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DIFFERENT APPROACHES TO DEFINE INDIVIDUAL AMINO ACID REQUIREMENTS

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■ **Abstract** A full review of the strengths and limitations of the various methods used to define amino acid requirements is provided. The focus is on the recent development of carbon oxidation techniques such as indicator amino acid oxidation and 24-h amino acid balance to determine dietary indispensable (essential) amino acid needs in adults. All approaches depend on the change in a metabolic parameter in response to graded intake of the test amino acid. In humans, the within-subject variance is less than the between-subject variance, which has led to an appreciation of the need to study each subject across a range of intakes, above and below the mean requirement level. The data can then be analyzed using two-phase linear regression crossover and a precise population mean requirement can be determined. Several approaches have been used to define the variance of the mean requirement. Finally, a minimally invasive indicator amino acid oxidation model has been developed which allows the determination of dietary essential amino acid requirements in children and other vulnerable populations.

CONTENTS

INTRODUCTION 102
METHODS TO DETERMINE AMINO ACID REQUIREMENTS 103
WHAT ARE THE NEEDS FOR PRIOR ADAPTATION TO THE LEVEL OF THE TEST AMINO ACID? 106
EXPERIMENTAL DESIGN AND DATA ANALYSIS CONSIDERATIONS IN STUDIES OF AMINO ACID REQUIREMENTS 107
Experimental Design Considerations 107
Data Analysis Considerations 108

OVERVIEW OF THE DATA AVAILABLE TO ESTIMATE ADULT DIETARY INDISPENSABLE (ESSENTIAL) AMINO ACID REQUIREMENT 108
APPLICATION OF CARBON OXIDATION TECHNIQUES TO THE DETERMINATION OF ESSENTIAL AMINO ACID REQUIREMENT IN CHILDHOOD 112
SUMMARY AND CONCLUSIONS 112

INTRODUCTION

The 20 amino acids for which t-RNAs exist are the components from which all proteins are synthesized (8). If they cannot be endogenously synthesized in sufficient amounts then they are needed in the diet (36). Classically in healthy adults nine amino acids are regarded as dietary essential (or indispensable) (Table 1). During growth or in disease several others are regarded as conditionally indispensable; in neonates only 5 out of the 20 can be regarded as fully indispensable (Table 1). If amino acids are not present in the right proportions then protein synthesis will be reduced (6, 55). Further, it has been shown that whole body protein breakdown is increased when less balanced mixtures of amino acids are fed (12). The endogenous amino acids then provide the missing amino acids for protein synthesis, but net protein accretion and nitrogen balance (retention) is lower when a less-than-ideal mixture of amino acids is fed, either as a protein or a mixture of free amino acids (12, 18).

The last time the topic of the determination of essential amino acid requirements was discussed in the *Annual Review of Nutrition* was in 1994 (17), at which time

TABLE 1 Categorizing dietary amino acids by their degree of indispensability[a]

Dispensable	Conditionally indispensable	Indispensable
Alanine	Arginine	Histidine
Aspartate	Cysteine	Isoleucine
Asparagine	Glutamine	Leucine
Glutamate	Glycine	Lysine
Serine	Proline	Methionine
	Tyrosine	Phenylalanine
		Threonine
		Tryptophan
		Valine

[a]Adapted from (36).

the question asked was, Can the controversy be resolved? The controversy being referred to was the discrepancy between the newer carbon oxidation estimates (3, 6, 32, 50, 53) and the older estimates based on nitrogen balance (16, 19, 27, 33, 35, 37, 40, 44–46). In this review we show how that controversy can now be resolved.

METHODS TO DETERMINE AMINO ACID REQUIREMENTS

Determination of amino acid requirements involves feeding of graded levels of the test amino acid to the subject and looking for a clearly definable change in a relevant biological parameter. Ideally a range of graded levels should be fed above and below the requirement point, as this allows for a more precise definition of the requirement level (see Experimental Design and Data Analysis Considerations in Studies of Amino Acid Requirements, below). Furthermore, a similar estimate of requirements should be obtained irrespective of the method used (see Figure 1). Fundamentally, all of the methods used are a surrogate for measuring protein synthesis, which is hard to measure directly. In Figure 1 three patterns of response are shown, in the relevant biological parameters, to graded intakes of the test amino acid. The first is in nitrogen balance and growth, where as increasing intakes of the test amino acid are given there is a progressive increase in growth or nitrogen balance until the requirement level is reached for the test amino acid, after which there is no further increase in growth or nitrogen balance. The second response is in plasma amino acid level, or in oxidation of the test amino acid [direct amino acid oxidation (DAAO)], or in the 24-h balance of the test amino acid (24-h DAAB). Initially there is no change for the second pattern of response, as graded levels of the test amino acid are fed below the requirement level. However, once the requirement level is reached there is a progressive (usually linear) increase in the outcome parameter. The third response is in plasma urea, or in the oxidation of

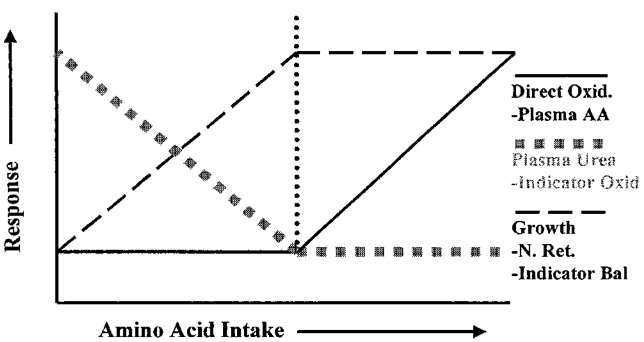

Figure 1 The three different patterns of metabolic responses to graded intakes of an essential amino acid.

an indicator amino acid [indicator amino acid oxidation (IAAO)], or in the 24-h oxidation of the indicator amino acid (24-h IAAO). For the third pattern of response, the outcome parameter falls as graded levels of the test amino acid are fed below requirement, until the requirement level is reached, after which there is no further change (6, 55). Twenty-four-hour indicator amino acid balance (IAAB) follows the same pattern as that of nitrogen balance and growth.

A similar estimate for the requirement of the test amino acid should be obtained regardless of which model is used, and indeed this is the case in piglets (20). Until 1986, all of the published estimates of essential amino acid requirements in adult humans had been obtained using either nitrogen balance or, for a few, change in plasma amino acid (16, 48, 52). In 1986 a series of DAAO studies were reported (29–31, 56) in which the estimate for requirement of the test (essential) amino acid was two to three times higher than estimates published by the Food and Agriculture Organization of the United Nations, World Health Organization, and United Nations University (FAO/WHO/UNU) (16). Not surprisingly, these higher new estimates were strongly criticized (33). The introduction of IAAO in 1993 (54) provided independent verification of the higher DAAO essential amino acid requirements. The key question was why were the nitrogen balance estimates, as summarized by the FAO/WHO/UNU (16), so low? This question was answered through a series of steps. First, it was shown that within-subject variance is lower than between-subject variance in adult humans (53, 54), which led to an appreciation that the same subject needed to be studied across a range of test amino acid levels, including the requirement level (53, 54). A careful review of the extensive literature, in which nitrogen balance was used as the outcome parameter to determine essential amino acid requirements (27, 44), revealed two studies (19, 40) in which subjects were examined across a range of intakes of the test amino acid. One of these studies (19), in which the lysine requirement of young women was examined, was reanalyzed using nonlinear regression and the addition of miscellaneous nitrogen losses (38). It was shown that as nitrogen balance was approached, the efficiency of nitrogen utilization was reduced, with the result that the requirement point (defined as the achievement of nitrogen balance) was shifted to the right, and a much higher estimate was obtained for lysine requirement. The reanalysis resulted in requirement estimates for lysine ranging from 18 to 30 mg/kg/d. By comparison, the requirement estimates for lysine obtained using various amino acid oxidation techniques ranged from 30 to 35 mg/kg/d (15, 21, 22, 30, 54). Similarly, we have conducted an identical analysis of the sulphur amino acid data of Reynolds et al. (40) and defined a total sulphur requirement in the range of 15 to 20 mg/kg/d (P.B. Pencharz & R.O. Ball, unpublished data). This range is comparable to our estimates obtained using indicator amino acid oxidation (see Table 3) (10). In essence, the discrepancy between the nitrogen balance estimates of essential amino acid requirements and the amino acid oxidation techniques has been removed.

Classically, outcome variables such as nitrogen balance and/or growth have been used in infants and children and nitrogen balance in adults (6, 17, 55). Both of these parameters are indirect and hence may be insensitive. Furthermore, the body urea pool may need seven to ten days to adapt (39). Because it is unacceptable to maintain infants and children on either deficient or excessive intakes for any period of time (5), alternative methods were needed. The change in the plasma level of the test amino acid has also been used to define essential amino acid requirements, most successfully for tryptophan (48, 52); however, on balance it is an insensitive method and has been supplanted by carbon oxidation methods. Although carbon oxidation methods had been previously developed in animals (20, 49), Vernon Young and coworkers (29–31, 56) pioneered the application of carbon oxidation methods to determine essential amino acids in adults. They used the direct oxidation approach, in which the test amino acid was also used as the tracer. Their initial studies were in the fed state only and questions were raised of whether this was an adequate representation of amino acid requirements on a 24-h basis, which led them to conduct 24-h direct oxidation amino acid balance studies (14).

A little earlier, a different carbon oxidation model was introduced (54) to study adult amino acid requirements, namely, IAAO. This model had first been developed and validated in growing pigs, against the classical techniques of nitrogen balance and growth (20). The IAAO model uses another essential amino acid, such as phenylalanine (in the presence of an excess of tyrosine), lysine, or leucine, as the tracer, which is thereby independent of the changes in levels of the intake of the test amino acid. Since there is no storage of amino acids, the indicator is partitioned between incorporation into protein or oxidation. In the early animal studies it had been shown that the IAAO method did not require adaptation to the level of the test amino acid, which is a basic requirement for using nitrogen balance to determine amino acid requirements. When we decided to adapt the IAAO to humans, one of the first steps was to study the effects of adaptation on the requirement estimate. This was done with subjects fed either 4.2 mg/kg/d or 14 mg/kg/d of phenylalanine for two nine-day periods, during which test levels of phenylalanine were fed on days three, six, and nine. No effect of prior adaptation to the two levels of phenylalanine was seen on the requirement for phenylalanine (53). Based on these studies and the earlier animal work, we concluded that prior adaptation to the level of the test amino acid was not needed. On the other hand, we have shown a need to adapt for two days to the level of protein used, prior to conducting an indicator study (47). These important steps enabled us to develop a minimally invasive indicator model to study amino acid requirements (5), and the model was then applied to determine the tyrosine needs of children with phenylketonuria (PKU) (4). Most recently, the concepts of 24-h amino acid balance have been applied to the indicator oxidation model (2, 22, 24). While this approach is theoretically the most satisfactory method yet developed to determine amino acid requirements, its major drawback is that subjects must be detained for 24 to 29 hours for each study, compared with eight to ten hours for the standard fed-state IAAO study. In addition, the users of the 24-h IAAB method still feel that adaptation to the level of the test amino acid is needed. Subjects in both the IAAO and IAAB studies are fed amino acid–based diets that are unpalatable. For the IAAO studies, subjects are on the test diet only the day of the oxidation study. Conversely, for the IAAB study, depending on the length of adaptation, subjects are on the amino acid–based test diet for five to seven days for each test level. Our group also introduced the use of two-phase linear crossover regression analysis to determine amino acid requirements (53, 54). This method of analysis requires that each subject be studied at six or seven levels of the test amino acid. In practical terms this means that subjects need to take the study diet for six or seven days using the IAAO model and between 30 and 49 days for the IAAB model, as it is presently used. Clearly the IAAB method is much more demanding and probably impractical in children.

Notwithstanding the different assumptions, strengths, and weaknesses of the various oxidation methods or nitrogen balance, when the subjects are fed at five or more graded levels of the test amino acid, similar values are obtained for lysine requirement (21, 22, 38, 54). We therefore moved to make the IAAO model as noninvasive as possible. We gave the tracer orally, rather than intravenously, and this change made no difference to the estimate of lysine requirement (21). Urine can be used to determine the enrichment of the tracer amino acid in arterialized blood (5). The one caveat is that the tracer must contain less than 0.2% contamination with the D-amino acid, which due to relative failure of renal tubular reabsorption is concentrated in urine (9). Further, there is limited or no need for prior adaptation to the level of the test amino acid, but a two-day adaptation to the level of the protein fed may be necessary (47). In the minimally invasive model (5), subjects do have to be adapted to the experimental diet for four hours to allow for equilibration of the background $^{13}CO_2$ enrichment in breath. This makes the total experimental day about nine hours—four hours for background equilibration and four to five hours for the oxidation study (5).

Children with inborn errors of amino acid metabolism are already on standardized dietary protein intakes, so in these subjects even adaptation to the level of protein intake is not needed. Although the minimally invasive model was originally developed to permit study of children with PKU (4), it has recently been used to determine total branched chain amino acids (BCAAs) in six- to ten-year-old healthy children (28). The way is now open to a more general application of this modified IAAO model for determining amino acid requirements in vulnerable groups such as infants, children, adolescents, and pregnant and lactating women (4–6).

WHAT ARE THE NEEDS FOR PRIOR ADAPTATION TO THE LEVEL OF THE TEST AMINO ACID?

Traditionally, the minimum adaptation period to a change in the test amino acid intake level was considered to be seven days. This comes from the use of nitrogen balance as the outcome variable and the fact it takes about seven days for urinary nitrogen excretion to achieve a new equilibrium (39). When direct amino acid oxidation or 24-h direct amino acid oxidation and balance is used, few days of adaptation may be required to each new level of test intake. The only experimental data on the subject is work in which we compared the estimate obtained of phenylalanine requirement in the same subjects adapted to either 4.2 or 14 mg phenylalanine/kg/d. Subjects were on each level for nine days and oxidation studies were conducted at days three, six, and nine. No effect of the day of study was detectable (53). Kurpad et al. (25) also compared seven days of adaptation with 21 days and showed no effect on the requirement estimate. As mentioned above, when the IAAO method was first being validated in piglets, it was shown that no prior adaptation was needed to the level of the test amino acid (20). The IAAO and the 24-h IAAB are based on the partitioning of the indicator amino acid either to protein synthesis or to oxidation (6, 20, 55) in which any adaptation occurs primarily at the acyl-t-RNA level, which adapts in less than four hours (8). An indirect way of addressing the fundamental question of whether prior adaptation affects the requirement estimate is to compare our results with those of Kurpad, Young, and coinvestigators (25), who use a seven-day adaptation period. Currently, two essential amino acids have been studied by both approaches: lysine (21, 22) and threonine (2, 24, 50). The respective results are 35 mg/kg/d compared with 30 mg/kg/d for lysine and 19 mg/kg/d compared with 15 mg/kg/d for threonine. The data are all in the same range and because of the invasive nature of the IAAB model, subjects are not studied at all levels of intake, whereas in the IAAO model all subjects were studied at each of the test intake levels. Further, the only published IAAB model study which uses what we regard as the minimum number of intake levels (six) to be able to fully apply regression analysis to determine the requirement breakpoint with any precision (see Data Analysis Considerations, below) is one which determines the threonine requirement in Indian subjects (24), and again each subject was not studied at all six levels. Nonetheless, the issue of the length of adaptation needs careful study. The minimally invasive model permits the determination of essential amino acid requirements in vulnerable groups such as children and has been used in children as young as three years of age (28).

EXPERIMENTAL DESIGN AND DATA ANALYSIS CONSIDERATIONS IN STUDIES OF AMINO ACID REQUIREMENTS

Experimental Design Considerations

It is desirable to study a range of intakes, below and above the predicted requirement level, to strengthen the regression estimates of requirement. The oxidation data fit a bilinear model as well, if not better, than they fit curvilinear models (53, 54). In a bilinear model one line has no (or minimal) slope and is essentially flat while the other line has a slope. In DAAO the first line is flat while the second line is sloped upwards, while in IAAO the first line has a downward slope and the second line has no slope (6). A strength of the IAAO and IAAB approaches is that the lowest test level can be zero, whereas for DAAO or 24-h DAAB the minimum intake of the test amino acid is set by how much isotope has to be used to obtain detectable $^{13}CO_2$ enrichment in breath. The minimum number of points to satisfactorily define a line is three. Therefore, if the bilinear model is to be used, a minimum of six test levels is needed, with three above the requirement breakpoint and three below. When there is no previous estimate of the breakpoint, or only a weak estimate, then a seventh level of intake may be needed to increase the likelihood of achieving a minimum of three levels on each line.

Data Analysis Considerations

Modern statistical software packages all have models for nonlinear regression. The user selects the model that best fits the data and thereby minimizes the error of the breakpoint to obtain a valid estimate of the mean requirement for the test amino acid. The bilinear (or two-phase linear regression crossover) model also provides the upper 95% confidence interval of the breakpoint (53, 54), which we have used as an estimate of the mean plus two standard deviations and hence an estimate of the safe level for a population (16); it is analogous to the recommended dietary allowance (RDA). The various carbon oxidation studies are expensive to conduct, and therefore the number of subjects studied is limited. The IAAO model is less invasive, and therefore it has been possible to study subjects, even children (4), at six or more levels. Conversely, even in the most extensive IAAB study (24), subjects were studied at only three of six test levels. In an effort to model the unequal allocation of subjects to intake levels, the authors conducted a simulation study using 1000 Monte Carlo simulations (24). Further work is necessary to refine the determination of the requirement breakpoint using regression techniques and even more so the definition of the variance of the breakpoint to obtain better estimates of population variance.

OVERVIEW OF THE DATA AVAILABLE TO ESTIMATE ADULT DIETARY INDISPENSABLE (ESSENTIAL) AMINO ACID REQUIREMENT

Regretfully, many of the earlier studies using nitrogen balance do not conform to the standards now regarded as necessary: (*a*) repeated measurements made in the same subject over a series of levels of the test amino acid to control for the large between-subject variance in humans and (*b*) the use of a series of test amino acid intakes above and below the estimates requirement level to precisely apply nonlinear regression techniques to define the mean population requirement level. The two studies that come closest are by Jones et al. (19) to determine lysine requirements and by Reynolds et al. (40) to define total sulphur amino acid needs.

The limitations of the earlier nitrogen balance studies (16, 27, 44) led Millward (33) and others (summarized in Reference 17) to develop concepts such as the "metabolic demand for amino acids" and to attempt to factorially determine essential amino acid needs. In our view these approaches are indirect and have been surpassed by the development of direct experimental approaches such the IAAO and IAAB. Millward and his group are also working to develop a ^{13}C-leucine oxidation and balance method to evaluate the utilization of proteins such as wheat and milk (34). This work is of great potential interest in the study of amino acid (in their case lysine) utilization in intact protein. However, since only one level of intake is used for each protein, it is hard to see how this approach can also be used for the purpose of defining amino acid requirements.

The strengths and limitations of the carbon oxidation and balance methods are summarized in Table 2. Clearly (from the theoretical point of view) IAAO and its extension to 24-h IAAB are the strongest. The mean requirement values are similar for the two amino acids (lysine and threonine) that have been studied using both methods. In addition, it is only in the most recent IAAB study that six levels of the test amino acid were used (24), but each subject was studied at only three test levels. Nonetheless, detailed inspection of the data suggests that if only the fed oxidation were used it would not alter the requirement estimate for threonine (24). It appears then that the fasted oxidation studies are not necessary when the indicator oxidation approach is used to determine essential amino acid requirements. In our view, the issue that does need resolution is the length of adaptation to each change in level of the test amino acid, a topic that is discussed in detail in a previous section (What are the Needs for Prior Adaptation to the Level of the Test Amino Acid?). Despite the theoretical and experimental design issues discussed above, a remarkable similarity between the mean (breakpoint) estimates can be derived from the original (fed state only) DAAO studies and 24-h DAAO, and balance with those from IAAO and IAAB.

IAAO can also be used to determine the needs of conditionally indispensable amino acids such as tyrosine (43) and cysteine (11). Sulphur amino acid needs were determined with (11) and without (10) the presence of excess cysteine, and it was demonstrated that cysteine can spare about 50% of methionine needs (11).

The existing carbon oxidation/balance studies are summarized in Table 3, including the number of subjects and test levels used. Areas for future research are the aromatic amino acids, where the present estimates range from 15 to 39 mg/kg/d. The 15 mg/kg/d estimate, derived from the work of Roberts et al. (43) and Zello et al. (53), is probably an underestimate, and the 39 mg/kg/d of Basile-Filho et al. (1) is likely an overestimate. There are no carbon oxidation studies of isoleucine requirement, and there are concerns about interactions between the three branched chain amino acids. We have tentative estimates of total BCAA requirements, which have only been published in abstract, of approximately 137 mg/kg/d for adults (41) and 145 mg/kg/d for children (28). The adult values, if confirmed, suggest that the current leucine and valine estimates listed in Table 3 may be low.

TABLE 2 Comparison of methods used to determine amino acid requirements

Method	**Strengths and limitations**
Growth	Useful only in rapidly growing young animals
Nitrogen balance	Classical method, but it needs great pains to make sure the balances are as precise as possible Requires 5 to 7 days adaptation to each level of amino acid
Direct amino acid oxidation (DAAO)	Unclear as to whether prior adaptation is needed—indeed, Zello et al. (51) suggest that at most only 2 days of adaptation are needed Can only be used to determine the requirements of amino acids whose carboxyl group is directly released to the bicarbonate pool and so will appear in breath Concern that the precursor pool from which oxidation takes place increases as the level of the test amino acid increases The question of whether fed state studies are representative of daily (24-h) needs
24-h DAAO	Unclear as to whether prior adaptation to the level of the test amino acid is needed, but adaptation to the experimental diet is needed for at least 4 hours prior to starting the study Can only be used to determine the requirements of amino acids whose carboxyl group is directly released to the bicarbonate pool and so will appear in breath. Also, concern that the precursor pool from which oxidation takes place increases as the level of the test amino acid increases
Indicator amino acid oxidation (IAAO)	There is still some debate as to the best indicator to use, and at present phenylalanine (in the presence of an excess of tyrosine), lysine, and leucine have been used; the only other possible choices are valine and isoleucine The pool from which oxidation takes place does not change in size as the level of the test amino acid is altered The evidence (discussed in the text) suggests that prior adaptation to the level of the test amino acid is not needed The requirements of any essential amino acid or conditionally indispensable amino acids can also be studied A full range of intakes (from zero and up) of the test amino acid can be used
24-h indicator amino acid balance (24-h IAAB)	A combination of the features of 24-h direct balance with the indicator oxidation approach and all of the theoretical advantages of IAAO plus the possible advantage of measuring indicator oxidation and balance in the fasted as well as in the fed state. So far its users have made its use more limited by still insisting on adaptation to the level of the test amino acid for 5 to 7 days. More invasive for the subjects and costly for the investigative team. Unless modified, its application will be limited to studies in healthy adult volunteers.

TABLE 3 Essential amino acid requirement estimates determined by carbon oxidation/balance methods

Amino acid	Author (reference #)	Method used[a] {number of levels}	Breakpoint ($mg/kg^{-1}/d^{-1}$)
Aromatic amino acids			
Phenylalanine	Zello (53)	DAAO {7} [41][b]	9.1
Tyrosine	Roberts (43)	IAAO {7} [42]	6.0
Total Aromatics	Zello plus Roberts (43, 53)		15.1
	Basile-Filho (1)	24-h Tyr Bal {3} [16]	39.0
Branched chain amino acids			
Leucine	Meguid (29)	DAAO reanalyzed {8} [52]	24.5
	El-Khoury (14)	24-h Leu balance {3} [10]	38.3
	Kurpad (23)	24-h Leu balance {4} [40]	40.0
Valine	Meguid (30)	DAAO reanalyzed {7} [37]	19.2
Lysine	Meridith (31)	DAAO reanalyzed {8} [28]	26.6
	Zello (54)	IAAO {7} [42]	36.9
	Kriengsinyos (21)	IAAO {5} [60]	35
	Kurpad (22)	24-h IAAB {4} [32]	29
	Kurpad (25)	24-h IAAB {4} [36]	31
	Duncan (13)	IAAO {6} [30]	45
Sulphur amino acids	Di Buono (10)	IAAO {6} [36]	12.6
Threonine	Zhao (56)	DAAO reanalyzed {7} [33]	13.5
	Wilson (50)	IAAO {7} [36]	19.0
	Borgonha (2)	24-h IAAB {3} [15]	15.0
	Kurpad (24)	24-h IAAB {6} [48]	15.0
Tryptophan	Lazaris-Brunner (26)	IAAO {8} [33]	4.0

[a]Reanalysis of the data was performed by P.B. Pencharz & R.O. Ball using two-phase linear crossover regression analysis and the data were presented in the published papers.

[b]The number of data points refers to the number of total observations within each study.

Abbreviations: DAAO, direct amino acid oxidation; IAAO, indicator amino acid oxidation; 24-h IAAB, indicator amino acid balance and oxidation.

Another point that deserves consideration is the high estimate of mean lysine requirements obtained by Duncan et al. (13) of 45 mg/kg/d as compared with 35 mg/kg/d obtained using the same IAAO model. Our original interpretation of these data was that feeding subjects at the rate of 0.8 g protein/kg/d did not alter the estimate of lysine requirement compared with similar subjects studied at a protein intake of 1 g protein/kg/d (54). However, when we studied this matter in more detail (21) we found that both the original results of Zello et al. (54) as well as the more recent results all in subjects receiving 1 g protein/kg/d are significantly lower than the estimates obtained by Duncan et al. (13) in subjects fed only 0.8 g protein/kg/d. We tentatively interpret these differences as that not all the subjects were in nitrogen equilibrium at a protein intake of 0.8 g protein/kg/d.

APPLICATION OF CARBON OXIDATION TECHNIQUES TO THE DETERMINATION OF ESSENTIAL AMINO ACID REQUIREMENT IN CHILDHOOD

The preexisting nitrogen balance data in childhood consists of work from three groups: Snyderman and colleagues for infants (45, 46), Pineda and colleagues for preschool children (37), and Nakagawa and colleagues for 10- to 12-year-old children (35). All these results predate and hence are included in the FAO/WHO/UNU 1985 report (16).

With the development of the minimally invasive IAAO model (5), it has proven possible to study essential amino acid requirements in children as young as three years of age. Published in full is a study of the tyrosine requirements of children with phenylketonuria (4). In these children, with blocked phenylalanine hydroxylase pathways, tyrosine became a conditionally indispensable dietary amino acid and a mean tyrosine requirement of 19 mg/kg/d was obtained (4). With a blocked hydroxylase pathway the requirements for phenylalanine are only those for protein synthesis. It would therefore be expected that phenylalanine requirements in these children would be lower than their tyrosine needs, and indeed they are at 14 mg/kg/d (7).

Conceptually, essential amino acid requirements in growing animals and hence also in children can be regarded as the amount needed for maintenance plus that needed for growth. In this regard the total BCAA needs of 145 mg/kg/d for children determined by Mager et al. (28) make sense in relationship to the values in adults of 137 mg/kg/d (41). The children studied were six to eight years old, an age at which the daily growth component is small and is roughly equal to the 8 mg/kg/d difference between the childhood and adult total BCAA estimates.

The lower age limitation of the minimally invasive model is due to an inability to collect expired breath from children under the age of three. An additional issue is the unpalatability of an amino acid–based diet. On the other hand, it is possible to conduct carbon oxidation studies in neonates since expired breath can be collected in a head box. To the best of our knowledge the only such study used to determine the requirement of an amino acid was one to determine tyrosine needs in parenterally fed neonates (42).

SUMMARY AND CONCLUSIONS

The introduction of DAAO in 1986 revolutionized the field of the determination of essential amino acid requirements in adults. However, it was not until 1993, with the application of IAAO to the determination of essential amino acid requirements in adults, that independent verification was obtained of the DAAO finding. Twenty-four-hour balance and oxidation studies have been added to both DAAO and IAAO, but it is not clear that the fasted studies are needed, at least in the IAAO model, to define the requirement breakpoint.

The IAAO model, including the two-phase linear crossover regression model, was developed in animal nutrition (20). Its introduction to human nutrition (54) has changed the experimental design of essential amino acid requirement studies and has permitted the precise definition of the mean population requirement. Further, the application of regression analysis to sets of the older nitrogen balance data with repeated measurements in the same adult has permitted reanalysis of these data and an explanation of the apparent discrepancy between the higher carbon oxidation estimates of essential amino acid requirements and the previous interpretation of the nitrogen balance data. The newer carbon oxidation estimates appear to provide reasonable estimates of essential amino acid needs, and the nitrogen balance data in which repeated measurements were made within the same individual can be reanalyzed to produce supportive data.

Finally, the development of the minimally invasive IAAO model now makes it possible to systematically determine the essential amino acid requirements in children and in other vulnerable groups, such as pregnant women and persons with chronic illnesses.

The *Annual Review of Nutrition* is online at http://nutr.annualreviews.org

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Annual Review of Nutrition
Volume 23, 2003

CONTENTS

FRONTISPIECE—*Frank Chytil* xiv

ROUGH AND ROCKY ROAD TO THE RETINOID REVOLUTION, *Frank Chytil* 1

MECHANISM AND REGULATION OF SELENOPROTEIN SYNTHESIS, *Donna M. Driscoll and Paul R. Copeland* 17

IRON STATUS AND NEURAL FUNCTIONING, *John L. Beard and James R. Connor* 41

INSIGHTS INTO THE PATHOGENESIS OF GALACTOSEMIA, *Nancy D. Leslie* 59

DIET AND NUTRITION IN POOR AND MINORITY COMMUNITIES IN THE UNITED STATES 100 YEARS AGO, *Robert Dirks* 81

DIFFERENT APPROACHES TO DEFINE INDIVIDUAL AMINO ACID REQUIREMENTS, *Paul B. Pencharz and Ronald O. Ball* 101

VITAMIN D AND ITS ANALOGS AS REGULATORS OF IMMUNE ACTIVATION AND ANTIGEN PRESENTATION, *Matthew D. Griffin, Nianzeng Xing, and Rajiv Kumar* 117

NUTRITION AND PREVENTION OF TYPE 2 DIABETES, *T. Costacou and E.J. Mayer-Davis* 147

BIOLOGIC MECHANISMS OF THE PROTECTIVE ROLE OF LUTEIN AND ZEAXANTHIN IN THE EYE, *Norman I. Krinsky, John T. Landrum, and Richard A. Bone* 171

NUTRITIONAL REGULATION OF MILK FAT SYNTHESIS, *Dale E. Bauman and J. Mikko Griinari* 203

TROPHIC AND CYTOPROTECTIVE NUTRITION FOR INTESTINAL ADAPTATION, MUCOSAL REPAIR, AND BARRIER FUNCTION, *Thomas R. Ziegler, Mary E. Evans, Concepción Fernández-Estívariz, and Dean P. Jones* 229

NUTRITION IN THE PERIOPERATIVE PATIENT, *Lyn Howard and Christopher Ashley* 263

PHYSIOLOGY AND MOLECULAR BIOLOGY OF DIETARY IRON ABSORPTION, *Silvia Miret, Robert J. Simpson, and Andrew T. McKie* 283

GUGULIPID: A NATURAL CHOLESTEROL-LOWERING AGENT, *Nancy L. Urizar and David D. Moore* 303

CHALLENGES AND APPROACHES TO REDUCING FOODBORNE ILLNESS, *Catherine E. Woteki and Brian D. Kineman* 315

DIETARY, EVOLUTIONARY, AND MODERNIZING INFLUENCES ON THE PREVALENCE OF TYPE 2 DIABETES, *Leslie Sue Lieberman* 345

IN VIVO MEASUREMENT OF FLUXES THROUGH METABOLIC PATHWAYS: THE MISSING LINK IN FUNCTIONAL GENOMICS AND PHARMACEUTICAL RESEARCH, *Marc K. Hellerstein* 379

COMMON ENDOCRINE CONTROL OF BODY WEIGHT, REPRODUCTION, AND BONE MASS, *Shu Takeda, Florent Elefteriou, and Gerard Karsenty* 403

INDEXES

Subject Index 413

Cumulative Index of Contributing Authors, Volumes 19–23 433

Cumulative Index of Chapter Titles, Volumes 19–23 436

ERRATA

An online log of corrections to *Annual Review of Nutrition* chapters (if any, 1997 to the present) may be found at http://nutr.annualreviews.org/