be the most reliable and noninvasive method for assessing polyphenol biomarkers (8, 9). Creatinine is a usual correction method when spot urine has been collected (2). However, although creatinine is an indicator of renal function (9), its concentrations vary greatly depending on sex, age, physical activity, renal function, and diet (9).

With regard to the analysis of total resveratrol metabolites, the methodology was explained in detail in the article by Urpi-Sarda et al (10). This study analyzed glucuronide and sulfate conjugates of resveratrol without enzymatic hydrolysis of samples. This biomarker of wine consumption is defined as the sum of 7 phase II resveratrol metabolites, namely the following: trans-resveratrol-3-O-glucuronide, cis-resveratrol-4'-O-glucuronide, cis-resveratrol-3-O-glucuronide, trans-resveratrol-4'-O-sulfate, trans-resveratrol-3-O-sulfate, cis-resveratrol-4'-O-sulfate, and cis-resveratrol-3-O-sulfate (2). The methodology was quantitatively adapted to this study to analyze the 24-h urine samples collected on the last day of the run-in period and the last day of each intervention. As far as we know, authentic standards of some resveratrol metabolites have recently become commercially available in Canada (Toronto Research Chemicals) and in France (Bertin Pharma). In the present study, such metabolites were quantified with standards acquired from Toronto Research Chemicals and are as follows: trans- and cis-resveratrol-3-O-glucuronide (98% purity each), cis-resveratrol-4'-O-glucuronide (96% purity), and trans-resveratrol-3-O-sulfate (98% purity).

The present study was designed to determine whether ethanol or wine polyphenol interventions are responsible for the regulation of resveratrol metabolism in humans. Clin Chem 2007;53:292–9.


The process through which the dietary requirement for any nutrient is set is an important but challenging task, both conceptually and practically. After a thorough critical review of the literature, Dewey et al (1) proposed a mean requirement for protein for healthy school-age children of 0.69 g·kg\(^{-1}\)·d\(^{-1}\) and a safe level of 0.86·kg\(^{-1}\)·d\(^{-1}\). The most recent international expert consultation on protein requirements by FAO/WHO proposed 0.75 and 0.92 g·kg\(^{-1}\)·d\(^{-1}\), respectively (2). The calculations on which these requirements are based derive from studies of intakes that allow infants and young children to grow and develop normally. In their recent article, Elango et al (3) report data derived from metabolic studies, which leads them to conclude that the mean requirement should be 1.3 ·kg\(^{-1}\)·d\(^{-1}\), with a safe protein requirement of 1.55·kg\(^{-1}\)·d\(^{-1}\). This represents a substantial increase from the previous recommendations of 50–90% and carries very serious implications for policy, health care, food supplies, and the social welfare of large groups of the population. It is incumbent on those suggesting any revision of this order to be clear about how any proposed change compares with existing guidance. Any proposed change should be considered critically and discussed in the context of existing knowledge and with recognition of any potential limitations in the methods used to derive alternate requirements.

Supported by the Ministerio de Ciencia e Innovacin [grants AGL2006-14228-C03-01 and -02-ALI, and Sara Borrell postdoctoral program (MUH-CD09/00134)], Spain. The authors declared no financial or personal conflicts of interest.

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doi: 10.3945/ajcn.112.037002.

Protein requirements and the indicator amino acid oxidation method

Dear Sir:

The process through which the dietary requirement for any nutrient is set is an important but challenging task, both conceptually and practically. After a thorough critical review of the literature, Dewey et al (1) proposed a mean requirement for protein for healthy school-age children of 0.69 g·kg\(^{-1}\)·d\(^{-1}\) and a safe level of 0.86·kg\(^{-1}\)·d\(^{-1}\). The most recent international expert consultation on protein requirements by FAO/WHO proposed 0.75 and 0.92 g·kg\(^{-1}\)·d\(^{-1}\), respectively (2). The calculations on which these requirements are based derive from studies of intakes that allow infants and young children to grow and develop normally. In their recent article, Elango et al (3) report data derived from metabolic studies, which leads them to conclude that the mean requirement should be 1.3 ·kg\(^{-1}\)·d\(^{-1}\), with a safe protein requirement of 1.55·kg\(^{-1}\)·d\(^{-1}\). This represents a substantial increase from the previous recommendations of 50–90% and carries very serious implications for policy, health care, food supplies, and the social welfare of large groups of the population. It is incumbent on those suggesting any revision of this order to be clear about how any proposed change compares with existing guidance. Any proposed change should be considered critically and discussed in the context of existing knowledge and with recognition of any potential limitations in the methods used to derive alternate requirements.
There are 3 important considerations for the proposals made by Elango et al: 1) the method adopted in the study, 2) the application of that method to the assessment of protein requirements, and 3) the interpretation of the data generated.

Elango et al (3) used an isotopic tracer to follow the path of amino acids in healthy school-age children under different dietary conditions and from that inferred their need for dietary protein. The method used in this study is known as the indicator amino acid oxidation (IAAO) approach. There are different ways in which experimental models based on the IAAO have been used, and it is important that they are not confused with each other. As discussed by Elango et al (3), the method has mainly been applied in a number of studies by this group to determine the needs for a single indispensable amino acid. The authors also note that the method has been used by others, quoting studies that include “indicator amino acid oxidation” in the titles (4, 5). The implication that this is the same method is in fact misleading because the studies to which they refer involve a quite different approach in which 13C-1 leucine balance is used as an indicator of protein or nitrogen balance in a 24-h study. The application of the method to determine the requirement for protein as in the current study (3) was first used in piglets (6) and then in male adults (7).

The most widely used application of the IAAO method in humans, to assess the requirement for an individual amino acid, involves acute feeding studies in subjects who have been fasted overnight. In this biological state, the individual uses dietary protein to support net tissue protein synthesis, which replaces tissue protein losses during fasting, and to meet the metabolic demand associated with the utilization of amino acids in a range of metabolic pathways. The experimental protocol involves subjects who consume on each study day repeated small meals containing fixed intakes of an amino acid mixture (at approximate protein requirement amounts), which contains the indicator amino acid, usually phenylalanine, together with variable amounts of the test amino acid under investigation. A [1-13C]-1-labeled phenylalanine tracer is given during the feeding period to trace the oxidation of the indicator amino acid. By adding a range of amounts of the test amino acid on separate study days, a dose-response relation of the indicator oxidation to the test amino acid is determined. An inadequate, limiting intake of the test amino acid leads to inefficient utilization of all other amino acids and hence their high rates of oxidation. As the amount of the test amino acid increases so that its availability matches and then exceeds requirements, all other amino acids are used with greater efficiency, leading to low or minimal rates of their oxidation. This pattern of oxidation of the amino acid mixture is shown by the oxidation of the indicator phenylalanine. The amount of intake of the test amino acid that enables efficient utilization of the dietary mixture is identified when the partitioning of the indicator amino acid shifts from higher to low and constant rates of oxidation, the breakpoint in the intake-oxidation curve. Thus, the metabolic fate of the indicator amino acid as judged by the oxidation of the 13C-labeled tracer is presumed to “indicate” that of all other amino acids in the amino acid mixture. In effect, the paradigm is the determination of the amount of intake of the test amino acid that renders the amino acid mixture fully competent to meet the demand, a response that can be measured during the few hours of the feeding study.

This is not the paradigm in studies aimed at evaluating the protein requirement reported in the study of Elango et al (3), in their previous study with adults (7), or in a piglet “validation” study (6). Although a familiar [1-13C]-phenylalanine oxidation breakpoint response to graded intakes of protein is reported in all of these articles, quite different considerations apply. In these studies the study design involves subjects given a standardized diet for 2 d followed, on each study day, by repeated small meals of a diet that was adequate for energy and other nutrients but that contain different amounts of protein (as an amino acid mixture based on egg protein), from inadequate to surplus. Across studies, the breakpoint in the oxidation of the phenylalanine indicator amino acid was determined. Crucially, on every study day with every amount of protein, all subjects were given the same fixed intake of the indicator phenylalanine (as well as fixed amounts of tyrosine). The critical consideration is what factors determine the breakpoint in the oxidation of the phenylalanine for this design of study: fixed indicator but varied protein. As shown in Figure 1, there is a response curve that superficially mirrors the oxidation data reported in these articles but that in fact reflects only the experimental design in terms of the concentration of the phenylalanine “indicator” relative to that which would be present if a balanced reference amino acid mixture had been fed at the different total protein intakes. Each point is calculated from the reported data in the studies in children (3) or in adults (7). Thus, the relative indicator content of the meals varies across a wide range, with a 5-fold excess of phenylalanine at the lower protein intakes, falling to only 20% of the reference at the highest protein intakes. As shown in Figure 1, it is only when the total intake is equivalent to −0.6 g · kg−1 · d−1 that the total amino acid mixture may be considered to be balanced in relation to the reference (egg) protein. The similarity of the shape of the tracer oxidation curve in the published article with that of the relative indicator concentration in the test meal protein intakes shown in Figure 1 points to the latter determining the former: that is, the rate of oxidation of the tracer does not “indicate” the oxidation of the rest of the dietary protein, only its own excess or limitation in relation to the overall pattern of the demand.

Elango et al (3) do not provide any data or provide information that informs on the overall rate of amino acid oxidation, or protein utilization in their studies. However, we can predict the response to this experimental design of feeding meals in which the protein content is low and the energy content sufficient, from other experimental meal feeding studies using 13C-1 leucine (8) and from metabolic considerations about the role of energy and protein in regulating protein balance. Under these conditions, compared with the fasting state, there is inhibition of proteolysis, a reduction in the overall concentration of amino acids in blood, and reduced rates of amino acid oxidation. The dietary protein appears to be fully used, and the negative balance associated with fasting is reduced by an amount proportional to the dietary protein supplied. Thus, in the studies by Elango et al (3), it is most likely that the meal protein will be fully used with low levels of overall amino acid oxidation at lower amounts of intake until it becomes limited by the relative availability of phenylalanine as the intake of protein approaches and exceeds −0.6 g · kg−1 · d−1. At this point, amino acid oxidation would start to rise as increasing amounts of the unbalanced, phenylalanine-limited amino acid mixture is fed. If an increasing amount of a balanced mixture of amino acids had been consumed, then utilization would be high and oxidation low, up to an intake at which the capacity for postprandial utilization was exceeded. Above this intake, oxidation would start to increase. The capacity for maximal postprandial utilization would be a reflection of the previous amount of dietary protein intake, which would have determined the extent of postabsorptive losses (fasting negative balance), an indication of the adaptive metabolic demand (9, 10). Thus, the response to meal feeding studies is determined by the recent amount of protein intake, and this in itself provides no information on the protein requirement. For these reasons, although the studies by Elango et al (3) might identify a breakpoint in the oxidation profile of the indicator amino acid, the identity of this breakpoint is mainly a reflection of the relative excess or limitation of the so-called indicator rather than the utilization of the protein meals or whether the “protein requirements” are being met.
supply of protein for vulnerable population groups has been used to developing countries is due to inadequate protein intakes."
The premises that the adult requirement values are too low and state that "it is unknown with no long-term validation studies, and they develop arguments that the adult requirement values are too low and state that "it is conceivable that the current high prevalence of stunting in the poor in South America is due to inadequate protein intakes." The premise that there is a nutritional problem that can be attributed to a limited supply of protein for vulnerable population groups has been used to justify a body of stable isotope literature relating to amino acid and protein requirements over the past few decades. However, unlike the adult requirement for protein, which is heavily dependent on evidence from nitrogen balance studies, for children we have an independent guide to estimates of the protein requirements based on their rate of growth, especially during infancy when the modest amount of protein provided from breast milk supports normal growth rates and comparable information with breast-milk substitutes. It was these data that guided both Dewey et al (1) and WHO/FAO/UNU (2) in their derivation of the protein requirements of preschool and older children. With low rates of growth in preadolescents (weight gain of \(-0.055 \text{ g kg}^{-1} \cdot \text{d}^{-1}\) compared with a 5-times greater rate of weight gain at 6 mo of age) (1, 2), any argument that the safe protein requirement for preadolescents, \(1.55 \text{ g kg}^{-1} \cdot \text{d}^{-1}\) (1), is greater than values determined for breast-fed infants at 6 mo of age, i.e., \(1.19 \text{ g kg}^{-1} \cdot \text{d}^{-1}\) (1) or \(1.14 \text{ g kg}^{-1} \cdot \text{d}^{-1}\) (2), should be accompanied by a convincing argument to justify the proposition. We know that infants, whether breast- or formula-fed, consume a diet with a much lower protein concentration than after weaning, at which time food intake comes to reflect the adult diet with its higher protein concentration (protein-to-energy ratio). This fact alone makes the identification of the minimum protein requirement of older children or adults as defined by WHO (2) very difficult to determine. The adaptive metabolic demand model of the protein requirement (9, 10) predicts that any acute or short-term balance study of the protein requirement will identify an intake close to the habitual protein intake with true minimum intakes only identifiable after long-term adaptation to lower intakes, and such studies are unlikely to be performed in children. Growth in length is now widely discussed as a functional indicator of dietary adequacy and is known to be nutritionally sensitive to intakes of protein, zinc, and other type 2 nutrients (11). Several studies have shown a particular effect of increased milk intake on linear growth in children (12), possibly mediated by insulin-like growth factor 1. It remains unclear whether the observed effect of milk on linear growth can be directly or indirectly attributed to its protein content, because similar effects have not been shown when equivalent amounts of protein were consumed as meat (13, 14). Clearly, the etiology of stunting and the protein requirements of children are important issues that need to be fully resolved. However, these issues cannot be resolved by the sort of studies reported by Elango et al (3).

Neither of the authors had a conflict of interest.

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14. Millward and Jackson’s Figure 1 is a hypothetical construct questioning the validity of our IAAO model. One particular assumption of the indicator model is that the concentration of the indicator does not change, something we reported earlier (7) where plasma phenylalanine and tyrosine concentrations do not change in response to changes in the test amino acid, in that case lysine. Their comments related to Figure 1 show that Millward and Jackson may not understand the fundamental concept behind the IAAO method—that the intake of the indicator amino acid must remain constant regardless of the intake of the other amino acids. The intake of the indicator must also be in excess of the requirement for protein synthesis so that, in no case, even at the highest intake of other amino acids or protein, will the intake of the indicator amino acid be deficient. The authors make the criticism that the methods and results must be wrong because “the rate of oxidation of the tracer does not ‘indicate’ the oxidation of the rest of the dietary protein, only its own excess or limitation in relation to the overall pattern of the demand.” In fact, Millward and Jackson are correct—the oxidation of the tracer reflects its excess in relation to the overall pattern of demand. This is why the method works and why it is an accurate method to measure amino acid and protein requirement. Millward and Jackson have confused the changes in oxidation that happen to the other amino acids at different intakes, from deficient to adequate, with what we have shown repeatedly happens to oxidation of the indicator amino acid when its intake is above requirements, is constant, and does not change across all the dietary treatments. In contrast to the statement by Millward and Jackson, this is not an unbalanced, phenylalanine-limited amino acid mixture. We clearly stated in our article (4) that the protein was a high-quality balanced amino acid mixture with excess phenylalanine intake; however, this statement appears to have been overlooked by Millward and Jackson. We have spent 30 y developing and validating the IAAO method in humans and animals. We have responded to questions and misunderstandings by conducting many additional experiments. After years of questioning our own method we have a great deal of confidence that the IAAO...