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# Broad-Spectrum Free-Form Amino Acid Supplements: Formulation Rationale, Mechanisms, Clinical Evidence and Dose Transferability of an 18-Component Formulation (Amino Complex)

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**Abstract:**

**Background**: Free-form amino acid supplements are marketed for muscle, metabolic, neurological, vascular, digestive and connective-tissue support, but the evidence derives almost entirely from single amino acids or complete essential-amino-acid mixtures at the gram scale. Broad-spectrum blends distributing a sub-gram total across many constituents have not been appraised.

**Objective**: To evaluate the rationale for this class and establish how far gram-scale ingredient evidence transfers to finished formulations of this type, with an 18-component composition as reference example.

**Methods**: Structured critical narrative review reported against SANRA and PRISMA-S. Five databases were searched from January 2016 to July 2026. Evidence was assigned to one of two layers — finished-product or ingredient-level — graded by Oxford CEBM level, and every efficacy dose recorded with its fold-difference from the reference quantity.

**Results**: The reference formulation supplies 897.5 mg of amino acids, 274 mg of them indispensable; L-threonine is absent. No study of any comparable finished formulation was identified. Mechanistic plausibility is strong: leucine and arginine each have a named molecular sensor upstream of mTORC1, and methionine is sensed indirectly through S-adenosylmethionine. Transferability is nonetheless limited by three independent factors: first-pass mucosal catabolism, near-complete for dietary glutamate and aspartate and 30–50 % for arginine and the branched-chain amino acids, together 44.9 % of the formulation; competitive rather than additive transport of a third of its mass at one blood–brain barrier carrier; and dose gaps of roughly five- to two-hundred-fold. Every constituent with a published reference value sits 35- to 1,000-fold below that ceiling.

**Conclusion**: Supplements of this class are best characterised as broad-spectrum dietary sources of amino acid substrates with wide safety margins and a coherent biochemical rationale; efficacy claims are not supportable at these quantities. Where the dose gap is narrowest, composition rather than total quantity is the operative constraint.

**Keywords:** free-form amino acids; Amino Complex; essential amino acids; branched-chain amino acids; taurine; L-serine; dose–response; splanchnic first-pass metabolism; large neutral amino acid transport; structure–function claims;

FSSAI

**Abbreviations:** BBB, blood–brain barrier; BCAA, branched-chain amino acid; CEBM, Centre for Evidence-Based Medicine; EAA, essential (indispensable) amino acid; EPA, eicosapentaenoic acid; FSSAI, Food Safety and Standards Authority of India; GlyNAC, glycine plus N-acetylcysteine; IAAO, indicator amino acid oxidation; LAT1, L-type amino acid transporter 1; LNAA, large neutral amino acid; MPS, muscle protein synthesis; mTORC1, mechanistic target of rapamycin complex 1; NOAEL, no-observed-adverse-effect level; NO, nitric oxide; OSL, observed safe level; SAM, S-adenosylmethionine; UL, tolerable upper intake level.

## 1. Introduction

Nine amino acids are indispensable for humans — histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine — with a further conditionally indispensable group whose endogenous synthesis may not meet demand [8, 9]. Amino acids are not merely protein monomers: the functional amino acid concept recognises them as obligate precursors for nitric oxide, glutathione, taurine, creatine and the monoamine neurotransmitters [10]. Several are also signalling molecules — leucine is sensed by Sestrin2 [3] and arginine by CASTOR1 [4], while methionine enters the same network indirectly through S-adenosylmethionine, the ligand for SAMTOR [5] — all converging on mTORC1 at the lysosomal surface [11].

This dual identity drives a large market in free-form amino acid supplements. Free crystalline amino acids bypass proteolysis and are absorbed directly, producing a faster, higher but more transient plasma rise than intact protein; the International Society of Sports Nutrition concludes that free indispensable amino acids stimulate muscle protein synthesis more effectively than an equivalent mass of protein, with the response beginning at 1.5–3.0 g and plateauing near 15–18 g [12].

Almost all of that evidence concerns a single amino acid or a complete indispensable-amino-acid mixture, at the gram scale. A commercially common alternative architecture — a broad-spectrum blend distributing a sub-gram total across fifteen or more constituents — remains unappraised. The question is not whether the constituents have physiological functions; they demonstrably do. It is whether a formulation delivering each at one to two orders of magnitude below its studied dose can reproduce the reported effects.

We address that question for the class, using as the reference example an 18-component composition marketed in the Indian nutraceutical sector under the FSSAI framework [13]. The nine claim domains appraised here — general wellness; sports, muscle and recovery; fatigue; cognition, mood and sleep; cardiometabolic health; glycaemic regulation; digestive health; pain and connective tissue; and antioxidant protection — are those recurring across the category rather than the claim set of any one brand. To avoid the failure mode of product-focused literature, an ingredient catalogue attaching the most favourable finding to each constituent, we separate finished-formulation from ingredient-level evidence, group the constituents into functional domains, and state the dose of every cited efficacy study beside the quantity present in the reference composition. The intended contribution is therefore methodological as well as descriptive.

## 2. Methods

Design and disclosure. Structured critical narrative review with reproducible literature identification. A systematic review was rejected because the objective requires integrating biochemical, pharmacokinetic, clinical, safety and regulatory evidence across eighteen ingredients and nine claim areas, with inherently heterogeneous interventions and outcomes. Reporting follows SANRA [1] and, so far as a narrative review permits, the PRISMA-S search-reporting items [2]. The article is written by research staff of a company that manufactures a formulation belonging to the class appraised; it is not an independent synthesis (Declarations).

Two-layer evidence model. Layer 1 is evidence for a finished broad-spectrum sub-gram formulation of this class; gram-scale complete indispensable-amino-acid supplements are a different design and enter at level 3 of the Section 7.3 taxonomy, not Layer 1; Layer 2 is ingredient-level evidence, recorded as physiological function, mechanistic evidence, human supplementation evidence, dose used, dose present in the reference composition, and the resulting strength of formulation-level inference. Layer 2 findings were not transferred to Layer 1 by default — the review's principal methodological safeguard.

Search, eligibility and appraisal. PubMed/MEDLINE, Cochrane, Scopus, Web of Science and Google Scholar (the last for citation tracking only), January 2016 to July 2026 with no restriction for landmark mechanistic work; final search 30 July 2026. Five search blocks were run — amino acid mixtures; muscle and exercise; metabolic, vascular and neurophysiological outcomes; safety; connective tissue — supplemented by reference-list screening, citation tracking, trial-registry searches and hand-searching of regulatory sources; full strategies are in Supplementary Material S1. Human trials, systematic reviews, relevant cohorts and pharmacokinetic and safety studies were eligible; mechanistic work was admitted for mechanism only. Excluded, or admitted only as labelled indirect evidence: parenteral administration; intact-protein studies without amino-acid-level analysis; multi-stimulant energy drinks; products in which the amino acid contribution cannot be isolated; and five substitutions that would misrepresent the evidence — D-aspartic acid for L-aspartic acid, glutamine for glutamic acid, N-acetylcysteine for L-cysteine, D-/DL-phenylalanine for L-phenylalanine, and D-serine for L-serine. Records were extracted into a matrix recording design, population, ingredient, chemical form, dose, duration, comparator, outcomes, adverse events, the reference quantity, the dose ratio, directness and limitations (S2), and graded by Oxford CEBM level (1a–5); molecular plausibility and clinical efficacy were never combined into one category. Every citation was verified against its primary PubMed entry, and one taurine paper was excluded as retracted.

Stated limitations. Per-stage record counts were not retained, so a PRISMA-2020 flow diagram [14] is not provided and no PRISMA compliance is claimed; search strategies were not peer-reviewed by an information specialist; no formal risk-of-bias instrument was applied, so a well-conducted and a compromised trial of the same design receive the same CEBM tier (Sections 5.1, 6.3); and the dated protocol prepared before searching (S4) was not deposited in a public repository, PROSPERO not accepting narrative reviews. As all authors are employees of the manufacturer, S1–S4 are provided so that the judgements below can be audited directly.

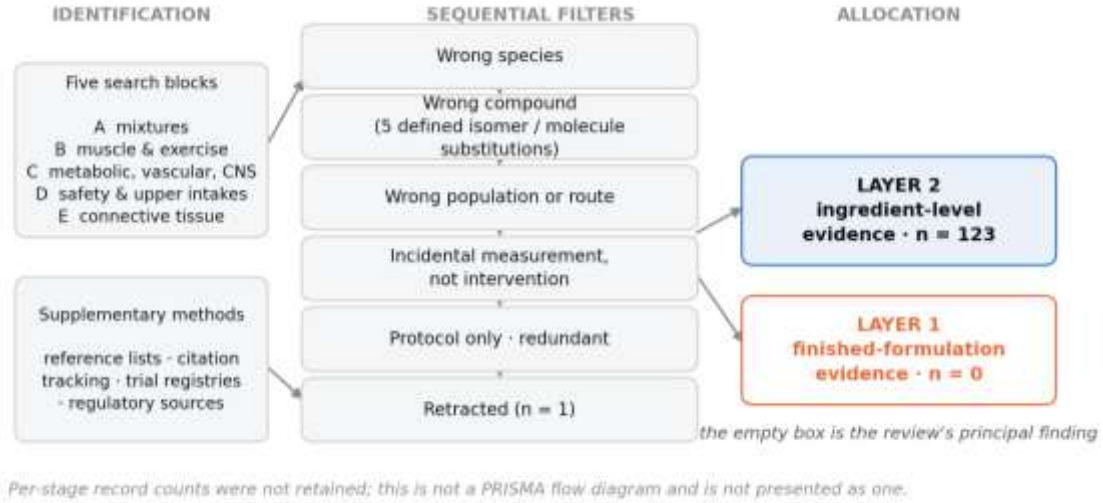


**Figure 1.** Evidence identification and appraisal schematic — deliberately not a PRISMA flow diagram, since per-stage record counts were not retained. Left: the five search blocks and supplementary methods. Centre: the sequential filters, each labelled with the dominant exclusion reason. Right: allocation of retained references to Layer 2 and to Layer 1, the empty box drawn to the same scale to make the asymmetry visible.

The principal search result is reported here rather than deferred: no study of any finished broad-spectrum sub-gram free-amino-acid formulation — of any design, in any database or language — was identified. Layer 1 is empty for the class as a whole, and every subsequent statement is therefore an ingredient-level inference. This is a gap in the literature rather than a feature of any one product, and it is why the appraisal below is framed as transferability rather than efficacy.

## 3. Formulation analysis

**Table 1**: Declared composition of the reference formulation per serving, with nutritional class and principal metabolic role.

| Amino acid | mg | Class | Principal role in this formulation |
|---|---|---|---|
| L-Glutamic acid | 137.0 | Dispensable | Principal enterocyte fuel; transamination hub; glutathione and GABA precursor; umami ligand |
| L-Arginine | 105.0 | Cond. indispensable | NO synthase substrate; urea cycle; mTORC1 sensing |
| Taurine | 86.0 | Non-proteinogenic | Osmoregulation; calcium handling; bile acid conjugation; mitochondrial function |
| L-Valine | 86.0 | Indispensable (BCAA) | Muscle oxidation; protein substrate; LAT1 competitor |
| L-Serine | 86.0 | Dispensable* | One-carbon metabolism; sphingolipid synthesis; cysteine and glycine precursor |
| L-Tyrosine | 65.0 | Cond. indispensable | Catecholamine precursor; LAT1 competitor |
| Glycine | 54.0 | Cond. indispensable | Glutathione co-substrate; collagen; inhibitory neurotransmitter; NMDA co-agonist |
| L-Lysine | 45.0 | Indispensable | Collagen cross-linking; carnitine synthesis |
| L-Tryptophan | 44.0 | Indispensable | Serotonin and melatonin precursor; kynurenine pathway; microbial indoles |
| L-Leucine | 36.0 | Indispensable (BCAA) | mTORC1 activation via Sestrin2 — the anabolic signal |
| L-Alanine | 32.5 | Dispensable | Glucose–alanine cycle; gluconeogenic substrate |
| L-Proline | 23.0 | Cond. indispensable | Collagen; arginine and polyamine interconversion |
| L-Aspartic acid | 23.0 | Dispensable | Malate–aspartate shuttle; urea cycle; nucleotide synthesis |
| L-Histidine | 23.0 | Indispensable | Histamine precursor; carnosine constituent; buffering |
| L-Isoleucine | 16.0 | Indispensable (BCAA) | Muscle oxidation; protein substrate |
| L-Cysteine | 12.0 | Cond. indispensable | Rate-limiting for glutathione; taurine precursor; mucin cross-linking |
| L-Methionine | 12.0 | Indispensable | SAM and methylation; transsulfuration; mTORC1 sensing |
| L-Phenylalanine | 12.0 | Indispensable | Tyrosine precursor; LAT1 competitor |
| Total | 897.5 | | |

*Classification follows the US Institute of Medicine reference intakes [9], which place arginine, cysteine, glutamine, glycine, proline and tyrosine in the conditionally indispensable category.* *L-Serine is classified

as dispensable by that standard; a reasoned case for reclassifying it as conditionally indispensable has been made on one-carbon and sphingolipid-metabolism grounds [15], but the standard classification is retained here.

**Table 2:** Derived quantities.

| Quantity | Value | Relevance |
|---|---|---|
| Total amino acids | 897.5 mg | ~1.1 % of a 1.2 g/kg/day protein target for 70 kg |
| Indispensable amino acids | 274 mg | 8 of 9 present; L-threonine absent |
| Branched-chain amino acids | 138 mg | Leu 36 + Ile 16 + Val 86 |
| BCAA ratio Leu : Ile : Val | 2.25: 1: 5.4 | Inverted vs conventional leucine-dominant 2:1:1 |
| LNAAs competing at LAT1 | 294 mg | Trp, Tyr, Phe, Leu, Ile, Val, Met, His |
| Collagen-relevant amino acids | 122 mg | Gly 54 + Lys 45 + Pro 23 |
| Sulfur amino acids | 24 mg | Met 12 + Cys 12, a 1:1 mass ratio |
| Dispensable, cond. indispensable and non-proteinogenic | 623.5 mg (69 %) | Includes taurine 86 mg; unlikely to be rate-limiting for protein anabolism |

Five features of this composition determine what can and cannot be concluded from the ingredient literature. Each is a design variable common to the class rather than a property peculiar to the reference example.

**3.1 Completeness of the indispensable set.** Broad-spectrum blends frequently carry eight of the nine indispensable amino acids, threonine being the one most often omitted; the reference formulation follows that pattern. A formulation omitting any indispensable amino acid cannot be described as complete, or as a substitute for dietary protein, at any dose. The point also bears on digestive positioning: threonine is a major and potentially limiting substrate for intestinal mucin synthesis, and in animal studies dietary restriction reduces the mucus barrier and increases paracellular permeability [16].

**3.2 Glutamic acid and glutamine are not interchangeable.** Glutamate is the largest constituent at 137 mg, and glutamine is absent — the usual arrangement in this category. The distinction matters: the clinical literature on amino acid support of the intestinal barrier is a glutamine literature at 5–30 g/day [17,18] and does not transfer to a glutamate-containing formulation. Glutamate has a different mechanism (Sections 4 and 6.7).

**3.3 Choice of nitric oxide precursor.** Where a blend addresses the nitric oxide pathway, it generally does so through arginine — 105 mg here — rather than citrulline. Oral arginine is substantially eliminated presystemically by arginase, which is why citrulline has displaced it as the preferred precursor [19]. Precursor selection is a design variable operating independently of dose.

**3.4 Branched-chain ratio.** Blends assembled for spectrum breadth rather than anabolic signalling often distribute the branched-chain fraction differently from purpose-built sports products: here valine exceeds leucine 2.4-fold, against the leucine-dominant 2:1:1 convention. That convention exists because leucine is the sensed anabolic signal [3] and, in humans, the leucine fraction rather than total protein sets the magnitude of the synthetic response [20, 21].

**3.5 Every constituent sits below its studied dose** — necessarily so, since a sub-gram total distributed across eighteen fractions cannot reach gram-scale single-ingredient doses. The gap is not uniform, ranging from roughly five-fold to two-hundred-fold, and in one instance the reference quantity exceeds a reported effective dose (Section 7).

Calibration against reference requirements. Where a label of this type declares percentage contributions to reference intakes, the spread across constituents is itself informative. For the reference formulation, declared against the Indian Council of Medical Research 2020 values for an adult woman, contributions range from 1.45 % for isoleucine and 1.68 % for leucine to 20 % for tryptophan, with no reference value

established for ten of the eighteen constituents. A broad-spectrum blend therefore does not contribute uniformly across the indispensable set. The spread also separates two questions that product literature tends to merge: tryptophan supplies the largest fraction of its nutritional requirement of any constituent here, and is nonetheless some twenty-three-fold below the lowest dose at which any functional effect has been reported (Section 7). Nutritional contribution and pharmacological sufficiency are different thresholds, and a formulation of this class can approach the first while remaining far from the second. The choice of reference base matters as much as the quantity: the same 36 mg of leucine is 1.68 % of the ICMR requirement but 0.66 % of the indicator-amino-acid-oxidation estimate for older adults [22], the two differing by the factor by which that method has revised the requirement upward.

Calibration against dietary protein. Indispensable amino acids are primarily responsible for the anabolic response to feeding: 18 g alone stimulated anabolism in older adults equivalently to 40 g of a balanced mixture containing the same 18 g plus 22 g of dispensable amino acids [23]. Against that benchmark, the 623.5 mg of dispensable, conditionally indispensable and non-protein genic constituents here is unlikely to be rate-limiting for anabolism. One large egg supplies roughly ten times the reference formulation's indispensable-amino-acid content, and whole-body protein turnover is of the order of 3–4 g/kg/day — some 250–300 times a single serving. A formulation of this size cannot be expected to influence whole-body amino acid flux and should not be described as doing so; a nutritional-adjunct framing is the accurate one.

## 4. Absorption, first-pass metabolism and systemic availability

First-pass metabolism determines whether any downstream mechanism can be engaged by an oral dose, and is therefore treated before the mechanistic domains.

Free amino acids require no proteolysis and are absorbed by class-specific, storable and mutually competitive transporters, bypassing the high-capacity peptide route used by intact protein. Competition therefore begins before absorption, not only at the blood–brain barrier: neutral and aromatic amino acids compete for the $B^0$-type apical system, and arginine, lysine and histidine for the cationic $b^{0,+}$ and $y^+L$ systems — essentially the same constituent set vulnerable to central transport competition, so the two constraints compound. The result is a sharper but more transient plasma peak, the pharmacokinetic basis of the position-stand conclusion favouring free indispensable amino acids [12].

The quantitatively decisive constraint is splanchnic extraction. The intestinal mucosa catabolises 30–50 % of dietary arginine and the branched-chain amino acids before they reach the portal circulation, and almost all dietary glutamate and aspartate [6]. Both classes are represented here: arginine 105 mg and the branched-chain amino acids 138 mg fall in the 30–50 % group, glutamate 137 mg and aspartate 23 mg in the near-complete group — together 403 mg, or 44.9 % of the total. Including the remaining constituents the same source lists as subject to appreciable mucosal catabolism (proline, methionine, lysine, phenylalanine, 92 mg) would raise the affected fraction to 495 mg or 55 %. For glutamate, the extraction is near-total: in piglet tracer studies ~95 % is metabolised in first pass by the gut mucosa, where it is the largest single contributor to intestinal ATP generation [24,25]. For arginine, human tracer work shows ~60 % first-pass conversion to urea and under 0.1 % of the dose reaching nitric oxide [26], consistent with presystemic intestinal arginase, the mechanism proposed in a crossover study in which oral citrulline at 3 g twice daily raised plasma arginine more effectively than arginine itself, though without improving flow-mediated dilatation [19]. Extraction is not simply loss: for glutamate it *is* the mechanism of action — the amino acid is consumed by the tissue it is intended to support (Section 6.7).

The second constraint completes the transport picture: competition at the blood–brain barrier. Eight constituents — tryptophan, tyrosine, phenylalanine, leucine, isoleucine, valine, methionine and histidine, together 294 mg — share the L-type transporter, so transport is competitive rather than additive: raising one lowers brain uptake of the others in proportion to their relative plasma concentrations [7], and the meaningful clinical index is the plasma tryptophan-to-large-neutral-amino-acid ratio rather than tryptophan intake [27]. A recent study inverts the logic of a multi-precursor blend: branched-chain amino acids with phenylalanine and methionine were used deliberately to *inhibit* tryptophan uptake, and they prolonged exercise duration more than branched-chain amino acids alone in a rat model, with an

uncontrolled human response-rate comparison alongside [28]. Such formulations supply tryptophan together with all its principal competitors, so two intended central effects work against one another.

The lowest dose of a free indispensable-amino-acid *mixture* shown to produce a measurable endpoint is 1.5 g [29]; single constituents have produced effects at lower doses still (Section 7). Whether 897.5 mg across eighteen constituents alters plasma amino acid concentrations at all is untested, and Section 9 specifies the study that would resolve it.

## 5. Mechanisms by functional domain

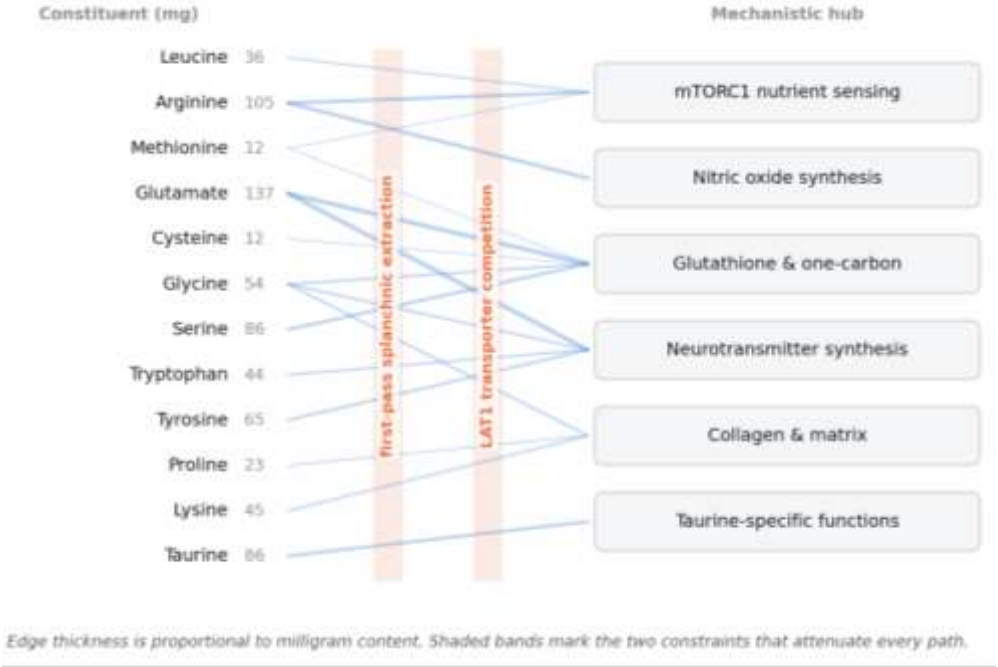


**Figure 2.** Mechanistic convergence: constituents as source nodes, edge thickness proportional to milligram content, converging on six hubs — mTORC1 nutrient sensing, nitric oxide synthesis, glutathione and one-carbon metabolism, neurotransmitter synthesis, collagen, and taurine-specific functions. Two shaded bands cross the edges, marking first-pass splanchnic extraction and LAT1 competition.

### 5.1 Indispensable amino acids, muscle protein synthesis and exercise metabolism

*Leucine 36, isoleucine 16, valine 86, lysine 45, methionine 12, histidine 23, phenylalanine 12, tryptophan 44 mg — 274 mg; threonine absent.*

Muscle protein synthesis requires both a signal and a substrate. The signal is leucine, sensed by Sestrin2, which inhibits GATOR2 in the leucine-free state [3]; arginine and methionine are sensed in parallel [4, 5], all converging on the lysosomal Rag–Regulator–GATOR machinery [11]. Two constituents typical of this class are sensed directly by named, structurally characterised nutrient sensors upstream of the principal anabolic regulator in mammalian cells, and a third enters the same network indirectly — the best-evidenced molecular-signalling statement available for formulations of this type. One qualification: SAMTOR binds S-adenosylmethionine rather than methionine itself, and whether 12 mg of methionine, roughly 2 % of habitual daily intake, raises cellular S-adenosylmethionine sufficiently to engage it is untested. The substrate requirement is separate, and is where isolated branched-chain amino acids fail: synthesis consumes all twenty amino acids, so a leucine signal without a complete indispensable supply cannot sustain net synthesis [30]. Broad-spectrum blends are constrained on both requirements, and the substrate constraint is categorical rather than quantitative: leucine content is 36 mg against a threshold the position stand places at 0.7–3 g [31], and an indispensable set lacking threonine cannot complete the substrate pool at any dose.

The dose picture is nonetheless more favourable than those figures suggest. In a parallel-group study of twenty-four women aged 65 ± 1 years, eight per arm, 1.5 g of a leucine-enriched indispensable-

amino-acid supplement providing only 0.6 g of leucine stimulated muscle protein synthesis as robustly as 40 g of whey protein, with negligible additional benefit at 6 g; the authors concluded that "composition of EAA, in particular the presence of leucine rather than amount is most crucial for anabolism" [29]. Four caveats attach, and are stated because much of this appraisal rests on the study: PubMed indexes it as observational rather than randomised, so it is graded CEBM 2b and not 1b; the arms comprised eight participants each; the endpoint is a tracer-derived fractional synthetic rate; and there was no untreated control. It is not an isolated result, however — the International Society of Sports Nutrition position stand independently places the stimulation threshold at 1.5–3.0 g [12].

The finding cuts both ways. It establishes that the relevant gap is ~5.5-fold on total indispensable amino acids and ~17-fold on leucine, within reach of reformulation, and simultaneously that if composition is the critical variable, composition is where formulations of this type are most readily improved, and where the reference example is furthest from the studied designs. Supporting evidence is consistent: 10 g of indispensable amino acids with 3.5 g leucine produced 33 % greater post-exercise synthesis than the same 10 g with 1.87 g [20], and leucine-matched supplements induced similar increases in synthesis irrespective of total protein, though the acute response in the exercised leg remained 46 % greater with the leucine-enriched supplement [21]. Against an indicator-amino-acid-oxidation leucine requirement of 78.5 mg/kg/day in older adults [22], 36 mg supplies well under 1 % of daily need.

For the branched-chain fraction specifically, the central finding is a dose condition: supplementation alleviates muscle damage only at more than 200 mg/kg/day for more than ten days, most effectively taken before the damaging exercise and where muscle damage is low-to-moderate [32] — over 14 g/day for a 70 kg adult, ~100-fold the reference content — and meta-regression found higher daily and total dose associated with larger effects on creative kinase at 48 h and soreness at 24 h, though not at other time points [33]. A recovery trial at 0.17 g/kg (~12 g) found no benefit on muscle damage or performance [34], and the most recent synthesis concludes that branched-chain-induced biochemical changes do not translate into functional endurance benefit [35].

**Assessment.** Mechanism strong; transferability at these quantities weak, and limited by composition more than by dose — this is the domain in which composition rather than total quantity is most clearly the binding constraint. Within it, the branched-chain fraction carries the widest gap of any claim in the muscle domain, at roughly one hundred-fold.

### 5.2 Nitrogen metabolism, the intestinal fate of glutamate, and nitric oxide

*Glutamic acid 137, alanine 32.5, aspartic acid 23, arginine 105, serine 86 mg — 383.5 mg.*

These constituents participate in transamination and interorgan nitrogen transport rather than signalling: alanine carries muscle nitrogen to the liver in the glucose–alanine cycle; aspartate operates the malate–aspartate shuttle; glutamate is the transamination hub and the immediate precursor of GABA and of glutathione; serine feeds one-carbon metabolism and the first committed step of sphingolipid synthesis.

The defensible mechanistic narrative for the largest constituent lies here rather than in systemic or central physiology. Glutamate is the dominant enterocyte fuel, and its near-complete first-pass extraction is the mechanism, not a limitation [24,25]. Luminal glutamate is sensed by gastric mucosal metabotropic glutamate receptors, triggering mucin and nitric oxide release, enterochromaffin-cell serotonin release and vagal afferent activity in the rat [36], while T1R1/T1R3 umami receptors on mouse gastric smooth muscle mediate relaxation directly, without nitric oxide or neural involvement [37]. Neither route requires systemic absorption, and they are therefore the mechanisms here that first-pass extraction does not undermine. Serine has a specific human correlate: when serine is limiting, serine palmitoyltransferase substitutes alanine, generating cytotoxic 1-deoxysphingolipids that independently predicted incident type 2 diabetes over eight years [38]. No interventional oral-serine dose–response study addressing this pathway exists.

Arginine is the nitric oxide synthase substrate. The field rests on three findings: that an intact endothelium is obligatory for acetylcholine-induced relaxation [39]; that endothelium-derived relaxing factor is nitric oxide [40]; and that endothelial cells synthesise nitric oxide specifically from L-arginine and not from D-arginine [41] — the isomer specificity on which this review's exclusion of D-isomer evidence rests. That exogenous arginine still augments production despite a synthase Michaelis constant far below

normal intracellular concentrations is explained by competitive inhibition from endogenous asymmetric dimethylarginine [42]. The paradox has a second limb more important for a supplement: arginase expression is proposed to rise in proportion to arginine supplementation, so that the more arginine is introduced, the more is destroyed [43]. That mechanism rests on a narrative synthesis rather than confirmed human dose–response data, and is offered as the most coherent explanation for the null and adverse findings in Section 6.5 rather than as an established quantitative relationship. It is also why citrulline, which escapes presystemic arginase, is preferred [19].

### 5.3 Neurotransmitter synthesis and central nervous system signalling

*Tryptophan 44, tyrosine 65, phenylalanine 12, glutamic acid 137, glycine 54, histidine 23, methionine 12, serine 86, taurine 86 mg.*

Tryptophan is hydroxylated and decarboxylated to serotonin and thence melatonin, with the competing kynurenine branch consuming most available tryptophan and upregulated by inflammation. Phenylalanine is hydroxylated to tyrosine and thence to the catecholamines; the clinical extreme is phenylketonuria [44]. Glutamate is the principal excitatory transmitter and the GABA substrate; glycine acts both as an inhibitory transmitter and as an obligatory NMDA co-agonist; histidine is decarboxylated to histamine. Methionine is the precursor of S-adenosylmethionine, whose exogenous administration has meta-analytic antidepressant support against placebo across 23 trials (standardised mean difference −0.58) [45], tempered by a Cochrane review finding no strong evidence of monotherapy superiority [46].

Two established constraints bound this entire domain. First, the eight large neutral amino acids compete for one carrier, so central effects are not additive and may be mutually offsetting [7]. Second, dietary glutamate does not raise brain glutamate: ingested monosodium glutamate produces no appreciable rise in blood glutamate except at experimentally excessive doses, and the intact barrier restricts passage even then [47]; expert consensus concurs [48], though the principal consensus statements in this area were produced by groups with glutamate-industry involvement. The corollary is symmetrical: 137 mg of glutamic acid is neither a central nervous system benefit nor a risk.

**Assessment.** Transferability at these quantities weak, and uniquely constrained by transporter competition in addition to dose. No cognition, mood or sleep claim is supportable for a formulation of this type.

### 5.4 Redox metabolism, sulfur amino acids, connective tissue and taurine

Glutathione is γ-glutamyl-cysteinyl-glycine, and all three constituent amino acids are present — glutamate 137, cysteine 12, glycine 54 mg — a stoichiometrically complete and coherent design choice. Cysteine derives from diet or from methionine via transsulfuration; taurine is a downstream cysteine product.

A methionine consideration runs opposite to usual supplement logic. Methionine restriction extends rodent lifespan independently of caloric restriction [49]; a systematic review of amino acid supplementation in cardiovascular and chronic kidney disease found arginine the most preventive and methionine the most adverse constituent, although only thirteen of sixty studies were human trials [50]; and methionine is the one indispensable amino acid with plausible clinical toxicity when cysteine synthesis lags, making stoichiometric balance the design consideration [51]. A 1:1 pairing of 12 mg methionine with 12 mg cysteine is therefore appropriately conservative and is a deliberate design feature.

Connective tissue. Collagen is approximately one-third glycine, with proline and hydroxyproline in the repeating triplet, and ascorbate-dependent lysis hydroxylation generating the hydroxylysine that permits cross-linking. Glycine, proline and lysine — 122 mg here — are precisely the three amino acids collagen synthesis consumes in greatest disproportion to their dietary abundance. The clinical literature is a gelatin and collagen-peptide literature and is unusually clean on dose (Section 6.8).

Taurine is not proteinogenic. A β-amino sulfonic acid present intracellularly at millimolar concentrations, it functions in osmoregulation, calcium handling, bile acid conjugation, mitochondrial translation and inhibitory neuromodulation. Its physiological necessity is established genetically: transporter-knockout mice show >90 % cardiac and skeletal muscle depletion, retinal degeneration and reduced exercise capacity [52]. Two points of hygiene attach. The 2023 report that taurine declines with age across species and that supplementation extends murine lifespan [53] must be paired with the subsequent

human study in 137 men aged 20–93 finding no association between circulating taurine and age, muscle mass, strength, performance or mitochondrial function [54]. And taurine is the one constituent for which a reported effective dose falls below the reference quantity (Section 7.2).

## 6. Clinical evidence by health domain

Layer 1 evidence is absent throughout and is not restated in each subsection.

**6.1 General wellness and nutritional support.** The only domain where the quantities are of the right order. Against WHO/FAO requirement patterns [8], the DIAAS framework [55], stable-isotope requirement studies — several of which have revised estimates upward, including in the Indian dietary context [56] — and a requirement literature that remains sparse across the life course [57], a spread of eighteen amino acids at these quantities is a supplementary contribution to intake. One nuance should be reported rather than filtered: enrichment with cysteine, glycine, arginine, glutamine and glutamate may favour cardiometabolic outcomes, whereas enrichment with leucine, isoleucine, valine, lysine and methionine may increase risk [58]; this formulation contains both groups. Inference: partially direct, defensible.

**6.2 Sports performance, muscle and recovery.** Fifteen grams of indispensable amino acids daily for twenty-four weeks with aerobic training improved strength in older adults but did not change lean mass [59]. A formulated indispensable-amino-acid supplement improved six-minute walk distance and grip and leg strength in older adults with low physical functioning [60] — the closest published analogue to a formulated product with a functional endpoint. Inference: indirect; not defensible at 138 mg branched-chain and 274 mg indispensable amino acids.

**6.3 Fatigue and energy metabolism.** The central fatigue hypothesis holds that a rising free-tryptophan-to-branched-chain ratio raises brain serotonin and contributes to fatigue [61,62], but has an often-omitted refutation in that pharmacological serotonin blockade failed to improve endurance [63]. Taurine has meta-analytic support for endurance at 1–6 g/day, effect size 0.40, with no dose–response within that range [64]. Histidine gives the most current signal: a single dose improved vigour and reduced fatigue, but only in an exploratory high-fatigue subgroup, the pre-specified whole-sample analysis being null [65]. Inference: indirect.

**6.4 Cognition, mood, sleep and central nervous system support.** Genuinely positive at gram doses and genuinely inapplicable at these. Tryptophan reduces wake after sleep onset at or above 1 g/day, on a meta-analysis of four studies, with no effect on other sleep components [66]; acute tryptophan depletion lowers mood only in those with a family history of depression or remitted major depression [67], so a general-population product should not expect a mood signal even at an adequate dose. Tyrosine benefits cognition only under acute stress with transiently depleted catecholamines [68], at 150 mg/kg twice [69] or a single 300 mg/kg dose [70] — 10.5 to 21 g for a 70 kg adult — while a single 2.0 g dose worsened cognitive flexibility under high load [71]. The glycine trial most often cited in this area used 3 g at bedtime and measured daytime fatigue and psychomotor vigilance under sleep restriction rather than sleep onset [72], and the lysine–arginine anxiety trial used 2.64 g/day of each in 108 adults over one week [73], a single small manufacturer-affiliated trial. The NMDA co-agonist literature concerns D-serine, not the L-serine present here [74]. Inference: indirect throughout, additionally constrained by transporter competition.

**6.5 Cardiometabolic and endothelial function.** Meta-analysis of thirteen trials found no significant pooled effect of arginine on post-ischaemic blood flow [75]; blood-pressure reductions require ≥4 g/day, independent of trial duration [76], the lowest effective dose located being 1.5–2 g/day for aerobic outcomes [77]. Two findings materially qualify this: in older adults with cardiovascular risk, arginine worsened microvascular reactive hyperaemia and executive function [78], and in acute myocardial infarction, 9 g/day added to standard therapy was stopped early, with six deaths in the arginine arm versus none on placebo [79]. With the arginase mechanism [43], these form a coherent caution, tempered only by the finding that benefit appeared where baseline fasting plasma arginine was low [80]. L-citrulline or watermelon intake, absent here, reduced systolic and diastolic pressure by 4.02 and 2.54 mmHg across fifteen trials in middle-aged and older adults [81]. Taurine at 1.5–6 g/day reduced heart rate 3.6 bpm and systolic pressure 4.0 mmHg [82], though a systematic review in which only one of eleven studies was high quality found the

pooled cardiac effect not significant [83]; the lowest cardiovascular dose located with any positive effect is 1.5 g/day, and on inflammatory and atherogenic indices rather than blood pressure or cardiac function [84]. Inference: indirect; the safety literature must accompany any mechanistic statement.

**6.6 Blood-glucose regulation.** This is the domain in which the observational literature points in the opposite direction to the proposed benefit, and an impartial appraisal has to record that. Amino acids are both insulinotropic and glucagonotropic, and protocols capable of altering postprandial glycaemia used boluses of 5–50 g. Higher circulating branched-chain, aromatic, alanine, glutamate, lysine and methionine concentrations associate with higher incident type 2 diabetes risk (hazard ratios 1.07–2.58) across 61 reports, 71,196 participants and 11,771 incident cases, while glycine, glutamine and betaine associate with lower risk [85]; in 5,181 men followed 7.4 years, nine amino acids including aspartate and glutamate associated with declining insulin secretion [86]. Nine of the eighteen constituents appear in those lists, as they would for most blends of comparable breadth. Three considerations temper this: these are endogenous concentrations reflecting a metabolic phenotype, not the consequence of a 12–137 mg oral dose; Mendelian randomisation in 619,372 individuals suggests marker rather than driver [87]; and 15 g/day of indispensable amino acids for twenty-two weeks had no effect on insulin sensitivity and was metabolically safe [88]. Histidine at 4 g/day for twelve weeks reduced insulin resistance in obese women [89]. No blood-glucose claim is supportable for a formulation of this type.

**6.7 Digestive and intestinal health.** This domain carries the strongest mechanistic case not dependent on systemic absorption, and the least transferable interventional evidence, because the two concern different molecules. Mechanistically: glutamate is the enterocyte's principal fuel, its ~95 % first-pass extraction in piglet tracer work being the mechanism [24,25]; luminal glutamate signals through gastric mucosal metabotropic receptors to trigger mucin and nitric oxide release and vagal afferent activity in the rat [36], and T1R1/T1R3 umami receptors on mouse gastric smooth muscle mediate relaxation by a route that is neither nitric-oxide- nor neurally-mediated [37], neither requiring systemic absorption; tryptophan-derived microbial indoles activate the aryl hydrocarbon receptor and interleukin-22 axis and confer mucosal protection from inflammation in mice [90]; and a narrative review of nutritional support for mucosal healing names arginine, glutamine, glutamate, threonine, methionine, serine and proline — nearly the reference formulation's exact subset, minus threonine — while stating plainly that the evidence is animal- and cell-derived [91]. What cannot be claimed: the human interventional literature is a glutamine literature — 15 g daily for eight weeks produced a ≥50-point severity reduction in 79.6 % versus 5.8 % of patients with post-infectious irritable bowel syndrome [17], and meta-analysis found a null pooled effect on permeability, with a reduction reported only in a subgroup above 30 g/day and in trials under two weeks [18]. These findings concern glutamine and do not transfer to a glutamate-containing formulation. Threonine content bears directly here for the same reason [16]. Inference: mechanism partially direct; efficacy indirect.

**6.8 Pain and connective-tissue recovery.** Most of the relevant pain literature is preclinical receptor pharmacology — spinal α3 glycine receptor potentiation reverses inflammatory hyperalgesia [92] — manipulations of receptors rather than dietary glycine, and no evidence was located that 54 mg of ingested glycine raises spinal extracellular glycine. The D-phenylalanine enkephalinase literature dates from the 1980s, rests on animal pharmacology with uncontrolled human case reports, and concerns the D-isomer [93]. Human causal data run in the depletion direction [94], dietary glutamate elimination trials in fibromyalgia are inconsistent [95, 96], and arginine in migraine is hypothetical, the better-established effect of nitric oxide donors being to trigger headache [97].

Against that background, one trial changes the assessment of this domain. In 120 adults with at least three months of both low-back and knee pain, 594 mg of L-serine with 149 mg of eicosapentaenoic acid daily for eight weeks significantly reduced validated low-back and knee scores, with Brief Pain Inventory measures 11–27 % better than placebo, no adverse events, and significance surviving multiplicity adjustment [98] — a properly powered double-blind trial at a sub-gram L-serine dose, approximately 7-fold from the reference quantity of 86 mg. Two constraints prevent it becoming a product claim: eicosapentaenoic acid is a co-intervention not present here, and the trial cannot separate the

contributions; and it is authored almost entirely by employees of an amino acid manufacturer, which should be disclosed on citation.

The connective-tissue literature adds a coherent adjacent case with the clearest dose–response in the review: in eight healthy men, vitamin C-enriched gelatin doubled circulating procollagen type I N-terminal propeptide at 15 g, the 5 g arm raising collagen content only in an *ex vivo* engineered-ligament assay [99]; hydrolysed collagen at 0, 15 and 30 g showed dose-dependent augmentation [100]; and a meta-analysis of four knee osteoarthritis trials (n = 507, all at high risk of bias) reports pain reduction, standardised mean difference −0.58 [101]. Inference: indirect, but the most promising lead in the review. The domain is better framed as connective tissue and musculoskeletal comfort than as pain management, and it is the strongest candidate for formulation-specific research.

**6.9 Antioxidant and cellular protection.** The glutathione precursor set is complete (Section 5.4), but the interventional evidence is combined glycine and N-acetylcysteine (GlyNAC) at glycine 1.33 mmol/kg/day — approximately 0.1 g/kg/day, or 7 g for a 70 kg adult [102] — plus an N-acetylcysteine component not present here, which corrected glutathione deficiency and improved oxidative stress, mitochondrial function, inflammation and physical function in older adults [103]. The randomised trial's full methods are not open-access, so the regimen is taken from the same group's published protocol and should be confirmed before the ratio is relied on. Inference: indirect, at a ~130-fold dose gap and without the N-acetylcysteine component.

## 7. Dose-relevance analysis

### 7.1 The dose gap

**Table 3. Quantity present compared with the lowest dose reported to produce an effect in a controlled human study.** Where more than one positive dose was identified for a constituent–claim pair, the value entered is the lowest dose at which a statistically significant effect was reported in the highest-tier available design. Evidence type: ▣ pooled estimate from a systematic review or meta-analysis; ◧ single trial; △ single-study or subgroup-derived figure requiring verification; unmarked, no dose comparison possible. Fold gaps are exposure comparisons, not effect estimates of comparable certainty.

| Constituent | Reference formulation | Claim domain | Lowest effective dose reported | Fold gap | Ref |
|---|---|---|---|---|---|
| Taurine △ | 86 mg | Muscular fatigue, antioxidants | 0.05 g (50 mg) pre-exercise | Reference quantity exceeds — unverified | [104] |
| Total indispensable AA ◧ | 274 mg | Muscle protein synthesis | 1.5 g leucine-enriched EAA (CEBM 2b) | ~5.5× | [29] |
| L-Serine ◧ | 86 mg | Chronic low-back and knee pain | 594 mg with 149 mg EPA (co-intervention absent here) | ~7× | [98] |
| L-Glutamic acid | 137 mg | Enterocyte fuel, umami-vagal | Not dose-defined; ~95 % first-pass | Mechanism, not dose | [24] |
| L-Arginine ▣ | 105 mg | Aerobic performance (chronic) | 1.5–2 g/day × 4–7 wk | ~14–19× | [77] |
| L-Leucine ◧ | 36 mg | MPS signal | 0.6 g within a leucine-enriched matrix (CEBM 2b) | ~17× | [29] |
| Taurine ◧ | 86 mg | Inflammatory and atherogenic indices | 1.5 g/day | ~17× | [84] |

| | | | | | |
|---|---|---|---|---|---|
| L-Tryptophan ▣ | 44 mg | Wake after sleep onset | 1 g/day | ~23× | [66] |
| L-Arginine ◧ | 105 mg | Anxiety, cortisol (with lysine) | 2.64 g/day | ~25× | [73] |
| L-Arginine ▣ | 105 mg | Blood pressure | 4 g/day | ~38× | [76] |
| Glycine ◧ | 54 mg | Daytime fatigue after sleep restriction | 3 g at bedtime | ~56× | [72] |
| L-Lysine ◧ | 45 mg | Anxiety, cortisol (with arginine) | 2.64 g/day | ~59× | [73] |
| Total BCAA ▣ | 138 mg | Muscle damage, soreness | >200 mg/kg/day (>14 g) × >10 d | ~100× | [32] |
| Gly + Pro + Lys ◧ | 122 mg | Collagen synthesis (PINP) | 15 g vitamin C-enriched gelatin | ~123× | [99] |
| Glycine △ | 54 mg | Glutathione, ageing (GlyNAC) | 1.33 mmol/kg/day (~7 g), with N-acetylcysteine | ~130× | [103,102] |
| L-Tyrosine ◧ | 65 mg | Cognition under acute stress | 150 mg/kg per dose (~10.5 g), given twice daily | ~160× | [69] |
| L-Histidine ◧ | 23 mg | Insulin resistance | 4 g/day | ~174× | [89] |
| L-Methionine | 12 mg | — | Restriction, not repletion, is the direction of evidence | n/a | [49] |

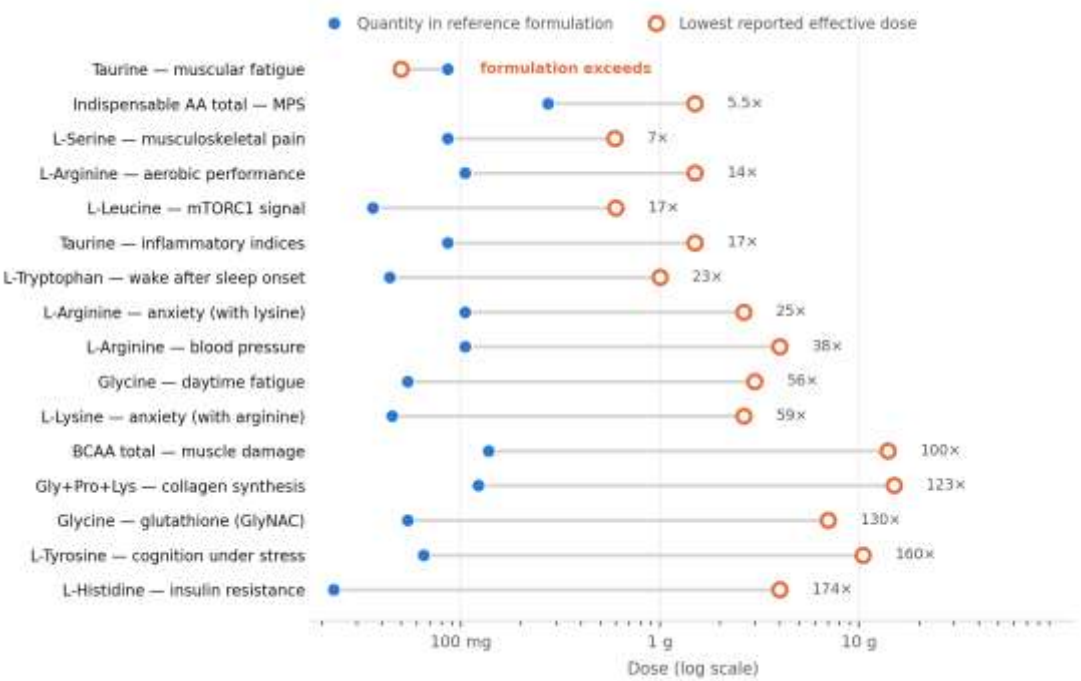


**Figure 3.** Dose gap for each constituent–claim pair in Table 3, on a base-10 logarithmic axis. Filled circle, quantity present in the reference formulation; open circle, lowest reported effective dose; line, the gap. Taurine is the only row in which the filled circle lies to the right of the open circle.

### 7.2 Interpretation, and the taurine exception

The gap is not uniform, and the range matters. It spans from the reference quantity exceeding a reported effective dose, through five- to twenty-fold for total indispensable amino acids, serine, arginine and leucine, to over one-hundred-fold for the branched-chain amino acids, the collagen amino acids, glycine-as-GlyNAC, tyrosine and histidine.

Where the gap is narrowest, composition rather than total quantity is the operative constraint. The 5.5-fold indispensable-amino-acid gap sits alongside an inverted leucine-to-valine ratio and absent threonine [29]; the 7-fold serine gap alongside an absent co-intervention [98]; the 14–19-fold arginine gap alongside absent citrulline [19]. In each of the three domains where dose comes closest to sufficiency, composition does more to determine the outcome than total quantity — the central analytical finding of this review, and one that applies to the class.

Threshold behaviour and non-monotonicity are both documented and pull in opposite directions. Muscle protein synthesis shows threshold behaviour, so a sub-threshold dose produces no effect rather than a proportionally smaller one [31]; branched-chain recovery outcomes show a dose-related gradient at the timepoints where any effect appears, so a milligram dose predicts none [33]; and taurine shows neither, with no dose–response across 1–6 g [64] and one review reporting an effect at 50 mg on strength-exercise fatigue markers while 6 g failed to lower lactate — different outcomes rather than a within-outcome dose comparison [104]. Sub-gram amino acid dosing therefore cannot be dismissed on general grounds; it must be assessed constituent by constituent, and for most constituents it has never been assessed at all.

That 50 mg of taurine before strength exercise reduced muscular fatigue and increased enzymatic antioxidants [104] is the only instance in which a reference quantity exceeds a reported effective dose, and it requires four qualifications: it rests on a single study within a ten-study systematic review, not a pooled estimate; it is biologically surprising, the rest of the literature operating at 1–6 g with no detected dose–response [64]; the outcomes are surrogate rather than clinical; and the pattern is non-monotonic. The primary study should be obtained, and the dose confirmed as 50 mg absolute before any reliance is placed on it. If it survives, it is the most defensible quantitative statement available for this class.

### 7.3 Four levels of inference

Every statement about a formulation of this class falls into one of four categories, and we suggest that label-substantiation documentation for such products adopt the same taxonomy: (1) established biochemical role — supportable for all eighteen constituents; (2) evidence from the individual amino acid — supportable with the dose disclosed; (3) evidence from a comparable mixture — supportable only for indispensable-amino-acid mixtures, with compositional differences stated; (4) evidence directly applicable to the finished product — empty.

## 8. Safety, interactions and quality

### 8.1 Declared serving basis and safety margin

Every margin in this section is computed on the basis of one serving per day. For the reference formulation, that basis is the declared label: one film-coated tablet of 0.9588 g, thirty per pack, recommended at one serving per day after meals. The 897.5 mg of amino acids therefore represent 93.6 % of tablet mass, the balance being the coating and the binding, diluent, disintegrant and antisticking excipients. Because the reference values against which margins are computed are all daily intakes, the margins scale inversely with labelled serving frequency: at two servings daily every margin would halve, taurine falling from 34.9-fold to 17.5-fold and the stated range becoming 17- to 500-fold. At the declared one serving per day, the margins in Table 4 stand as computed, and any future change to labelled serving frequency requires them to be recomputed.

Most amino acids lack a formal tolerable upper intake level — the European Food Safety Authority has set none for any individual amino acid [105] — so safety is governed by the observed safe level and highest observed intake framework [106]. Against the no-observed-adverse-effect levels established by the International Council on Amino Acid Science programme and its successors, which

cover the proteinogenic constituents [107], and the observed safe level for taurine [108], every constituent sits between approximately 35-fold and 1,000-fold below its ceiling on the declared single-serving basis. The narrowest margin is taurine at 34.9-fold, the widest phenylalanine at 1,000-fold.

**Table 4**: Safety margins, contraindications and interactions.

| Constituent | Reference formulation | Published NOAEL / OSL | Margin | Cautions |
|---|---|---|---|---|
| L-Arginine | 105 mg | Single-dose NOAEL ~7.5 g [109]; OSL 20 g/day [108]; 30 g/day × 90 d tolerated [110] | ~71× (against the most conservative value) | Adverse vascular and cognitive effects at gram doses in at-risk older adults [78]; excess mortality at 9 g/day post-infarction [79] |
| Taurine | 86 mg | OSL 3 g/day [108] | ~35× (narrowest margin) | None at nutritional doses; the 2008 OSL is lower than doses used without adverse signal in cardiovascular trials [82], so the margin is conservative |
| L-Serine | 86 mg | 12 g/day [111] | ~140× | — |
| L-Leucine | 36 mg | UL ~30–35 g/day [112] | ~970× | Ammonia rise only above ~550 mg/kg/day |
| L-Lysine | 45 mg | 6.0 g/day [113] | ~133× | Gastrointestinal symptoms are dose-limiting |
| L-Tryptophan | 44 mg | UL 4.5 g/day [107] | ~102× | Caution with serotonergic medication (8.2) |
| L-Histidine | 23 mg | 8 g/day [114]; >24 g/day adverse [115] | ~350× | Inadvisable in liver disease [116] |
| L-Phenylalanine | 12 mg | 12 g/day [111] | ~1,000× | Absolute contraindication in phenylketonuria [44] |
| L-Methionine | 12 mg | 3.2 g/day [107] | ~267× | Plausible toxicity if cysteine synthesis lags [51]; most adverse constituent in cardiorenal disease [50] |
| L-Glutamic acid | 137 mg | ADI "not specified" per an industry-sponsored workshop [117]; EFSA has proposed a group ADI of 30 mg/kg/day (~2.1 g at 70 kg); habitual intake 10–20 g/day [25] | ~1 % of habitual intake; ~6.5 % of the proposed EFSA ADI | Attributed symptoms rarely reproduced under blinded challenge at dietary doses [118] |
| Glycine | 54 mg | Concerns only above ~500 mg/kg [119] | >600× | Extrapolated from *in vitro* and animal cytotoxicity data in a narrative review; no human NOAEL established |

Margins are computed on the declared basis of one tablet daily (Section 8.1) and scale inversely with any increase in labelled serving frequency. Reference values are not commensurate: upper intake levels are derived for chronic intake, no-observed-adverse-effect levels come from defined-duration dosing trials,

and observed safe levels are the highest intakes for which no adverse effect has been reported rather than thresholds. The column is a margin-of-exposure indication, not a single graded scale.

### 8.2 Interactions and special populations

Gastrointestinal symptoms are dose-limiting for most amino acids, though a systematic review of oral arginine found no significant increase in gastrointestinal symptom risk at 2–30 g, pooling 23 of 34 included trials in 647 healthy participants [109]. None of the interactions below is expected to be clinically meaningful at milligram quantities; each is listed because precautionary labelling is inexpensive relative to the consequence of omitting it.

Serotonergic medication. A fatal case of serotonin syndrome with concurrent syndrome of inappropriate antidiuresis and profound hyponatraemia occurred in a patient on amitriptyline and chlorphenamine who self-administered duloxetine, with possible L-tryptophan supplementation [120]; the dose was not quantified and causal attribution is weak, so the case supports precautionary labelling rather than a dose-specific risk statement at 44 mg. A label advisory for users on selective serotonin or serotonin–noradrenaline reuptake inhibitors, monoamine oxidase inhibitors, triptans or tramadol is prudent.

Cardiovascular, glycaemic and neurological co-medication. Arginine and taurine lower blood pressure at gram doses [76,82] and amino acids are both insulinotropic and glucagonotropic, but no additive effect is expected at these quantities and 15 g/day of indispensable amino acids for twenty-two weeks did not alter insulin sensitivity [88]; the label should nonetheless imply neither a blood-pressure nor a blood-glucose action, and users on antihypertensive or glucose-lowering therapy should be advised to consult a clinician. Arginine is a nitric oxide precursor and nitric oxide donors are established headache triggers [97], though at 105 mg with under 0.1 % reaching nitric oxide [26] a trigger effect is implausible; and large neutral amino acids compete with levodopa for transport, making a two-hour spacing advisory a low-cost precaution.

Impairment, inherited disorders, paediatric use and pregnancy. Amino acid loads increase nitrogenous waste and warrant caution in advanced chronic kidney disease, where methionine is the constituent of most concern [50]; histidine supplementation is described as inadvisable in liver disease [116]; phenylketonuria is an absolute contraindication irrespective of the 12 mg quantity; and homocystinuria, maple syrup urine disease and urea cycle disorders warrant supervision. Requirement data are themselves sparse in children, pregnancy and lactation [57], and no data exist for this formulation in those groups. Long-duration single-ingredient data are reassuring: 30 g/day of arginine for ninety days was safe [110].

### 8.3 Regulatory and quality considerations

All eighteen constituents appear in the Schedules to the Indian Food Safety and Standards (Health Supplements, Nutraceuticals, Food for Special Dietary Use, Food for Special Medical Purpose, Functional Food and Novel Food) Regulations, 2016, as amended, which set no maximum daily quantity for individual amino acids but require that the rationale for ingredient combinations be supported by scientific and technical evidence [13]. The schedule entry for each constituent should be cited from the current consolidated text at the point of label filing, since the schedules are amended periodically; taurine warrants separate treatment, being non-proteinogenic and subject to category-specific conditions in India. High-dose amino acid safety must be established in human studies because animal toxicity testing is frequently unsuitable for this class [121]. The US structure–function framework is the relevant international comparator [122] but is not directly transferable: the Indian framework operates through positive schedules with a combination-rationale requirement, whereas the US framework permits any lawfully marketed dietary ingredient subject to notification and a disclaimer.

Excipient selection is a jurisdictional rather than an active-ingredient question, and is worth separating from the amino acid schedules. Film-coated tablets in this category commonly use titanium dioxide as an opacifier and colour. It remains permitted with restrictions in India, but the European Food Safety Authority concluded in May 2021 that it could no longer be considered safe as a food additive, and Commission Regulation (EU) 2022/63 removed it from the Union list with effect from 7 August 2022. A coating system that is compliant in the domestic market may therefore be non-compliant in an export

market, and the coating specification should be reviewed against the destination jurisdiction rather than assumed to travel with the formulation.

The most important quality reference in this field concerns an event rather than a specification. The 1989 epidemic of eosinophilia–myalgia syndrome associated with L-tryptophan was attributed to manufacturing impurities rather than to tryptophan itself — the canonical illustration that identity and purity specifications, rather than intrinsic toxicology, determine the safety of an amino acid supplement [123]. The minimum specification is therefore: quantitative amino acid assay by ion-exchange chromatography or UPLC-MS/MS against the certificate of analysis; chiral chromatography confirming L-isomer content, not optional given that this review excluded D-aspartic acid, D-serine and D-phenylalanine evidence precisely because the isomers differ pharmacologically; related-substance and process-impurity limits, with particular attention to tryptophan; content uniformity and a stability programme accounting for the differing hygroscopicity and oxidative susceptibility of free amino acids; heavy metal, microbiological and residual-solvent limits; and declaration of raw-material provenance and production route for each constituent, since fermentation-derived, synthetic and hydrolysate-derived amino acids carry different allergen profiles.

## 9. Claim support, research gaps and priorities

**Table 5**: Evidence strength and defensible wording by proposed benefit.

| Proposed benefit | Best ingredient-level evidence | CEBM | Directness | Defensible wording | Avoid |
|---|---|---|---|---|---|
| General wellness/nutrition | Requirement patterns; IAAO studies; DIAAS | 1a/5 | Partially direct | "Provides a spectrum of dietary amino acids contributing to daily intake" | "Complete amino acid formula" |
| Sports, muscle, recovery | Leucine-threshold and EAA dose–response | 1a/5 | Indirect | "Supplies substrates involved in muscle protein turnover" | "Promotes muscle growth" |
| Fatigue, energy | Central fatigue hypothesis; taurine; one histidine RCT | 1a/1b | Indirect | "Amino acids participate in energy metabolism" | "Reduces fatigue" |
| Cognition, mood, sleep, CNS | Glycine 3 g; tryptophan 1 g; tyrosine 21 g; Lys+Arg 2.64 g | 1a/1b | Indirect | "Amino acids are precursors of neurotransmitters" | "Improves sleep/mood/memory" |
| Cardiometabolic, endothelial | Arginine and taurine meta-analyses | 1a | Indirect | "Arginine is the physiological substrate for nitric oxide synthesis. No blood-pressure or cardiovascular effect is claimed or implied." | "Lowers blood pressure"; "improves circulation" |
| Blood glucose | Insulinotropic physiology; contradicted by cohorts | 1a/2/3 | Indirect, partly contradicted | Nothing beyond participation in glucose metabolism | "Regulates blood sugar" |

| | | | | | |
|---|---|---|---|---|---|
| Digestive health | Glutamate as enterocyte fuel; glutamine trials do not transfer | 1b/5 | Mechanism partly direct | "Supplies substrates used by intestinal mucosal cells" | "Protects against digestive disorders" |
| Pain, connective tissue | L-serine 594 mg + EPA; collagen meta-analyses | 1b/1a | Indirect | "L-serine has been investigated for musculoskeletal comfort in combination with eicosapentaenoic acid, which such formulations do not typically contain; the effect of L-serine alone is not established." | "Relieves pain"; "repairs joints"; citing the L-serine trial without the co-intervention disclosure |
| Antioxidant protection | Complete glutathione precursor set; GlyNAC at ~7 g glycine | 1b | Indirect | "Supplies the three amino acid precursors of glutathione" | "Antioxidant protection". The GlyNAC trial must not be cited in consumer-facing material: the glycine dose is ~130-fold the quantity present, and N-acetylcysteine is absent. |
| Finished product | None identified | — | — | Nothing | Any efficacy statement |

All wordings assume the declared one-tablet-daily basis of Section 8.1 and must be re-checked against any change to labelled serving frequency before filing.


**Figure 4.** Evidence pyramid for the class. The apex — formulation-specific trials — is empty. Below it: trials of comparable formulated mixtures, sparse and limited by compositional differences; randomised

trials of individual constituents, almost all at gram scale; observational evidence, which for nine constituents carries associations with elevated rather than reduced risk; and a mechanistic base tier, the largest by count.

**Gaps.** No trial of the finished formulation. No pharmacokinetic data for this or any comparable sub-gram broad-spectrum blend. No evidence of synergy, which therefore remains a hypothesis rather than a rationale. Threonine absent. Leucine and total indispensable-amino-acid exposure low and compositionally unbalanced. Dose–response data below 1 g essentially absent for every constituent except taurine, where the single low-dose data point requires verification. No interventional oral L-serine dose–response study addressing the sphingolipid pathway [38].

**Priority 1 — analytical confirmation per batch**, as specified in Section 8.3, with stereochemical purity non-negotiable.

**Priority 2 — single-dose pharmacokinetics: the highest-value missing study, and inexpensive.** Randomised placebo-controlled crossover in twenty to twenty-four healthy adults, plasma amino acid profiles at 0–240 minutes after one serving; primary endpoints maximum concentration and area under the curve for the eight large neutral amino acids plus arginine, taurine and serine, secondary the plasma tryptophan-to-large-neutral-amino-acid ratio [27]. This answers the question the review turns on — whether 897.5 mg across eighteen constituents measurably alters plasma amino acid concentrations at all — and a null result is as useful as a positive one, because it would redirect development toward reformulation rather than an efficacy trial that could not succeed.

**Priority 3 — steady-state study.** Four weeks of daily dosing with fasting amino acid profile, tryptophan ratio, safety chemistry and tolerability.

**Priority 4 — one efficacy trial in the indication with the most plausible low-dose mechanism.** On this evidence that is gastrointestinal comfort and function, the enterocyte-glutamate and umami-vagal mechanisms being the only ones here that do not require systemic absorption [24,36]: randomised, double-blind, placebo-controlled, twelve weeks, in adults with functional gastrointestinal symptoms, with a validated symptom score and a permeability marker. The second choice is musculoskeletal comfort, following the L-serine precedent [98].

**Priority 5** — verification of the taurine low-dose finding (Section 7.2) before any reliance on it.

Design variables identified by the dose comparison. Four compositional variables most affect what a formulation of this class could support, and are stated as testable hypotheses rather than validated improvements: add L-threonine, completing the indispensable set and addressing the mucin argument [16]; raise leucine relative to valine, since composition matters more than amount [29]; add or substitute L-citrulline, which escapes presystemic arginase [19] and shows the largest blood-pressure effect in combination with arginine [81]; and raise L-serine toward 500–600 mg, optionally with eicosapentaenoic acid, which would place the formulation at rather than below a published effective dose [98].

## 10. Conclusion

The reference formulation supplies eighteen free amino acids totalling 897.5 mg per serving — 274 mg indispensable, 138 mg branched-chain, 122 mg collagen-relevant, 24 mg sulfur amino acids, 86 mg taurine, threonine absent — a composition typical of the class. Such formulations are physiologically coherent: they supply the three precursors of glutathione in stoichiometrically complete form, the substrates collagen synthesis consumes in greatest disproportion, the precursors of the serotonergic, catecholaminergic, histaminergic and inhibitory transmitter systems, the substrate for nitric oxide synthesis, the principal oxidative fuel of the enterocyte, and two amino acids sensed directly by named regulators upstream of mTORC1 with a third entering the same network indirectly.

No study of any finished formulation of this class exists, so every conclusion is an ingredient-level inference, and three factors limit how far such inferences carry: first-pass mucosal catabolism, removing 30–50 % of arginine and the branched-chain amino acids and almost all dietary glutamate and aspartate, together 403 mg; competitive rather than additive transport, first at the shared apical carriers and again at

the blood–brain barrier, where a third of the formulation's mass shares one transporter; and dose. The gap spans roughly five-fold to two-hundred-fold, narrowest for total indispensable amino acids, L-serine, arginine and leucine, widest for histidine, tyrosine, glycine and the collagen amino acids. In the three domains where it is narrowest, compositional variables — branched-chain ratio, completeness of the indispensable set, choice of nitric oxide precursor, and the presence or absence of a co-administered agent — do more to determine the outcome than total quantity does. That is a more useful finding than a flat statement of underdosing, because composition is a design choice rather than a fixed constraint.

On the declared single-serving basis every constituent for which a published reference value exists sits 35- to 1,000-fold below that ceiling, the methionine content is conservatively low in a direction both the geroscience and cardiorenal literatures favour, and the glutamate content is about 1 % of habitual dietary exposure; those margins are computed on the declared basis of one tablet daily and scale inversely with any increase in labelled serving frequency. The quality risk in this product class lies not in intrinsic toxicology but in raw-material identity, stereochemical purity and impurity control.

Within the FSSAI framework, the defensible position for a formulation of this class is that it is a broad-spectrum dietary source of amino acid substrates relevant to muscle, metabolic, neurological, vascular, digestive and connective-tissue physiology; claims of clinical efficacy are not supportable at these quantities and should be presented as proposed benefits pending formulation-specific study. Two leads justify that study rather than merely deferring it, both with explicit caveats: reduced muscular fatigue reported at a taurine dose below the reference quantity, requiring verification against its primary source; and relief of chronic low-back and knee pain at a sub-gram L-serine dose in combination with a fatty acid such formulations do not typically contain.

The broader observation is that the low-dose amino acid literature is not so much negative as absent. Dose–response data below one gram are almost entirely lacking, threshold and non-monotonic behaviour are both documented, and no systematic appraisal of sub-gram broad-spectrum blends has previously been published. Whether such formulations can be justified is an open empirical question, and answering it requires the formulation-specific work this review has been obliged throughout to identify as missing.

## Declarations

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**Competing interests.** All authors are employees of Biotex Life Solutions Pvt. Ltd., which manufactures Amino Complex, the 18-component formulation used as the reference example throughout. This is disclosed in full as a potential conflict of interest. The review states explicitly where evidence is weak, mixed, contradictory or absent, and where its own numbers rest on unverified single studies; it includes the null, negative and adverse findings identified; it identifies and excludes a retracted publication; and it makes no claim of clinical efficacy for the finished product. No commercial function of the manufacturer influenced study selection, appraisal or interpretation.

**Author contributions.** All authors jointly conceived the scope, designed the search strategy and two-layer evidence model, contributed to the ingredient-physiology, mechanism and safety sections, participated in the evidence appraisal and dose relevance analyses, and coordinated and drafted the manuscript. All authors critically revised and approved the final version, met all four ICMJE criteria for authorship, and take responsibility for the work.

**Data availability.** No new datasets were generated. Full search strategies (S1), the evidence-extraction matrix (S2), the list of records excluded on verification (S3), and the protocol (S4) are provided as supplementary material. Composition, serving size and excipient declarations for the reference formulation are those of its published label and are reproduced in Table 1 and Section 8.1.

**Ethics and consent.** Not applicable; a review of published literature involving no human participants, identifiable data or animals.

**Disclaimer.** This review appraises broad-spectrum sub-gram free-amino-acid supplements as a class, using Amino Complex, an 18-component composition manufactured by Biotex Life Solutions Pvt. Ltd., as the reference example. Its findings apply to the constituent amino acids as classes of nutraceutical ingredients and to the design of such formulations generally, not to any finished product, none of which has been evaluated in a clinical trial. No claim is made that any product diagnoses, treats, cures or prevents any disease. Structure–function statements must remain within applicable FSSAI regulations and be supported by the certificate of analysis for each production batch.

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