*29, 30*). It may also explain why calcium citrate malate supplements are more effective for bone health than other calcium salts (*31*).

The importance of achieving low net renal acid excretion is that once the potential acidifying influence of dietary protein is balanced by the alkalizing effect of the dietary potassium intake, protein can exert an independently beneficial effect through its insulin-like growth factor-1 (IGF-1)-mediated anabolic influences on bone. Such effects have been clearly shown on bone length growth rates in animal studies (*32, 33*) and are likely explanations of the beneficial effects of additional protein on height growth in children (*34*). However, more recent studies have shown that in elderly populations protein supplements increase serum IGF-1 levels and attenuate proximal femur bone loss in patients with recent hip fracture (*35*). The magnitude and importance of the bone protein pool are such that a positive effect of protein on bone is not surprising. Apart from any IGF-1-mediated effects there is considerable evidence for a limitation on the synthesis of glycine (*36*), which accounts for 25% of collagen, so that competition for glycine between collagen and its other important metabolic demands might prevent its reutilization during bone collagen turnover. What is not yet clear is the relation between these IGF-1-mediated anabolic influences acting on the matrix and the parathyroid hormone-mediated responses to any protein-derived, acid-stimulated, increased urinary calcium loss, but it has been suggested that, in the absence of sufficient dietary alkali to neutralize the protein-derived acid, net calcium loss ensues and the anabolic drive of dietary protein on the bone matrix is ineffective in maintaining bone mineral density (*37*). Taken together, it does appear that dietary protein as part of a well-balanced diet is most likely to be

beneficial for bone, possibly at dietary levels in excess of the recommended intake.

13.3 Kidney stones

A second potential consequence of high-protein diets, which has been extensively discussed in the literature, is an increased occurrence of renal stones. Renal stones occur very commonly, and have been estimated to affect 12% of the United States population at some time (*38*). The urine contains high concentrations of calcium and oxalate, which can accumulate in the kidney as calcium oxalate stones, the most common form of renal stone. Initial studies showed that an increase in dietary animal protein resulted in an elevation of urinary calcium and oxalate, which was estimated to increase the risk of forming stones by 250% (*39, 40*). Moreover, prospective studies of the effect of dietary calcium and other nutrients on the risk of kidney stones showed that a higher intake of calcium decreased, and a higher intake of animal protein increased, the risk of stones (*41, 42*). More recent prospective studies have involved very large numbers of subjects: 96 245 women in the Nurses Health Study II (*43*), and 45 619 in a follow-up after 14 years (*44*). These have confirmed the negative effect of calcium intake on stone formation, but for animal protein intake the effect has been less clear, being either non-significant (*43*), or significant only in men, in whom it was positively related (*44*). However, intervention studies have shown that a 38% reduction in protein intake by calciuric patients resulted in an improvement in their lithogenic profile (*45*). In another study (*46*), idiopathic calcium stone formers and healthy controls were given a normal protein diet (approximately 80 g/day from meat and fish) for 5 days, followed by a high-protein diet (approximately 185 g/day from meat and fish) for a further 5 days. The rate of oxalate excretion in the controls did not change significantly, whereas in the idiopathic calcium stone formers there was an increase in oxalate excretion, particularly in those that were classified as having "mild metabolic hyperoxaluria". As urinary oxalate is thought to be a promoter of calcium oxalate stones (*47*), this can be taken as evidence that a diet high in animal protein favours the formation of kidney stones in sensitive subjects. A recent 5-year clinical trial in 120 men with idiopathic hypercalciuria compared two diets, one with normal calcium but restricted animal protein (52 g/day) and salt (50 mmol/day), and the other with low calcium (*48*). The occurrence of stones in men on the animal-protein-restricted diet was half that of men on the low-calcium diet. However, although this result confirms that stone incidence is diet-related, it does not enable the role of protein intake to be discerned, as the total protein (1.2 g/kg per day) did not differ between the two diets, and calcium was varied. Moreover, although it has been believed that protein of animal origin will result in more acid urine because of the

higher sulfur amino acid content (*49, 50*), a comparison of diets containing protein from mainly animal or mainly plant sources showed no differences in the Tiselius risk index, a measure of calcium oxalate precipitability (*51*).

In conclusion, although some studies suggest that high animal protein intake might increase the risk of kidney stones, particularly in those subjects who are classified as idiopathic calcium stone formers, as yet no clear conclusions can be drawn since dietary effects are apparent only in studies with very large differences in protein intakes (i.e. >185 g/day compared with 80 g/day). Moreover, it is not yet clear whether there is a difference between proteins of animal versus plant origin. In fact, the sulfur amino acid content of cereals and most plant proteins (except for legumes) is similar to that of meat and dairy products. Certainly, prospective studies involving very large numbers of subjects have not produced a clear-cut relationship between animal protein intake and stone incidence. However, to allow for the present uncertainty, it is recommended that in order to minimize the risk of kidney stones in patients who are at risk, the diet should ideally provide at least the safe level (0.83 g/kg per day), but not excessive amounts (i.e. less than 1.4 g/kg per day), preferably from vegetable sources.

13.4 Cardiovascular disease

There is a complex relationship between protein intake and cardiovascular disease which has yet to be fully resolved. There is a body of experimental studies in rodents pointing to animal protein intakes being more hypercholesterolaemic and atherogenic compared with intakes of vegetable protein, especially when fed as part of cholesterol-free, purified diets (*52*). However, this effect is not observed in other species, such as pigs and humans (*53*), and as yet no convincing mechanisms have been identified. Moreover, evidence has accumulated from human studies that diets with a higher proportion of protein are beneficial for the heart (*54, 55*). Analysis of the data from the Nurses Health Study, which included 14 years of follow-up of 80 082 women aged 34 to 59 years, showed a moderate inverse correlation between protein intake and incidence of ischaemic heart disease (*56*). Furthermore, this association was apparent for both animal and vegetable protein.

As yet no consensus has been reached about whether such associations showing protective effect of protein for cardiovascular disease are causal and no convincing potential mechanisms have been proposed. However, the strongest associations relate to a protective influence of protein intake on raised blood pressure. Raised blood pressure is a major risk factor for coronary heart disease and the major risk factor for stroke, and is largely environmental in origin, with dietary sodium and alcohol intakes having direct effects, and potassium intake an inverse influence. Inverse relationships

between protein intake and blood pressure have been reported from various countries (e.g. *57, 58*). Several reviewers have assembled evidence showing an inverse relationship between protein intake and blood pressure. Obarzanek, Velletri & Cutler (*54*) list nine cross-sectional surveys of American and British adults showing that increased protein intake lowers blood pressure, one American study showing the same relationship with vegetable protein, and three studies in China and Japan showing the same relationship with animal protein. The more recent relevant literature has been reviewed by Elliott (*59*), listing 25 reports of cross-sectional analyses from 18 different studies, most of which found a significant inverse association in at least one analysis, although some did not. Notably the NHANES III study found no relationship but did show an attenuation of the age-related increase in blood pressure by protein intake (*60*). Most recently, a full meta-analysis of nine population-based studies has been reported showing a convincing cross-sectional inverse association between dietary protein intake and blood pressure (*61*). However, longitudinal studies of dietary protein or change in dietary protein in relation to change in blood pressure or incidence of hypertension have been inconclusive (*59, 60*).

Elliott (*59*) cautions about over-interpretation of these studies, many of which were not designed to examine the question of diet and blood pressure, and which varied markedly in design and analysis. One specific problem flows from lack of control for energy intake, which is an important potential source of bias. Because high blood pressure is associated with overweight, and overweight people tend to underreport energy intake, a spurious inverse association between low protein intake and blood pressure might result.

Trials of protein supplementation, protein restriction, or substitution of meat for vegetarian products have generally given varying and inconsistent results, although interventions with soy protein have largely shown positive beneficial influences.

Overall, while it seems certain that protein intakes are not harmful for blood pressure, with cross-sectional population studies clearly showing benefit of increasing protein intakes, some caution is probably still justified since dietary associations can be confounded by highly correlated nutrients for which no adjustment has been made. Part of the difficulty is that the underlying mechanism by which dietary protein influences blood pressure is largely unknown, although several hypotheses have been proposed. Animal models have indicated that dietary protein intake could increase renal concentrating ability and induce increases in renal plasma flow, glomerular filtration rate and sodium excretion in the short term, and increases in renal size, renal plasma flow and glomerular filtration rate in the long term. Individual amino acids have been proposed as mediators of metabolic

effects, such as L-arginine through its influence on nitric oxide synthesis, and cysteine through its influence on nitric oxide turnover and metabolism. An effect of soy protein might be related to an influence of isoflavones from soya on the endothelium, although recent trials have reported that isoflavones alone, without soya supplementation, had no effect on blood pressure. One potential mechanism not raised to date is through a homocysteine-lowering effect of dietary protein. Although methionine is the precursor of homocysteine, so that increased protein and consequent methionine intakes might be thought to increase homocysteine concentrations, protein intakes appear to be inversely related to homocysteine levels (*62*). However, assuming that the disposal of homocysteine via the transulfuration pathway is adaptive, with the capacity increasing with methionine intakes, an inverse association between protein intake and homocysteine concentrations is not entirely unexpected.

This is an obvious area for further research aimed at identifying causality and, if causality exists, determining whether the effect is attributable to proteins of plant or animal origin.

13.5 Cancer

As the incidence of cancer is clearly influenced by environment, the role of diet in the development and growth of malignant tumours has received much attention, although the unequivocal identification of dietary influences has proved most difficult. Furthermore, whereas there have been many large-scale studies to investigate the roles of specific foods or food sources, as well as energy substrates and micronutrients, on specific cancers, few have examined dietary protein specifically. Thus potential influences of protein have to be surmised from studies examining the major protein-containing food groups such as meat, dairy foods, eggs and fish. Recent large studies have shown that high intake of red and processed meat is associated with greater incidence of colorectal cancer (*63, 64*), that meat and dairy consumption do not influence the incidence of gastric cancer (*65*), and that vegetable and fruit consumption reduces the risk of breast cancer (*66*). However, care is needed in the interpretation of these studies because of potential confounding influences. Thus, in the study of meat consumption in the Cancer Prevention Study II nutrition cohort (*64*), the association of high intake of red and processed meat with higher risk of colon cancer was observed after adjusting for age and energy intake but not after further adjustment for body mass index, cigarette smoking, and other covariates.

The most likely way of identifying disease linkage is when study groups are most closely matched apart from the variable of interest. This was the case in studies of vegetarians where meat or fish intakes were the variables of

interest and all subjects were recruited from population groups with similar healthy lifestyles. In an analysis of the combined data from five prospective studies of death rates from common diseases in vegetarians (no meat or fish, 27 808 vegetarians studied) compared with non-vegetarians with similar (healthy) lifestyles (48 364 non-vegetarians studied), the only difference identified was that mortality from ischaemic heart disease was 24% lower in vegetarians than in non-vegetarians, possibly through a lower blood cholesterol level in the vegetarians (*67*). Importantly, within these cohorts of healthy adults there was no significant difference between vegetarians and non-vegetarians in mortality from cerebrovascular disease, stomach cancer, colorectal cancer, lung cancer, breast cancer, prostate cancer, or all other causes combined.

As for protein per se, this was examined in relation to breast cancer mortality in the 88 647 women covered by the Nurses Health Study (*68*). Since protein intakes have been associated with increasing circulating insulin-like growth factor-1 levels, which in turn have been reported to show a striking relationship with breast cancer risk among premenopausal women in the Nurses Health Study (*69*), an influence of dietary protein on relative risk of breast cancer might be predicted. However, no effect of total protein or of animal or vegetable protein was detected, although processed meat other than bacon or sausages was shown to be associated with greater risk. A lack of significant association between cancer mortality and protein from animal or vegetable sources was shown in a prospective study of 29 017 women in the Iowa Women's Health Study (*70*).

Overall, the evidence indicates that there is little effect of total protein intake on the incidence of cancer, but that specific foods, such as red or processed meat, might increase the risk relative to vegetable protein sources. However, it has been reported that high dietary protein results in better survival in women with breast cancer (*71*).

13.6 Is there a maximum limit of dietary protein intake?

As indicated above, in developed countries most people consume substantially more protein than the safe level, especially through consumption of meat-based diets at energy intakes required to meet the demands of high levels of physical activity, or with supplementary protein intakes often consumed by young men attempting to increase their musculature. Typical intakes are up to 3.0 g/kg from food (*72*) with an extra 1 g/kg from supplements. This is equivalent to 320 g/day for an 80-kg male, and at energy intakes which match an expenditure of twice the basal metabolic rate (i.e. 3800 kcal/day). This implies an overall protein:energy ratio of the diet of 34% (see *72*). Such intakes are similar to those involved in studies of the impact of dietary

protein intake on nitrogen balance, where protein intakes were increased to 200–300 g/day for 2 months (*73*). Such practices are almost certainly ineffective in terms of gain of muscle mass, even though substantial nitrogen retention is often reported (*73, 74*). Where measurements of muscle mass or protein concentration are made no changes are identified (*75*), suggesting the apparent gain in body nitrogen to be an artefact of the nitrogen balance method at these very high intakes, possibly with an unmeasured source of loss of nitrogen (*71, 76*).

While there have been no systematic investigations of the safety of such high intakes (at least to the current available knowledge), it must be assumed, given that such dietary habits are widespread, that any untoward effects are subtle, long-term and unreported. The most widely quoted potential problems relate to renal function and damage, but as discussed above the evidence for such claims in otherwise healthy individuals does not stand up to scrutiny. Similarly, any adverse impact on bone mineral balance would appear to be more than adequately balanced by the positive influence of weight-bearing exercise in strength training, judging by most reports of high bone mineral content in power athletes.

Thus there is scant information that would help identify an upper limit to the capacity to metabolize protein by healthy individuals, or the symptoms that might result from exceeding such a level. One study which attempted to identify the maximum amount of protein that can be metabolized examined the maximum rate of urea synthesis in response to increasing meal protein portions, by healthy subjects and patients with renal insufficiency (*77*). The study found that an increase in the protein content of a meal was followed by an increase in urea synthesis, but only up to a certain level. Above this level of intake, the rate of urea synthesis continued at the same high level for a longer period, until the excess of dietary nitrogen had been eliminated. This high rate of urea synthesis might be taken as representative of the maximum rate of nitrogen intake that can be processed by the liver and kidneys. However, the value obtained, which corresponds to an intake of approximately 230 g protein/day, is within the range of intakes discussed above, which were not apparently associated with any ill effects. Given the adaptive nature of amino acid oxidation and urea synthesis (see section 2), the intake value identified may have underestimated the true maximum, as the subjects in the study of Rudman et al. (*77*) were not adapted to the diet prior to study.

Additional evidence for a maximum limit for dietary protein can be derived from historical records and anthropological studies, and these provide evidence that very high protein intakes might be toxic. In their analysis of the older literature, Speth & Spielmann (*78*) noted that consumption of more than about 45% of the dietary energy as protein led to nausea and diarrhoea within

3 days and to death in a few weeks, a condition known as "rabbit starvation". Rabbit meat has a very low fat content, so consumption of enough rabbit meat to satisfy energy requirements resulted in very high protein intake (*79*). The effects of a diet with a very high protein:energy ratio were studied experimentally in two Arctic explorers who were monitored closely for a year while consuming an exclusively meat diet (*79–82*). They remained fit and healthy during this period, but when one of them ate lean meat only (about 60% of energy as protein), the symptoms of "rabbit starvation" soon developed. These symptoms were rapidly reversed when the fat content of the diet was restored (15–25% of energy as protein). This is consistent with analyses of the archaeological evidence of the dietary practices of hunter-gatherer populations, as well as present-day hunter-gatherers, which have suggested that humans avoid protein intakes in excess of about 40% of dietary energy, even when consuming mainly meat (*83, 84*).

Clearly, in early life, metabolic capacity to handle protein may be less well developed. Although it is believed that amino acids do constitute a major part of the substrates for fetal metabolism, postpartum, especially in preterm neonates, there is clear evidence that very high protein intakes can be harmful. In one study, 304 preterm infants were given diets containing either 3.0–3.6 g/kg per day or 6.0–7.2 g/kg per day of cows' milk protein (*85*). These intakes are 1.5–1.8 and 3.0–3.6 times the estimated milk protein intake of a breastfed newborn (*86*). The higher protein intake resulted in more fever, lethargy and poor feeding than the lower protein group, but also in higher plasma protein levels and less oedema. At 3 years of age, and again at 6 years of age, the children were followed up with physical and psychological testing (*87, 88*), which revealed an increased incidence of strabismus and low IQ scores in the children with lowest birth weight who had been fed the high-protein diet. Also, severely malnourished infants presenting with the characteristic pathologies of kwashiorkor cannot tolerate the high-protein formulas which are often misguidedly administered on admission, and which are associated with high levels of mortality.

13.7 Conclusions

Current knowledge of the relationship between protein intake and health is insufficient to enable clear recommendations about either optimal intakes for long-term health or to define a safe upper limit. In pregnancy, supplements of protein per se as distinct from mixed protein and energy supplements have been reported to reduce birth weight (*89*). For infants and young children, while additional protein can improve linear growth where pre-existing diets are nutritionally poor in terms of protein and other important nutrients, there is no evidence of benefit of additional protein above that found in otherwise nutritionally complete diets. For adults, risks and benefits in terms of

multifactorial diseases are complex, and there is insufficient evidence to draw specific conclusions. The fact that our current models of protein and energy requirements identify sedentary elderly people as most likely to be at risk from protein deficiency (see section 5), together with the evidence of a beneficial effect of dietary protein on bone in elderly people, suggests that attention should be given to the provision of protein-dense foods to this particular population group.

As for a safe upper limit for adults, we can be reasonably confident that an intake of twice the recommended intake, previously identified as a safe upper limit, is likely to be safe given that it equates to intakes of physically active individuals consuming average mixed diets who would otherwise be identified as having healthy lifestyles. It is also clear that there is an upper limit to the protein content of food, which is identifiable by the individual in terms of the nausea and diarrhoea of "rabbit starvation", although exactly what that limit is has not been identified. Many individuals consume intakes of 3–4 times the recommended intake, possibly for relatively long periods of time, without (presumably) exhibiting such symptoms; while no specific evidence for harm, can be identified neither the fact nor such intakes are risk-free can be insured. Given the lack of evidence of benefit in terms of athletic performance or physique, it might be prudent to avoid such intakes (*90*). Protein is the most satiating macronutrient, and protein supplements may lead to suboptimal intakes of those starchy foods essential for both performance and long-term health, and insufficient dietary alkali derived from fruit and vegetables to buffer the protein-derived acid load, with adverse effects on bone. High-protein diets can both increase exercise-induced amino acid oxidation, especially in untrained individuals and those with an inadequate energy intake, and increase risk of negative nitrogen balance and loss of lean body mass between training periods when high intakes are reduced (*91*). Clearly, with minimum protein intakes to maintain appropriate body composition and function that are probably much lower than intakes of individuals with healthy lifestyles consuming usual mixed diets, there is a need to improve our understanding of the relationship between protein intakes and overall health. This is a particularly important area for future research.

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14. Summary of requirements

The aim of this section is to summarize the estimates for protein and amino acids derived in sections 7–9, on the basis of the principles discussed in sections 2–4.

14.1 Derivation of requirements

The derivation of corrections to be applied for different diets is described in section 6.

14.1.1 *Safe intake for individuals and populations*

The protein requirement is derived as an average (or median) value for the population, with its variance.

For an individual, a *safe individual intake* has been defined as the 97.5th percentile of the distribution of individual requirements, nominally the average + 1.96SD. Thus any individual receiving such an intake will have a very low (<2.5%) risk of deficiency (intake < requirement).

For a population, a *safe population intake* cannot be defined as a simple function of the mean requirement. This is because risk of deficiency is influenced by the distributions of both individual requirements and intakes. In most circumstances this value will be greater than the safe individual intake, and in the usual circumstance where the SD of the intake is >SD of the requirement, the safe population intake will approximate to a value which is somewhat greater than the requirement + 1.96SD of intake.

14.1.2 *Precision of estimates*

As with previous reports, because calculations in sections 7–9 have been made with a greater degree of precision, or apparent precision, than can ever be useful in practice, values reported in this summary are rounded to two significant figures.

14.1.3 *Age ranges*

The age range used for adults is in most cases 18 years to old age, except where the protein requirement is related to energy requirement, as in section 5. For children, somewhat narrower ranges have been adopted. Even so, they embrace a very wide span of body weights. For example, for boys 3–5 years old, the acceptable range of body weight extends from 12 kg (3 years, 5th percentile) to 23 kg (5 years, 95th percentile). Therefore, if greater precision is needed, the more detailed tables in section 9 should be consulted.

14.1.4 *Relation to body weight*

Protein requirements are derived as amounts per kg body weight of subjects whose weight is within the acceptable range for height (adults) or age (children). The requirements per person within the acceptable ranges of body weights may either be based on the actual weight or normalized to the median weight for height or age, as given in the appropriate tables, according to the objectives for which they are to be used. It should be stressed, however, that for children, in whom the range of body weight within acceptable limits for age (10th–90th percentiles) is very wide, the latter approach will considerably underestimate or overestimate the requirements of those at the extremes of the distribution.

14.1.5 *Corrections for characteristics of the diet*

A correction may have to be made for protein quality in terms of the protein digestibility-corrected amino acid score value, which takes into account digestibility and amino acid score (see section 6). As in the previous report, it is recommended that when comparisons are being made between requirements and dietary intakes, these corrections should be applied to the diet rather than to the values for requirements. The reason for this is that it will facilitate aggregated comparisons between intakes and requirements, for example for a family unit, when different members of the family consume different diets. However, there are situations in which the user may find it more convenient to make the correction in the traditional way, i.e. by adjusting the estimate of requirement.

14.2 Protein requirements of adults

The protein requirements of adult men and women of various body weights are shown in Table 46. For adults, the protein requirement per kg body weight is considered to be the same for both sexes, at all ages, and for all body weights within the acceptable range. The value accepted for the safe level of intake is 0.83 g/kg per day, for proteins with a protein digestibility-corrected amino acid score value of 1.0. No safe upper limit has been identified, and it is

Table 46
Safe level of protein intake for adult men and women[a]

Body weight (kg)	Safe level of protein intake (g/kg per day)[b]
40	33
45	37
50	42
55	46
60	50
65	54
70	58
75	62
80	66

[a] All ages >18 years.
[b] 0.83 g/kg per day of protein with a protein digestibility-corrected amino acid score value of 1.0.

unlikely that intakes of twice the safe level are associated with any risk. However, caution is advised to those contemplating the very high intakes of 3–4 times the safe intake, since such intakes approach the tolerable upper limit and cannot be assumed to be risk-free.

14.3 Protein requirements of infants, children and adolescents

The protein requirements of infants, children and adolescent boys and girls are shown in Table 47. It is recommended that the calculation of protein requirements for this age group should be made in two steps: first, the requirement per kg should be obtained, according to the age range; second, this should be multiplied either by the actual weight or by the median weight for age (*1*) to obtain the total requirement.

The body weights shown in Table 47 are presented as a guideline, to be used when actual weights are not known. Within each age range there may be an almost 2-fold variation in acceptable body weight. For more detailed calculation of the requirements of children, if actual weights are not available, the user should obtain the median weight at the actual age from the WHO tables (*1*).

Adjustments for protein quality, according to age, should be made as set out below (section 14.7).

Table 47
Safe level of protein intake for infants, children and adolescent boys and girls

Age (years)	Boys			Girls		
	Weight [a] (kg)	Safe level of protein intake[b] (g/kg/day)	Safe level of protein intake (g/day)	Weight[a] (kg)	Safe level of protein intake[b] (g/kg/day)	Safe level of protein intake (g/day)
0.5	7.8	1.31	10.2	7.2	1.31	9.4
1	10.2	1.14	11.6	9.5	1.14	10.8
1.5	11.5	1.03	11.8	10.8	1.03	11.1
2	12.3	0.97	11.9	11.8	0.97	11.4
3	14.6	0.90	13.1	14.1	0.90	12.7
4–6	19.7	0.87	17.1	18.6	0.87	16.2
7–10	28.1	0.92	25.9	28.5	0.92	26.2
11–14	45.0	0.90	40.5	46.1	0.89	41.0
15–18	66.5	0.87	57.9	56.4	0.84	47.4

[a] WHO reference values (*1*).
[b] From Tables 33a and 33b.

14.4 Protein requirements of women during pregnancy and lactation

The extra protein requirements for pregnancy and lactation are shown in Table 48 as extra daily intakes of protein and of food with the appropriate protein:energy ratio. In the previous report, a single value for extra protein was recommended throughout pregnancy. More recent body-composition measurements do not show any maternal storage in early pregnancy, thus increasing amounts are recommended for each trimester. The additional protein should be taken by consuming more of a normal diet, rather than as supplements.

Table 48
Extra protein requirements for pregnancy and lactation

	Safe intake (g/day)	Additional energy requirement (kJ/day)	Protein:energy ratio
Pregnancy trimester			
1	1	375	0.04
2	10	1200	0.11
3	31	1950	0.23
Lactation			
First 6 months	19	2800	0.11
After 6 months	13	1925	0.11

14.5 Amino acid requirements and scoring pattern of adults

Amino acid requirements are shown in Table 49 as mg/kg body weight per day, and as the requirement pattern (mg/g protein) calculated from average requirements for amino acids and protein (0.66 g/kg per day).

14.6 Amino acid requirements and scoring pattern of infants, children and adolescents

Amino acid requirements for infants and children are derived by using a factorial model, based on the estimated dietary provision for maintenance and growth. It is assumed that the maintenance requirement pattern is the same as that for adults, and that the growth requirement provides for tissue deposition with the reported composition of whole-body protein. Values for both the requirements (mg/kg per day) and the requirement pattern (mg/g protein, calculated from the average requirement values for amino acids and protein) are shown in Table 50 for the nutritionally important amino acids.

14.7 Corrections for protein quality of the diet

When intakes of specific diets are being calculated to match requirements, or when diets are being assessed in terms of their adequacy, adjustments need to be made for protein quality to assess available protein, as follows:

- The total protein content of the diet = total nitrogen × 6.25.
- The available protein in the diet = total protein × protein digestibility corrected amino acid score value (digestibility factor × amino acid score).

Table 49
Amino acid requirements of adults[a]

Amino acid	mg/kg per day	mg/g protein
Histidine	10	15
Isoleucine	20	30
Leucine	39	59
Lysine	30	45
Methionine	10	16
Cystine	4	6
Methionine + cysteine	15	22
Phenylalanine + tyrosine	25	30
Threonine	15	23
Tryptophan	4	6
Valine 26	26	39

[a] From Table 23.

Table 50
Amino acid requirements of infants, children and adolescents[a]

Age (years)	Lysine	Sulfur amino acids	Threonine	Trypto-phan	Lysine	Sulfur amino acids	Threonine	Trypto-phan
	(mg/kg per day)				(mg/g protein)			
0.5	64	31	34	9.5	57	28	31	8.5
1–2	45	22	23	6.4	52	26	27	7.4
3–10	35	18	18	4.8	48	24	25	6.6
11–14	35	17	18	4.8	48	23	25	6.5
15–18	33	16	17	4.5	47	23	24	6.3
>18	30	15	15	4.0	45	22	23	6.0

[a] From Table 36.

- The digestibility factor is the best available estimate of the true digestibility (e.g. Table 5, section 6).
- The amino acid score is calculated from the amino acid pattern of the digestible dietary protein intake as a percentage of the appropriate reference pattern for each age group (see Tables 28 and 42).

These corrected dietary values are then used either as requirements or as utilizable protein intakes.

14.8 Protein density of foods and the protein:energy ratio of the requirements

The protein:energy ratio of the requirements expressed as a function of age, body weight, sex and physical activity level allow the required protein density of foods to be identified as a function of lifestyle, size, age and sex. This shows (see Table 4, section 5) that reference protein:energy ratios range from 0.048 for a 2.5-year-old infant to 0.128 for a large sedentary older adult female. Thus, for any diet considered to be limiting in protein, the population groups most likely to be at risk are elderly people, especially sedentary women. This means that while calculated protein requirements for elderly people are not different from those for younger adults, unless the elderly people are physically active they will require more energy-dense food.

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15. Research needs

The advances made in this report reflect a more thorough analysis of nitrogen balance studies, mainly organized at the time of the previous report (*1*), the benefit of new stable isotope studies of protein and amino acid utilization and balance, better information on body composition in infancy and childhood, and a better understanding of the statistical estimation of the risk of deficiency and the consequent definition of safe individual and population intakes. However, many questions remain unanswered. We need to understand how the human organism interacts with dietary protein in food, i.e. the systems biology of the protein and amino acid components of the human organism in relation to appropriate and acceptable growth, development, weight maintenance, as well as long-term overall health and well-being throughout life. Recognition of the deficiencies in the existing knowledge base helps the identification of potential areas for future research and investigation by the wider scientific and academic communities. The deliberations and recommendations of experts in this important sphere carry much weight within the academic community, as well as for research funding bodies, international agencies and bilateral donors.

The following recommendations for future research are based on the topics and issues that were identified and considered during the discussions at the preliminary working group sessions, during the Consultation and during the subsequent preparation of this document. Some of them were identified previously in the 1985 report (*1*) and are repeated here because they remain unsolved problems. However, as the present Consultation acknowledged, it is not enough to come up with a "wish-list" of research topics, without prioritizing what needs to be done. With resources becoming increasingly limited, the experts recognized that it would be futile either to outline research needs too broadly or to attempt to include every conceivable topic that may be relevant to the issues raised during their deliberations. The Consultation recognized the need to make judgements on priorities when they stated: "We need to prioritize our recommendations so as not to dilute the strength of our requests." In defining research priorities, no attempt was made to group topics in relation to the disciplines and facilities needed for answering them, since most require a combination of approaches. However, the needs of regulatory

authorities for more extensive review in relation to protein quality evaluation have been separately identified.

Whereas more nitrogen balance data informing better about the extent of relationships and interrelationships between climate, sex, age and protein source would be very useful, it is clear that, as concluded by Munro (*2*) in 1985, "Measurements of safe levels of protein intake by zero balance (adults)…have achieved their potential and this approach is unlikely to yield significant further revisions of requirements." Clearly, much more information is needed about the metabolic responses to variation in protein intake, especially the possibility and extent of adaptive mechanisms that might allow the requirement to be met by intakes that are lower than those currently recommended. Whatever the explanation for the apparent responses of healthy adults to varying protein intakes reviewed here, it is clear that they raise important questions about the usefulness of apparent nitrogen equilibrium as the major criterion of dietary protein adequacy. There is a clear and important task for research to explore new approaches and criteria for defining protein requirements that are not dependent on the achievement of balance in short-term experiments. Ultimately, the task is to identify protein intakes that not only enable appropriate body composition to be achieved and maintained, but also enable long-term health and well-being.

15.1 Recommendations for future research

1. The requirement for protein has of necessity been derived from studies of nitrogen balance, yet the data are widely scattered and many aspects of this approach remain unclear. It is an insensitive tool to use in defining protein requirements in adults and children, since it is derived by subtracting one large number (excretion) from another (intake). Moreover, the large pool of urea in body water means that there is an extended delay before any change in nitrogen input or output becomes fully apparent. Research should be undertaken to meet the urgent need for better techniques for assessing body protein homeostasis and balance that are sensitive enough to detect small changes that might be of significance for health. In addition, to gain a better understanding of the processes that determine the "maintenance" and "efficiency of protein deposition" values is needed, so that more accurate determinations of requirement might be made, and protein utilization might be optimized.

2. The development of animal models for human nutrition and metabolism should be a high priority. Protein and amino acid requirements are increasingly well understood in monogastric mammals used for agriculture. Extended dietary studies in human subjects are by their nature difficult to perform with the necessary level of precision, and animals

can be controlled more effectively over long periods of study. Whereas studies of animals will not give direct evidence of human requirements, particularly as in most young animals, unlike humans, growth is the dominant influence on requirement, they will enable techniques to be developed and mechanisms to be examined, and will help in identifying and minimizing sources of error that will be relevant to human studies.

3. The subject of adaptation has been raised in this report, yet little is known about the ability of humans to adapt to varying intakes of protein, particularly to low protein intake. A better understanding of the adaptive capability of humans is necessary, so that the true impact of protein deficiency for both the individual and the population can be assessed. Further work should be undertaken in this area. Not only would an adaptive process enable balance to be achieved at lower levels of intake than currently recommended, but also any adaptive responses of the requirement to habitual intakes would result in some correlation between intake and requirements. The implications of this for calculation of a safe individual intake and risk of deficiency within a population need to be explored. Also, to what extent do variations between individuals in their apparent requirements for protein reflect habitual protein intakes and incomplete adaptation during nitrogen balance studies?

4. On the basis of the very limited analysis reported here, there is a considerable proportion of the population with relatively low energy requirements and consequent low food intakes, such as sedentary elderly people consuming a diet typical of many developing countries, who will not meet their safe intakes of protein. Such populations may not be entirely risk-free on diets typical of developed countries. The requirements model that has been used implies that they will be unable to maintain their body protein content because of a low total protein intake, as well as because of a lysine limitation. Research should explore whether nitrogen equilibrium can be achieved at intakes of both protein and nutritionally limiting amino acids, such as lysine, below the safe intakes defined here, and whether there is any adverse cost in terms of body composition and function.

5. It is clear that intakes of more than twice the current safe adult level of protein intake are likely to occur widely in subjects with "healthy" physically active lifestyles. At the same time there is emerging information on the apparently beneficial effect of protein intakes in excess of the safe level for lowering blood pressure, reducing risk of ischaemic heart disease, and – at least within a mixed diet – improving bone health. It is clearly urgent to identify whether such associations are causal, what the mechanisms are, and what the dose response is. This is relevant to the

emerging issue of the significance of food-derived peptides. Thus specific peptides derived from certain animal proteins may be functionally important, e.g. as blood pressure regulators, as anti-ulcer agents, or in appetite regulation. This raises the important question of whether food proteins need to be distinguished for reasons other than their amino acid content. Future research should enable protein requirements to be better defined as intakes which not only enable appropriate body composition to be achieved and maintained, but also enable long-term health and well-being in terms of reduced risk of specific multifactorial diseases that currently affect a high proportion of the population. Our understanding remains poor in most of these areas, yet they represent problems of clear practical importance.

6. Research should be undertaken on the extent to which manipulation of dietary protein intakes above the recommended safe intake can enable better control of body weight in populations with declining levels of energy expenditure. Evidence in support of this has been accumulating, but more information on the mechanisms involved is needed.

7. The analysis of risk of deficiency of the new requirement values in section 12 is made on a population basis, considering only subpopulations varying in terms of their energy requirements, i.e. age, sex, size, and lifestyle. Clearly, more detailed work should be carried out on the application of these recommendations to specific populations, in particular to help clarify the difficult issues relating to individual and population requirements, and the development of individual and population requirements for "abnormal situations", which may be very common.

8. In this report, the consideration of protein and amino acid requirements has been largely independent of consideration of other nutrients in the diet which can markedly influence the effectiveness of dietary protein or amino acid utilization. These influences include energy, discussed here to a limited extent, and micronutrient intake. Given the current concerns about obesity on the one hand and inadequate micronutrient status on the other, research should be carried out by those with an understanding of protein nutrition and metabolism, in collaboration with experts on these other areas of importance, to explore more complex interactions. For example, in some critical population groups in the United Kingdom, there is reasonable evidence that poor B-vitamin status is common. Will this have implications for protein requirements? What is the quantitative impact of marginal or inadequate intakes of other nutrients? A future research agenda should explore the extent to which effective utilization of dietary protein might be seriously constrained by marginal status of other nutrients. The outcomes of relevance should include functional

measures, and markers of the activity of intermediary metabolism, especially that related to the synthesis of dispensable amino acids.

9. For pregnancy, an increased requirement for protein has been identified by employing a factorial model based only on a satisfactory birth weight, which is derived from very limited information, none of which is related to outcome in terms of the health of the newborn in either the short or the longer term. Pregnant women often appear to have intakes of both energy and protein which are far less than recommended amounts. Given the considerable current interest in the relationship between nutritional exposure during pregnancy, optimal fetal development and longer-term risk of chronic disease, any uncertainty over protein requirements would be an obvious shortcoming. This is all the more so given the evidence from supplementation studies that additional protein during pregnancy can be detrimental to the offspring. Do adjustments in the requirement occur that have not so far been identified? If they do occur, what is the cost to the mother and infant, before and after birth? These questions are of great scientific and public health importance. More research should be carried out on how dietary protein or individual amino acids modulate genetic expression, including epigenetic changes, in the short and longer term – as integral aspects of programming and later responses to environmental challenge.

10. In the case of infants and children, the Consultation identified lower requirements for protein than in the previous report. This reflects a more rational consideration of the implications of breast-milk intakes and the recent experimental evidence on infant growth with low-protein formulas. Nevertheless, in formulating the factorial model, judgements have been made, so further research in validating the elements of the factorial model should clearly be undertaken. The estimates need to be tested in the field, as far as is feasible in programmes designed to examine the relationships between habitual intakes and defined functions such as growth, resistance to infection, and physical and mental development.

11. The relationship between dietary intakes of protein and energy on the one hand, and the development of the individual or the prevalence in the community of different forms of protein–energy malnutrition on the other hand, was highlighted in the previous report. While there is general agreement that the etiology of the kwashiorkor syndrome is unlikely to reflect primarily protein deficiency, it is likely that an imbalance between protein and energy intakes could contribute to stunting. More research should be undertaken into dietary and environmental factors that constrain net protein deposition, leading to reduced height growth (stunting), as well as those that constrain net deposition leading to reduced muscle

mass or the selective partitioning of energy to adipose tissue rather than lean tissue. Are the protein requirements for growth in height greater than those for weight gain? Research should identify the metabolic or functional consequences of these constraints which translate to poorer health in the short and longer term. Such objective biochemical or physiological indices of marginal protein malnutrition are required in order to quantify a cost function relating to deficit intakes so that the prevalence of protein deficiency states can be better defined.

12. Knowledge of the requirements for resistance to and recovery from infections under the conditions that prevail in many communities is poor and should be a priority subject for further research, especially in relation to the current HIV epidemic in developing countries.

13. The revised amino acid requirements largely reflect the introduction of stable isotope tracer techniques. Although understanding has improved, it is by no means complete. For lysine in particular, there remains a need to reconcile the new adult maintenance requirement identified in this report with the considerably lower intakes deemed adequate in some of the older literature. So far there has been very little use of the isotopic techniques in infants, children and adolescents, and further research on amino acid requirements using isotopic methods should be undertaken to test the validity of the factorial model by which the present requirements have been derived.

14. As discussed in section 6, the proportion of total dispensable:indispensable amino acids is relatively unexplored, but potentially important influence on the extent to which dietary proteins can meet the requirement. Specifically, research should be undertaken to determine the upper limits of synthesis of dispensable amino acids, the conditions under which the need might exceed this capacity, and the extent to which this capacity might be determined by specific micronutrient status. Also, research should be undertaken to explore the relative importance of supplies of conditionally indispensable amino acids during critical periods of normal human development and during the stress of disease.

15. Research should be undertaken to determine whether supplementation with micronutrients can enhance the capacity of metabolic pathways that have been constrained as a result of programming during earlier life.

15.2 Regulatory issues

This report has endorsed the protein digestibility-corrected amino acid score method of protein quality evaluation, as identified in the 1991 FAO technical report (*3*), with some minor modifications to the calculation method. Several

issues which require further consideration have also been identified. These concerns include the digestibility of proteins, especially the extent to which new information on the origins of faecal nitrogen in relation to the diet requires a re-evaluation of the assumed digestibility of foods and diets. The concept of protein quality values in excess of 100 has also been suggested as a protein quality index for specific protein sources. These concerns are particularly important in relation to regulation within the food and ingredient industry. While they are outside the scope of this report, their resolution is urgently required through a new separate expert review.

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Annex. Statistical procedures

Derivation of reference protein:energy ratio, individual diets

The derivation of the reference protein:energy ratio for individual diets is drawn from the 1985 report (*1*). The following equation, derived from the equations presented by Beaton & Swiss (*2*) based on the arguments and approach of Lorstad (*3*), will provide an estimate of the protein:energy ratio that ensures, with whatever probability is desired, that a diet with the calculated protein:energy ratio will meet or exceed the actual protein requirements on the condition that enough is consumed to meet the energy requirements of the randomly selected individual.

$$R_a = \frac{E^2}{E^2 - Z_a^2 S_E^2} \cdot \left[\frac{P}{E} - \frac{Z_a{}^2 \; r \; S_P \; S_E}{E^2} + \frac{Z_a}{E} \cdot Q \right]$$

$$\text{and } Q = \sqrt{S_P^2 - \frac{2r \; P \; S_E \; S_P}{E} + \frac{P^2 \; S_E^2}{E^2} - \frac{Z_a^2 \; S_P^2 \; S_E^2}{E^2}(1 - r^2)}$$

where

R_α is the value of the protein:energy ratio requirement that would be expected to be exceeded by a certain proportion (α) of individuals (changing α alters the probability of adequacy or inadequacy of the ratio for the random individual);

Z_α is the standardized normal deviation above which α of the distribution lies (e.g. $Z_{0.025} = 1.96$);

E is the average energy requirement for the specified class of individual (specified by age, weight, activity, etc.);

P is the average protein requirement for the specified class of individual, expressed as energy equivalents;

S_E is the standard deviation of energy requirements;

S_P is the standard deviation of protein requirements;

r is the correlation between energy and protein requirements among individuals in the specified class.

Note that the equation is written in two parts for convenience only.

Estimation of distribution parameters

Given data that are assumed to represent the requirements of a representative sample of a population of interest, it is necessary to identify that distribution and characterize it by estimating its parameters. With a large amount of data, a specific family of distributions can usually be chosen and the parameters estimated (*4*). In general, however, not enough data exist. Two specific aspects of the requirement distributions are needed: an estimate of the centre or midpoint of the distribution and an estimate of the between-individual variability. In deriving estimates of the centre and variability of the requirement distributions, it is necessary to pay special attention to the shape of the distribution. The standard statistics used to summarize normally distributed variables (the mean and standard deviation) are particularly misleading if the variable of interest is either skewed (not symmetrical) or kurtotic (peaked or flat – having less or more of its area in its tails when compared with the normal distribution) (*5*).

The centre or middle of the requirement distribution is of primary importance. The median is defined as the level that divides a population in half; it has practical utility in the estimation of population prevalence. If the distribution can be assumed to be normally distributed, the arithmetic average or mean can be used as an estimate of the median, since this is both easy to calculate and provides an entrée to powerful statistical testing. This was not the case for protein requirements, which appear to be both skewed and kurtotic, suggesting that the median should be used as the reference midpoint of individual requirements. However, given the relatively large amount of data available, it was possible to perform a logarithmic transformation of the data that produced a normal distribution and permitted the use of standard parameter estimation techniques.

Regression

Regression is a general statistical technique that is used to explore relationships between a single continuous (outcome or dependent) variable, such as energy expenditure or nitrogen balance, and one or more other variables (independent, either continuous or dichotomous) such as age or weight. Given a general mathematical formula (a model, such as a linear relationship: $y = a + bx$), the regression procedure estimates the coefficients for the particular model (values of a and b) which best fit the data; measures how well the model fits the data (R^2 and standard error of fit); and estimates how

precisely the coefficients are known (standard error of *a* and of *b*). Thus regression has three goals: (i) description of the relationships and estimation of the coefficients; (ii) evaluation of how well the overall model fits the data; and (iii) evaluation of the individual, bivariate relationships in terms of whether the individual characteristics are statistically significantly related to the outcome variable.

The process is of necessity iterative, involving fitting various subsets of the variables that are under consideration. An initial model is chosen and fitted, and then is judged either to fit the data and well mimic the phenomena under study, or not to fit the data. Then another model is examined, one that is simpler if an explanatory variable could be eliminated, or one that is more complex if little of the total variability is explained. Once a satisfactory model is found, the fitted coefficients can be used to estimate the average or expected outcome at specific levels of the independent variables, and the estimated variability in the coefficients used to estimate the error in the ultimate predictions. While tools exist to assist the investigator in searching for an optimal model, the process optimally involves an interaction between biological insights into the phenomenon and statistical insights into the results of the computer programs that are used to do the complex calculations. One type of automated procedure that is frequently used is stepwise regression, which automatically searches a class of similar models, but this is restricted to use in exploring linear models. Descriptions of the procedures of regression and their practical applications can be found elsewhere (*5, 6*), as can more technical discussions (*7*). Many statistical programs exist to conduct the actual work (SPSS and SAS are two of the more complete and widely used programming systems).

The essential components of the regression process are: choosing a model (a specific form of mathematical equation – see below for examples); finding the specific coefficients that produce the best fit between the model and the data (where "best" fit is usually defined as the model which comes closest to the data in the sense of minimizing the squared discrepancy) and examining how good that fit is by looking at the summary statistics of the overall fit and of the individual coefficients, and at the residuals (the differences between the fit and the data for each data point) to check for regions or individuals for which the model seems inappropriate. This careful examination of the residuals is essential, especially at the extreme range of the data, to see if the model fits data similarly over whole range.

Mathematical models

The choice of the mathematical equation that might represent the data is key to regression, and several were used in the present report:

Linear model

This model, which represents the relationship between the outcome and independent variables as a straight line, is the usual default model, the starting point for a modelling exploration:

$$y = A + Bx + Cz + \ldots$$

where A is the intercept (the value of y when x, z, etc. are all zero) and B, C, etc. summarize the relationships between the outcome and the individual independent variables.

If a satisfactory linear model cannot be determined, non-linear models must be examined. Their problems are twofold: there is an infinite variety of non-linear relationships and no generally agreed default; and the actual fitting procedure is very complicated and requires careful implementation and interpretation.

The following represent some of the more commonly used non-linear models:

General logistic model

This model represents a sigmoidal relationship

$$y = A + (D - A) / \{1 + \exp(-B(x - C))\}$$

where A is the upper asymptote, D is the lower asymptote, C centres the curve, and B estimates how quickly the curve moves between the asymptotes.

Monomolecular model

This model represents an exponential relationship with a single, upper asymptote

$$y = A - \exp\{-B\,(x - C)\}$$

where A is the upper asymptote, C centres the curve, and B estimates how quickly the curve rises.

Biphasic linear model

This model represents a linear increase to a breakpoint and then another linear change:

Nitrogen balance = $A + Bx$ when x is below the breakpoint, or

$C + Dx$ when x is above the breakpoint,

where B and C are the slopes of the two "phases" and the breakpoint is (A–C)/(B–D).

Each of these models can be extended to include more dependent variables.

Regression output

The output of any regression program includes four general areas:

Estimates of the coefficients and their standard errors

These are usually in reference to the general formulation:

$$y = B_0 + B_1 x_1 + B_2 x_2 + B_3 x_3 + \ldots$$

and are reported as Bs and standard error of the Bs.

Estimates of how well the data and the model agree overall

Standard error of the estimate or of the fit: estimates, in the units of the dependent variable, how much variability remains in the data when the variability explained by the independent variables is taken into account. It is essentially the standard deviation of the residuals.

R^2 (coefficient of determination): estimates, as a proportion or percentage, how much of the variability in the dependent variable is removed by inclusion of the independent variables.

Estimates of how well the model and subsets of the data agree

Cook's distances, Studentized ranges, point leverages: these are measures of the influence of individual points and are useful in focusing the investigator on specific areas of the data that need closer examination. The basic strategy should be to carefully examine the residuals and how they relate to the individual variables.

Estimates of the interrelationships between the independent variables

Correlation of the coefficient estimates: these estimate the redundancy among the explanatory variables and are used for refining estimates of the precision of the final model.

Collinearity diagnostics: these give an indication of whether fitting problems may arise because of correlation between the independent variables. The detection of these problems is aided by examination of collinearity diagnostics.

Regression assumptions

While regression has a number of formal assumptions, the most important for its application are:

- The data represent the phenomenon of interest (this is why the initial gathering and screening of the data are critically important).

- The model is biologically reasonable. It must be kept in mind that the statistical fitting procedure involves fitting of the data and not the phenomena, and that the result is descriptive and not causal. The fundamental point is that the choice of the form of the model is not strictly a statistical decision but should be based on biological considerations.
- The underlying error is normally distributed. While technically regression assumes linearity, constant variance, and independence of the variables, the most important assumption is that of normality of error. This assumption justifies the use of the least squares method and gives access to powerful statistical testing techniques. While non-parametric regression approaches do exist, the general ubiquity and power of the normal distribution has led it to be the default assumption for model building. It is, however, an assumption. The distribution of the residuals must be examined to ensure that non-normality is not a major problem.This is especially important when examining non-linear models.

Analysis of variance

Analysis of variance (ANOVA) is a general statistical technique that is used primarily to explore whether subgroups of data (e.g. different sexes) have different mean values for some outcome variable. It is routinely used to test hypotheses about the equality of different subgroup means, and is a generalization of (and includes as a special case) the simple Student t-test. The technique operates by considering that the value of each data point in a data set can be written as the sum of an overall mean (of all the data) plus an amount that the specific subgroup mean differs from the overall mean plus an amount that the specific point differs from the mean of its specific subgroup (often termed error). For an arbitrary data point, y, in a specific subgroup, this decomposition can be written as:

y = overall mean + (subgroup mean – grand mean) + (y − subgroup mean).

Summing this formulation over all the data points gives a decomposition of the total variability of the data into that between and within subgroups. Statistical tests exist to determine whether the subgroup means are sufficiently different, compared with their within-subgroup variability, to declare that the subgroups differ statistically. The ANOVA approach was used by the Consultation to partition the variability of the protein requirement data in order to isolate the variability of major interest, the between-individual variability.

ANOVA is based on the assumption of normally distributed error. It is a robust procedure (not being very sensitive to slight departures from that assumption) for comparing subgroups, but for estimating components of

variance, it is sensitive to skewness and extreme outliers. For this reason, the trimmed logarithm of protein requirement was analysed.

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