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Toward a Dynamic Model of Indispensable Amino Acid Requirements of the Adult Human: A Factorial Estimate of Oxidative Amino Acid Losses

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A B S T R A C T

Background: Consensus regarding the required intake of indispensable amino acids (IDAAs) and protein [representing total amino acids (AAs)] in the adult is lacking. Oxidation is a major, although not exclusive, source of IDAA loss in humans body and a primary factor determining requirements; a quantitative understanding of oxidative IDAA losses is required.

Objectives: This study aimed to develop a factorial diurnal model of total oxidative IDAA and protein losses in the adult human.

Methods: A factorial diurnal model of oxidative losses of protein and each IDAA at maintenance was developed by estimating the magnitude and variability of sources of oxidative loss from existing literature: inevitable catabolism (constitutive oxidation of each absorbed dietary AA), and protein turnover in the postprandial and postabsorptive states. Total oxidative losses were calculated by summing individual losses, validated against published independent nitrogen balance data and compared with current IDAA requirements.

Results: The factorial model predicted minimum oxidative total AA losses of 390 ± 60 mg/kg BW/d, 59% of the estimated average requirement for protein. Inevitable AA oxidation and oxidation associated with postabsorptive protein turnover were the major sources of the oxidative loss for protein, at 40% and 44%, respectively. Summed oxidative IDAA losses ranged from 64% (isoleucine) to 91% (tryptophan) of current requirements. Total oxidative losses predicted by the model were significant predictors of actual experimental oxidative losses obtained by nitrogen balance ($R^2 = 0.66$; $P = 0.049$).

Conclusions: The use of a factorial model for estimation of minimum IDAA and protein oxidative losses in the adult human provides an essential starting point for an updated understanding of protein and IDAA requirements. Further iterations of the model will estimate total protein and IDAA requirements, and account for variations in dietary protein quantity and quality, as well as different populations and physiologic states. Additional data, especially for inevitable oxidation in humans, and particularly with respect to individual IDAAs, are needed.

Keywords: factorial model, oxidative amino acid loss, amino acid requirements, protein requirements, protein turnover

Introduction

Dietary protein and its constituent amino acids (AAs) play essential roles in many aspects of human health, including the maintenance of muscle mass, metabolic regulation, and bone health [1,2]. While average minimum requirements for the dietary essential amino acids [*indispensable amino acids (*IDAAs)] and total protein (representing total AAs) have been established [3], the specific requirement for an individual is dependent on age, lean body mass, dietary protein source, and physical activity

Abbreviations: AA, amino acid; AAA, aromatic amino acid; BW, body weight; DAA, dispensable amino acid; EAR, estimated average requirement; GIT, gastrointestinal tract; IAAO, indicator amino acid oxidation; IDAA, indispensable amino acid; IO, inevitable catabolism; NB, nitrogen balance; PA, postabsorptive; PB, protein breakdown; PB_{PA}, protein breakdown in the postabsorptive period; PB_{PP}, protein breakdown in the postprandial period; PP, postprandial; PS, protein synthesis; PTO_{PA}, oxidative amino acid losses associated with postabsorptive protein turnover; PTO_{PP}, oxidative amino acid losses associated with postprandial protein turnover; TID, true ileal digestibility; TOL, total oxidative loss; UUN, urinary urea nitrogen; UUN_{EXP}, experimental urinary urea nitrogen; UUN_{HG}, urinary urea nitrogen due to ammonia absorption across the hindgut; UUN_{IO}, urinary urea nitrogen due to inevitable oxidation; UUN_{PA}, urinary urea nitrogen due to oxidation associated with postabsorptive protein breakdown; UUN_{PP}, urinary urea nitrogen due to oxidation associated with postprandial protein breakdown; UUN_{PRE}, predicted urinary urea nitrogen.

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level, among other factors. The current recommendation for the minimum dietary intake of protein required for nitrogen balance (NB) in adults [the estimated average requirement (EAR)] is 0.66 g protein/kg body weight (BW)/d [3], based on a meta-analysis [4] of empirical data from NB studies. While this level of intake is expected to account for maintenance AA losses, as well as any inefficiencies in the digestion, absorption, and metabolic utilization of the consumed dietary protein [3], the estimate has been criticized as being too low [5,6], with updated analyses of the EAR using the indicator amino acid oxidation (IAAO) methodology providing higher requirement values ranging from 0.87 to 0.93 g protein/kg BW/d [7]. The current recommendations for the IDAAs [3,8] are based largely on isotopic methodology, with a reanalysis of early NB data included for some IDAAs. However, both these methods require assumptions and have limitations [2,3,9–13], and the complexities of protein metabolism, some of which are not entirely understood, make an estimation of IDAA and protein requirements inherently difficult [3]. The result is an overall lack of consensus in recommendations for both minimum protein and IDAA intake. A mathematical (factorial) model to allow prediction of the minimum requirement of each IDAA and total protein, capable of addressing such complexities and applicable to different populations and physiologic states, would be useful and would provide a framework to assess the relative importance of underlying metabolic processes as well as the mechanisms underpinning their variation.

Based on a substantial body of research [14–20], a summary of the major metabolic processes contributing to the requirement for IDAAs and protein is shown in Figure 1 [16,19,21,22]. Losses of AAs arise mainly from integumental losses, urinary losses of free AAs, gastrointestinal losses, and oxidative losses, the latter of which are likely to have the greatest magnitude. These oxidative losses occur within a diurnal framework [15–17,23] including distinct losses during each of the postabsorptive (PA) and postprandial (PP) phases. During the PA period, protein breakdown (PB) exceeds protein synthesis (PS) to provide IDAAs for the maintenance of protein body mass in crucial tissues and organs, producing a negative protein balance. Postprandially, protein intake must be sufficient to allow a rate of PS that exceeds PB, to both counterbalance PA losses and provide for any losses occurring during the fed state. As each source of loss is influenced by multiple factors, including the quantity and quality of dietary protein consumed, the provision of clear recommendations regarding IDAA and protein intake for each population group relies on an updated understanding of each source of IDAA loss in a protein-containing diet, taking into account the nuances of protein metabolism. We have previously published a quantitative model for the gastrointestinal AA losses [24] and as a major source of loss, an estimate of minimum daily oxidative losses is an essential next step toward developing a comprehensive model of AA metabolism in the adult human.

Oxidative AA losses are variable across the diurnal cycle and occur in the adult human dependently and independently of

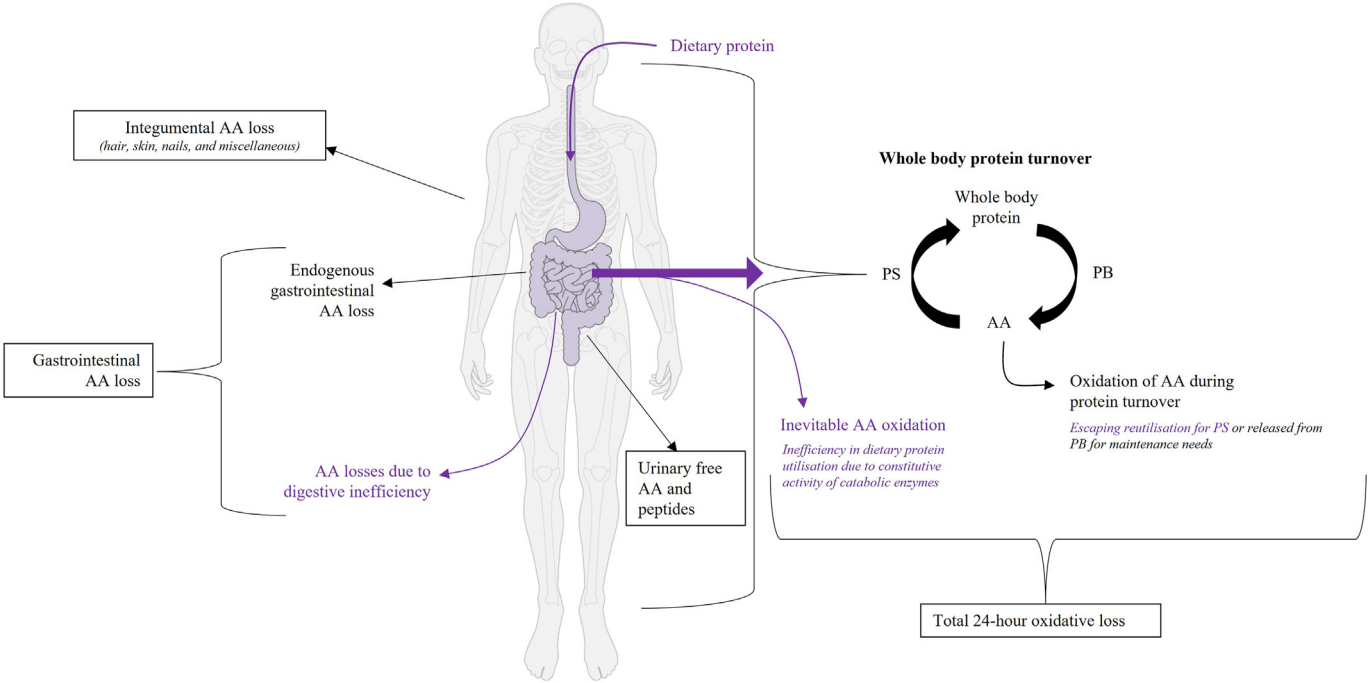


FIGURE 1. Amino acid (AA) losses contributing to the maintenance dietary protein requirement for the adult human. Losses specific to the postprandial phase are shown in purple. Digestive losses vary with the dietary protein source. Absorbed dietary AA are subject to inevitable AA catabolism [19,21] due to the existence of constitutively active catabolic enzyme systems in the cell. AA escaping inevitable catabolism enter protein turnover [16,22], the tightly regulated coordination of protein breakdown and protein synthesis. During the postabsorptive period, protein breakdown provides AA for maintenance at a rate determined by the first-limiting AA; however, AA in excess to requirements for the first-limiting AA will be catabolized. Postprandially, recycling efficiency is expected to be high although a small amount of AA from protein breakdown likely escapes reutilization for protein synthesis. In addition to oxidative AA losses, dietary protein must replace endogenous AA losses that occur via the export of AA into the gastrointestinal tract, the excretion of urinary free AA and peptides, the shedding of skin, hair, and nails, and the secretion of miscellaneous body fluids [3,16,19].

protein turnover (Figure 1). It appears that following digestion and absorption, a proportion of each absorbed dietary AA is oxidized, regardless of the overall level of AA intake or express energetic need (ATP supply). A number of animal studies have shown that 15%–30% [21] of the dietary absorbed first-limiting IDAA is irreversibly catabolized. The term inevitable catabolism has been used [19,21,25] to describe this phenomenon, for which a currently accepted explanation is the existence of constitutively active catabolic enzyme systems in the cell, and AA catabolism, both by mammalian and bacterial enzymes, in the epithelial tissue. There is always a degree of catabolic loss of the first-limiting AA, even when the absorbed amount is below the amount required for maximum PS (a biological threshold above which excess AAs will be catabolized), and there is a surfeit of nonprotein energy (indicating that any express energetic requirement for AA catabolism is low). Thus, even in situations where it might be expected that the absorbed amount of the first-limiting AA may be preserved, there is still some catabolic loss of this AA. Dietary AAs escaping inevitable oxidation during uptake are largely channeled to PS for the maintenance of whole-body protein, including covering AA losses from AA oxidation associated with protein turnover, which occurs as a function of PB in both the PP and PA periods. While protein turnover describes the tightly regulated and coordinated process of PS and PB, with the reutilization of AA directly from PB for PS, this reutilization is not 100% efficient, and a proportion of these AAs is oxidized, the magnitude of which is proportional to the rate of PB [21,22]. In the fed state, the direct recycling of AA from PB into PS, without significant exposure to enzymes involved in AA oxidation, is expected to create a high AA reutilization efficiency [22,26], with published estimates ranging from 2% to 10% of PB [22]. In the fasted state, however, PB occurs in a purposeful manner, to provide AA explicitly needed for maintenance requirements, as well as for oxidative processes such as gluconeogenesis [20,27]. A recently published model estimated the total oxidative loss of AA in the PA state in adult humans at 20% of PB [28]. While direct, physiologically relevant measurements for each source of oxidative loss have been published, these data have not been combined into a factorial model of AA metabolism in humans.

In an effort to move closer to a dynamic understanding of IDAA and protein requirements, we developed a factorial diurnal model for minimum total oxidative losses, representative of utilization inefficiency. The model is based upon maintenance protein and IDAA oxidative losses from the human body, determined under conditions whereby subjects receive a protein-containing diet providing total protein and IDAAs at amounts commensurate with current estimates of requirements. Losses due to inevitable catabolism and each of PA and PP PBs are estimated and summed to determine the total minimum oxidative loss of protein and each IDAA. The primary aim of developing the model was the provision of insight into the magnitude and variable nature of oxidative IDAA and protein losses, to contribute to a larger dynamic model that will underpin an understanding of population and physiologic state specific IDAA and protein requirements. While focused on humans, the modeling approach also has application to animal nutrition, for the optimization of feed efficiency (as dietary protein utilization) and subsequent reduction of excess nitrogen emissions. The model also serves the purpose of identifying key gaps in current knowledge.

Methods

Conceptual summary

The model was designed to predict oxidative losses of IDAAs and protein at a maintenance protein intake in an adult male human and is depicted schematically in Figure 2. The state of maintenance is defined in this study based on a diet where protein and each AA [IDAA or dispensable amino acid (DAA)] are provided commensurate with minimum requirements (Figure 2A) so that there is no additional oxidative loss due to the provision of dietary protein in excess of the requirement (Figure 2B), provision of the first-limiting AA below requirements (Figure 2C), or an imbalance between the amounts of provided IDAAs and DAAs as required for PS (that is, a difference between amounts of each AA provided and that incorporated into body protein), nor no net gain in body protein. This scenario allows for a definitive estimate of the minimum oxidative loss for dietary protein and each IDAA. Three separate sources of oxidative loss were characterized (Figure 2, light gray boxes): inevitable oxidation (IO) due to constitutive catabolism of AA during absorption and uptake into the body cells, losses due to the oxidation of AA arising from PB in the postabsorptive (PTO_{PA}) state, and losses due to the oxidation of AA arising from PA in the postprandial state (PTO_{PP}). The model is also based on the rationale that AAs entering protein turnover are only those that have not been lost due to inevitable catabolism (Figure 1). Thus, losses due to the oxidation of AAs arising from PB refer to AAs that have escaped inevitable catabolism, been incorporated into protein via PS, and then oxidized as a function of the breakdown of body protein.

The modeling process followed the 4 steps:

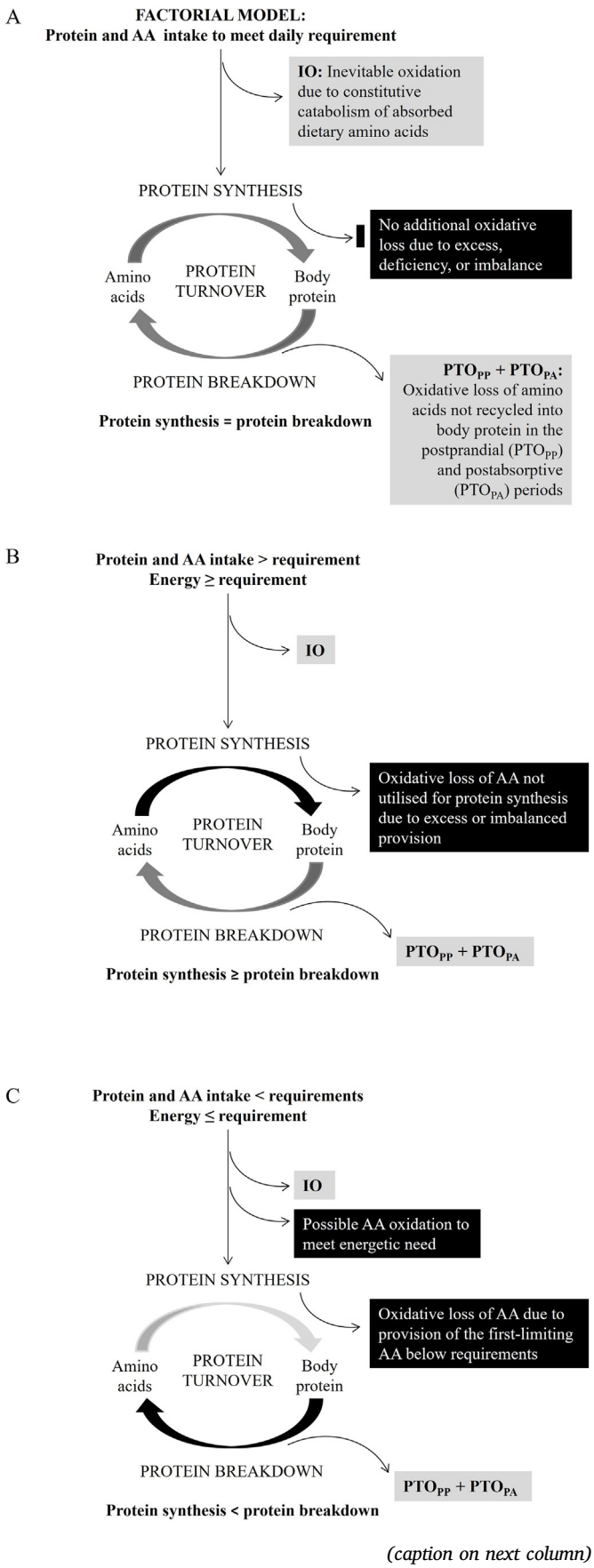
- Step 1: Estimation of IO
- Step 2: Estimation of PTO_{PA}
- Step 3: Estimation of PTO_{PP}
- Step 4: Summation of these 3 oxidative losses to determine the total oxidative losses (TOLs) associated with maintenance (TOL, Equation 1).

$$TOL = IO + PTO_{PA} + PTO_{PP} \quad (1)$$

Estimates for the magnitude of each source of oxidative loss (IO, PTO_{PA}, and PTO_{PP}) were obtained following a comprehensive search of the literature and modeling where necessary, with subsequent calculation of the mean and SD for each model parameter.

Step 1: Estimation of IO (oxidation due to constitutive AA catabolism)

IO was expressed as a percentage of the absorbed amount of the first-limiting dietary IDAA [19,21]. Although constitutive AA catabolism provides an important source of energy for the gastrointestinal tract (GIT) and body, it reduces the availability of the absorbed AA for PS and thus the ability of the diet to replenish maintenance AA losses and is therefore a major process underlying inefficiency in the utilization of dietary AA for PS. The process of IO is in contrast to that of preferential AA oxidation [19,21], where AAs are specifically catabolized to match an ATP need. Thus, IO occurs irrespective of there being sufficient dietary energy for the purpose of ATP production and is reflected as a constant in the model. Preferential AA



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catabolism is not represented in this model as it is assumed that protein will be consumed along with adequate amounts of energy from carbohydrates and fat. Since the presently described factorial model was designed to determine the minimum amount of dietary protein and each IDAA required for NB, it is also based on a diet providing protein and each IDAA at the minimum required amount; thus, the rate of IO for a first-limiting IDAA is the only diet-related loss due to utilization inefficiency for that IDAA. Subsequent iterations of the model will address IDAA loss (oxidation) due to the provision of IDAA in excess of requirements and due to dietary AA imbalance.

An estimation of IO was made using data from studies evaluating the efficiency of utilization of absorbed lysine and threonine in the growing pig [29,30] (Supplemental Table 1). While IO in humans can be related to losses due to splanchnic metabolism, estimates of splanchnic loss usually include losses due to both oxidation and incorporation into splanchnic protein and no studies directly investigating IO in humans have been reported. Serial slaughter data for the growing pig were selected for animals retaining body nitrogen below their potential rate of nitrogen retention, at first-limiting AA intakes of 70% to 90% of requirements and with sufficient energy intake. The mean value chosen for IO, expressed as a percentage of the absorbed first-limiting dietary AA in the growing pig, was 29% ± 2%. This value is consistent with a mean IO of 26% ± 5% found in the growing rat, based on the analysis of each IDAA, as the first-limiting IDAA, individually, and determined by direct measurement at near maximal PS [31]. Similar values have also been reported in the chicken [32,33]. There is some evidence that IO may vary between AAs and is influenced by the level of AA intake [23,31]. This is not addressed in this study, and the value of IO was assumed to be the same for all AAs. Subsequent models will address variations in IO as more data become available.

Since IO is defined as a percentage loss, relative to the amount of absorbed AA, IO was calculated as 295% ± 2% of available protein or AA (Equation *2*). Absorbed protein or AA was defined as the intake multiplied by the true ileal digestibility (TID) of that protein or AA. For example, if the TID of a protein is 90% (such as for cooked egg protein) [34,35], then the overall estimated value for IO would be 29% of (90% of) protein intake. TID is defined as apparent AA digestibility corrected for basal gut AA losses determined after giving a protein-free diet and is the most appropriate measure of digestibility to assess the bioavailability of AAs in foods for humans [36].

$$IO = 0.29 \times (\text{AA or protein intake} \times \text{TID}) \quad (2)$$

FIGURE 2. Schematic showing the rationale underpinning the factorial model of oxidative amino acid (AA) losses at maintenance (A), where total oxidative AA losses are the sum of inevitable oxidation (IO) and oxidation associated with protein breakdown in each of the postprandial (PTO_{PP}) and postabsorptive (PTO_{PA}) states (gray boxes). The rationale in (A) is that upon which the factorial model is based. There is no additional oxidative loss due to the provision of AAs in excess of requirements (B), lower than requirements (C), preferential catabolism due to an energetic need (C), or an imbalance in AAs provided compared with the composition of body protein (B, C; black boxes). Protein synthesis and breakdown are equal, and there is no net gain or loss in body protein.

For the purpose of the model, maintenance intakes for protein and the IDAAs were based on the current recommendation for the minimum dietary intake of high-quality protein required for N balance of 0.66 g protein/kg BW/d [3], and the current daily intake recommendations for the IDAAs [3], respectively.

Step 2: Estimation of PTOPA (total AA oxidation arising from body protein turnover during the PA period)

To estimate PTO_{PA}, data were collected from stable isotope tracer studies conducted for the determination of the dietary requirement for a first-limiting AA [37–44] (Supplemental Table 2). Selection was limited to studies conducted on young (younger than 60 y), healthy adult males, to reduce heterogeneity in PS measurements due to differences in age, sex, and health status. Within the selected studies, only data where the first-limiting IDAA intake was 90%–100% of the current recommended intake, while dietary protein and all other IDAAs were provided at greater than the maintenance requirement, were used. Since the rate of protein turnover depends on the availability of the first-limiting IDAA, the rates of oxidation, as well as PB and PS, were assumed to be close to the maintenance level.

Stable isotope tracer methodology provides measures of flux (Q) and oxidation (O) for a tracee IDAA (usually lysine, leucine, or phenylalanine). From these values, rates of AA release from PB and incorporation into PS can be estimated, using the relationship $Q = PB + I = PS + O$ [45,46], where I represents dietary intake of that IDAA. For each parameter, data for an individual IDAA is divided by the proportion of that IDAA in whole-body protein to translate the individual IDAA value into a total protein value. According to the abovementioned relationship, the estimation of PB is carried out by subtracting I from Q. In the PA state, there is no dietary IDAA intake, and the flux of an IDAA will stem solely from PB and the intravenous tracer AA [46].

Stable isotope tracer data [37–44] were used to estimate the oxidative loss of IDAA in the PA period, given as a proportion of determined PB in that period. Thus, only studies presenting complete numerical data for AA flux and oxidation were included. For each data set, tracee IDAA oxidation presented for the PA period was expressed as a proportion of the release of that IDAA from PB in the PA period (PB_{PA}), within the same experiment and the mean rate of oxidation, given as a function of PB_{PA} (PTO_{PA}) was calculated. Mean IDAA oxidation during the PA period was found to be 11% ± 2% of IDAA release from PB_{PA}, based on 10 observations from 6 independent studies and defined as follows:

$$PTO_{PA} = 0.11 \times PB_{PA} \quad (3)$$

To allow estimation of PTO_{PA}, it was then necessary to derive an estimate of PB_{PA}. Using selected isotope tracer data [37–44, 47], PB was determined at a mean rate of PB per hour for the PA period. The PA and PP periods were defined as 8 and 16 h, respectively. These periods were based on a 12-h feeding window, followed by a further 4 h of PP metabolism [48], resulting in 16 h spent in the PP state. The determined mean rate of PB_{PA} per hour was multiplied by 8 h, to give a total value for PB_{PA} (per 8-h fasted period) of 1.41 g/kg BW/d. Therefore, the value of PTO_{PA} for use in the factorial model (according to Equation *3*) was 155.1 mg protein/kg BW/d. PTO_{PA} losses for the individual IDAAs (eg, PTO_{PA} for lysine, PTO_{PA} for valine) were calculated using the AA composition of whole-body protein [8], assuming that all IDAA from body protein are catabolized equally per unit of protein available for catabolism.

Step 3: Estimation of PTOPP—total AA oxidation arising from body protein turnover in the PP period

While the flux of an IDAA will stem solely from PB and intravenous tracer AA in the PA state, in the PP state, IDAA flux represents AA release from PB, dietary AA, and the reabsorption of AA across the GIT [49]. Determination of the dietary AA component requires unproven assumptions, and the magnitude of the reabsorbed AA relies on the extrapolation of limited data from measurements in animals [24]. Therefore, while IDAA and total protein oxidation associated with PTO in the PA state have been determined directly from 24-h isotope tracer AA data, IDAA and total protein oxidation associated with protein turnover in the PP state (PTO_{PP}) were estimated in this study, indirectly.

There are limited data available to provide an estimate of PTO_{PP}. In the fasted state, AA from PB that are not recycled back into PS are oxidized due to either recycling inefficiency or meeting maintenance needs; in the fed state, however, dietary AAs largely provide for maintenance needs, and recycling inefficiency is expected to decrease given an increase in the rate of PS [28]. Thus, PTO_{PP} will include AA oxidation due to recycling inefficiency only and likely at a decreased rate compared to that occurring in the PA state. A recent estimate of AA oxidation associated with direct recycling inefficiency from PA protein turnover, based on measured rates in the skin and muscle of human subjects, was 5% of PB [28], 25% of total postabsorptive oxidative losses (PTO_{PA}), estimated at 20% of PB. These losses include both recycling inefficiency and maintenance oxidation. It is considered, therefore, based on these observations that PTO_{PP}, which comprises solely of losses due to recycling inefficiency, lies somewhere between 0% and 5% of AA released from PB in the fed state. The estimation of PTO_{PA} in the current model based on IAAO data are 11% of PB. Applying the proportion of recycling inefficiency as 25% of total PTO_{PA} [28] to these data produces a recycling inefficiency of 2.75%. An independent estimate of recycling inefficiency, based on extrapolation of a relationship between urinary urea excretion and protein intake for the growing pig in the fed state is 2% [21,22]. The mean of these available data were used to provide an estimate of for oxidative AA losses associated with protein turnover in the PP state, of 2.4% ± 2%, as follows:

$$PTO_{PP} = 0.024 \times PB_{PP} \quad (4)$$

The magnitude of PB_{PP} (protein breakdown during the PP period) was estimated by subtracting the rate of PB_{PA} from determined (24-h) total protein breakdown over 24 h. PS (24 h) served as a proxy for 24-h PB; while protein turnover is usually reported with respect to PS, the complete process describes the tightly regulated and coordinated processes of both PS and PB, which will have an equal rate over 24 h in a state of net protein balance [17,23], although the individual rates of PS and PB vary during this time [17,23]. An estimate of the mean 24-h rate of PB in adult male subjects consuming close to the EAR for protein (0.66 g/kg BW/d) is 4 g/kg BW/d [50–52]. Since PB_{PA} was estimated in this study to be 1.41 g/kg BW (over 8 h) [37–41,44, 53], PB_{PP} was set as the difference, at 2.59 g/kg BW (over 16 h). Using this estimated rate, the resulting value for PTO_{PP} for use in

the factorial model was 62.2 mg protein/kg BW/d. PTO_{PP} losses for the individual IDAAs were calculated using the AA composition of whole-body protein [8].

Step 4: Summation of component losses to estimate total oxidative losses

Total oxidative losses for an adult male at maintenance were estimated as outlined:

$$TOL = [0.29 \times (AA \text{ or protein intake} \times TID)] + (0.11 \times PB_{PA}) + (0.024 \times PB_{PP}) \quad (5)$$

Protein intake at maintenance was defined relative to the current EAR of 0.66 g/kg BW/d [3]. Intake for each of the IDAAs was based on the current recommended IDAA requirements [3]. TID was based on the TID for cooked egg protein of 0.90, due to egg protein being a well-characterized reference protein that has an AA profile allowing almost complete utilization [54]. It was assumed that the TID for egg protein would apply to all IDAAs.

Assessment of the accuracy of the model predictions

Assessment of the accuracy of TOL predicted by the model was performed by comparison of model predictions with actual oxidation derived from 24-h urinary urea nitrogen (UUN) data from human NB studies where egg protein was fed as the sole source of dietary protein at the maintenance requirement [55–58].

While a wealth of UUN data for subjects consuming varied intakes of different dietary protein sources is available in published NB studies, not all NB data, and not all dietary protein sources, were suitable for the assessment of TOL. In NB experiments, graded levels of a specific dietary protein are fed, each with a unique IDAA and DAA profile that is defined by a specific first-limiting IDAA. PS will take place up to the level defined by the first-limiting IDAA only, and IDAAs in excess of this level will either be transaminated to provide DAAs limiting for PS, or be oxidized [59]. Thus, the balance between IDAAs and DAAs of a specific protein source will determine the extent of AA oxidation due to AA excess. Egg protein is a well-characterized reference protein that has an IDAA profile allowing almost complete utilization [54] and has a high ratio of IDAAs to DAAs (almost 50:50); any IDAAs present above the amounts required for PS are transaminated to provide required DAAs [60]. Data from NB studies using egg protein were therefore expected to be representative of AA oxidative losses for a diet providing an ideal balance of AAs, with minimal excess AA oxidation, either from an imbalance between IDAAs and DAAs or an excess supply of dietary AAs. It was also expected that AA oxidation in these studies would be comparable with that in the selected IAAO oxidation data used for the estimation of PTO_{PA}, as the tracer and first-limiting IDAAs were provided at the maintenance requirement in these studies, with no oxidation due to imbalance or excess provision. NB studies selected for assessment of the model [55–58] met a strict set of criteria, as follows: the NB studies were from the meta-analysis by Rand et al. [4]; the studies reported UUN data; dietary egg protein was the sole dietary protein source; egg protein intake was at the maintenance requirement level; an energy-sufficient diet was fed; the subject group comprised healthy young adult males only. Only data for egg protein intakes falling in the range estimated to provide NB (0.44–0.77 g/kg BW/d via linear regression analysis) [55–58, 61–64] were selected.

To directly compare model predictions to actual experimental urinary urea nitrogen data (UUN_{EXP}), conversion to the nitrogen equivalent was carried out using a factor of 6.25 for each source of oxidative loss to give urinary urea nitrogen from inevitable oxidation (UUN_{IO}), urinary urea nitrogen from protein turnover in the postabsorptive state (UUN_{PA}), and urinary urea nitrogen from protein turnover in the postprandial state (UUN_{PP}). In addition, a small amount of UUN stems from ammonia absorption across the hindgut due primarily to the bacterial metabolism of undigested IDAA (UUN_{HG}) [65,66]. UUN due to ammonia absorption across the hindgut was estimated based on an observed mean 6% difference that exists between true ileal and true fecal dietary protein digestibilities in humans [67]. That is, there is a loss of 6% of undigested dietary AA between the terminal ileum and the end of the rectum. Given that there is little absorption of AA across the hindgut mucosa [68], this 6% AA loss can be assumed to be due to bacterial metabolism and ammonia production. Moreover, it has been shown that around 20% of hindgut ammonia nitrogen appears as UUN [66,69]. The contribution of hindgut bacterial AA metabolism to UUN was thus estimated at 20% of a 6% dietary intake loss, equivalent to 1.2% of dietary nitrogen intake (Equation *6*).

$$UUN_{HG} = 0.012 \times \text{dietary N intake} \quad (6)$$

The components of 24-h UUN predicted by the model (UUN_{PRE}) can be described as follows:.

$$UUN_{PRE} = UUN_{IO} + UUN_{PA} + UUN_{PP} + UUN_{HG} = (0.29 \times \text{dietary N intake} \times TID) + [0.11 \times (PB_{PA}/6.25)] + [0.023 \times (PB_{PP}/6.25)] + (0.012 \times \text{dietary N intake}) \quad (7)$$

Agreement between UUN_{PRE} and UUN_{EXP} for each reported dietary intake was assessed by plotting each as a function of N intake, as well as Bland–Altman analysis [70], where the mean difference (±SD) between predicted and experimental data was calculated, and simple linear regression. Normality of the difference between the means was assessed via the Shapiro–Wilk test, appropriate for small sample sizes [71,72]. The difference between predicted and experimental data ($UUN_{PRE} - UUN_{EXP}$) was plotted as a function of the mean of predicted and experimental data (($UUN_{PRE} + UUN_{EXP}$)/2), to provide a visual analysis of the variation in the mean difference according to the magnitude of UUN.

Sensitivity analysis

The effect of variation in each oxidative loss parameter of the factorial model (IO, PTO_{PA}, and PTO_{PP}) on the TOL for total protein and each IDAA was determined by altering the magnitude of each oxidative loss parameter independently, in 5% increments, with a total variation range of ±20%. For each parameter, TOL was plotted as a function of the variation and the slope of the line determined. The slope of the line (*b*) represents the level of change in TOL per each 1% variation in an oxidative loss parameter.

Statistical analysis

All data plotting, trendline fitting, and statistical analyses were carried out using Microsoft Excel (version 2407) or SPSS (version 29.0.2.0) software. Where applicable, parameter estimates, and model predictions are shown ±SD. The 95% CI was

calculated as the mean ±1.96 SD. The SD of TOL was determined as the square root (SQRT) of the sum of the squares of all parameter SDs as follows:

$$SD(TOL) = SQRT[SD(IO)^2 + SD(PTO_{PA})^2 + SD(PTO_{PP})^2] \quad (8)$$

Results

Predicted oxidative losses of protein and the individual IDAAs at maintenance

The predicted (model) values relating to IO, PTO_{PA}, and PTO_{PP}, as well as TOL for protein and each of the IDAAs, are shown in Table 1. Total oxidative losses for protein at the current recommended intake for maintenance were 389.5 ± 60.2 mg/kg BW/d, 59.0% of the current EAR for protein. For the IDAAs, total oxidative losses (based on the current recommended IDAA intakes) ranged from 3.7 (tryptophan) to 26.5 (leucine) mg/kg BW/d. The percentage contribution to the recommended intake (requirement) varied among IDAAs, ranging from 64.1% (isoleucine) to 91.3% (tryptophan). The relative contributions of each source of oxidative AA loss to TOL are presented in Figure 3. PTO_{PA} was the major source of oxidative loss for all IDAAs, ranging from 42% (isoleucine) to 51% [tryptophan; aromatic amino acids (AAAs)] of TOL. The percentage contribution of PTO_{PA} to TOL for total IDAAs was 47%. For total protein, PTO_{PA} was 40% of TOL, second to IO (44%). IO was responsible for 34% of TOL for total IDAAs. Losses from PTO_{PP} were higher for IDAAs (from 17% to 20%) compared to total protein (16%) when expressed as a percentage of TOL.

Sensitivity analysis

Figure 3 shows the effect that variation in each oxidative loss parameter of the factorial model had on the TOL for total protein (Figure 4A) and total IDAAs (Figure 4B), with full results for all model components presented in Table 2. The slope of the line (*b*) represents the magnitude of the change in TOL for every 1% change in a model parameter. Variation in losses due to PTO_{PA} and IO had the largest effect on TOL for total protein, with a ±1% change altering the TOL for protein by ±1.55 and 1.72 mg/kg BW/d, respectively. PTO_{PA} had the largest impact on TOL for total IDAAs, with a parameter variation of ±1% leading to a change of ±0.65 mg/kg BW/d, as well as the most notable impact on TOL for every individual IDAA. Change in PTO_{PP} losses had the least effect on TOL without deviation.

Assessment of the accuracy of model predictions

Comparison of the factorial model predictions for TOL (PTO_{PA}, PTO_{PP}, and IO collectively), given as units of UUN with independent measures of 24-h UUN for adults at protein maintenance (Equation *4*), is shown in Table 3 and Figure 5. The factorial model predicted UUN derived from 24-h protein turnover (UUN_{PP} + UUN_{PA}) to be 25.8 mg N/kg BW/d, with UUN_{IO} and UUN_{HG} varying as a function of nitrogen (protein) intake (Table 3). There was a mean difference between experimental and predicted total UUN of 7.9 ± 6.5 mg N/kg BW/d (Figure 5A), indicating a positive bias of the model equivalent to 49.4 mg protein/kg BW/d or 3.7 g protein/d for a 75-kg adult male. The mean difference was normally distributed (Shapiro–Wilk test, $P = 0.257$). There was a decreasing trend in the difference per measurement, with an overestimation of mean UUN at low

TABLE 1
Factorial model predictions of oxidative protein and IDAA losses in the adult human[1], including oxidative losses from postabsorptive and postprandial protein turnover, and inevitable AA oxidation.

Protein/AA	Current recommended intake[2]	PTO_{PA}[3]	PTO_{PP}[4]	IO[5]	Total oxidative losses	
					TOL[6]	Percentage of requirement
	mg/kg BW/d					
Total protein	660	155.1 (28.2)	62.2 (51.8)	172.3 (11.9)	389.5 (60.2)	59.0
IDAA						
Histidine	10	4.2 (0.8)	1.7 (1.4)	2.6 (0.2)	8.5 (1.6)	84.8
Isoleucine	20	5.4 (1.0)	2.2 (1.8)	5.2 (0.4)	12.8 (2.1)	64.1
Leucine	39	11.6 (2.1)	4.7 (3.9)	10.2 (0.7)	26.5 (4.5)	67.9
Lysine	30	11.3 (2.1)	4.5 (3.8)	7.8 (0.5)	23.7 (4.3)	79.0
SAA	15	5.4 (1.0)	2.2 (1.8)	3.9 (0.3)	11.5 (2.1)	76.8
AAA	25	11.3 (2.1)	4.5 (3.8)	6.5 (0.5)	22.4 (4.3)	89.5
Threonine	15	6.5 (1.2)	2.6 (2.2)	3.9 (0.3)	13.0 (2.5)	86.9
Tryptophan	4	1.9 (0.3)	0.7 (0.6)	1.0 (0.1)	3.7 (0.7)	91.3
Valine	26	7.6 (1.4)	3.0 (2.5)	6.8 (0.5)	17.4 (2.9)	67.0
Total IDAAs	184	65.3 (11.9)	26.2 (21.8)	48.0 (3.3)	139.5 (25.0)	75.8

Abbreviations: AA, amino acid; AAA, aromatic amino acid (phenylalanine and tyrosine); IDAA, indispensable amino acid; IO, inevitable oxidation; PTO_{PA}, oxidative AA losses associated with postabsorptive protein turnover; PTO_{PP}, oxidative AA losses associated with postprandial protein turnover; SAA, sulfur amino acid (methionine and cysteine); TOL, total oxidative loss.

[1] Values are means (SD), in mg/kg BW/d.

[2] Current maintenance requirements for protein and the IDAAs as defined by the WHO [3].

[3] PTO_{PA} for total protein was calculated as 11% of whole-body protein breakdown during the postabsorptive period (1.41 g/kg BW/d; Equation *3*), with the composition of whole-body protein [8] applied to the calculation for individual AAs.

[4] PTO_{PP} for total protein was calculated as 2.4% of whole-body protein breakdown during the postabsorptive period (2.59 g/kg BW/d) (Equation *4*), with the composition of whole-body protein [8] applied to the calculation for individual AAs.

[5] IO was set at 28.7% of absorbed dietary intake for protein and IDAAs (Equation *2*). IO for total IDAAs was the sum of IO values for each IDAA.

[6] TOL was calculated as the sum of PTO_{PA}, PTO_{PP}, and IO for each of protein, total IDAAs, and individual IDAAs.

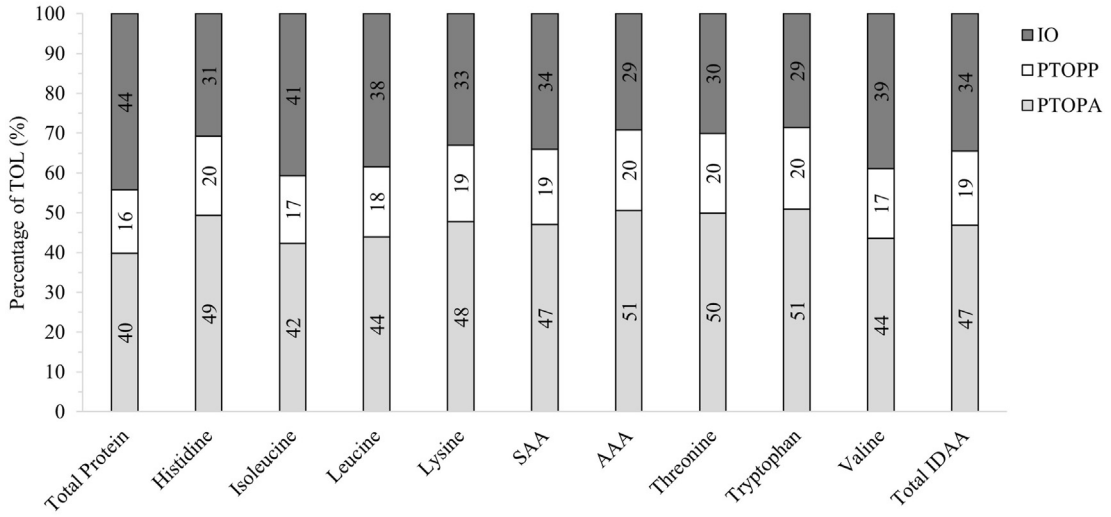


FIGURE 3. Distribution of predicted oxidative losses making up the total oxidative loss (TOL) for total protein, each IDAA, and total IDAAs. TOL was set to 1.0, and the loss resulting from each model parameter was expressed as a fraction of of TOL. AAA, aromatic amino acid (phenylalanine and tyrosine); IDAA, indispensable amino acid; IO, inevitable oxidation; SAA, sulfur amino acid (methionine and cysteine); PTO_{PA}, oxidative amino acid losses associated with postabsorptive protein turnover; PTO_{PP}, oxidative amino acid losses associated with postprandial protein turnover.

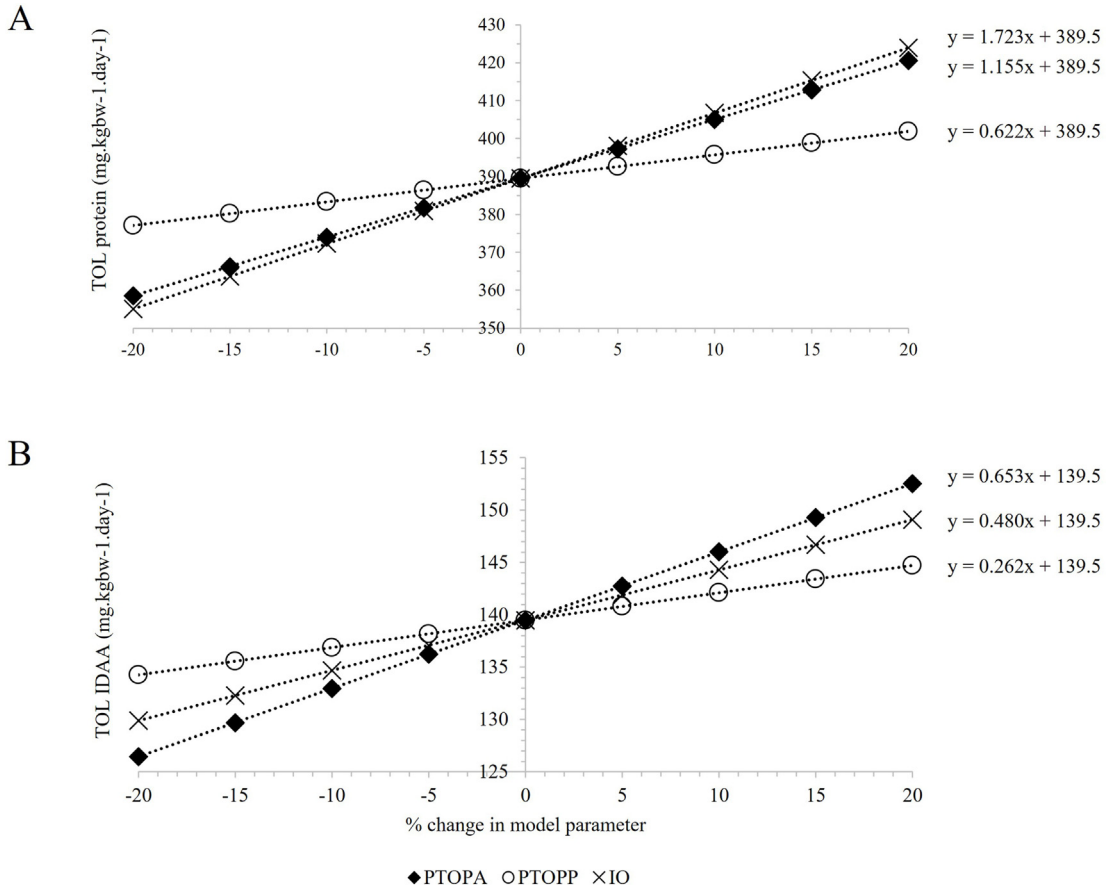


FIGURE 4. Sensitivity of total oxidative loss (TOL) for (A) protein and (B) total IDAAs to percentage variation in each model parameter. For each parameter, the slope of the trendline (*b*) represents the absolute change in TOL for a ±1% variation in that parameter, with the equation of the trendline as follows: $y = bx + c$. IDAA, indispensable amino acid; IO, inevitable oxidation; PTO_{PA}, oxidative amino acid losses associated with postabsorptive protein turnover; PTO_{PP}, oxidative amino acid losses associated with postprandial protein turnover.

TABLE 2
Sensitivity analysis for total protein, total IDAAs, and each individual IDAA, showing the change in predicted TOLs per each 1% change in an individual model parameter.

Protein/AA	TOL	Change in TOL per 1% change in model parameter (mg/kg BW/d)		
		PTO_{PA}	PTO_{PP}	IO
Total protein	389.5	1.55	0.62	1.72
IDAA				
Histidine	8.5	0.04	0.02	0.03
Isoleucine	12.8	0.05	0.02	0.05
Leucine	26.5	0.12	0.05	0.10
Lysine	23.7	0.11	0.05	0.08
SAA	11.5	0.05	0.02	0.04
AAA	22.4	0.11	0.05	0.07
Threonine	13.0	0.07	0.03	0.04
Tryptophan	3.7	0.02	0.01	0.01
Valine	17.4	0.08	0.03	0.07
Total IDAA	139.5	0.65	0.26	0.48

Abbreviations: AA, amino acid; AAA, aromatic amino acid (phenylalanine and tyrosine); IDAA, indispensable amino acid; IO, inevitable oxidation; PTO_{PA}, oxidative AA losses associated with postabsorptive protein turnover; PTO_{PP}, oxidative AA losses associated with postprandial protein turnover; SAA, sulfur amino acid (methionine and cysteine); TOL, total oxidative loss.

values and with accuracy increasing at higher values. This trend indicates a nonconstant difference between measurements, reducing the applicability for limits of agreement, which are not therefore shown. Regression analysis showed that the modeled values were able to statistically significantly predict experimental values [$F(1,4) = 7.85$; $P = 0.049$; $R^2 = 0.662$) (Figure 5B). Closest predictions to experimental data were obtained at the highest measured nitrogen intake of 84.2 mg/kg BW/d (Figure 5C), corresponding to an egg protein intake of 0.625 g/kg BW/d. Exact agreement between experimental and predicted data (based on intersection of experimental and predicted lines of best fit) was obtained at an egg protein intake of 0.635 g/kg BW/d, within the range of required intakes published for egg protein.

Discussion

The factorial model presented in this study was designed to allow prediction of the minimum AA loss at maintenance arising from various metabolic processes associated with oxidation of protein and each IDAA in the adult human. The model provides part of a larger, dynamic factorial model describing population and physiologic state specific protein and IDAA requirements.

Despite over 6 decades of research, the minimum dietary protein and IDAA requirements of the adult human are yet to be fully understood, and disagreement regarding the required dietary intake of protein and each IDAA for NB remains. Although the factorial model described in this study relies upon several assumptions and is based upon a conceptual diet that provides protein and each IDAA at the amount required for N balance, it is valuable in producing quantitative insight into biological processes that both give rise to a high proportion of protein needs and are variable according to the nuances of protein metabolism. The quantitative values of several of the model parameters are known with a relatively high degree of uncertainty, but the model, nevertheless, provides a framework to begin to understand the relative importance of the constitutive metabolic processes.

Total modeled oxidative losses of protein were 390 mg/kg BW/d, 59% of the current total protein requirement estimate, comprising primarily of losses occurring during PA protein turnover and due to inevitable AA oxidation. The factorial model addresses minimum oxidative losses only, occurring within a modeled conceptual diet providing the minimum quantity of dietary protein necessary for basal metabolism. AAs are absorbed commensurate with requirements, and digestive losses are limited to those expected with a diet consisting solely of high-quality protein sources. The modeled diet assumes that there is no oxidation due to the consumption of AAs in excess of the AA

TABLE 3
Assessment of the factorial model by comparison of predicted total oxidative protein loss (as UUN) with actual (experimental) data from published human NB studies.

Study	Experimental NB data				Factorial model predictions				
	N intake	Protein intake	Total urinary N	UUN_{EXP}	IO[1]	UUN_{PP}[2]	UUN_{PA}[3]	UUN_{HG}[4]	UUN_{PRE}[5]
	mg N/kg BW/d	mg/kg BW/d	mg N/kg BW/d						
Young et al., 1973 [58]	80	0.5	58.2	41.6[6]	20.9	9.9	24.8	0.96	56.5[6]
Young et al., 1984 [57]	80	0.5	73.8	56.5[6]	20.9	9.9	24.8	0.96	56.5[6]
Komatsu et al., 1983 [56]	75.2	0.47	64.9	44.7[6]	19.6	9.9	24.8	0.90	55.2[6]
	100	0.625	84.2	61.8[6]	26.1	9.9	24.8	1.20	62.0[6]
Huang and Lin 1982 [55]	72	0.45	63.9	40.8[6]	18.8	9.9	24.8	0.86	54.4[6]
	88	0.55	71.5	50.8[6]	23.0	9.9	24.8	1.06	58.7[6]

Abbreviations: BW, birth weight; IO, inevitable oxidation; NB, nitrogen balance; TID, true ileal digestibility; UUN, urinary urea nitrogen; UUN_{EXP}, experimental urinary urea nitrogen; UUN_{HG}, urinary urea nitrogen due to ammonia absorption across the hindgut; UUN_{IO}, urinary urea nitrogen due to inevitable oxidation; UUN_{PA}, urinary urea nitrogen due to oxidation associated with postabsorptive protein breakdown; UUN_{PP}, urinary urea nitrogen due to oxidation associated with postprandial protein breakdown; UUN_{PRE}, predicted urinary urea nitrogen.

[1] IO was calculated as 0.287 × (dietary N intake × TID), using a TID for cooked egg protein of 0.9 (34, 35).

[2] UUN_{PP} was calculated as 0.023 × (PB_{PP}/6.25), using a value for PB_{PP} of 2.59 g/kg BW/d.

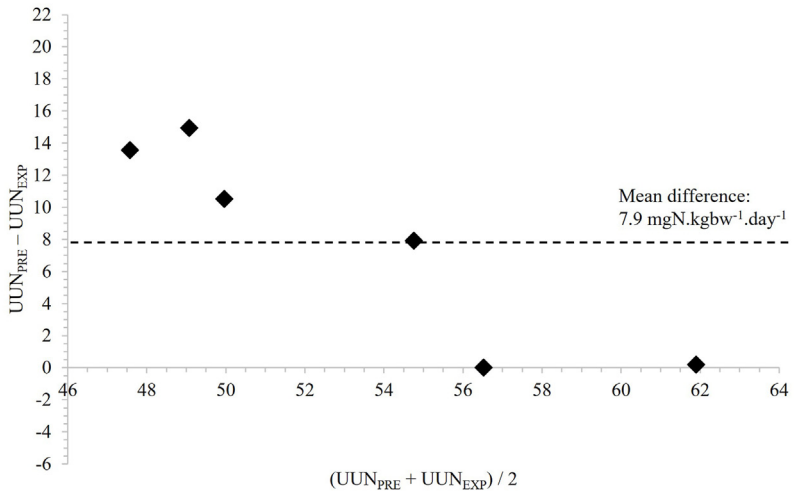
[3] UUN_{PA} was calculated as 0.11 × (PB_{PA}/6.25), using a value for PB_{PP} of 1.41 g/kg BW/d.

[4] UUN_{HG} was calculated as 0.012 × dietary N intake (Equation *6*).

[5] UUN_{PRE} was calculated as the sum of UUN_{IO} + UUN_{PA} + UUN_{PP} + UUN_{HG} (Equation *7*).

[6] Actual (experimental, UUN_{EXP}) and predicted (UUN_{PRE}) UUN values.

A



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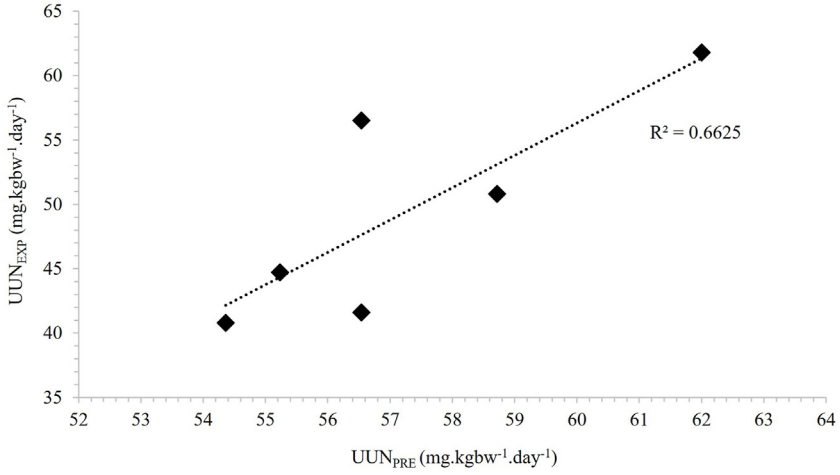

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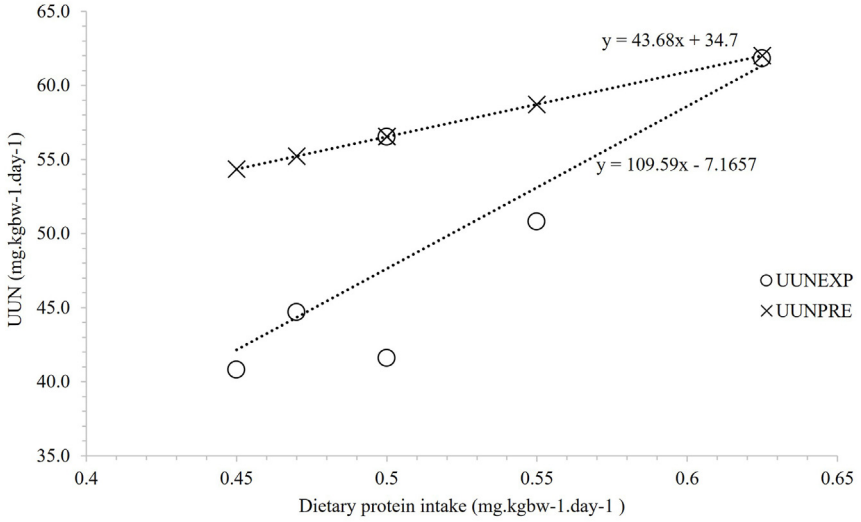

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requirements for maintenance. High-quality dietary protein has a high utilization efficiency based on an AA composition where most excess IDAAs can be transaminated to form DAAs, and there is little oxidation due to AA imbalance. However, the standard human diet does not fit within these constraints and additional oxidative losses, over and above those due to inevitable catabolism and protein turnover, due to the consumption of mixed protein sources at intakes over the EAR, can be expected. In addition, both IO and PTO are influenced by multiple factors, including dietary protein quantity and quality, hormonal changes, pregnancy and lactation, percentage lean muscle mass, exercise training, and more. The magnitude of IO is a direct function of the amount of dietary protein intake [19,21]. Assuming 90% digestibility, an increase in dietary protein intake from the EAR of 0.66 g/kg BW/d to the current safe recommended dietary protein intake of 0.8 g/kg BW/d would increase IO by 2.7 g protein/d for a 75-kg adult; an increase to an intake of 1.2 g/kg BW/d would increase IO by 10.5 g protein/d for that same adult. On the contrary, IO appears to decrease with decreasing dietary AA intake, suggesting adaptation to account for AA deficiency [21]. An acute increase in protein intake will increase PS while decreasing the rate of protein breakdown [73], while resistance exercise increases both PS and protein breakdown [74]. Further, muscle PS is proportional to total muscle mass [1] and sex differences in muscle protein metabolism have been identified, which appear to depend on life-stage, particularly puberty, pregnancy, and menopause [75–77]. The factorial modeling of protein and IDAA oxidation specifically in females is needed. Subsequent versions of the model will estimate oxidative losses in mixed diets differing in protein quality and quantity and for different populations, demographics, and physiologic states.

In addition to oxidative losses, daily dietary protein intake must also provide for integumental AA losses, GIT AA losses (including both losses during digestion and endogenous losses due to the continual shedding of the gut lining), and urinary free AA losses, as well as quantitatively small specific losses for some AAs. On the basis of the difference between the modeled oxidative losses and current maintenance requirements of 0.66 g/kg BW/d, these additional losses are estimated to be a minimum of 270.5 mg protein/kg BW/d. However, and as is the case for oxidative losses, each of these losses is affected by multiple factors. For example, along with minimum dietary protein, the current model is based on a diet containing negligible dietary fiber and/or antinutritional factors, whereas increased amounts of protein, fiber and antinutritional factors have been shown to significantly increase GIT endogenous AA losses [78,79]. Importantly, protein digestibility differs according to source; a diet containing high-quality protein (such as eggs and meat, TID of ~0.9) [34,35] will be subject to lower intestinal losses than one containing protein from lower quality sources (such as legumes; TID of ~0.7) [80]. An accurate estimate of both minimum dietary protein needs and an intake that will optimize nutrition and health goals relies upon an understanding and integration of all losses and all moderating factors. The current model is an essential step in this process.

The predominant role of dietary protein is to provide IDAAs for PS, balancing out PA protein breakdown and maintaining muscle mass. In contrast to TOLs for protein, which were found to be a moderate proportion of requirements at amounts that might be expected for a state of maintenance, oxidative losses for the IDAAs ranged from 64.1% (isoleucine) to as high as 91.3% (tryptophan) of current recommended intakes, with PA protein turnover the major source of loss for all IDAAs. As for dietary protein, AA intake must also account for digestive, integumental, and urinary losses. While just over 40% of the recommended protein intake remained to meet these needs, according to the model predictions, only 24% of total IDAAs (range from 9.0% to 36%) would be left to provide for nonoxidative needs. This discrepancy between total protein and IDAA oxidation would suggest 1 of the 2 scenarios. On one hand, the current minimum requirement for dietary protein, reflected by the EAR, may be driven by an imbalance between IDAAs and DAAs in plant-based protein sources, compared with the current model that is focused on a diet containing high-quality protein. At current protein and IDAA recommended intakes, the IDAA:DAA ratio is 28:72, while high-quality protein sources have an IDAA:DAA ratio closer to 50:50, allowing for almost complete utilization and reducing AA oxidation due to imbalance. However, NB studies feeding egg protein as the sole protein source [55–58,61,64] have produced a minimum dietary protein requirement ranging between 0.44 [58] and 0.77 g/kg BW/d [63], suggesting that even when protein quality is high, requirements are close to those currently recommended. On the other hand, the current requirement, at least for some IDAAs, may be too low. For example, according to the calculations presented, only 9.0% of tryptophan intake would be available to meet nonoxidative losses, which would become negligible if digestive losses of 10% (based on a TID of 0.9 for egg protein) [34,35] were taken into account. Further, each of the IDAAs have specific physiologic roles that have not been accounted for in the model. For example, ~3% of dietary tryptophan intake is used for serotonin synthesis [81], suggesting that the minimum required intake for tryptophan may be higher than that currently recommended. TOLs for the AAAs,

FIGURE 5. Assessment of the accuracy of the factorial model for predicting total oxidative protein loss (TOL) by comparison with actual experimental UUN data (UUN_{EXP}). UUN data were taken from NB studies used in the meta-analysis by Rand et al. [4], with feeding of between 0.44 and 0.77 g/kg BW/d egg protein as the sole dietary protein source. TOL was expressed as predicted UUN (UUN_{PRE}) via the following equation: $UUN_{PRE} = (0.29 \times \text{dietary N intake} \times \text{TID}) + [0.11 \times (PB_{PA}/6.25)] + [0.024 \times (PB_{PP}/6.25)] + (0.012 \times \text{dietary N intake})$ (Equation *7*). (A) Comparison of UUN_{PRE} and UUN_{EXP} via Bland–Altman analysis, with the difference between predicted and experimental data ($UUN_{PRE} - UUN_{EXP}$) plotted as a function of the mean of predicted and experimental data (($UUN_{PRE} + UUN_{EXP}$)/2). The mean difference was determined to be 7.9 mg N/kg BW/d (solid line). (B) Simple linear regression with factorial estimates of UUN (UUN_{PRE}) at each protein intake as the dependent variable and experimental values of UUN (UUN_{EXP}) per each protein intake as the predictor variable. The factorial model was able to predict experimental UUN values with significant accuracy ($P = 0.049$; $R^2 = 0.662$). (C) UUN as a function of protein intake for both predicted (UUN_{PRE}) and experimental (UUN_{EXP}) data. A line of best fit was fitted to each data set, and exact agreement was determined as the intersect of the 2 lines of best fit; this occurred at a protein intake of 0.635 g/kg BW/d. UUN, urinary urea nitrogen; UUN_{PRE}, urinary urea nitrogen predicted by the factorial model; UUN_{EXP}, published values of experimental urinary urea nitrogen.

essential for catecholamine synthesis [82], were estimated at 89.5% of requirements. The current recommendation for the AAAs is set at 25 mg/kg BW/d, the midpoint of a range of estimates (from 9 to 39 mg/kg BW/d), all of which contain considerable uncertainty; the model suggests that the optimal AAA intake may be at the higher end of this range. Amino acids with nonprotein roles, termed functional AAs in recent literature, have been suggested to hold therapeutic potential and include both IDAAs and DAAs. While increased understanding is required, these roles may influence the IDAA:DAA ratio that provides for maximum utilization efficiency, particularly within different health and/or disease states [83]. For threonine, TOLs were estimated to be 86.9% of requirements, and in a previous model of gut endogenous AA loss (EGL) [22], threonine needs due to EGL were predicted to contribute 97% of the current FAO requirement [3]. According to these 2 models, minimum threonine requirements may be closer to double what is currently recommended, in line with early IAAO estimates of 19–26 mg/kg BW/d [84]. The factorial model therefore supports previous conjecture [22] that the current daily requirement for threonine, which plays an important role in GIT mucosal health and immune defense [85], is too low. Yet, more recent IAAO studies have suggested threonine requirements between 10.5 and 12.1 mg/kg BW/d [86]. Additional research is needed to better determine and understand all IDAA requirements. It is also important to consider that each source of oxidative loss as defined by the factorial model may differ between AAs. While the model assumes that the rate of AA oxidation as a proportion of protein breakdown is constant for each IDAA, and a function of body protein composition only, there is likely to be variation between different AAs, based on the physiologic role of each. Similarly, rates of IO have been found to be nonconstant in animals. Direct measurements in rats showed an IO rate upward of 50% for methionine [31], explained by the role of methionine as a donor of methyl groups (eg, nucleic acid synthesis) and the source of sulfur for the formation of cysteine, taurine, and sulfate. In addition, the rate of gastrointestinal catabolism has been found to differ between IDAAs in the piglet [87]. As the specific nonprotein needs of each IDAA may have a direct impact on the magnitude of IO for each, and therefore the total requirement, further understanding is needed for this key physiologic process [19,21]. Updated versions of the factorial model with increased detail and complexity are warranted as more information becomes available.

The estimation of each oxidative loss parameter (IO, PTO_{PA}, and PTO_{PP}) within the factorial model contains a degree of inherent error. Each of the oxidative losses were based either on direct data from nonhuman mammalian species or indirect estimation in humans, potentially reducing the reliability of these parameters and the factorial model overall. However, comparison of the magnitude of IO, PTO_{PA}, and PTO_{PP}, as combined contributors of predicted UUN, with experimental UUN data, showed that the factorial model is able to significantly ($P < 0.05$) predict a realistic value for total oxidative AA loss. This finding gives confidence in the model overall, despite there being relatively few data points. While the model overpredicted mean TOLs compared with UUN data by Bland–Altman analysis, this overprediction is in line with criticisms that the NB methodology tends to underestimate N loss and overestimate N provision [3,12,13]. Consistent with this, accuracy of the model increased at higher levels of oxidative loss, indicative of increased protein intake. There was exact agreement between the predicted and experimental UUN data at a protein intake of 0.635 g/kg BW/d, which is within the range of egg protein intakes determined for NB and close to the current EAR. The assessment methods rely on the assumption that oxidative losses from the experimentally derived data are devoid of contributions from imbalanced dietary AAs or the consumption of protein in excess of minimum requirements. Both of these assumptions appear to be met in the assessment exercises, where egg protein was fed in controlled amounts or feeding was eliminated altogether. While previous models for oxidative loss, based on the PP protein utilization efficiency of high-quality protein sources, were estimated to be ~70%–80% of protein intake in humans [12], these values were calculated over 8 h, compared with the 24-h period of this model. The assessment and comparisons suggest that the diurnal factorial model presented in this study is an accurate overall representation of TOLs in the adult human at maintenance. Further testing of the model predictions compared with experimental data are required for model refinement, such as at nonmaintenance protein intakes, and subsequent development.

The factorial model defines and estimates the oxidative losses of IO, PTO_{PA} and PTO_{PP} separately, since each source of loss is influenced by factors that will affect total protein and IDAA requirements, creating a foundation for their subsequent modeling. However, the methods used for assessment of the accuracy of the factorial model were not designed to test IO, PTO_{PA}, and PTO_{PP} as individual components, highlighting the need for further study into the magnitude of these parameters in the adult human. Values for IO were estimated using data from growing animals due to a lack of direct measurement in adult humans. While the application of these data to adult human protein requirements has limitations, it is worth noting that values for inevitable catabolism of the absorbed dietary first-limiting AA were similar between 2 mammals (pigs and rats) and share a remarkable similarity with that measured in chickens [32,33]. This congruency between different mammals, and between mammals and birds, suggests that the process of IO may be similar across animal species and increases confidence in the currently used estimate of IO. Indirect values for IO, determined from data reported in human metabolic studies, were found to be in the order of 20%–30% [44,88–93], in agreement with the estimate used in this study. A notable assumption is that the published measurements of IO exclude oxidation associated with protein turnover. If the published IO data were to include protein-turnover associated AA oxidation, the factorial model would overestimate total oxidative AA losses. However, in the specific data selected for the estimation of IO [29,30], inevitable AA catabolism was calculated using a correction for AA losses occurring as a function of maintenance requirements, which include protein turnover–associated oxidative AA losses. Although this correction was derived statistically, rather than by direct measurement, these data are expected to represent IO, in isolation from other oxidative AA losses. The model prediction of PTO_{PA}, at 11% of the rate of protein breakdown over the PA period, is just over half the amount of a previously published estimate for PA protein turnover–associated oxidative AA loss [28]. However, these data were based on protein breakdown in muscle and skin, where the data used for the factorial model were taken from measurements of whole-body protein turnover.

Of the 3 sources of oxidative AA loss within the model, there is the least confidence in estimates of PTO_{PP}, due to there being limited data available. In other recent modeling of AA utilization by our group, a value for PTO_{PA} of 23% of protein breakdown has been applied, based on certain measures of the oxidative flux of AAs in the PA state. The latter work has taken a less compartmentalized approach to the oxidative losses and has assumed as a starting point that oxidative losses determined in the PA state can be applied generally. In this study, a different approach to estimating PTO_{PA} was taken, strictly ensuring that rates of protein oxidation, breakdown, and synthesis were at maintenance levels. If however, the higher experimental estimate of PTO_{PA} of 23% was used in the presently described model, our overall conclusions do not change, but TOLs for protein increase to 85% of the current EAR, while for some IDAAs (isoleucine and valine), the TOLs are close to 100% of the respective EAR, and for the sulfur amino acids (histidine, leucine, and lysine) and the AAAs (threonine and tryptophan), the TOLs account for more (~120%) of the respective current requirement value. This underlines the sensitivity of the model to key model parameters and the need for more refined and accurate data. Direct measurement of oxidative AA losses associated with protein turnover in the fed state has not been performed. Some predictions of PTO_{PP} are as high as 10% of protein turnover [25] and estimates from published IAAO data are, on average, 7% [37–44,47]. These data were not used, largely due to inherent methodologic error. If applied to the model, the increased values for PTO_{PP} would put total oxidative AA losses at >100% of current requirements. While data reporting the fractional oxidation of labeled AAs provided orally with a meal have been published [44,94], these data will include oxidative losses due to both IO and PTO_{PP}. Correction of these data for IO might be expected to provide an estimate for PTO_{PP}, with such calculations producing PTO_{PP} values from 1 to 5% of PP protein breakdown, in line with the current model. As these data are subject to a number of unproven assumptions and limitations [24,49], they were not included within the model estimate of PTO_{PP}. Despite these limitations, the model suggests that PTO_{PP} plays a minor role in total minimum oxidative losses. It is also important to note that the magnitude of protein turnover will influence that of PTO_{PA} and PTO_{PP}. While all data in this article were taken close to maximal rates of PS, which is appropriate for modeling at maintenance, PS and protein turnover will be reduced at lower protein intakes, decreasing protein turnover–associated losses, and also possibly increasing recycling efficiency. The model assumes that IO, PTO_{PP}, and PTO_{PA} are independent and that there is no interaction between them. While the model is based on a scenario where the intake of each IDAA is perfectly matched to requirements, it is possible that the magnitude and rate of each variable will influence that of the other variables; for example, if the rate of IO differs for each IDAA, this may affect the availability of each IDAA for PS, impacting the rate of protein turnover and, by default, oxidative losses as a proportion of this. The known reduction in whole-body protein turnover that occurs during adaptation to low protein intakes [95] may involve an interaction between different sources of oxidative loss. These factors highlight the importance of understanding the relationship between protein and IDAA intake and protein turnover, as well as the individual components of loss, for a true determination of protein and IDAA requirements.

In conclusion, we developed a diurnal factorial model that provides reasonably accurate predictions for the minimum oxidative losses of protein and the IDAAs in the adult male human on the basis of currently understood physiology, contributing to an increased understanding of both baseline protein and IDAA requirements and the key factors moderating these. The model indicates that oxidative IDAA losses account for a notable proportion of protein and EAR requirements and calls into question whether current recommendations for some IDAAs, particularly tryptophan, the AAAs, and threonine, are sufficient. AA losses from the body are not constant, but vary with food intake, and the composition (chemical and ingredient) of the diet consumed. Additional lifestyle factors and/or stages and demographic characteristics such as physical exercise, psychologic stress, sex, puberty, pregnancy, and aging can also influence the rate of protein turnover and oxidative AA losses [3,96,97]. All of these influences will be captured in further expanded versions of the presently described model to represent the dynamics of human protein metabolism in response to lifestyle and diet. The power of the modeling approach is that such variation can be described, and protein and IDAA requirement values determined for specific states.

Author contributions

The authors' responsibilities were as follows—CSS, PJM: were responsible for study design and modeling; CSS: was responsible for manuscript preparation, writing, and statistical analysis; CSS, PJM, RRW: were responsible for determining the final content of the manuscript; and all authors: have read and approved the manuscript.

Conflicts of interest

RRW is a shareholder in The Amino Company holds United States patents for essential amino acid compositions and has received grants from the National Cattleman's Beef Association.

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Data availability

Data described in the manuscript will be made available upon request pending application and approval.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tjnut.2024.10.049.

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