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No protein intake compensation for insufficient indispensable amino acid intake with a low-protein diet for 12 days

Eveline A Martens^{1*}, Sze-Yen Tan², Richard D Mattes² and Margriet S Westerterp-Plantenga¹

Abstract

Background: Protein quality evaluation aims to determine the capacity of food sources and diets to meet protein and indispensable amino acid (IAA) requirements. This study determined whether nitrogen balance was affected and whether dietary IAA were adequately obtained from the ad libitum consumption of diets at three levels of protein from different primary sources for 12 days.

Methods: Two 12-day randomized crossover design trials were conducted in healthy subjects [$n = 70/67$ (M/F); age: 19-70 y; BMI: 18.2-38.7 kg/m²]. The relative dietary protein content was lower than [5% of energy (En%)], similar to (15En%), and higher than (30En%) customary diets. These diets had a limited variety of protein sources, containing wheat protein as a single protein source (5En%-protein diet) or 5En% from wheat protein with 10En% (15En%-protein diets) or 25En% (30En%-protein diets) added from whey with α -lactalbumin, soy or beef protein.

Results: There was a dose-dependent increase in nitrogen excretion with increasing dietary protein content, irrespective of the protein sources ($P = 0.001$). Nitrogen balance was maintained on the 5En%-protein diet, and was positive on the 15En%- and 30En%-protein diets ($P < 0.001$) over 12 days. Protein intake from the 5En%-protein diet did not reach the amount necessary to meet the calculated minimal IAA requirements, but IAA were sufficiently obtained from the 15En%- and 30En%-protein diets. In the 15En%- and 30En%-protein conditions, a higher protein intake from the soy-containing diets than from the whey with α -lactalbumin or beef containing diets was needed to meet the minimal IAA requirements.

Conclusion: Protein intake did not compensate for an insufficient indispensable amino acid intake with a low-protein diet for 12 days.

Trial registration: These trials were registered at clinicaltrials.gov as NCT01320189 and NCT01646749.

Keywords: Protein intake, Nitrogen balance, Indispensable amino acids, DIAAS, Protein source

Background

Meeting metabolic needs for amino acids is crucial to ensure health [1]. Amino acids provide nitrogen and carbon skeletons for tissue protein synthesis and for the production of nitrogenous compounds involved in a range of bodily functions [2]. Many types of amino acids are needed physiologically but some cannot be synthesized in the body and must therefore be obtained

through the diet; these amino acids are known as the dietary indispensable amino acids (IAA). Protein quality evaluation aims to determine the capacity of food sources and diets to meet protein and IAA requirements. Currently, nitrogen balance studies are primarily applied to define protein requirements as the degree to which protein intake compensates for obligatory losses of nitrogen from several pathways including amino acid breakdown [1,3]. However, nitrogen balance alone may be insufficient and other factors should be considered when determining protein requirement. For example, protein sources may influence the efficiency of protein utilization, and diets containing protein of lower quality

* Correspondence: eap.martens@maastrichtuniversity.nl

¹Department of Human Biology, Nutrition and Toxicology Research Institute Maastricht (NUTRIM), Maastricht University, PO Box 616, 6200, MD Maastricht, The Netherlands

Full list of author information is available at the end of the article

are associated with increased losses of nitrogen [2,4,5]. Furthermore, the observation of a non-linear relationship between protein intake and nitrogen balance indicates the existence of metabolic adjustments to changes in protein intake [2,6]. The overall requirements of amino acids should consider adaptive components, such as net protein deposition and oxidative losses [3].

Due to a large inter-individual variability, even during energy balance and after metabolic adaptation, nitrogen balance studies are not sufficiently accurate to determine whether dietary protein intake meets IAA requirements [1,3,7]. In the context of protein-diets, the digestible IAA score (DIAAS) enables the determination whether dietary protein intake is sufficient for the metabolic needs for the individual IAA. The DIAAS permits the definition of protein quality by determination of the extent to which dietary IAA are provided in proportion to the requirements [1].

Recently, it has been proposed that humans either consume relatively more energy from a low-protein diet [8], or relatively less from a high-protein diet [9,10]. In these studies, complete protein leveraging was absent [8-10]. It is unclear whether protein intake compensates for the protein-proportions to maintain nitrogen balance or to meet a sufficient amount of IAA. Compensatory intake of protein for obligatory loss of nitrogen is essential for normal growth and development in organisms [1,3]. The proposed mechanism to prevent negative nitrogen balance may be related to an observed signaling pathway that detects amino acid depletion [11-19]. This study determined whether nitrogen balance was affected and whether dietary IAA were adequately obtained from the ad libitum consumption of diets at three levels of protein from different primary sources for 12 days. Data from two dietary intervention trials conducted over 12 consecutive days were combined and analyzed [9,10]. The relative dietary protein content was lower than [5% of energy (En%)], similar to (15En%), and higher than (30En%) customary diets. These diets had a limited variety of protein sources, containing wheat protein as single protein source (5En%-protein diet) or 5En% from wheat protein with 10En% (15En%-protein diets) or 25En% (30En%-protein diets) added from whey with α -lactalbumin, soy or beef protein.

Methods

Results from a trial on whey protein with α -lactalbumin ($n = 39$) and soy protein ($n = 40$) at Maastricht University [9], and a two-center trial on beef protein ($n = 58$) at Maastricht University and at Purdue University [10] were combined for analysis. These studies were conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Medical Ethical Committees of

Maastricht University and Purdue University. Written informed consent was obtained from all subjects. The study on whey with α -lactalbumin and soy protein was registered as NCT01320189 and that on beef protein as NCT01646749 at clinicaltrials.gov. Data on energy and protein intake of the separate studies has been previously published [9,10]. The present study encompasses the analyses on the nitrogen balances and on the DIAAS.

Study subjects

One hundred and eighty-six subjects were recruited, of whom 28 dropped out due to lack of time. Four subjects were excluded from the data analysis because of non-compliance, as shown by urinary nitrogen excretion. Overall, 137 subjects (70 men and 67 women) were included in the final data analysis. BMI ranged from 18.2-38.7 kg/m² and age from 19 to 70 y [9,10]. Subjects underwent screening that included anthropometric measurements and the completion of questionnaires eliciting information about health, smoking behavior, use of medications, alcohol consumption, physical activity, eating behavior and liking of the study meals. Subjects were non-smoking, not using more than moderate amounts of alcohol (>10 drinks/wk), were weight stable (body weight change < 3 kg during the prior 6 mo and had no planned weight change during the study period), were not using medication or supplements except for oral contraceptives in women, and rated the taste of the meals as acceptable [Visual analog scale (VAS) score for liking: ≥ 50 mm] [9,10]. Individual daily energy requirements were calculated as the basal metabolic rate calculated with the formula of Harris and Benedict [20], times the physical activity level based on the Baecke Activity Questionnaire or on the two validated questions physical activity at work and at leisure time [21,22].

Study design

In the two single-blind, randomized crossover design trials, the relative protein content included 5En% from wheat protein (5En%-protein diet), and 5En% from wheat protein with 10En% (15En%-protein diets) or 25En% (30En%-protein diets) from whey with α -lactalbumin (Hiprotal Whey Protein Alpha; DOMO, FrieslandCampina), soy protein (SUPRO Soy Protein Isolate; Solae LLC) or beef protein. Subjects were randomly assigned to diets containing whey protein with α -lactalbumin or the soy protein in trial one and all consumed beef protein in the second trial. The order of the three treatment arms for protein energy content was random for all subjects. Each dietary intervention lasted for a period of 12 consecutive days, in which subjects visited one of the universities for ad libitum consumption of breakfasts, lunches and dinners [9,10]. Prior work indicated that this period of intervention is long enough to reliably measure possible effects

on energy and macronutrient intake [23]. The wash-out period between the test sessions was ~6 wk to minimize carry-over effects and to take menstrual cycle phase effects into account in women on energy intake [24,25].

Diet composition

Each protein-diet consisted of 5En% from wheat flour protein. The 5En%-protein condition contained wheat flour as a single protein source. In the 15%-protein conditions, the protein content was comprised of 33% (5En%) of wheat flour protein and of 67% (10En%) of either whey plus α -lactalbumin, soy or beef protein. Dairy protein was comprised of 70% of whey protein and of 30% of α -lactalbumin. Protein content of the 30En%-protein conditions comprised of 17% (5En%) of wheat flour protein and of 83% (25En%) of either beef, whey plus α -lactalbumin or soy protein. Again, dairy protein content was comprised of 70% of whey protein and of 30% of α -lactalbumin. The fat content between the meals and between the conditions was maintained at a constant proportion (35 En%) to prevent possible effects of energy density and palatability on energy intake [26-28]. The resulting diet compositions were 5/60/35 En% from protein/carbohydrate/fat (low-protein), 15/50/35 En% (medium-protein), and 30/35/35 En% (high-protein). Moreover, the fiber content was comparable between the conditions.

Biomarker of protein intake and nitrogen balance

Nitrogen excretion, measured from 24-h urine collections at baseline (day 0) and at days 5 and 11, was used as a crude estimate of protein intake. Urine was collected in 2-L urine bottles containing 10 mL of diluted hydrochloric acid (4 mmol/L) to prevent nitrogen loss through evaporation. Collection started after the first voiding in the morning on the collection days at 8.00 h, and lasted through the first voiding on the next day at 8.00 h. The total volume of the 24-h urine was recorded. Urine was gently mixed, and samples were taken and frozen at -20°C until analysis. Nitrogen concentrations were measured with an elemental analyser (CHN-O-Rapid, Heraeus, Hanau, Germany, in the Netherlands, and Integra COBAS 400 plus, Roche Diagnostics GmbH, Rotkreuz, Switzerland, in the USA). Total nitrogen output was calculated as 24-h urinary nitrogen plus 10% to account for normal losses via feces and other losses [9,10]. Nitrogen balance was calculated as the difference between nitrogen excretion and nitrogen intake.

Protein intake

Each meal was weighed to the nearest gram before it was provided to the subjects. Leftovers were reweighed to permit calculation of protein intake for each subject. Weights of provided snack items were also recorded, and the portions returned uneaten were weighed to

determine the protein intake from the snacks per subject. Mean protein intake was calculated as the sum of protein intake from meals and from snacks [9,10].

DIAAS

The IAA compositions of wheat protein and that of beef protein in the provided diets were obtained from the United States Department of Agricultural data base [29] and from a FAO publication [30] (Additional file 1). Amino acid analyses of specific food products provided the dietary IAA compositions of whey, α -lactalbumin and soy protein (Additional file 1) [31]. The IAA content of the protein-diets was calculated as the sum of IAA of the separate proteins in the diets. Subsequently, the IAA content of the protein-diets was corrected for digestibility (Additional file 2) [1]. Since fecal protein represents largely bacterial protein, determination of undigested protein at the end of the ileum is a more reliable measure for the digestibility of dietary protein [32-34]. Furthermore, the predicted ileal amino acid content needs to be corrected for ileal endogenous amino acids that are voided in the digestive tract. When the coefficients of amino acid digestibility are corrected for the endogenous amino acids, the resultant digestibility coefficients are considered as 'true' coefficients [1,32,33]. Differences have been observed in the ileal digestibility of different proteins, as well as between different amino acids within each type of protein [1,32,35]. Therefore, in this study, the digestible IAA content was calculated for each individual IAA within each of the protein sources (wheat, whey with α -lactalbumin, soy, and beef) as the dietary IAA content (mg/g protein) multiplied by the corresponding IAA true ileal digestibility coefficient. True ileal digestibility coefficients were obtained from a compound table representing observations in humans, pigs and rats from single studies or as means across studies [33]. In general, digestibility coefficients for humans are used. When human data were not available, predictions for humans based on data obtained from growing pigs or rats were used, and then followed by digestibility coefficients for growing pigs or rats. For wheat flour protein, a predicted value obtained from pig true ileal amino acid digestibility was used, based on the equations of Deglaire and Moughan and from the endogenous amino acid losses determined using the protein-free diet method [32-34]. A determined value in humans was available for the whey with α -lactalbumin protein concentrate, whereby endogenous amino acid losses were determined using the protein-free diet method [32-34,36]. Also for soy protein isolate, amino acid digestibility coefficients corrected for endogenous amino acid losses were determined using the protein-free diet method. These were available from a human study by Moughan *et al.* [32-34,36]. Since specific studies on beef protein are scarce, the amino

acid digestibility coefficients of meat protein hydrolysate determined in rats with endogenous amino acid losses determined using the enzyme hydrolyzed casein/ultrafiltration method, were used as representatives [33].

The minimal IAA requirements are the minimal intakes of IAA to avoid deficiencies [37,38]. These represent the profile of IAA intake to fulfill the obligatory role of maintenance. The DIAAS for the minimal IAA intakes were calculated for each IAA per protein-diet with the following formula (Additional file 3):

$$\text{DIAAS (\%)} = 100 \times \left[\frac{\text{(mg of the digestible dietary IAA in 1 g of the dietary protein)}}{\text{(mg of the same dietary IAA in 1 g of the reference protein)}} \right] [1].$$

The DIAAS of a protein reflects the availability of the most limiting IAA relative to the minimal requirement. The lowest DIAAS indicates that the corresponding IAA is the most limiting IAA of the protein, and this score is used as the DIAAS for that protein. The minimal IAA scores for a reference protein were calculated with a minimal protein requirement value of 0.66 g.kg body weight (BW).d⁻¹ [1] (Additional file 3). In our study, the minimal DIAAS for each IAA within the different protein-diets was calculated (Table 1). Thus, the lowest score represents the DIAAS for the corresponding protein-diet.

In order to assess whether subjects met their minimal IAA requirements by consuming the different protein-diets, the protein intake to meet these requirements was calculated per diet (Table 2).

$$\begin{aligned} &\text{Protein intake to meet dietary IAA requirements (g.kgBW.d}^{-1}\text{)} \\ &= [\text{assumed dietary IAA requirement (minimal:} \\ &\quad \text{0.66 g.kgBW.d}^{-1}\text{)}/\text{DIAAS}]. \end{aligned}$$

Statistical analysis

SPSS version 20 for Macintosh OS X (SPSS Inc.) was used to perform statistical analyses. Differences in subject characteristics between protein groups were assessed using Factorial ANOVA. Factorial ANOVAs with repeated measures were applied to test whether nitrogen excretion and nitrogen balance changed in response to the dietary interventions within protein groups. Differences were regarded as statistically significant if $P < 0.05$. Data are presented as mean \pm SD.

Possible effects of protein source on nitrogen excretion and nitrogen balance were evaluated by calculating the differences in response elicited by the different protein diets within each protein group. Kruskal-Wallis tests were used to evaluate whether these differences were affected by source of protein. Mann-Whitney tests were used to follow-up noted differences. The Monte Carlo method was applied to estimate the significance of the Mann-Whitney tests. Effect sizes for the Mann-Whitney tests were calculated by dividing the obtained z-scores by the corresponding number of subjects. A Bonferroni correction was applied so all effects are reported at a $0.05 / 3 = 0.017$ level of significance. Data are presented as median \pm range. Differences were regarded as statistically significant if $P < 0.05$.

Results

Subject characteristics

Subject characteristics are summarized per protein group in Table 3. Subject characteristics did not differ between the protein groups.

Biomarker of protein intake and nitrogen balance

There was a dose-dependent increase in nitrogen excretion with increasing dietary protein content, irrespective of the protein sources ($P = 0.001$, Table 4). In each condition,

Table 1 Dietary IAA reference ratios for minimal IAA and DIAAS per protein diet

	His	Ile	Leu	Lys	SAA	AAA	Thr	Val	Trp	DIAAS
										% (IAA)
5En% wheat	1.26	1.06	1.05	0.53	1.68	1.36	1.00	0.94	1.62	53 (Lys)
5En% wheat + 10En%:										
Whey + α -lac	1.19	1.63	1.45	1.47	2.00	1.50	1.90	1.13	3.06	113 (Val)
Soy	1.29	1.14	1.00	0.84	1.09	1.01	1.10	0.89	1.54	84 (Lys)
Beef	1.78	1.38	1.23	1.44	1.65	1.79	1.55	1.12	1.33	112 (Val)
5En% wheat + 25En%:										
Whey + α -lac	1.17	1.77	1.55	1.69	2.08	1.53	2.12	1.18	3.41	117 (His)
Soy	1.31	1.18	1.00	0.93	0.96	1.02	1.14	0.89	1.53	89 (Val)
Beef	1.93	1.47	1.30	1.68	1.66	1.92	1.71	1.18	1.28	118 (Val)

α -lac, α -lactalbumin; AAA, aromatic amino acids (phenylalanine + tyrosine); DIAAS, digestible indispensable amino acid score; En%, percentage of energy; IAA, indispensable amino acid; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; SAA, sulphur amino acids (cysteine + methionine); Thr, threonine; Trp, tryptophan; Val, valine.

Table 2 Calculated protein intake to meet minimal IAA requirements and measured intake over 12 days

	Calculated <i>g.kgBW.d⁻¹</i>	Measured	Calculated <i>g/d</i>	Measured
5En% wheat	1.24	0.39	87.6	27.5
15En%				
5En% wheat + 10En%:				
Whey + α -lac	0.58	1.01	40.0	67.5
Soy	0.78	0.98	57.8	70.7
Beef	0.59	1.18	41.0	81.2
30En%				
5En% wheat + 25En%:				
Whey + α -lac	0.56	1.61	38.3	108.4
Soy	0.74	1.56	54.5	113.4
Beef	0.56	1.96	38.9	134.5

α -lac, α -lactalbumin; BW, body weight; En%, percentage of energy; IAA, indispensable amino acid.

no significant difference between the urine collection at days 5 and 11 were observed, which indicates stable protein intakes within the conditions over the 12-d test periods [9,10]. Nitrogen balance did not differ significantly from zero on the 5En%-protein-diet, and was positive on the 15En%- and 30En%-protein diets ($P = 0.001$, Table 4). The differences in nitrogen excretion and balance between the protein-diets were not affected by the sources of protein or by age.

Table 3 Subject characteristics of the whey with α -lactalbumin, soy and beef protein groups

	Whey protein	Soy protein	Beef protein
Participants, M/F (n)	16/23	24/26	30/28
Age (y)	35 \pm 17	34 \pm 19	33 \pm 16
Age range (y)	18 – 70	18 – 69	19 – 70
Height (cm)	172 \pm 11	174 \pm 10	169 \pm 10
BW (kg)	68.6 \pm 13.8	73.6 \pm 12.3	69.8 \pm 11.8
BMI (kg/m ²)	23.1 \pm 3.5	24.3 \pm 3.2	24.4 \pm 4.0
BMI range (kg/m ²)	18.2 – 33.9	18.1 – 33.4	18.7 – 38.7
BMR (MJ/d)	6.5 \pm 1.2	6.9 \pm 1.0	6.7 \pm 1.0
PAL	1.76 \pm 0.15	1.75 \pm 0.14	1.73 \pm 0.16
DER (MJ/d)	11.4 \pm 2.0	12.1 \pm 2.1	11.5 \pm 2.1
TFEQ Dietary restraint score	6 \pm 4	5 \pm 3	6 \pm 4
TFEQ Disinhibition score	4 \pm 2	4 \pm 3	4 \pm 3
TFEQ Hunger score	4 \pm 2	4 \pm 3	4 \pm 3

Values shown as means \pm SDs.

There were no significant differences between the protein groups (factorial ANOVA).

BMR, basal metabolic rate; BW, body weight; DER, daily energy requirement; PAL, physical activity level; TFEQ, Three-Factor Eating Questionnaire.

Protein intake and DIAAS

The DIAAS for minimal IAA intake are displayed for the 5En%-protein diet, and for the 15En% and 30En%-protein diets containing whey with α -lactalbumin, soy or beef protein separately (Additional file 3). Measured protein intake from the 5En%-wheat-protein diet was lower than the intake necessary to meet the calculated minimal IAA requirements (Table 2). Measured protein intake from the 15En%- and 30En%-protein diets was sufficient to meet minimal IAA requirements, irrespective of protein source. Age did not have an effect on differences in protein intake between conditions.

In line with previous data on amino acid composition, wheat flour protein was deficient in lysine, and had a lower total IAA content than whey with α -lactalbumin, soy and beef protein (Additional file 1). A characteristic of whey and α -lactalbumin protein was the relatively high abundance of leucine and lysine. Soy protein was lower in the sulphur amino acids (SAA) cysteine and methionine. The 15En%- and 30En%-soy protein diets had a relatively lower IAA content than diets with comparable protein levels from whey with α -lactalbumin or beef sources (Additional file 2).

For the 5En%-protein diet, the lowest minimal DIAAS was obtained for lysine. Valine and histidine were the most limiting IAA of the 15En%- and 30En%-protein diets containing whey with α -lactalbumin protein (Table 1). For the 15 En%- and 30En%-protein diets containing soy, the most limiting IAA were lysine and valine respectively. The minimal DIAAS for the diets containing 15En% and 30En% from beef protein were ascribed to valine. DIAAS for minimal IAA intake were higher on the 15En%- and 30En%-protein diets compared with the 5En%-protein diet. All the added protein sources tested were relatively high quality protein sources, but the DIAAS for minimal IAA intake only exceeded 100% for the whey with α -lactalbumin and beef containing diets. In the 15En%- and 30En%-protein conditions, a higher protein intake from the soy-containing diets than from the whey with α -lactalbumin or beef containing diets was needed to meet the minimal IAA requirements.

Discussion

This study determined whether nitrogen balance was affected and whether dietary IAA were adequately obtained from the ad libitum consumption of diets at three levels of protein from different primary sources for 12 days. The successful implementation of the dietary protein intervention on each condition in both trials was confirmed by urinary nitrogen concentrations. There was a dose-dependent increase in nitrogen excretion with increasing dietary protein content, irrespective of the protein sources. Despite protein intake with the 5En%-protein diet being below the minimal protein requirement

Table 4 Nitrogen excretion and nitrogen balance per protein diet

	Whey protein (n = 39)	Soy protein (n = 40)	Beef protein (n = 58)
N excretion day 11 (mg.kgBW.d⁻¹)			
5En% (5En% wheat)	71.2 ± 30.6 ^a	70.8 ± 22.8 ^a	67.9 ± 21.6 ^a
15En% (5En% wheat + 10En%)	124.8 ± 59.0 ^b	112.2 ± 47.9 ^b	138.0 ± 56.8 ^b
30En% (5En% wheat + 25En%)	203.0 ± 104.6 ^c	191.0 ± 79.9 ^c	217.4 ± 109.7 ^c
N balance day 11 (mg.kgBW.d⁻¹)			
5En% (5En% wheat)	4.2 ± 39.1 ^a	-6.9 ± 28.3 ^a	3.5 ± 27.6 ^a
15En% (5En% wheat + 10En%)	47.6 ± 52.1 ^b	48.5 ± 43.5 ^b	50.1 ± 60.4 ^b
30En% (5En% wheat + 25En%)	73.5 ± 69.6 ^c	71.5 ± 53.9 ^c	95.8 ± 110.1 ^c

Values shown as means ± SDs.

Values with different superscript letters indicate significant differences between diets that differed in relative protein content, $P < 0.05$ (in the whey, in the soy, or in the beef protein diets, repeated-measures ANOVA with Bonferroni correction for pairwise post-hoc comparisons).

There were no significant differences between the protein groups (Non-parametric tests).

BW, body weight; En%, percentage of energy; N, nitrogen P, protein.

of 0.66 g.kgBW.d⁻¹ [1], it was sufficient to maintain nitrogen balance over 12 days. Nitrogen balance was positive on the 15En%- and 30En%-protein diets, irrespective of dietary protein composition. However, protein intake from the 5En%-protein diet did not reach the amount necessary to meet the calculated minimal IAA requirements, but IAA were sufficiently obtained from the 15En%- and 30En%-protein diets. In the 15En%- and 30En%-protein conditions, a higher protein intake from the soy-containing diets than from the whey with α -lactalbumin or beef containing diets was needed to meet the minimal IAA requirements.

Data of this study suggest that the lower concentration of protein provided with the 5En%-protein diet was not outside the range that is feasible to maintain nitrogen balance over 12 days. The Adaptive Demands model developed by Millward may provide an explanation for this observation by proposing that the metabolic demand for amino acids comprises a fixed component and a variable adaptive component [39]. Short-term changes in protein intake are likely within the adaptive range [3]. Adaptations in protein and amino acid metabolism to changes in protein intake largely occur via changes in whole body protein turnover and amino acid oxidation [2,40]. Changes in amino acid oxidation were reflected as decreased and increased nitrogen excretion in response to the low- and high-protein diets respectively. The activity of the enzymes that regulates: 1) transamination, 2) the disposal of the carbon skeletons in intermediary metabolism, and 3) the disposal of nitrogen through the urea cycle was increased in response to high protein intake [41,42]. Nevertheless, a positive nitrogen balance was observed in the present study following the high-protein diets despite increased enzyme activities. This is in line with earlier observations [2,43-45], but does not automatically reflect an increase in protein anabolism [3]. The capacity of the body to increase amino acid anabolism through an increase in lean body mass is limited [3].

Only interventions using diets high in specific IAA, such as leucine, might be able to stimulate protein synthesis in specific target groups [46,47]. Therefore, a transient retention or loss of body nitrogen because of a labile pool of body nitrogen may contribute to adaptations in amino acid metabolism in response to changes in protein intake [48]. Transient adaptive mechanisms may be distinguished from mechanisms that maintain homeostasis in the body in the longer-term.

The calculated DIAAS for the 5En%-protein diet were well below 100%, which confirms that wheat is a low-quality protein. Especially at this low protein density, the intake of lysine was inadequate for satisfying the minimal IAA requirements. Protein intake should exceed 0.66 g.kgBW.d⁻¹ to reach the minimal IAA intake. Consequently, measured intake from wheat protein being 0.39 g.kgBW.d⁻¹ with the 5En%-protein diet means that minimal IAA requirements were not met. Protein intake was not spontaneously adjusted to reach the calculated optimal intake level of IAA. To meet the calculated minimal DIAAS level, subjects should have consumed ~63 MJ a day, from this diet. Interestingly, in animals, the detection of reduced concentrations of IAA in the anterior piriform cortex in the brain may result in deacetylation of the cognate transfer RNA [15]. Subsequent activation of general amino acid nonderepressing kinase 2 may phosphorylate eukaryotic initiation factor 2 α , a factor involved in the control of the initiation of translation in protein synthesis. This may lead to behavioral responses including under-consumption of diets that lack a minimal amount of IAA [13,16,18]. The findings of this study do not show a behavioral response in humans [9,10]. The lower concentration of protein provided in the present trials was not outside the range that is feasible to adjust protein intake. Nevertheless, a study [49] has documented acute food-choice compensation after low-protein meals in humans. After a low-protein meal, an increase in wanting and task-related signaling in the hypothalamus

has been related to increased protein intake in a subsequent meal [49]. Griffioen-Roose et al. [50] showed in a 4-day study that subjects increased their protein intake in a compensatory way during ad libitum feeding after a low-protein diet (5En% from protein). Since no shift towards a higher energy intake from the 5En% protein diet compared with the 15En% protein diet was observed (9, 10), the insufficient amount of IAA clearly does not trigger a possible compensatory protein intake over 12 days. Observations from studies in developing countries show health deterioration related to low protein intake, especially of IAA, at intakes < 0.66 g.kg BW.d⁻¹ [51]. Thus, although we conclude that protein intake did not compensate for an insufficient IAA intake with a low-protein diet for 12 days, we do not propose that low-protein diets are sustainable.

Increasing the relative dietary protein content from whey with α -lactalbumin, soy or beef protein resulted in an improved protein quality of the diets. However, the higher DIAAS of the whey with α -lactalbumin and beef diets did not affect total daily protein intake differently compared with the lower DIAAS of the soy diets. Minimal requirements for IAA were reached with the ad libitum intake of each 15En% and 30En%-protein diet. However, a higher protein intake from the soy-containing diets than from the whey with α -lactalbumin or beef containing diets was needed to meet the minimal IAA requirements. This corresponds with the recommendations for vegetarians to consume more protein or to include a combination of different plant protein sources in the diet. Furthermore, a larger amount of protein from whey with α -lactalbumin or beef was available to fulfill roles beyond the obligatory role of maintenance. Since the subjects in this study were healthy, one of these roles may be the stimulation of muscle protein synthesis. The relative high abundance of leucine in whey with α -lactalbumin protein may be beneficial to stimulate muscle protein synthesis [46].

The DIAAS can be applied in practice to examine protein quality of food products or mixed diets. However, it should be emphasized that the calculations of the DIAAS rely on some assumptions. First, the use of digestibility coefficients based on animal data is unavoidable when human data is not available. Second, the calculated protein intake necessary to meet minimal IAA requirements is based on the assumptions of a daily minimal protein requirement. This value may vary depending on dietary protein composition and should be adjusted according to subject-specific protein requirements. Factors influencing protein and amino acid metabolism, subsequently affecting protein and IAA requirements, should be further elucidated. Longer-term intervention studies with measurements of blood

concentrations of amino acids and other key factors in appetite regulation, whole body protein turnover and muscle protein synthesis would provide more insight in the changes in protein and amino acid metabolism in response to dietary protein intake. Furthermore, possible IAA sensing pathways involved in the regulation of energy and protein metabolism in humans remain to be investigated.

Conclusion

To summarize, nitrogen balance was maintained on the 5En%-protein diet, and was positive on the 15En%- and 30En%-protein diets over 12 days. Despite this, the protein intake from the 5En%-protein diet did not reach the amount necessary to meet the calculated minimal IAA requirements, but IAA were sufficiently obtained from the 15En%- and 30En%-protein diets. In the 15En%- and 30En%-protein conditions, a higher protein intake from the soy-containing diets than from the whey with α -lactalbumin or beef containing diets was needed to meet the minimal IAA requirements. In conclusion, protein intake did not compensate for an insufficient indispensable amino acid intake with a low-protein diet for 12 days.

Additional files

Additional file 1: IAA composition per type of protein.

Additional file 2: True ileal digestible IAA and total IAA content per protein diet.

Additional file 3: IAA reference pattern for minimal requirements.

Abbreviations

BW: Body weight; DIAAS: Digestible indispensable amino acid score; En%: Percentage of energy; IAA: Indispensable amino acids; VAS: Visual analog scale.

Competing interests

All of the authors declare that they have no competing interest.

Authors' contributions

The study was designed by MSWP, RDM, EAM, and SYT. EAM analyzed the data, performed the statistical analyses, and wrote the paper. MSWP, RDM, and SYT contributed to the interpretation of the data and reviewed the manuscript. MSWP had primary responsibility for final content. All authors read and approved the final manuscript.

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Author details

¹Department of Human Biology, Nutrition and Toxicology Research Institute Maastricht (NUTRIM), Maastricht University, PO Box 616, 6200, MD Maastricht, The Netherlands. ²Department of Nutrition Science, Purdue University, West Lafayette, IN 47905, USA.

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