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ORIGINAL ARTICLE

Modelling of protein turnover provides insight for metabolic demands on those specific amino acids utilised at disproportionately faster rates than other amino acids

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Abstract

The nitrogen balance is regulated by factors such as diet, physical activity, age, pathogenic challenges, and climatic conditions. A paradigm was developed from published recommended rates of protein intake (g/kg/day) with corresponding rates of endogenous protein turnover and excretion, to extrapolate amino acid balances under various conditions. The average proportions of amino acids in the ingested proteins representing a well-balanced diet were used to assess intake and an average human composition profile from five major high-turnover proteins in the body to assess endogenous protein turnover. The amino acid excretion profiles for urine and sweat were constructed for males and females from published data. The model calculated the nitrogen balances for a range of amino acids to determine the amino acid requirements to support daily exertion. Histidine, serine, glycine, and ornithine were in negative balances in males and females and this potential deficit was greater in the higher body-mass ranges. Conversely, leucine, isoleucine, and valine were conserved during nitrogen flux and resulted in positive balances. The model was run under a scenario of high demand for the synthesis of IgG during a response to an infectious challenge which indicated that these were increased requirements for tyrosine, threonine, and valine. It was concluded that these amino acids represent points of limitation to anabolic metabolism by restriction of their supply at critical times of demand. This would especially occur under conditions of fitness training, maintaining intensive exercise regimes, facilitating responses to pathogenic challenge, or recovery from injury.

Keywords Amino acids · Protein turnover · Nitrogen balance · Metabolic homeostasis · Metabolic modelling

Introduction

The study of nitrogen balance has been extensively researched, revealing complex relationships between various pools of metabolites that act to maintain metabolic support for homeostasis and exercise activities (el-Khoury et al. 1994; Fielding and Parkington 2002; Millward 2004; Millward et al. 1996; Poortmans et al. 2012). Assessment of the nitrogen balance is difficult, as it needs to encompass protein turnover in tissues, organs and muscles as well as requirements for energy metabolism and the utilisation of amino acids in numerous biochemical pathways and regulatory systems. The rates of utilisation depend on factors such as genetics as well as diet, physical activity, pathogenic challenges, and climate. Notwithstanding these variations, consensus evaluations of nitrogen turnover have been determined, whereby 0.6 g/kg/day of protein would be sufficient to achieve a zero nitrogen balance. It has thus been recommended that 0.75–0.8 g/kg/day would be a minimum recommended daily intake for adults, but would be higher in babies and children undergoing critical growth phases (Tessari 2006). These values increase on the basis of rates of physical exercise activity, whereby adults undertaking muscle strength training would require 1.6–1.7 g/kg/day and those undertaking endurance training would require 1.2–1.4 g/kg/day (Dunstan et al. 2016; Fielding and Parkington 2002; Poortmans et al. 2012). The assessment of protein

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loss is more difficult to assess, because the waste nitrogen from energy metabolism is excreted as urea, ammonia, and creatinine. Amino acids and these waste products can also be lost directly via urine, faeces, and sweat, with the latter including contributions from natural moisturising factors leached from the skin via sweating, sebum, and desquamation (Dunstan et al. 2016).

Management of nitrogen balance could have a direct influence on fatigue, both in the healthy and unwell populations. Fatigue is experienced frequently in the healthy public from daily exertion which can vary greatly throughout the week for individuals with demanding work profiles, care commitments, fitness training, sports events and excursions. This type of fatigue is referred to as "peripheral" fatigue and results from impairment and micro-damage of muscle tissue, but can also occur as a result of low-level immune responses to acute infections (Prinsen et al. 2015). Simplistically, fatigue can be easily defined as "difficulty in initiating or sustaining voluntary activities" (Chaudhuri and Behan 2004). The logical approach to a scenario of increased exercise loading has been to increase protein intake to counter the higher rates of protein turnover. This generally has efficacy, but raises some interesting research questions when one considers that certain amino acids are utilised at disproportionally faster rates than others. The composition of dietary protein does not necessarily reflect the differential usage rates and thus taking in sufficient protein to meet the demands of a few, highly utilised amino acids, may leave others in surplus. The body does not effectively store amino acids, and therefore, there is potential for them to be used in oxidation for energy metabolism or converted to fatty acids.

Six key amino acids have been shown to be lost in sweat at concentrations much higher (4–20 fold) than those found in the plasma (Dunstan et al. 2016). These amino acids were identified as histidine, serine, glycine, ornithine, lysine, and aspartic acid. The concentrations of these amino acids were higher in sweat from females compared with males, suggesting that females may be more susceptible to fatigue from altered nitrogen homeostasis (Dunstan et al. 2017). It was also shown that the average losses of histidine, glycine, serine, and lysine comprised nearly 60% of amino acids excreted through urine (Dunstan et al. 2017). The combined losses of these key amino acids from urination and sweating were modelled under various scenarios ranging from sedentary- to medium-level exertion. It was demonstrated that the losses of these amino acids become very extensive, as exertion levels rise (Dunstan et al. 2017).

Serine, glycine, and aspartic acid can all be produced in the body and are thus deemed non-essential. However, under periods of elevated exertion and activity or during periods of pathogenic challenge, the body may not be able to meet the demand for these amino acids by the way of endogenous supply and ingested proteins (de Koning et al. 2003; Jackson 1991; Meléndez-Hevia et al. 2009; Rezaei et al. 2013; Wang et al. 2013). These amino acids are required in non-nutritive metabolic pathways which make them very difficult to quantify despite their considerable avenues of utilisation. Examples of the metabolic functions of amino acids have been summarised for glycine, serine, histidine, lysine, ornithine, and aspartic acid in Table 1. The plasma maintains a reservoir of amino acids which services the supply of these nutrients to muscles, tissues, and organs in the body. The composition of amino acids is maintained under homeostasis via hormonal regulation of anabolic (human growth hormone and insulin-like growth factor) and catabolic (cortisol and cytokines) processes, all of which are influenced by protein ingestion and bodily activity.

The goal of this investigation was to model the dietary intake of amino acids via a range of protein sources in regard to published rates of protein turnover and excretion. On the premise that certain key amino acids are utilised and excreted at higher rates than others, the data were then adjusted to include average rates of excretion of selected amino acids which have been reported in the literature. This simple approach can provide insight as to how deficits in certain amino acids can build over time with constant daily exertion. The model was then used to demonstrate potential localised demands (a) in muscles during a 60 min exercise session and (2) in bone marrow for IgG production during an infectious challenge.

Methods

The general interactions and flow of protein nitrogen have been well summarised by Tessari (2006). To assess the total daily nitrogen losses, it was proposed that this will be 56 mg/kg/day based on general average excretion rates of 36 mg/kg/day from urine, 12 mg/kg/day from faeces, and 8 mg/kg/day from a combination of saliva, desquamations, sweat, sebum, hair, and nails (Tessari 2006). This represents the obligatory nitrogen loss (ONL) which would represent 3.92 g N for a 70 kg person, and if one assumed an equivalence factor of 6.25 g protein per g of nitrogen, then this would represent 24.5 g protein per day.

It was determined that 0.6 g protein/kg/day is the level required to deliver a "0" nitrogen balance without growth and increased muscle mass (FAO 1985; Poortmans et al. 2012; Waterlow 1984; Waterlow and Jackson 1981). It was thus proposed that 0.75–0.8 g protein/kg/day would be a minimum recommended daily intake for adults (Tessari 2006) and these values would increase to 1.2–1.4 g protein/kg/day for endurance athletes and 1.6–1.7 g protein/kg/day for those undertaking muscle strength training (Fielding and Parkington 2002; Poortmans et al. 2012). The standard rate used for this modelling procedure was set at 1.2 g protein/kg/

Table 1 Summary of the proteinogenic and metabolic utilisation profiles for those amino acids identified as being utilised at disproportionately faster rates than other amino acids in humans

Glycine	
Represents 11.5% of all amino acids	Wu (2010a, b)
Represents 20% of the amino acids in proteins	
Represents 33% of amino acids in collagen which is the most abundant family of proteins in the body	Wu (2009)
Used as a humectant in the natural moisturising factor in the skin	Scott et al. (1982)
Inhibitory nerve transmitter	Rajendra et al. (1997)
Glycine modulates Ca^{2+} levels in leukocytes and macrophages regulating the production of cytokines, and the generation of superoxide for immune function	Zhong et al. (2003)
Conjugation with bile acids with a key role in digestion and absorption of fatty acids, lipophilic nutrients and lipid-soluble vitamins	Hafkenscheid and Hectors (1975)
Found in major pathways of metabolism	Hall (1998)
Purines for generating nucleosides, nucleotides, RNA and DNA	
Haem for haemoglobin and cytochromes critical for oxygen transport/CO_2 removal and mitochondrial electron transport	Dai et al. (2013)
Creatine which participates in energy metabolism within muscles and nerves	
Glutathione which is the most abundant low molecular weight thiol and the major anti-oxidant in cells	Wu et al. (2004)
Present in sweat and urine at significantly higher concentrations than found in plasma	Dunstan et al. (2016, 2017)
Source	
It is technically classified as non-essential as it can be synthesised by the body	Darling et al. (1999), Wu (2010a)
Glycine synthesis can be insufficient to meet body demands leading to sub-optimal growth, impaired immune responses	Jackson (1991), Meléndez-Hevia et al. (2009), Rezaei et al. (2013)
It has been thus deemed "conditionally essential"	Wang et al. (2013), Wu (2010a, b)
Formation of glycine from serine is important for generating N^5–N^{10}-methylene tetrahydrofolate	Stover et al. (1997)
Serine	
Used as a humectant in the natural moisturising factor in the skin	Scott et al. (1982)
Found in numerous major pathways of metabolism	
Predominant source of one carbon (methyl) groups for de novo synthesis of purine nucleotides and certain pyrimidines (thymidine)	Snell et al. (1987)
Folate metabolism (5,10-methylenetetrahydrofolate)	
Has prominent role in cell proliferation (growth and repair)	Eagle (1959)
Precursor for synthesis of, cysteine, taurine, phosphatidylserine, ceramide	de Koning et al. (2003)
Also produces glycine (see above—also an inhibitory nerve transmitter) and D-serine (neuromodulator) and contributes greatly to cerebral function	
Contributor to liver gluconeogenesis	
Sulphur metabolism for the synthesis of glycosphingolipids and steroid hormones	Felig (1975), Felig et al. (1969)
Glutathione (via cysteine and glycine)	Johnson and Duran (2001)
Phosphoglycerols, sphingolipidis and glycolipids (phosphatidylserine)	de Koning et al. (2003)
Present in sweat and urine at significantly higher concentrations than found in plasma	Dunstan et al. (2016, 2017)
Source	
Non-essential as it can be synthesised by the body	
Serine synthesis can be insufficient to meet body demands leading to sub-optimal growth	Maxwell et al. (1956)
Addition of non-essential serine can instigate improved growth in human cells	Eagle (1959)
Classified as conditionally essential	
Histidine	
Used as the precursor for urocanic acid which is a humectant working as part of the natural moisturising factor on the skin	Scott et al. (1982), Rawlings and Harding (2004)
Is an essential amino acids in humans	
Required for synthesis of haemoglobin	Kopple and Swendseid (1975)

Table 1 (continued)

Deficit in diet can lead to anaemia	Clemens et al. (1984), Cooperman and Lopez (2002)
Histidine can stabilise oxyhaemoglobin and C)-bound haemoglobin	
Required for the formation of carnosine	Bauchart et al. (2007), Tamaki et al. (1984)
Carnosine is highly concentrated in the muscles and brain	
Acts as an anti-oxidant, chelating agent,	Reddy et al. (2005)
Acts as an anti-glycating agent to reduce the rate of progression of certain degenerative diseases	Hobart et al. (2004)
Required for the formation of histamine	
Facilitates inflammatory immune response	
Stimulates gastric secretion	
Neurotransmission roles	Nieto-Alamilla et al. (2016)
Histidine is a major component in actin and myosin families of muscle proteins	
Methylated to form 3-methyl-histidine	
Anti-inflammatory and anti-secretory properties	
Histidine acts as an anti-oxidant by scavenging the hydroxyl radical and singlet oxygen	Raghavan et al. (1989), Wade and Tucker (1998)
Present in sweat and urine at significantly higher concentrations than found in plasma	Dunstan et al. (2016, 2017)
Source	
An essential amino acid not made by humans	
Dietary proteins (meat and vegetable)	
Via ingested carnosine from meat	
Ornithine	
Not proteinogenic	
Centrally involved in the urea cycle	Sugino et al. (2008)
It is recycled and effectively acts as a catalyst for the removal of excess N as urea	Rodwell (2000)
Increased ornithine levels improves urea cycle function in liver	Briggs and Freedland (1976)
Acts as precursor for the generation of arginine for subsequent use in metabolism	
Arginine is the source for the production of nitric oxide (NO)	Pokrovskiy et al. (2011)
The production of NO is limited by the availability of arginine	
NO is the signal molecule responsible for eliciting vasodilation	
Promotes growth hormone release by stimulating the pituitary gland	Bucci et al. (1990), Evain-Brion et al. (1982)
Indirect promotion of the metabolism of proteins, lipids and carbohydrates	Davidson (1987)
Attenuates physical fatigue by	
Increasing efficiency of energy consumption	Sugino et al. (2008)
Promoting excretion of urea	
Ornithine stimulates afferent vagal nerves and activates the central nervous system	
Activated sympathetic neurotransmission to adipose tissues and accelerated energy expenditure	Konishi et al. (2015)
Decreased food intake (appetite suppression)	
Increased fat utilisation in brown adipose tissue	
Present in sweat at significantly higher concentrations than found in plasma	Dunstan et al. (2016)
Source	
Non-essential as it can be synthesised by the body	
Ornithine synthesis can be insufficient to meet body demands leading to	
Sub-optimal nitrogen excretion	
Inefficient energy metabolism	
Could be classified as conditionally essential	
Lysine	
Required for synthesis of proteins	Harmeyer (2002)
Lysine can be readily modified in proteins to give key structural properties	
Collagen—is the largest family of proteins in the body	Yamauchi and Sricholpech (2012)
Lysine provides cross-linking capacity	

Table 1 (continued)

Essential for formation and repair of soft tissues in the body	
Recovery from exercise and injury	
Precursor for the synthesis of Carnitine	
High concentrations in muscle tissues and other tissues	
Facilitates mitochondrial oxidation of long chain fatty acids for energy production	
Branch chain amino acid metabolism	Hoppel (2003)
Enhances calcium uptake and retention	
Lysine enhances the intestinal uptake and renal conservation of Ca^{2+}	Civitelli et al. (1992)
Present in sweat and urine at significantly higher concentrations than found in plasma	Dunstan et al. (2016, 2017)
Source	
An essential amino acid not made by humans	
Dietary proteins (plants, eggs and meat)	
Aspartic acid	
Required for synthesis of proteins	
Participates in	
The urea cycle for the removal of nitrogen as urea	
Gluconeogenesis	
Energy metabolism via the TCA cycle	
Synthesis of purine bases (nucleosides, nucleotides, RNA and DNA)	
Can act as a neurotransmitter (NMDA receptors)	Chen et al. (2005)
Forms D-aspartic acid principally in the pituitary gland and testes, which regulates	Topo et al. (2009)
Release and synthesis of luteinizing hormone and testosterone	
Used as a humectant in the natural moisturising factor in the skin	Rawlings et al. (1994), Scott et al. (1982)
Present in sweat at significantly higher concentrations than found in plasma	Dunstan et al. (2016)
Source	
Non-essential as it can be synthesised by the body	
Aspartic acid synthesis can be insufficient to meet body demands leading to sub-optimal growth	
Could be classified as conditionally essential	

day (Tessari 2006). To develop the protein utilisation aspects of the model, a number of parameters were fixed based on literature reference values: protein turnover rate was 5.7 g/kg/day (Poortmans et al. 2012; Waterlow 1984; Waterlow and Jackson 1981); 15% protein intake oxidised for metabolism; 75% of protein intake used for protein synthesis; 27% of protein turnover oxidised for metabolism; 67% of protein turnover used for protein synthesis; 6–10% of amino acids from the proteins would be utilised in various metabolic pathways; obligatory N excretion losses as protein equivalents 350 mg/kg/day (i.e., 56 × 6.25) (Tessari 2006).

The next stage of development for the model was to extrapolate from the assessment of protein flux to amino acid transitions which is difficult when considering the highly variable amino acid compositions of different proteins. A simplistic approach for modelling was developed to estimate the nitrogen balances of the group of 6 amino acids shown to be lost at disproportionately faster rates: serine, glycine, histidine, lysine and aspartic acid as well as the non-proteinogenic ornithine, for comparison with the non-essential glutamine/glutamic acid (Glx), alanine and proline, as well as the essential leucine, isoleucine, valine, threonine, methionine, tyrosine, and phenylalanine. A representative human protein composition of these amino acids was estimated by averaging the percentage abundances of amino acids from five highly abundant human proteins: collagen (Chung and Miller 1974), albumin (Spahr and Edsall 1964), haemoglobin (Stein 1958; Stein et al. 1957), actin (Carsten 1963; Raszkowski et al. 1977), and myosin (Raszkowski et al. 1977), as shown in Table 2. A representative ingested protein composition of these amino acids was similarly estimated by averaging the percentage abundances of amino acids from meat and plant sources, assuming a well-balanced diet (Table 2). The meat source itself was averaged from beef (Samicho et al. 2013), pork (Okrouhlá et al. 2006), chicken (Rossi et al. 2009), and fish (Diniz et al. 2013), whereas the plant composition was averaged from peas (Pownall et al. 2010), rice (Carvalho et al. 2013), beans (Carvalho et al. 2013), and wheat (Chen and Bushuk 1970) (Supplementary data, Table A1). This provided the means to assign quantitative values for amino acid utilisation using the average meat/plant percentages to extrapolate the intake of amino acids via

Table 2 Average percentage relative abundance compositions of selected amino acids in human protein composition (Table A1) and dietary sources (Table A1)

Average % contents of key amino acids in human and dietary proteins														
Av comparisons	His (%)	Ser (%)	Gly (%)	Lys (%)	Asp (%)	Glx (%)	Leu (%)	Ile (%)	Val (%)	Thr (%)	Met (%)	Tyr (%)	Phe (%)	Pro (%)	Ala (%)
Meat	2.7	4.1	5.1	8.2	8.7	12.8	7.0	4.3	4.6	4.1	2.3	3.1	4.3	3.7	5.2
Plant	2.3	5.6	4.8	5.1	9.4	21.6	8.8	4.4	5.4	3.5	1.6	3.5	5.4	7.4	4.9
Av meat + plant	2.5	4.9	5.0	6.6	9.0	17.2	7.9	4.4	5.0	3.8	1.9	3.3	4.9	5.6	5.0
Human	**3.2**	**4.5**	**10.6**	**8.0**	**8.6**	**11.7**	**8.7**	**3.6**	**5.3**	**4.8**	**1.9**	**2.9**	**4.0**	**8.1**	**8.2**
Adjustment for an IgG response	3.1	5.3	9.4	8.0	8.6	11.8	8.7	3.4	5.9	5.3	1.8	3.5	4.1	7.9	7.4

The values in bold were the final values used for the human profile of amino acids

ingested proteins and the human average percentages to estimate the contributions from endogenous protein turnover.

The development of the excretion component of the model was the most difficult to conceptualise. Evaluations of nitrogen balance by measures of total nitrogen flux have established generally accepted partitions for nitrogen flow (Poortmans et al. 2012; Tessari 2006; Waterlow 1984). Extrapolating them to protein quantities has its uses for evaluating protein requirements, but drilling down to amino acid fluxes is problematic. The first option is to assume that amino acids would be lost at the same proportions, as they are ingested in proteins. This is extremely unlikely for several reasons, since: certain amino acids are used in multiple biochemical pathways in addition to protein synthesis; endogenous production of non-essential amino acids will skew the intake proportions; and certain amino acids are lost at disproportionately higher rates in urine and sweat than other amino acids (Dunstan et al. 2016, 2017). To counter this dilemma, the model was designed to generate two sets of estimates regarding input and output balances for each of the amino acids. The first estimate assumed that the excretion profile of amino acids mirrored the average protein composition of ingested proteins and the second estimate utilised excretion profiles that were adjusted to reflect concentrations measured in urine and sweat (Dunstan et al. 2016, 2017). Separate models were established for males and females, because significantly, different excretion characteristics were noted between the sexes (Dunstan et al. 2017). The design of the model allowed comparisons to be made between the sexes while demonstrating relationships between increasing body mass and the rates of protein intake and utilisation. The parameters used and their corresponding rationale are summarised in Table 3. The urine profiles previously reported in a healthy population group of males and females: $n = 30$ were also used to test the model (Jones et al. 2005).

The model was then used to estimate protein utilisation rates for an 80 kg male with a protein intake rate at 0.6 g/kg/day to generate a theoretical 0 nitrogen balance. It was argued that 90% of this intake would be utilised for oxidation and synthesis of new proteins [15% and 75%, respectively, (Tessari 2006)] and that this would constitute the rate of "minimal protein utilisation" (MPU) required from the diet per day for maintenance. This rate could be considered to represent a minimal estimate of the extra demand on endogenous protein turnover required during periods of exercise when ingestion is not possible. The nitrogen balance of amino acids was thus estimated in the context of amino acid utilisation during exercise by setting the protein intake rate to 0, and adding the rate of MPU to the endogenous protein turnover rate in muscle proteins. In this way, the model could be tuned to identify those amino acids inducing the greatest demand on muscle protein turnover during exercise.

Table 3 Summary of the parameters used in the modelling of amino acid fluxes based on protein intake, turnover, metabolism, and excretion

Parameter		Rationale
Fixed values		
Protein turnover rates	3 g/kg/day	3 g/kg/day for healthy adults (Poortmans et al. 2012)
% of protein intake directly oxidised for metabolism	15%	15–20 g per 100–110 g dietary intake
% of protein intake used for protein synthesis	75%	70–80 g per 100–110 g dietary intake
% of protein turnover oxidised for metabolism	27%	80 g per 300 g proteins turned over
% of protein turnover used for protein synthesis	67%	206–211 g per 300 g proteins turned over
Obligatory *N* excretion losses as protein equivalents:	350 mg/K/day	225 mg protein/kg/day from urine
		75 mg protein/kg/day from faeces
		50 mg protein/kg/day from a combination of saliva, desquamations, sweat, sebum, hair and nails (Tessari 2006)
Amino acid composition of protein sources		
Ingested proteins	% Amino acids	Average meat/plant composition (Table 2)
Endogenous protein turnover	% Amino acids	Average human composition (Table 2)
(1) Excretion losses assuming excretion profile mirrors average protein composition		1. Amino acids are excreted in the same proportions as they appear within ingested proteins
Amino acids metabolised or excreted via urine and sweat*		
Ingested proteins	% Amino acids	Average meat/plant composition (Table 2)
Endogenous protein turnover	% Amino acids	Average human protein composition (Table 2)
(2) Excretion losses based on measured average compositions in urine and sweat	% Amino acids	2. Amino acids are excreted in different proportions to those from the ingested proteins
Amino acids metabolised or excreted via urine and sweat*		
Ingested proteins	% Amino acids	Average % abundance reported in urine and sweat (Table A3, sup data)
Endogenous protein turnover	% Amino acids	Average % abundance reported in urine and sweat (Table A3, sup data) (Dunstan et al. 2017)
Variable parameters		For testing by the model:
Sex	Male/female 70 kg	Sex differences in excretion characteristics in urine and sweat (Dunstan et al. 2017). Modelled on the same body mass
Body mass	70–100 kg male	
	50–80 kg female	Influence of body mass on nitrogen balance for specific amino acids with differential sex ranges
Protein intake		Three levels tested
	0.8 g/kg/day	0.75–0.8 g/kg/day for healthy adults (Male only presented)
	1.2 g/kg/day	1.2–1.4 g/kg/day for endurance athletes
	1.6 g/kg/day	1.6–1.7 g/kg/day muscle strength training (Fielding and Parkington 2002; Poortmans et al. 2012; Tessari 2006)
Protein synthesis/turnover	3–7 g/kg/day	Five levels tested. Total estimated protein synthesis per day in adult is 3 g/kg/day (Poortmans et al. 2012)

*Amino acids excreted in faeces were modelled using the average human protein composition (Table A1), the obligatory nitrogen losses (Table A2) for both sets, as summarised in Table A3

Results and discussion

A simplistic model was developed utilising published rates of protein intake, oxidation, protein synthesis, and excretion, to investigate the nitrogen balance in terms of the throughput of individual amino acids. The first appraisal involved using the model to evaluate the fluxes for a 70 kg male and a 70 kg female with a protein intake set at 1.2 g/kg/day and a protein synthesis rate of 5.7 g/kg/day, while the remaining fixed variables were set, as shown in Table 3. The resulting calculations of protein intake, turnover, and utilisation have been presented for the 70 kg person, as shown in Table 4.

These values were extrapolated from units of protein into amino acids using average intake compositions from plant and meat proteins and turnover/excretion compositions from human proteins. The average percentage composition of amino acids in the plant/meat proteins was used to estimate the daily specific gram intake of the selected amino acids

Table 4 Daily utilisation of protein resources for a 70 kg individual has been calculated by assuming a protein intake of 1.2 g protein/kg/day and fixed rates of utilisation by oxidation, protein synthesis, and excretory losses

70 kg Individual	Protein contributions (g)	Oxidised (g)	Protein synthesis (g)	Excretion losses (g)	Miscellaneous metabolism
Protein intake	84	13	63	4	4
Protein turnover contribution	399	108	266	20	5
Totals	483	120	329	25	9

from (A) 84 g of dietary protein and (B) 399 g of protein turnover, as shown in Table 5. The total daily usage of each amino acid was then calculated to predict losses from (C) protein synthesis, (D) oxidation metabolism and (E) excretion. These values are entered as negative, because they represent utilisation of resources and thus summing the input and output values from (A) to (E) generated the nitrogen balance (F). There are no separate estimates at this stage between males and females, because fluxes were determined on a g/kg/day basis.

The values of nitrogen balance calculated for each of the amino acids in (F) were based on the assumption that the amino acids were utilised and excreted in the same proportions that they were ingested or provided from endogenous protein turnover. In this context, the nitrogen balance values were all positive and collectively (+ 8.3 g) represented 1.7% of the daily utilisation of proteins (483 g). However, certain amino acids are used in multiple biochemical pathways and are excreted at disproportionately faster rates than other amino acids, as shown in Table 1. The skewed rates of utilisation for metabolism are very difficult to estimate, and thus, this parameter could not be effectively adjusted. However, the model could be altered to include adjustments to represent more realistic rates of excretion via urine and sweat (Dunstan et al. 2016, 2017). The adjusted excretion rates for males (G) and females (H) resulted in enhanced losses of histidine, serine, glycine, and ornithine shown by more negative nitrogen balance assessments (Table 5). As a consequence, the net balances of proline, aspartic acid, phenylalanine, leucine, isoleucine, and valine all increased substantially [compared with (F)].

An external source of urine data was also tested for a mixed group of 11 healthy males and 19 healthy females (Jones et al. 2005), yielding similar results with negative adjusted nitrogen balances observed for histidine (− 1.4), serine (− 0.8), glycine (− 4.4), and ornithine (− 0.4) with all others in positive balance (supplementary Table A4). The skewed losses of histidine, serine, glycine, and ornithine could thus be viewed as potential sacrificial losses to conserve the remainder of the amino acids.

The adjusted nitrogen balances for the amino acids were compared between the sexes in Fig. 1 which showed that the males have a generally higher output of histidine, whereas the females had higher outputs of glycine and serine. The model is not perfect, because it has not included adjustments for skewed losses of amino acids from utilisation in metabolic pathways and losses via faeces. However, it does show that the skewed nature of excretion of certain amino acids at faster rates than others has impact on demand for those amino acids. Deficits in histidine can lead to anaemia and has been shown to be critical for the formation of haemoglobin and red blood cells (Clemens et al. 1984; Cooperman and Lopez 2002; Kopple and Swendseid 1975). Histidine is also required for the formation of the dipeptide carnosine (β-alanyl-L-histidine) which is highly concentrated in the muscles and brain (Bauchart et al. 2007; Reddy et al. 2005; Tamaki et al. 1984). The ingestion of protein is not the only source of histidine. When consuming meat, one is also taking in substantial quantities of carnosine which is not necessarily included in the evaluation of "protein". Most of the ingested carnosine (70–80%) is broken down to histidine and β-alanine in the digestive tract prior to absorption (Bauchart et al. 2007; Tamaki et al. 1984). It was concluded that consumption of meat, especially for athletes, was vital to meet protein turnover demands to supply sufficient histidine. It also raises the question as to whether replacement of meat with cheaper whey proteins is sufficient to meet the demands of exercise or recovery from ill-health and pathogenic challenge.

Serine, glycine, and histidine are the major components lost in urine and sweat. Serine is the direct metabolic precursor to glycine synthesis, and together, these amino acids contribute to numerous metabolic roles in the body, as summarised in Table 1. The human body has the capacity to synthesise serine and glycine on demand, but under certain conditions, it may not be able to keep up with demand (Darling et al. 1999; Eagle 1959; Jackson 1991; Maxwell et al. 1956; Meléndez-Hevia et al. 2009; Rezaei et al. 2013; Stover et al. 1997; Wang et al. 2013; Wu 2010a, b).

The model was then applied to looking at the influence of body masses on males (70–100 kg) and females (50–80 kg) in Fig. 2. Effectively, it demonstrates that the heavier the person (male or female), the greater the negative balances are for histidine, serine, glycine, and ornithine and the greater the apparent accumulations of the remaining amino acids, particularly leucine, aspartic acid, and proline. Thus, the demands on intake of histidine are more substantial in the higher weight ranges and there is a requirement for the

Table 5 Predicted levels of amino acids for a 70 kg male and a 70 kg female with a protein intake set at 1.2 g/kg/day and a protein turnover rate of 5.7 g/kg/day which was generated by the model from protein intake and protein turnover as well as the predicted fluxes into energy metabolism, protein synthesis, and excretion via urine, faeces, and sweat, as shown in Table 3

Amino acid (AA) calculated on the average composition in food proteins or turnover of body proteins (Table 2)	(A) Amino acids from protein intake Total: = $\%AA_{Food} \times 84$ g/day	(B) Amino acids from endogenous protein turnover Total: = $\%AA_{body} \times 399$ g/day	(C) Usage of amino acids for protein synthesis = − (75% × A)– (67% × B)	(D) Usage of amino acids for oxidation = − (15% × A) – (27% × B)	(E) Excretion of amino acids at 350 mg/kg/day at the same proportions as protein composition = $-(\frac{84}{483} \times \%AA_{Food} \times BW \times 0.35) - (\frac{399}{483} \times \%AA_{body} \times BW \times 0.35)$	*(F) Nitrogen Balance* $=A+B+C+D+E$	(G) Male Excretion losses based on measured values in urine and sweat $= -(\frac{84}{483} \times \%AA_{Male} \times BW \times 0.35) - (\frac{399}{483} \times \%AA_{Male} \times BW \times 0.35)$	*Male Adjusted nitrogen balance* g/day calculated on the average outputs measured in urine and sweat (Table A3) $=A+B+C+D+G$	(H) Female Excretion losses based on measured values in urine and sweat $= -(\frac{84}{483} \times \%AA_{Fem\ exc} \times BW \times 0.35) - (\frac{399}{483} \times \%AA_{Fem\ exc} \times BW \times 0.35)$	*Female Adjusted nitrogen balance* g/day calculated on the average outputs measured in urine and sweat (Table A3) $=A+B+C+D+H$
Histidine	2.1	12.9	− 10.2	− 3.8	− 0.8	*0.3*	− 5.0	***− 3.9***	− 3.7	***− 2.7***
Serine	4.1	17.8	− 14.9	− 5.4	− 1.1	*0.4*	− 2.0	***− 0.4***	− 2.3	***− 0.7***
Glycine	4.2	42.1	− 31.2	− 12.0	− 2.3	*0.7*	− 4.2	***− 1.1***	− 4.9	***− 1.8***
Ornithine					0.0	*0.0*	− 0.4	***− 0.4***	− 0.3	***− 0.3***
Lysine	5.6	32.0	− 25.5	− 9.5	− 1.9	*0.7*	− 1.5	*1.1*	− 1.2	*1.3*
Threonine	3.2	19.1	− 15.1	− 5.6	− 1.1	*0.4*	− 0.8	*0.7*	− 0.9	*0.6*
Valine	4.2	21.0	− 17.2	− 6.3	− 1.3	*0.5*	− 0.8	*1.0*	− 0.9	*0.9*
Leucine	6.6	34.8	− 28.1	− 10.4	− 2.1	*0.8*	− 0.9	*2.0*	− 0.9	*2.0*
Isoleucine	3.7	14.6	− 12.4	− 4.5	− 0.9	*0.4*	− 0.5	*0.7*	− 0.6	*0.7*
(Glx)	14.4	46.5	− 41.8	− 14.7	− 3.1	*1.3*	− 2.6	*1.8*	− 2.4	*2.0*
Aspartic acid	7.6	34.3	− 28.5	− 10.4	− 2.1	*0.8*	− 0.6	*2.4*	− 0.6	*2.3*
methionine	1.6	7.6	− 6.3	− 2.3	− 0.5	*0.2*	− 0.1	*0.5*	− 0.1	*0.5*
Tyrosine	2.8	11.5	− 9.7	− 3.5	− 0.7	*0.3*	− 0.5	*0.5*	− 0.5	*0.5*
Phenylalanine	4.1	16.1	− 13.8	− 5.0	− 1.0	*0.4*	− 0.4	*1.0*	− 0.4	*1.1*
Proline	4.7	32.3	− 25.1	− 9.4	− 1.9	*0.6*	− 0.4	*2.1*	− 0.5	*2.1*
Alanine	4.2	32.5	− 24.9	− 9.4	− 1.9	*0.6*	− 1.6	*0.9*	− 1.6	*0.9*

Table values have been rounded for presentation

The italic headings were used to isolate those columns of results that were generated using the updated data in the model for published rates of excretion

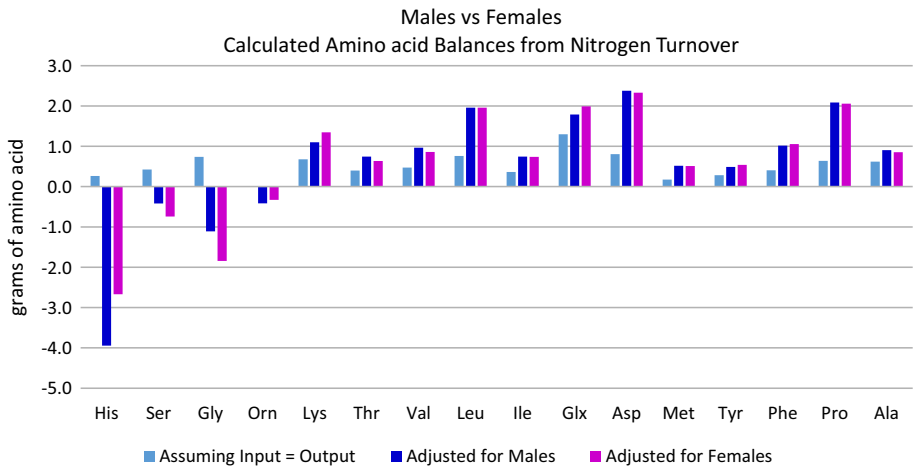

Fig. 1 Comparison of the nitrogen balances for each of the amino acids assessed in the model for males and females determined at 70 kg with a protein intake of 1.2 g/kg/day and a protein turnover rate of 5.7 g/kg/day. The first series (assuming input = output) shows the positive nitrogen balances calculated for all the amino acids on the basis that they would be excreted in the same proportions in which they would be generated as a resource from ingested proteins and from endogenous protein turnover. The series for males and females show the nitrogen balances calculated for all the amino acids when the model used realistic excretion rates for each amino acid which had been previously measured in males and females (Dunstan et al. 2016, 2017)

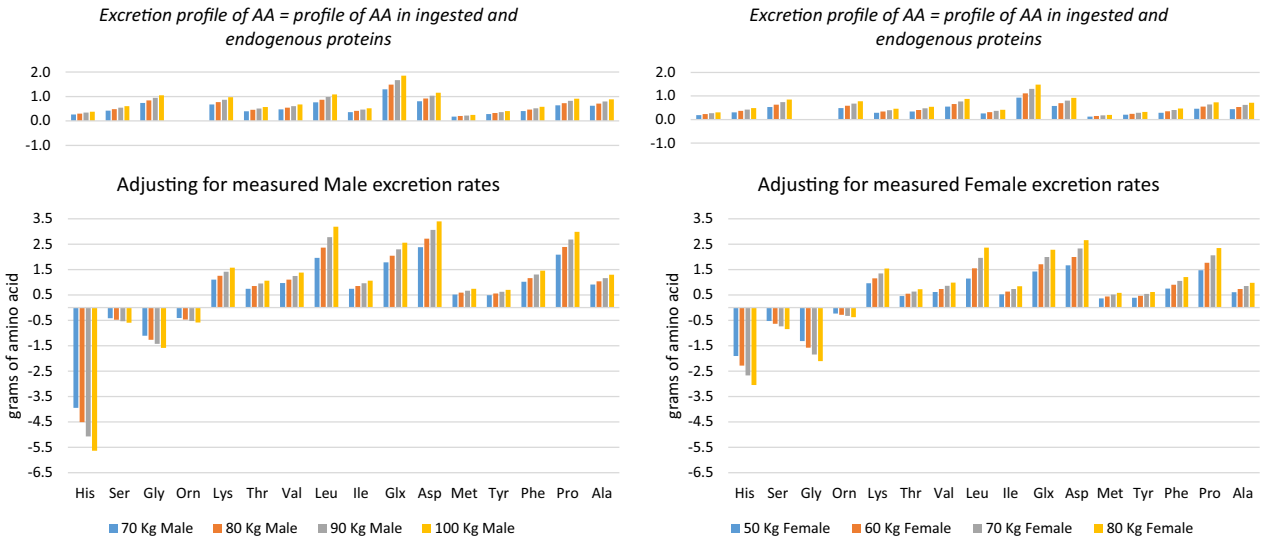

Fig. 2 Comparison of the nitrogen balances for each of the amino acids assessed in the model for males determined at 70, 80, 90, and 100 kg and for females at 50, 60, 70, and 80 kg with a protein intake of 1.2 g/kg/day and a protein turnover rate of 5.7 g/kg/day. The top graphs show the positive nitrogen balances calculated for all the amino acids on the basis that they would be excreted in the same proportions in which they would be generated from ingested proteins and from endogenous protein turnover. The lower graphs represent the nitrogen balances calculated for all the amino acids when the model used realistic excretion rates for each amino acid which had been previously measured in males and females (Dunstan et al. 2016, 2017)

enhanced provision of glycine, serine, and ornithine from endogenous metabolism. The net result for the essential amino acids would appear to be that there is some conservation of the essential amino acids in the heavier weight ranges. However, it should be noted that amino acids cannot be "stored"—they are either incorporated into proteins, utilised in oxidative or other metabolic pathways, converted to fats, or excreted. It is evident from the modelling in Fig. 2 that there would be a higher risk of having insufficient histidine, glycine, and serine in the heavier weight ranges. These components are, therefore, more likely to become limiting factors to certain aspects of metabolism and anabolism. If

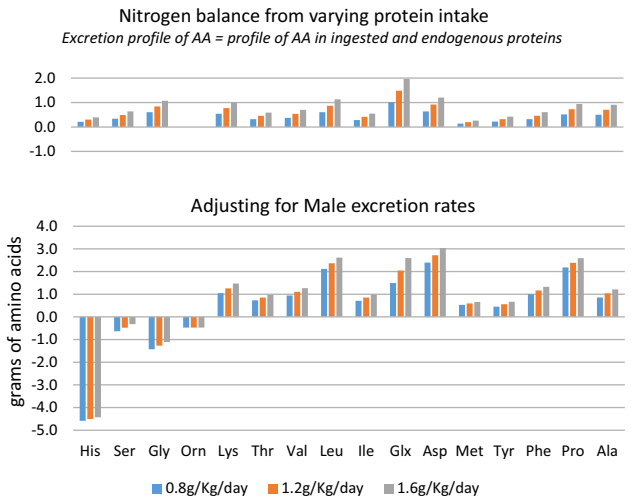

Fig. 3 Comparison of the nitrogen balances for each of the amino acids assessed in the model for 80 kg males determined at a protein intake of 0.8, 1.2, and 1.6 g/kg/day and a protein turnover rate of 5.7 g/kg/day. The top graph shows the positive nitrogen balances calculated for all the amino acids on the basis that they would be excreted in the same proportions in which they would be generated from ingested proteins and from endogenous protein turnover. The lower graph represents the nitrogen balances calculated for all the amino acids when the model used realistic excretion rates for each amino acid which had been previously measured in males and females (Dunstan et al. 2016, 2017)

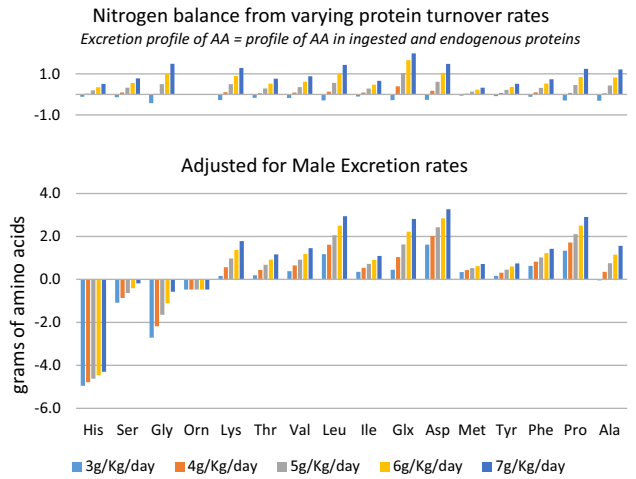

Fig. 4 Comparison of the nitrogen balances for each of the amino acids assessed in the model for 80 kg males determined at protein turnover rates of 3, 4, 5, 6, and 7 g/kg/day with a protein intake of 1.2 g/kg/day. The top graph shows the positive nitrogen balances calculated for all the amino acids on the basis that they would be excreted in the same proportions in which they would be generated from ingested proteins and from endogenous protein turnover. The lower graph represents the nitrogen balances calculated for all the amino acids when the model used realistic excretion rates for each amino acid which had been previously measured in males and females (Dunstan et al. 2016, 2017)

these factors were limiting for protein synthesis, then the accumulated essential amino acids may not be utilised as required in protein synthesis, and subsequently, they would be oxidised, converted to fat, or excreted. This could provide a novel insight as to why people in the higher weight range would find it harder to lose weight, even if they were eating balanced diets with appropriate protein and carbohydrate content with low fat.

To consider this further, the influence on increasing protein intake was assessed in the model for an 80 kg male in Fig. 3, where increasing protein intake has some minimal beneficial influence on serine and glycine but virtually no impact on histidine. Conversely, increasing the protein intake led to increasing the positive balance of the other amino acids, but, if histidine, serine and glycine were in short supply, this could potentially limit synthesis of proteins and the surplus essential amino acid components would be oxidised, converted to fat stores or excreted.

Increasing the protein synthesis rates might be expected to occur as exercise demand increases with increasing intensity of training. As expected, the higher rates of protein synthesis and turnover shown in Fig. 4 led to more histidine, serine, and glycine being recycled via endogenous protein turnover, and thus, the nitrogen balance for these three components improved (Fig. 4). However, increased protein turnover also leads to an increased availability of the remaining amino acids. If any of the amino acids lost at disproportionately faster rates via metabolism and excretion pathways became limiting for protein synthesis due to the negative nitrogen balance, then the essential amino acids will be lost to oxidation, converted to fat or excreted. Once processed and stored as fats, the essential amino acids cannot be reconstructed and are permanently lost as a critical resource.

Investigations of nitrogen balance have been largely focussed on a daily requirement schedule. When undertaking an extended period of exercise which prohibits protein intake, the body is subjected to a phase of intensified demand to provide endogenous resources to support the activity. The model was run to ascertain the immediate amino acid demands on endogenous protein turnover during exercise without protein ingestion. The results in Fig. 5 show that there were negative nitrogen balances for most of the amino acids, where Glx showed the greatest impact. These outcomes would represent the shortfall from operating without ingestion of protein as well as the compensation for high losses in sweat of specific components. During the post-exercise recovery phase, muscle protein turnover will continue for hours after exertion to support the recuperation and repair processes (Martin-Rosset 2008). Digestion is limited for hours after exercise, and thus, exogenous supply of protein amino acids is not readily forthcoming (Brouns et al. 1987; Burton et al. 2004; Butterfield 1987; Martin-Rosset

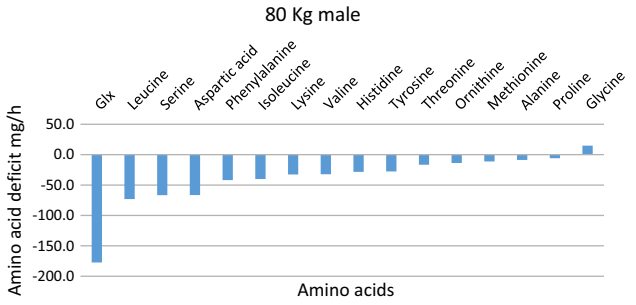

Fig. 5 Negative nitrogen balances of amino acids estimated by the model (mg/h) during prolonged exercise with no ingestion of protein and no excretion via urine or faeces. Under this scenario, there is an increased demand on muscle protein turnover to supply the amino acid resources to support exercise

2008; van Wijck et al. 2013; Williams et al. 1996). Even if digestion were fully operational, it would take hours before the proteins would be fully broken down for utilisation of the amino acids by the body. However, the absorption of free amino acids is not affected by moderate to severe exercise (Cammack et al. 1982). These modelling evaluations thus provided a premise for developing a supplementation strategy to replenish those amino acids in negative balance (histidine, serine, glycine, and ornithine). This approach would better sustain a positive nitrogen balance, and facilitate anabolic metabolism for recovery and repair processes. The essential amino acids released from endogenous protein turnover would not then be surplus to demand and lost as "wastage", because the limiting factors for anabolism have been replaced. Direct replenishment of these amino acids may also be feasible in certain endurance sports and may have substantial long-term benefits in maintaining muscle integrity and reducing damage.

In the scenario where an individual is responding to an infectious challenge, the production of IgG represents a primary immune response, where 66–95% is derived from the bone marrow and the remainder in the spleen. It has been estimated that this represents around 960 mg per day for a healthy 80 kg individual and this could double in those people with activated immune systems (Thornton et al. 1996). This rate of synthesis of IgG in the healthy individual represents around 0.3% of the whole body protein synthesis and adjustments in this context represent negligible impact on the whole body amino acid utilisation profile. However, since the bone marrow represents around 2.6 kg of the body mass (Fliedner et al. 2002) and is responsible for most of the IgG synthesis, there would be very high localised demand for specific amino acids in response to pathogenic challenge. This may have a net draining effect on the plasma supply of these amino acid resources. Appraisal of the amino acid composition of the IgG heavy chain indicated that the major components include serine (9.1%), valine (8.4%), threonine (7.4%), and tyrosine (6.1%) (Chaplin et al. 1965) which are present at 1.5–2 times their average composition calculated from the other five body proteins (see Table 2). On this basis, the model was run to evaluate the potential impact on amino acid demand within the bone marrow by slightly increasing the specific rates of utilisation of these amino acids for protein synthesis from endogenous resources, as shown in Table 2 (see also supplementary Table A1). The nitrogen balances for the amino acids under these conditions are summarised in Fig. 6 for comparison with the balances under normal healthy conditions.

It was apparent that the host defence response would generate higher levels of demand for serine, tyrosine, threonine, and valine, resulting in their negative nitrogen balances. Serine can potentially be generated endogenously, but under a critical requirement for a rapid response, the body may not be able to keep up with demand. Tyrosine can be synthesised from phenylalanine, but phenylalanine is an essential amino acid, and both may become limiting during this IgG response. Valine is an essential branch chain amino acid and must come from exogenous supply. The body instigates a catabolic response to pathogenic challenge to assist in provision of the necessary substrates from the turnover of muscle

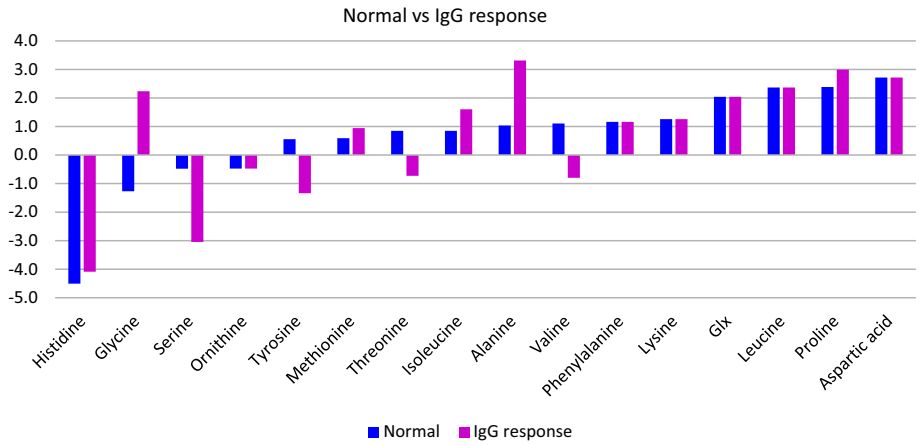

Fig. 6 Comparison of the nitrogen balances for each of the amino acids assessed in a healthy 80 kg male compared with an equivalent male mounting an IgG response to pathogenic challenge. The amino acid utilisation rates were adjusted to represent the specific increases in demand for the major components of the IgG heavy chain, as shown in Table 2

proteins required for supporting the immune defence. This is instigated because a rapid and efficient response is required to ensure that the infection is curtailed before becoming too well established. The extent of protein synthesis may well have been underestimated for supporting the response to pathogenic challenge, and this current modelling demonstrates that certain amino acids may become locally limiting under periods of heavy demand for production of large quantities of specific proteins for immune function or repair and recovery from injury.

It was concluded that it is important to focus on meeting the amino acid requirements of people under various regimes of activities, rather than generally just considering crude protein requirements. The negative balances observed in this model identified histidine, glycine, serine, and ornithine as potentially limiting factors for anabolic metabolism, especially under conditions of fitness training and maintaining intensive exercise regimes. If an individual is suffering an infectious challenge, then this model would suggest that tyrosine, threonine and valine should also be supplemented. The grams per day in negative balance (Table 5) provide a guide for the proportions and quantities of amino acids for specific supplementation. This model approach provides a basis for explaining how a daily provision of just 5 g of amino acids, representing 6% of the daily protein intake, could provide benefit if taken immediately after or during exercise, or during pathogenic challenge. People in higher weight ranges would have greater demands on the supply of these amino acids to sustain a positive nitrogen balance.

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Compliance with ethical standards

Conflict of interest The authors declare they have no conflict of interest.

Ethics statement No actual experiments were performed on humans or animals for this study. All data for modelling were derived from published resources and acknowledged appropriately.

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