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 commercially available in Canada (Toronto Research Chemicals) and in France (Bertin Pharma). In the present study, such metabolites were 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 soluble inflammatory mediators. We agree with Yang et al that the wine polyphenol interventions are responsible for the regulation of resveratrol-3-purity each), 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 commercially available in Canada (Toronto Research Chemicals) and in France (Bertin Pharma). In the present study, such metabolites were 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 soluble inflammatory mediators. We agree with Yang et al that the effect of each RW polyphenol on vascular function is intriguing and deserves further investigation.

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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 $^{13}$C-labeled tracer is presumed to “indicate” that of all other amino acids in the amino acid mixture fully competent to meet the demand, a response of the amount of intake of the test amino acid that renders the breakpoint in the intake-oxidation curve. Thus, the metabolic fate of intake of the test amino acid that enables efficient utilization of the amino acid mixture. In effect, the paradigm is the determination of the amount of intake of the test amino acid that renders the breakpoint in the intake-oxidation curve. Thus, the metabolic fate of intake of the test amino acid that enables efficient utilization of the amino acid mixture is shown by the oxidation of the indicator phenylalanine. The amount 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 $^{13}$C-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 enables efficient utilization of the amino acid mixture.
growth, especially during infancy when the modest amount of protein is 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 g·kg⁻¹·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 g·kg⁻¹·d⁻¹ (1), is greater than values determined for breast-fed infants at 6 mo of age, i.e., 1.19 g·kg⁻¹·d⁻¹ (1) or 1.14 g·kg⁻¹·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).

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Reply to DJ Millward and AA Jackson

Dear Sir:

We appreciate the opportunity to respond to the detailed letter submitted by Millward and Jackson. The most recent international expert consultation on protein requirements for healthy school-aged children, by the FAO/WHO (1), presented data that were based principally on nitrogen balance. As we have shown previously, nitrogen balance analyzed by using linear regression underestimates adult protein requirements by ∼20% (2). It was the limitations of nitrogen balance that led us to apply an independent method to determine protein requirements in adults. In addition, we did a thorough reanalysis of the existing nitrogen balance data by using 2-phase linear regression crossover analysis (rather than monolinear regression). The mean estimates of protein requirement were 0.91 g · kg⁻¹ · d⁻¹ derived from the reanalysis of the nitrogen balance data and 0.93 g · kg⁻¹ · d⁻¹ using indicator amino acid oxidation. The 2 independent estimates are remarkably similar. Hence, when we studied the protein requirements in children, we carefully reviewed the literature. The 2007 expert committee statement (1) was of little help, except for the study reported by Gattas et al (3). They studied their subjects at 4 different protein intakes—0.6, 0.8, 1.0, and 1.2 g · kg⁻¹ · d⁻¹—and showed a linear increase in nitrogen balance in response to protein intake. Unfortunately, they did not study higher protein intake amounts, and hence it was not possible to apply 2-phase linear regression analysis to the data (as was possible with the much larger adult nitrogen balance data set). Nonetheless, by using monolinear regression Gattas et al (3) reported a mean protein requirement of 0.94 and a population-safe requirement of 1.2 g · kg⁻¹ · d⁻¹, values that are significantly higher than the FAO/WHO values of 0.76 and 0.95 g · kg⁻¹ · d⁻¹, respectively. In light of the observations in our adult studies, that nitrogen balance analyzed by monolinear regression underestimates protein requirements by 20%, we reported in Table 4 of our recent article (4) that the corrected protein requirement estimates derived from nitrogen balance should be 1.13 and 1.44 g · kg⁻¹ · d⁻¹, which are similar to our experimentally determined values using indicator amino acid oxidation (IAAO) of 1.3 and 1.55 g · kg⁻¹ · d⁻¹.

It is clear from their letter that Millward and Jackson have reservations about the application of the IAAO approach. However, it is clear from the comparison of the IAAO results to reanalyzed nitrogen balance data that the 2 approaches give comparable results. One of the points raised by Millward and Jackson relates to the fact that plasma amino acid enrichments or their surrogate urinary amino acid enrichments may not reflect the precursor pool where oxidation takes place. Because the indicator amino acid used is phenylalanine, the precursor pool is the hepatocyte. Early in the validation of the IAAO technique, Ball and Bayley (5) showed that recovery of radioactivity from 1,14C-phenylalanine in liver tissue was inversely related to radioactivity in expired carbon dioxide. More recently in human adults using apo B-100 to determine intrahepatocyte enrichments of phenylalanine and tyrosine, we showed that, at maintenance phenylalanine intakes, the breakpoint in phenylalanine hydroxylation to tyrosine corresponded with the requirement for tyrosine determined by using oxidation of 1,14C-lysine as an indicator amino acid (6). Hence, the dietary breakpoint for the test amino acid measured by enrichment in liver protein is not different from that determined from enrichment in breath.

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...