REVIEW

Are there dietary requirements for dispensable amino acids and if so, how do we assess requirements?

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Purpose of review
Nonessential amino acids (NEAAs) represent a relevant portion of dietary protein(s), yet their requirement(s) has not been determined. Despite their nature as dispensable substrates, should either shortage of any NEAA precursor or impaired synthetic reactions occur, NEAA dietary intake may become insufficient. The purpose of this review is to discuss recent hypotheses and data on individual NEAA requirements and metabolism.

Recent findings
A minimum total NEAA requirement can simply be estimated by subtraction of essential amino acid (EAA) total RDAs, from recommended 'safe' protein intake. By this calculation, NEAA intake would account for two to three times that of the EAAs, under nitrogen-balance conditions. Although the α-amino-nitrogen of the NEAAs is 'not essential', yet it must be furnished by a common pool contributed by both EAAs and NEAAs. Thus, an increased demand for NEAAs may deprive the α-amino-nitrogen body pool(s) possibly limiting the NEAA de novo synthesis itself. Conversely, shortage of NEAAs may require more EAAs to maintain the nitrogen pool. Conditions of increased requirements could those of unbalanced diets, EAA intake below RDA, pregnancy, or else. In addition, the 'obligatory nitrogen losses' may consume NEAAs too. A novel approach to estimate NEAA 'requirements' in humans is proposed.

Summary
Methods to estimate NEAA requirements in humans should be the object of further studies.

Keywords
body protein replenishment, de novo production, nitrogen balance, obligatory nitrogen loss, requirements

INTRODUCTION

By definition, the term 'dispensable' applied to a substrate, in this case to an amino acid, indicates the capacity of the organism to synthesize that substrate endogenously, thus being theoretically 'dispensable' from diet for its provision.

The term 'dispensable' is often considered equivalent to, and/or exchangeable with, the term 'nonessential'. However, the significance of the latter may include a broader concept, that is, the need or not of a particular substrate for the body's metabolism, therefore, not just limited to the self-production capacity of the body. As a matter of fact, the body requires both 'essential' and 'nonessential' compounds for its maintenance as well as for its metabolic demands. The evaluation of amino acid requirements is crucial in both physiological and pathological conditions [1▪▪,2], in order to guarantee an appropriate diet to maintain or to rescue health.

That some 'requirement' of nonessential amino acids (NEAAs) may actually exist, simply comes from the concept of 'recommended intakes' of both total protein and essential amino acids (EAAs). International agencies agreed to set a safe protein intake at 0.8 g/kg body weight per day in adult man, whereas the sum of the recommended daily allowance (RDA) of the essential ('nondispensable') amino acids is ~0.2 g/kg body weight per day. The latter approximately represents the rough mean among the value reported by WHO (World Health Organization)/FAO

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KEY POINTS

- Nonessential amino acids (NEAAs) are important components of the diet.
- Although NEAAs are dispensable substrates, their de-novo production may impinge on essential amino acid availability and requirements.
- NEAAs are extensively interconverted.
- NEAAs are involved in the production of nonamino acidic substances, as well as they may contribute to obligatory nitrogen losses (ONL).

(Food and Agriculture Organization)/UNU (United Nations University), that derived from the ^{13}C amino acid oxidation method, and that computed from the factorial method, as described in refs. [1▪▪] and [3▪▪]. Therefore, the resulting NEAA 'requirements' would be ~0.6 g/kg body weight per day. It should be observed that such a 'preliminary' approach to estimate indirectly NEAA 'requirements', depends on the fact that, at nitrogen balance, a given amount of dietary proteins (preferably of high quality), including the EAAs, is required. Such an amount implies the concurrent intake of the NEAAs contained in the protein. Conversely, should nitrogen balance studies be carried out using EAA-enriched mixtures rather than natural proteins, these indirect estimates of the NEAA 'requirements' would be different.

Alternatively, a 'safe' daily protein intake can be derived from the factorial approach, that is, the summation, of the 'obligatory nitrogen losses' (ONL) [1▪▪] (see also below), resulting in 0.66 g protein/kg body weight per day. This figure is derived from the estimated average requirement (EAR) of protein (0.44 g/kg body weight per day), increased by ~30% to account for the nonlinearity between nitrogen intake and balance. Therefore, the resulting 'average' NEAA requirement would become ~0.46 g/kg body weight per day.

In Table 1, the theoretical combinations of the indirectly calculated NEAA figures, using either one of the safe protein intake values, as well as each of the three EAA RDAs values, are reported. The proportions of both EAA and NEAA requirements, in respect to total protein intake, estimated with either of the two above-reported values of safe protein intake, are also shown in Fig. 1.

Thus, a certain amount of total NEAAs (between two and three times that of the EAAs) needs actually to be assumed daily to match requirements for nitrogen balance. Therefore, although conceptually speaking the NEAAs are 'not essential', that is, 'dispensable' amino acids, they significantly and quantitatively contribute to the nitrogen-containing compounds required for body needs. Conversely, removing all the NEAAs from the diet would greatly limit the provision of the nitrogen necessary to achieve nitrogen balance.

An estimation of individual NEAA 'requirements' is, however, far from being defined. Such an uncertainty derives from objective, intrinsic difficulties involved in such a complex issue. However, taking into account these limitations, some hypotheses for an estimation of NEAA requirements are here presented and discussed.

INTRINSIC DIFFICULTIES IN THE DEFINITION OF NONESSENTIAL AMINO ACID 'REQUIREMENTS'

Any attempt to define individual NEAA requirements is hampered and/or biased by any of the following limitations:

(1) First of all, any assessment of NEAA requirement (from dietary sources) by definition may appear intrinsically contradictory, that is, an oxymoron, given the 'nonessential' nature of NEAAs;
(2) Even if any such investigation is undertaken, the network encompassing the relationships

Table 1. Calculated requirements of nonessential amino acids from combinations of two levels of recommended protein intake and three recommended daily allowance values for the essential amino acids

		Calculated NEAA 'requirement'	
	EAA RDAs (g/kg BW)	At 'safe protein intake' (0.8 g/kg/BW)	To compensate for ONL (0.66 g/kg/BW)
Agency/method			
WHO/FAO/UNU	0.184	0.616	0.476
^{13}C-leucine	0.193	0.607	0.467
ONL	0.221	0.579	0.439

The total EAA RDAs reported from either the ^{13}C-leucine or the ONL methods have been completed, as for the EAAs missing in each methods, after the corrections/recalculations described in ref. [3▪▪]. BW, body weight; EAA, essential amino acid; NEAA, nonessential amino acid; ONL, obligatory nitrogen losses; RDA, recommended daily allowance.

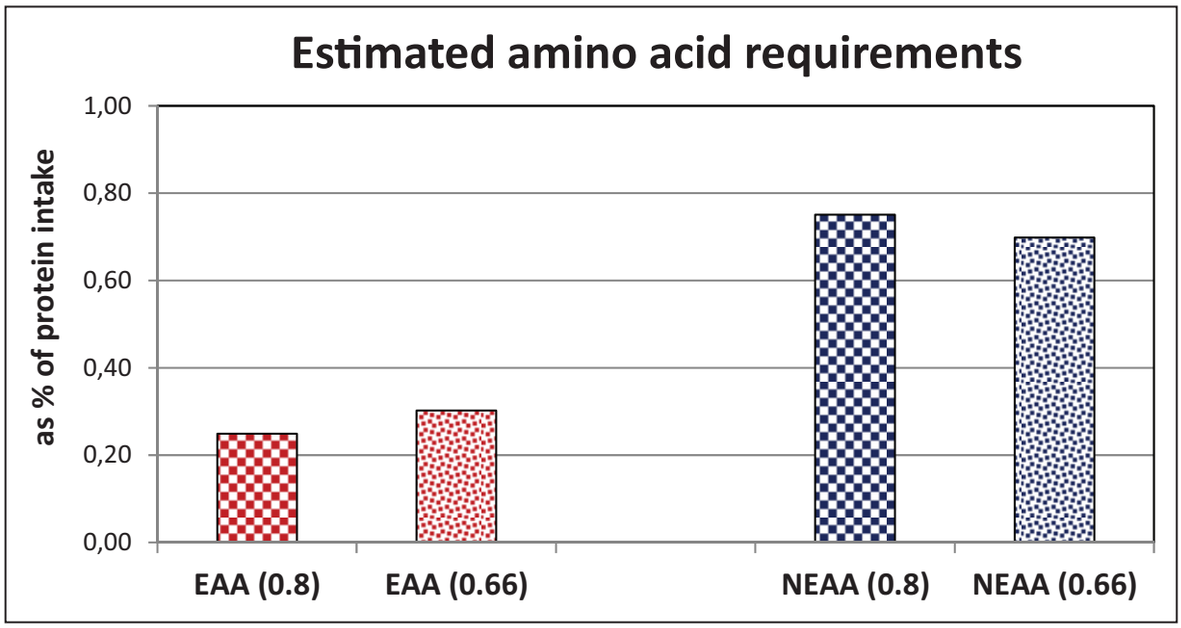

FIGURE 1. Estimated recommended daily requirements of the sum of the essential amino acids, and calculated estimates of requirements of the sum of the nonessential amino acids. Data from [1▪▪]. The data are reported as percentage of total protein intake, as indicated either by international agencies (at the level of 0.8 g/kg body weight/day) or using the obligatory nitrogen loss approach (at the level of 0.66 g/kg body weight/day [1▪▪]). The total EAA RDAs reported from either the ^{13}C-leucine or the ONL methods have been completed, as for the EAAs missing in each methods, after the corrections/recalculations described in ref. [1▪▪,3▪▪]. EAA, essential amino acids; NEAA, nonessential amino acids; OLN, obligatory nitrogen loss; RDA, recommended daily allowance.

between the NEAAs and their precursors, as well as their products, is very complex. Although in theory each of the metabolic steps leading to the production of a NEAA are known, their rates *in vivo* may prove to be extremely difficult, if not impossible, to be accurately quantified.

(3) Although selected deficiencies in any of NEAA precursor substrate(s) may develop *in vivo*, they could yet remain undetectable and/or be compensated by homeostatic mechanisms.

(4) The availability of precursor(s) of NEAAs may not directly translate into the quantitative generation of the product, because enzyme activity may vary and/or be differently regulated by multiple coenzyme and cofactors.

(5) As the NEAAs are extensively interconverted into each other, largely in the gut [4▪▪], any effort to identify individual deficiencies may prove to be of limited significance.

(6) *In vivo* clinical conditions of selected deficiencies of any NEAA are unknown, with the exception of some inherited diseases of amino acid metabolism, in which, however, the clinically observed effects are mainly because of substrate accumulation rather than to downstream deficiencies [5].

(7) Therefore, the design of *in vivo* studies aiming at testing selected deficiencies may prove extremely complex and virtually unfeasible.

For the above mentioned, and probably also for other, reasons, the current mantra is that the definition of the 'requirement' of the NEAAs would not represent a major issue in metabolic investigations.

THE CONCEPT OF THE OBLIGATORY NITROGEN LOSS AND THE NONESSENTIAL AMINO ACIDS

The obligatory nitrogen losses (ONL) are those associated to the disposal of nitrogen-containing compounds, that cannot be entirely abolished even following nitrogen-free diets [1▪▪]. The ONL is estimated to be about 54 ± 8 (SD) mg nitrogen/kg/day, with an upper 'safe' limit of 70 (i.e. mean + 2SD). The major portion (37 mg nitrogen/kg/day, i.e. ~70% of ONL), is lost in the urines, 12 mg nitrogen/kg/day (~22%) in the feces, the remaining portion through cutaneous exfoliation and other losses. Urinary losses are mainly because of urea (60–75% of total), ammonia (~11–12%), uric acid (2–3%), creatinine (5–17%), and other undetermined losses.

It is virtually impossible to figure out what could be the contribution of the NEAAs to each of such irreversible losses, that could themselves indicate some nondispensability of NEAAs as precursors. For instance, urea is a common sink of the ammonia derived from glutamate degradation, on turn the transamination product of most amino acids (both EAA and NEAA). As regard sweat losses, however, it has been reported that six amino acids, four NEAAs (serine, glycine, ornithine, and aspartic acid) and two EAAs (histidine and lysine) are lost in sweat at concentrations much higher (4–20-fold) than those found in plasma [6▪]. Therefore, such a disproportionate loss of NEAAs in sweat should be considered.

THEORETICAL METHODS TO ESTIMATE NONESSENTIAL AMINO ACID REQUIREMENTS

Some potential methods to estimate NEAAs requirements are briefly outlined below.

(1) Total NEAA 'requirements' could be roughly estimated as outlined above. To get a further insight into the requirements of individual NEAAs, one line of reasoning could be that to correlate the amino acid composition of (total) body proteins, with individual amino acid requirements. Although such an assumption could be viewed as 'naive' and rough, it could represent at starting point at least. As the EAA RDAs are known, a relationship between the individual EAA RDA and their abundance in body proteins could be established. Such a relationship could then be employed to back-calculate the use of each NEAA at least for body protein 'replenishment'. In order to estimate the individual amino acid composition of the human body, a factorial approach, that is, the summation of body protein amino acid content of published data, can be performed [3▪▪]. Using this methods, minimum requirements of individual amino acid usage for body protein replenishment were calculated [3▪▪].
(2) Another theoretical approach could be to calculate a sort of NEAA requirement from the composition of a diet providing both the RDAs of each EAAs and the recommended daily protein intake to achieve nitrogen balance. Such a calculation is strictly diet-dependent, and it can end up into wide different NEAA amounts, given the extremely large difference in amino acid content of foods [7▪].
(3) Finally, should the amino acid composition of each of the ONL components be available, the 'requirements' of individual NEAAs could be roughly estimated.

Clearly, any of the above reported theoretical possibilities are limited by the large interconversion occurring among the NEAAs, with the possible substitution of one amino acid for another, to furnish the theoretical daily requirement. Thus, any calculated figure of each individual NEAA could fluctuate inversely to that of other NEAAs.

RELATIONSHIPS BETWEEN ESSENTIAL AMINO ACID AND NONESSENTIAL AMINO ACID REQUIREMENTS

Nonessential amino acids may (partially) replace essential ones in specific dietary conditions as well as in some metabolic activities.

In normal and heat-stressed broiler chickens, exposed to methionine intake 30% greater than requirements, the same increase of both animal growth and Cox-1 mitochondrial respiratory chain enzymes (MRCEs) activity, as well as the reduced food intake, induced by the excess methionine intake, could be reproduced by replacement of the excess methionine with betaine [8]. Under these peculiar experimental conditions, betaine can thus become methionine-sparing.

Methionine contributes to the methyl-carbon pool for the synthesis of methyl-containing compounds. Therefore, its limitation in the diet could apparently increase the requirements of NEAAs as well as of other compounds, that feed the methyl-carbon pools too [9]. Conversely, dietary methionine deficiency results in increased protein breakdown, to provide endogenous methionine as compensation for its dietary deficiency. In neonate piglets, when experimentally-deprived of the remethylation precursors folate, betaine and choline, plasma concentrations of these methyl-donors were markedly decreased. Following their replacement in the diet, both transmethylation and remethylation rates were increased, as reflection of recovered methionine synthesis from homocysteine, whereas transulfuration was unaffected, confirming the prominent role of the methyl donors in sustaining the methionine cycle, while unaffecting the catabolic pathway. NEAA 'apparent' requirements may be affected also by the experimental modulation of their catabolism, as shown by the genetic ablation of catabolic enzyme(s) of arginase II as regards arginine [10]. Provision of a NEAA precursor may be more efficient than that of the amino acid itself as regards its systemic availability [11].

CONDITIONS OF INCREASE OF 'APPARENT' NONESSENTIAL AMINO ACID REQUIREMENTS

NEAA requirement might be increased in some physiological conditions, as it occurs in pregnancy. Protein requirement is increased in pregnancy by about an extra +0.22 g protein/kg/day [12]. Conversely, the recommended extra average EAA intake in the pregnant woman is +0.068 g/kg/day [12]. By applying the same line of reasoning described above [3▪▪], should such an increased EAA requirement be met by adequate protein intake, an increase by +0.152 g/kg/day of NEAAs would ensue, possibly suggesting an apparent increase of dispensable amino acids requirement as well. Conversely, should the EAA RDA in pregnancy not be met by an adequate intake, a relative, greater deficiency of NEAA would ensue, given the role of EAAs as precursors of some NEAAs.

In a recent interesting study performed in pigs [13▪], irrespective of the feeding level, the animals responded to a reduction of EAA content of the diet by increasing their food intake (therefore, increasing NEAA intake), as a compensatory mechanism to achieve a normal EAA intake.

A similar result was reported in another study [14▪▪], where the impact of six diets, with various EAA/NEAA ratios, was evaluated on body composition and the risk of developing tissue wasting in mice. Animals consuming NEAA-based diets, although exhibiting an increase in food and calorie intake, experienced the most severe weight loss. Also a diet with a moderate NEAA excess in respect to the EAAs induced catabolism and generalized body wasting, similar to those observed with a diet containing NEAA alone. In contrast, EAA-containing diets were associated with a decrease in body weight. Taken together, these data suggest that a deficiency of EAAs, either absolute or in respect to NEAAs, led the animals to increase food intake but induced a catabolic state, underlying the key effects of the EAAs to maintain growth as well as a good nutritional state, while keeping NEAA intake at the minimum required.

SPECIFIC CONDITIONS WHERE AN INCREASE OF NONESSENTIAL AMINO ACID INTAKE COULD BE RECOMMENDED

Irrespective of whether or not NEAAs are 'essential', in a number of clinical conditions their intake could be increased. Nonessential amino acids prevented gastric cancer cells from glucose starvation-induced apoptosis [15]. As increased apoptosis can sustain the survival of malignant cells, such an effect may prove to be useful in clinical conditions. In end-stage renal disease (ESRD), transsulfuration is reduced limiting de novo cysteine synthesis. This defect could be corrected by increased methionine supply [16].

OVERVIEW OF THE METABOLISM OF DISPENSABLE, NONESSENTIAL AMINO ACIDS

From a structural standpoint, the terms 'dispensable' and/or 'nonessential' is usually referred to the carbon skeleton. However, also the α-amino groups, as well as an additional amide group in glutamine and asparagine, or a guanidine group in arginine derived from common nitrogen pool(s) of the body are required to complete the synthesis of NEAAs. All these nitrogen sources are in the form of 'organic nitrogen', that is itself associated to life. As a matter of fact, the body cannot handle/utilize inorganic nitrogen for its metabolic reactions.

The synthesis of the carbon skeleton of EAAs involves some biochemical steps that are not available in human cells, because some reactions (oxidation/decaboxylation/hydroxylation) in their degradative pathways are irreversible, and no alternative routes for their re-synthesis are available. Therefore, the carbon skeleton must be derived only from diet or, alternatively, provided by the carbon skeleton of their keto-analogues of the EAAs in specific nutritional formulas, completed by transamination to furnish the α-amino group.

The carbon skeleton of NEAAs can be either newly produced or derived from that of other amino acids (including the EAAs). Glucose, glycerol, intermediates of glucose metabolism (pyruvate), as well as of Krebs cycle (oxaloacetate, α-ketoglutarate), provide the carbon skeletons of alanine, aspartate, asparagine, glutamate, glutamine, ornithine (and arginine), proline, serine, and glycine [1▪▪]. Cysteine synthesis (via cystathionine) requires serine and homocysteine (in turn derived from methionine metabolism), whereas tyrosine (a conditionally dispensable amino acid) can be produced from phenylalanine hydroxylation.

The carbon end-products of NEAA metabolism are pyruvate (for alanine, cysteine, and serine), oxaloacetate (for aspartate), ketones (for tyrosine), α-ketoglutarate (for glutamate and ornithine), 3-phosphoglycerate (for serine), and carbon dioxide (for glycine) [1▪▪]. Therefore, some of these substrates are both precursors and products in NEAA metabolism, i.e. a reversible flux between these substrates and NEAAs occurs extensively. However, should the availability of NEAA precursors be impaired, a relative deficiency of these compounds could develop.

Table 2. Exchange of amino nitrogen among nonessential amino acids

Donor of the amino group	Amino acid	Nitrogen-containing end product
Glutamate	Alanine	Glutamate
Citrulline + Aspartate	Arginine	Ornithine (+urea)
Glutamate	Aspartate	Glutamate
Aspartate + Glutamine	Asparagine	Aspartate (+NH_3)
Methionine (Homocisteine)	Cysteine	glutathione
Serine	Glycine	NH_3
Alanine, Glutamine, Aspartate	Glutamate	Alanine, Glutamine, Aspartate
Glutamate	Glutamine	Glutamate
Arginine	Ornithine	Glutamate, Citrulline
Glutamate-γ semialdeide, Ornithine	Proline	Glutamate
Glutamate, Alanine	Serine	Glutamate

The α-amino group predominantly derives from transamination with other amino acids. Transaminations are fully reversible. Only glycine does not contain an α-amino group, rather a condensation product between CO_2 and NH_3. The α-amino group for the de novo synthesis of the NEAAs is mainly donated by glutamate and alanine [1▪▪]. Glutamate is at the core of transamination reactions. The amino group necessary for its synthesis through α-ketoglutarate amination, can derive from many amino acids (the branched chain amino acids, BCAAs, alanine, aspartate, glutamine). In Table 2, the exchange of NEAA nitrogen from donors to nitrogen-containing end products, is summarized. Therefore, the α-amino nitrogen required to complete NEAA synthesis derives from a common pool that should be maintained at equilibrium to guarantee nitrogen balance. Such a nitrogen pool is shared with the essential amino acids too.

Transamination is, therefore, a key control point in the de novo synthesis of the NEAAs. From the seminal study by Matthews *et al.* (see ref. [1▪▪] for review), leucine transamination to its α-ketoanalogue, α-ketoisocaproic acid (KIC), accounted for a significant fraction, that is, 50%, of leucine carbon flux, and for 36% of leucine nitrogen flux. From our own study [17▪], leucine deamination to KIC, accounted for 70% of leucine carbon flux, and for 50% of leucine nitrogen flux, moderately greater than those previously reported, likely because of a different isotopic approach.

Between 13 and 21% of circulating alanine nitrogen derived from leucine [18] in the postabsorptive state, in turn representing ∼28% of the leucine nitrogen going to alanine, and 13% of alanine nitrogen. It was calculated that ∼40% of circulating plasma alanine derives from endogenous protein, the remaining 60% from de novo synthesis, at least 20% from leucine alone. Therefore, should the contribution of isoleucine and valine nitrogen be similar to that of leucine, the BCAA would yield a minimum of ∼60% of the nitrogen required for alanine synthesis. In the de novo glutamine synthesis, nitrogen transfer from leucine to glutamine represented ∼20% of leucine's nitrogen, and 9% of glutamine's α-amino nitrogen [18].

These directly measured rates underline the major contribution of the amino nitrogen derived from EAA transamination in de novo synthesis of some key NEAAs.

Which organs and/or tissues are predominantly responsible for transamination *in vivo* in humans? This issue was addressed in a series of studies performed by combining leucine and KIC isotope infusions, with organ catheterization of the leg, the splanchnic area and the kidney [17▪]. The leg (approximated to skeletal muscle) appears in absolute terms the major site of leucine transamination (∼60 and ∼80% of whole body deamination and reamination rates, respectively), followed by the splanchnic area (∼15% of both rates) and by the kidney (∼12 and ∼18%, respectively) (Fig. 2). Should the high absolute leucine transamination rates of skeletal muscle be associated to alanine (and glutamine) de novo synthesis, these recent data confirm and substantiate the muscle–liver alanine cycle originally proposed by Felig *et al.* decades ago (see ref. [1▪▪] for review). As leucine (and the BCAAs) transamination may donate the amino group to synthetize glutamine from glutamate in muscle too, the same considerations can be extended to de novo glutamine synthesis.

The de novo synthesis of alanine and glycine accounted for about 80% of their flux in the postabsorptive state [19]. Insulin (with maintenance of

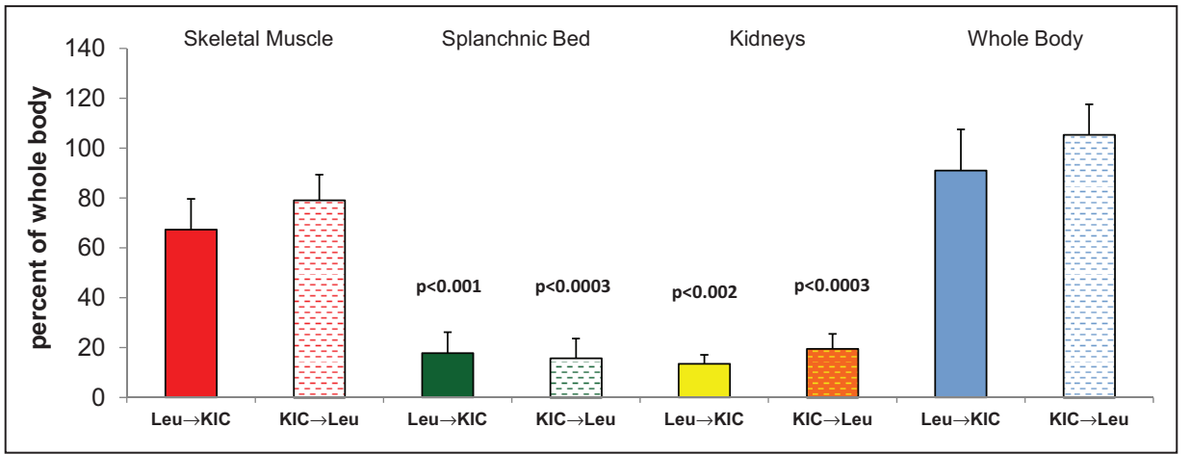

FIGURE 2. Percent contributions, to the whole body, of leucine deamination to α-ketoisocaproate, KIC (Leu→KIC), and of KIC reamination to leucine (KIC→Leu), across total skeletal muscle ($n = 10$), the Splanchnic Bed ($n = 8$) and the kidneys ($n = 7$), as well as the sum of the percentages of the three organs. The reported levels of statistical significance indicate the differences (by the one-way ANOVA and the Newman–Keuls post hoc test) between rates in either the Splanchnic Bed or the kidneys, and the corresponding ones in skeletal muscle. The sum of either deamination or reamination are not significantly different from 100% ($P > 0.7$). Data are shown as means ± SE. Reprinted with permission from ref. [17▪]. KIC, α-ketoisocaproic acid.

euglycemia) apparently prevented the glucose-induced increase in the de novo alanine synthesis [19], compatible with an insulin-induced suppression of amino nitrogen transfer by transamination. These findings agree with our recently reported finding that insulin suppresses leucine transamination in humans [20▪].

Both alanine (+50 and +230%) and glutamine de novo syntheses are increased by both acute and chronic cortisol administration, whereas leucine flux (an index of protein breakdown) is increased only by ~15%. This suggests that the amino group derived from transamination of leucine (and likely of the BCAAs) for the de novo alanine and glutamine synthesis, should have derived from leucine transamination rather than just from increased leucine (and BCAA) release from protein breakdown.

Table 3. Some important products synthetized from nonessential amino acid catabolism, and metabolic fate (either irreversible or reversible)

	Products	
Amino acid	**Irreversible pathway**	**Reversible pathway**
Arginine	Creatin(in)e, nitric oxide	
Aspartate		Purines and pyrimidines
Cysteine	Taurine	Glutathione
Glutamate	GABA (brain)	Glutathione
Glutamine		Purines and pyrimidines
Glycine	Creatine, porphyrins	Glutathione, purines,
Serine	Ethanolamine and choline	One carbon metabolism
Tyrosine	Catecholamines	Thyroid hormones

Adapted and modified from Table 2.7 of ref. [6▪].

OTHER END PRODUCTS GENERATED FROM AMINO ACID METABOLISM

A number of additional compounds are originated from the NEAAs, as reported in Table 3. The pathways leading to the synthesis of these compounds are in some instances irreversible, thus, contributing to the ONL [1▪▪] (see below). Furthermore, the gut microbiota metabolizes dietary amino acids to products that can enter common and yet poorly investigated metabolic pools [21]. Therefore, the theoretical 'requirement' of NEAAs would also include these other irreversible losses and/or metabolic end products.

CONCLUSION

Any estimate *in vivo* for humans of the 'requirements' of individual NEAAs is basically biased by their chemical nature of neosynthesizable substrates, as well as by the extensive interconversion. In addition, clinical 'models' of selected NEAA deficiencies are not available. However, from the simple subtraction of EAA RDAs from recommended 'safe' protein intake, some requirement of NEAAs as a whole could be suggested. Under certain conditions,

dietary intake of NEAAs could be insufficient to meet requirements. New approaches are required to investigate in depth such a complex issue.

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Conflicts of interest

There are no conflicts of interest.

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- ■ of special interest
- ■■ of outstanding interest

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