Eur J Nutr (2007) 46:1–11
DOI 10.1007/s00394-006-0623-x

ORIGINAL CONTRIBUTION

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Estimation of the metabolizable energy equivalence of dietary proteins

Abstract *Background* Protein contributes significantly to the human daily energy budget. The high diversity in composition, digestibility and dietary proportion complicates the estimation of its actual energy contribution. In practical terms we continue using the energy equivalents estimated by Atwater. This results in a persistent source of imprecision in the calculation of dietary energy that at least can be partially corrected. *Aim of the study* We used experimentally obtained data to compute an algorithm that will allow to estimate the gross energy content of a protein which composition is known. The relationship between gross energy (i.e. bomb calorimeter-derived) of protein is not a direct correlate of its metabolic efficacy as energy supplier. Thus we estimated the metabolic energy yield (i.e. ATP equivalents) of amino acid residues, using the data to compute the estimated protein metabolic energy yield. Both approaches were to be used to propose a corrected protein energy equivalence factor that will increase the precision in the calculation of dietary protein energy, especially when information on protein composition is available. *Methods* The gross energy content of amino acids was measured with a bomb-calorimeter, and compared with that of glucose. Amino acid estimated metabolizable energy yield, in moles of ATP per mol of amino acid residue, was also calculated. The net heat yield of all amino acids were used to compute the theoretical heat production of albumin, collagen, gelatin and whole rat protein, which gross energy was also measured experimentally. The mean estimated energy yield (both gross and metabolizable) for each amino acid residue were used to compute the theoretical energy of a number of dietary protein sources which composition was available in the literature. *Results* Calculated energy yield of a few selected proteins coincided with the data directly measured in the bomb calorimeter. The computed gross energy yield and metabolizable energy yield for a number of dietary protein sources was estimated. There were minor differences between both parameters: the proportion of aromatic and branched chain amino acids was the main factor affecting the gross energy yield of a given protein; conversely, the higher proportion of nitrogen, especially, but not exclusively, related to arginine and glycine content correlated with lower metabolizable energy. These parameters, corrected by the gross and metabolizable energy of glucose were used to compute a mean energy equivalence for dietary protein: 19 kJ/g protein (i.e. 4.55 kcal/g protein). This value, higher than the current Atwater factor, does not include protein digestibility (as Atwater value did), but included the cost of nitrogen excretion. *Conclusions* The methodology presented allows the approximate calculation of both the purported heat production of a protein (pure or mixture) for which we know its amino acid composition (and even get a good estimate if we only know its proportion of nitrogen), and its metabolic energy equivalence. We

Received: 15 March 2006
Accepted: 12 October 2006
Published online: 9 November 2006

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EJN 623

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also propose the use of a new energy correlate of dietary protein; this can be further tuned if the proportion of nitrogen in the protein is known, and even further if its amino acid composition is available. As a consequence of its application to dietary proteins, their energy yield may be higher than usually considered, which may influence the calculations of energy balance.

Key words protein energy – dietary protein – protein metabolism – protein energy density – calorimetry

Introduction

Protein is an essential component of diet. Dietary amino acids are released during the digestive processes, assimilated and used to sustain body protein turnover, to provide N for other molecules or, simply, as energy substrate. Under conditions of active turnover with no changes in the body protein mass, it is often assumed that the energy of the protein ingested is eventually and completely used to fuel body processes.

There are important differences between the metabolism of protein and those of carbohydrate and lipids, not only derived of the mass of substrate to process, but mainly from the complexity of the metabolic pathways implied in protein amino acid catabolism, and the need to dispose of their sulphur and, especially, nitrogen, in safe form. The latter process requires energy to detoxify the ammonia derived from amino groups, a process endeavored essentially through the purine nucleotide and urea cycles.

Amino acid metabolism is constrained by the huge tasks of maintaining full availability of all amino acids in all cells at any time, as well as the overall preservation of nitrogen and the hydrocarbon moieties of essential amino acids. In addition, the diversity of structures requires a wide diversity of pathways, which have not evolved to the high degree of energy efficiency of lipid and carbohydrate metabolism. This diversity, together with the necessarily limited flow of substrates through the amino acid pathways, results in limitations on metabolic control, essentially centred in the regulation of N disposal, and the preservation of the critical availability of some amino acids. Thus, the precise calculation of metabolic energy equivalence of dietary protein is fairly difficult, since it may depend on the efficiency of digestive and assimilative processes, the previous N and amino acid status of the organism, and the variable need for protein synthesis and other N-containing compounds. The panorama is further complicated by the variable but relevant loss of non-urea N in urine [1, 2], and by changes in the rates of oxidation of amino acids depending on the physiological phase or energy status [3]. This is compounded by our limited knowledge of the actual metabolic pathways of a number of amino acids in humans [4].

The complexity of protein structure and the lack of adequate reliable methods for the estimation of protein in composite systems (foods), and the evaluation of their energy content, results in the continuance of centuries-old methods, including the use of bomb-calorimeters, with some corrections, such as those of Atwater [5]. However, protein amino acid composition is variable, as is, too, the energy yield of amino acid residues, and, to a lesser extent, the energy output obtained from them in the bomb calorimeter.

In the present study we have analysed the relationships between amino acid energy content and the energy that we can obtain in their catabolism, as well as to check whether the widely used energy conversion factors provide an adequate information on the energy contribution of dietary protein; we intended also to derive practical applications from this relationship.

Experimental

Amino acid metabolizable energy (ATP-equivalent) yield

The estimation of the metabolic energy yield of the protein amino acids was based on their known metabolism in the mammal. All usable energy has been converted into ATP equivalents. ATP is the basic quantum of metabolic energy transfer in the cell. For that reason we have expressed here the metabolizable energy content in ATP-equivalence units. In this context, all reactions yielding ATP or a similar energy-rich nucleotide have equated to ATP. Reduced coenzymes have been translated into their most probable ATP equivalences, i.e. NADH and NADPH formation has been assumed to correspond to 3 ATP moles per mol transformed, irrespective of the compartment of the cell. Reduced flavonoid coenzymes have been assumed to yield only 2 ATP moles per mol transformed. One-carbon fragments have been assumed to be completely oxidized to carbon dioxide, and the reducing power generated has been also translated into ATP equivalents. Substrate oxidation through oxidases or oxygenases has not been considered to yield usable energy (i.e. ATP).

In all cases, it has been assumed that amino acids were used through the most common catabolic

pathways; when two or more alternative pathways coexist, the calculation has taken into account that considered most probable; the probability of alternative pathways has been introduced by taking a ponderated mean of the ATP yields through the different pathways.

For calculation purposes it has been assumed that amino nitrogen has been largely converted into ammonia through the purine–nucleotide cycle [6, 7]; all N (i.e. ammonia and amino groups) has been assumed to be disposed of as urea, and the hydrocarbon skeletons have been completely oxidized up to CO_2 and water, except where indicated otherwise (such as the excretion of an energy-containing compound, such as phenylpyruvate or creatinine). The theoretically efficient liver glutamate dehydrogenase pathway has been not taken in consideration because of the high K_M for glutamate of the liver enzyme [8], and the known postprandial inter-organ traffic of N from the peripheral tissues (i.e. muscle) to the splanchnic bed organs mainly in the form of alanine and glutamine [9, 10].

The energy costs of transport and translocation of amino acids have not been taken into account because of the intrinsic difficulties and variability of their estimation; but also because their ATP use is no different from that of any other metabolic process. Table 1 shows the ATP-equivalent yield of protein amino acids.

Amino acid bomb calorimetry

Amino acids were obtained from Sigma (St Louis, MO USA); purity was higher than 98%. All samples were dehydrated for 48 h at 65°C prior to weighing (0.5–1 g); the samples were spread on a piece of fluffy pure cellulose paper (20–30 mg) to facilitate combustion; cellulose paper energy yield was 17.22 ± 0.47 kJ/g (mean ± sem). Analyses of caloric content were done on an adiabatic bomb calorimeter (C-7000 Ika, Heitersheim, Germany); the heat of combustion of the cellulose paper used was subtracted. Analyses were carried out in triplicate, with an intrinsic variation from sample to sample in the range of 2–3%. Data for Leu and Ile ought to be equivalent because of their identical empiric formulas, but slight deviations were observed in the experimental data, probably due to impurities: 0.085 MJ/mol—i.e. 2.4% of Leu data—in the comparison between Leu and Ile.

The experimental data on amino acid calorimetry were used to determine the caloric equivalence of the amino acid residue as it stands in a protein chain. The mean molecular weight of an amino acid residue is that of the amino acid minus the water formed in the establishment of the peptide bond. When an amino acid is oxidized in the bomb calorimeter, the heat released is equivalent to that of the residue, since the difference is just water, which H is already completely oxidized. However, part of the energy has been used to vaporize the water that marks the difference between amino acid and amino acid residue. Since the heat of evaporation of water (at 100°C) is 2.26 kJ/g, we can estimate the heat of vaporization corresponding to each mole of amino acid and add this value (40.68 kJ/mol) to the bomb calorimeter-derived experimental data to obtain the combustion heat of the given amino acid residue (Table 2).

This approach was checked by oxidizing in parallel samples of Gly (Sigma), the dipeptide glycyl-glycine (Boehringer Mannheim, Germany) and the tripeptide glycyl-glycyl-glycine (Sigma); table 3 shows the results. The maximal difference between mean molar heat yields of Gly and triglycine was 1.6% of the Gly value; after correction by water vaporisation, the difference between means was reduced to 0.5%.

Since in many protein analyses, the Glu and Gln or Asp and Asn content are presented combined (i.e. Glx and Asx) because of methodological problems, and often giving the amide N as a separate entity, we estimated the "energy yield" of the amide group as the mean of the differences between Glu and Gln and Asp and Asn heat production. This figure has been included in the Tables as a separate entity.

Comparison of amino acid and glycosyl energy yields

In order to compare the data with the standard cellular energy substrate, glucose Sigma), was also subjected to the bomb calorimeter, adjusting the values for glucose residues as indicated for amino acids. The energy yield was in the same range than the classical Atwater data [5]. The bomb calorimeter analysis of glucose allowed us to introduce a factor of comparison between the individual amino acids and this main cellular fuel. Glucose metabolism generates a mean energy equivalence of 38 ATP moles per mol (Embden–Meyerhoff–Parnas pathway). Figure 1 shows the relationships between total oxidative energy produced in the bomb calorimeter by amino acids (and glucose) compared with their mean metabolic energy yield as ATP equivalents. All amino acids (and glucose) are well above the standard-conditions ATP energy-equivalence line, with arginine (contains an urea moiety, yielding no ATP) and tryptophan (most of its molecular energy content is wasted through the action of oxidases) being the amino acids with higher crude energy-ATP equivalent production relationships. For most amino acids, the efficiency in converting its crude chemical energy content into metabolizable and

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Table 1 Catabolic pathways and approximate ATP-equivalent energy yield of protein amino acids in man

AA	Flow	Pathways	ATP	Mean ATP
Gly	60	[Gly-cleavage system] → methylen-THF → methenil-THF → formate	4	**0.9**
	25	[trans to] glyoxylate → glycolate	−2.5	
	10	[via UC conj with Arg] to creatine → creatine-P → creatinine[a]	−6.5	
	5	other: excretion intact or as peptides, conjugation of xenobiotics and hormones	–	
Ala	100	[trans to] pyr → AcCoA → [KC]	15.5	**15.5**
Ser	90	[Ser-dehydratase to] pyr → AcCoA → [KC]	13	**13.0**
	5	[trans to] pyr-OH → AcCoA → [KC]	19.5	
	5	[cleavage to] Gly + 1C	7	
Cys	90	[trans + desulfuration to] pyr → AcCoA → [KC]	15.5	**14.3**
	10	other: conversion to taurine	3	
Met	100	[conjugated with ATP] → SAdMet → [de-metylation (→ 1C) to] Hcys → [conjugated with Ser] → cystationine → (pyr → AcCoA → [KC]) + oxobutyrate → propionylCoA → succ → OAA → pyr → AcCoA → [KC]	24.5	**24.5**
Thr	95	[Thr dehydratase to] oxobutyrate → propionylCoA → succ → OAA → pyr → AcCoA → [KC]	19	**19**
	5	[Thr aldolase to] Gly + acetaldehyde	18	
Asp	50	[trans to] OAA → pyr → AcCoA → [KC]	16	**16.5**
	50	[PNC or UC] fumarate → OAA → pyr → AcCoA → [KC]	17	
Asn	100	[asparaginase to] Asp	14	**14**
Glu	97	[trans to] oxoglutarate] → OAA → pyr → AcCoA → [KC]	24.5	**24.5**
	3	[decarboxylation to] γ-aminobutyrate → succ → OAA → pyr → AcCoA → [KC]	23.5	
Gln	100	[glutaminase to] Glu	23	**23**
Pro	50	[dehydrogen to] pyrrolin-carboxylate → Glu	30.5	**27.6**
	45	[oxid to] pyrrolin-carboxylate → Glu	27.5	
	5	excretion intact or as peptides	–	
Hyp	45	[dehydrogen to] pyrrolin-carboxylate[b] → erythro OH-glu → OH-oxoglutarate → (glyoxylate → glycolate) + (pyr → AcCoA → [KC])	18.5	**15.3**
	45	[oxid to] pyrrolin-carboxylate[b] → erythro OH-glu → OH-oxoglutarate → (glyoxylate → glycolate) + (pyr → AcCoA → [KC])	15.5	
	10	excretion intact or as peptides	–	
Val	100	[trans to] oxoisovalerate → isobutyrylCoA → [BO] → succCoA → succ → OAA → pyr → AcCoA → [KC]	31.5	**31.5**
Leu	100	[trans to] oxoisocaproate → [BO] → (AcCoA → [KC]) + AcAc → AcCoA → [KC]	39.5	**39.5**
Ile	100	[trans to] oxomethylvalerate → methylbutyrylCoA → [BO] → (AcCoA → [KC]) + propionylCoA → succ → OAA → pyr → AcCoA → [KC]	40.5	**40.5**
Lys	90	[conj with oxoglutarate] → saccharopine → (Glu) + oxoadipate → AcAcCoA → AcCoA → [KC]	36	**35.8**
	10	[deamination to] pipecolate → oxoadipate → AcAcCoA → AcCoA → [KC]	33.5	
His	95	[deamination to] urocanate → formiminoGlu → (1C) + Glu	20.5	**19.5**
	5	other: excretion/conversion to histamine	–	
Arg	100	[UC to] Orn → Glu-semialdehyde → pyrrolin-carboxylate → Glu	28	**28**
Trp	70	[oxid to] kynurenine (+ formate) → (Ala → pyr → AcCoA → [KC]) + oxoadipate → crotonyl-CoA → [BO] → AcCoA → [KC]	44.5	**31.3**
	30	[trans to] indol-pyruvate → indol-acetate (excreted)	0.5	
Tyr	95	[trans to] OH-phenyl-pyruvate → homogentisate → (fumarate → OAA → pyr → AcCoA → [KC]) + AcAc → AcCoA → [KC]	41.5	**39.3**
	5	other: [via dihydroxyphenylalanine. incorporation into] melanins, or catecholamines; or excretion	−3	
Phe	95	[oxid to] Tyr	38.5	**36.6**
	5	other: excretion as phenylpyruvate; synthesis of melanins or catecholamines	0.5	

The percentages of flow of each amino acid through the pathways indicated here have been assumed for a situation in which amino acid metabolism is neither depressed nor specially stimulated, i.e. corresponds to a dietary situation in which protein accounts for 10–15% of the dietary energy needs, and that there is not a deficit nor a large excess of energy. Growth, senescence, pregnancy, illness, starvation, overfeeding and metabolic derangements deeply modulate the metabolic utilization of amino acids, and, consequently may significantly alter the flow of these substrates along the described pathways

[a] The "flow" for glycine into creatinine reflects about half the daily excretion of creatinine, since we assumed that about one half of excreted creatinine may be derived from dietary creatine

[b] Alternate possibility of oxidase or dehydrogenase in the initial step (±3 ATP)

"AA" = amino acid; "flow" indicates the percentage of the available dietary amino acid that is metabolized or used through the indicated pathway; "pathways" indicate, in a succinct way, the path followed by the amino acid molecule up to excreted products; the "ATP" column indicates the estimated number of ATP moles or ATP-equivalent derived energy (i.e. reducing power translated into ATP units) per mol of amino acid metabolized through the indicated pathway; "mean ATP" represents the ponderated ATP-equivalent yield (i.e. in moles per mol of amino acid) taking into account the yield of each pathway and its "flow"

Pathways: oxid = oxidation; trans = transamination; 1C = one-carbon pool; conj = conjugation; PNC = purine–nucleotide cycle; UC = urea cycle; KC = Krebs cycle; BO = β-oxidation; OAA = oxaloacetate; pyr = pyruvate; AcCoA = acetyl-CoA; succ = succinate; AcAc = acetoacetate; succCoa = succinyl-CoA; AcAcCoA = acetoacetyl-CoA

A number of minor (albeit important) pathways has not been included because of their assumed scant quantitative significance

Table 2 Heat yield of amino acids, and calculation of their caloric equivalence in heat and ATP

AA	Residue MW	kJ/g AA	kJ/g res	MJ/mol	mol ATP/mol	mmol ATP/g res	mol ATP/MJ res	g N/g res
Gly	57.1	12.75 ± 0.13	17.49	0.999	0.9	15.8	0.90	0.245
Ala	71.1	17.87 ± 0.10	22.97	1.633	15.5	218.0	9.49	0.197
Ser	87.1	13.43 ± 0.12	16.67	1.452	13.0	149.3	8.95	0.161
Cys	103.2	18.15 ± 0.05	21.71	2.241	14.3	138.6	6.38	0.136
Met	131.2	23.62 ± 0.19	27.17	3.564	24.5	186.7	6.87	0.107
Thr	101.1	17.40 ± 0.12	20.91	2.113	19.0	187.9	8.99	0.138
Asp	115.1	11.49 ± 0.25	13.64	1.570	16.5	143.4	10.51	0.122
Asn	114.1	14.21 ± 0.10	16.81	1.917	14.0	122.7	7.30	0.245
Glu	129.1	15.19 ± 0.08	17.62	2.275	24.5	189.8	10.77	0.108
Gln	128.1	17.47 ± 0.08	20.24	2.592	23.0	179.4	8.87	0.219
Pro	97.1	23.24 ± 0.10	27.97	2.716	27.6	284.2	10.16	0.144
Hyp	113.1	19.34 ± 0.12	22.78	2.576	15.3	135.3	5.94	0.124
Val	99.2	24.67 ± 0.23	29.56	2.930	31.5	317.5	10.74	0.141
Leu	113.2	27.23 ± 0.20	31.91	3.613	39.5	348.9	10.93	0.124
Ile	113.2	26.58 ± 0.39	31.17	3.528	40.5	357.8	11.48	0.124
Lys	128.2	20.28 ± 0.33	23.45	3.006	35.8	279.3	11.91	0.218
His	137.2	20.25 ± 0.13	23.20	3.183	19.5	142.1	6.13	0.204
Arg	156.2	21.07 ± 0.01	23.76	3.711	28.0	179.3	7.54	0.359
Trp	186.2	27.55 ± 0.07	30.43	5.666	31.3	168.1	5.52	0.150
Tyr	163.2	24.23 ± 0.23	27.16	4.432	39.3	240.8	8.87	0.086
Phe	147.2	27.86 ± 0.11	31.54	4.643	36.6	248.6	7.88	0.095
Amide	44.0	2.50	2.90	0.186	−1.0	−22.7	–	0.318
Glucose	162.1	15.50 ± 0.60	17.47	2.832	38.0	234.4	13.42	0.0

"AA" = amino acid; "residue MW" = molecular weight of the amino acid residue; "kJ/g AA" = direct measured energy yield of crystalline amino acids in the bomb calorimeter in kJ per g of amino acid; "kJ/g res" = mean gross energy per g of amino acid residues in the bomb calorimeter, corrected for vaporisation of the water molecule that established the difference between amino acid and amino acid residue; "MJ/mol" = energy yield per mole of amino acids (or amino acid residues); "mmol ATP/mol" = calculated moles of ATP-equivalent metabolizable energy yield per of mole of amino acid or amino acid residue (calculated using the values derived in Table 1); "mmol ATP/g res" = calculated mmoles of ATP-equivalent metabolizable energy yield per g of amino acid residue; "mol ATP/MJ res" = calculated moles of ATP-equivalent metabolizable energy per MJ of gross energy (i.e. bomb-calorimeter-measured) of amino acid residue; "g N/g res" = grams of N contained (i.e. needing to be excreted) per gram of amino acid residue
The last row contains the data for glucose (and glycosyl residues) presented in comparable units to those of amino acids

usable energy (i.e. ATP equivalents) is lower than for glucose, but in some instances fairly close (glutamate, alanine, branched-chain, etc.) corresponding to the amino acids being metabolized essentially through pathways common to carbohydrates and lipids.

All amino acids gave ATP-equivalent values lower than glucose for a given heat production; most amino acids contained more energy per residue weight than glucose (Fig. 2). However, when the ratio of ATP-equivalent energy versus heat production were compared, only Ile, Leu and Tyr showed slightly higher values than glucose; the contribution of Gly was very low.

Table 3 Heat released in the oxidation of glycine peptides

Compound	MJ/mol	MJ/mol Gly res
Gly	0.958 ± 0.010	0.999
Gly-Gly	1.946 ± 0.011	0.994
Gly-Gly-Gly	2.899 ± 0.023	0.994

"MJ/mol" = bomb calorimeter energy yield of the compound; "MJ/mol Gly res" = energy yield of the compound per unit of Gly residue; the heat of vaporisation of water (0.041 MJ/mol) was added to the experimental data (once to Gly-Gly and twice to Gly-Gly-Gly) before dividing the results by the number of glycine residues

Direct bomb calorimetry of proteins

Using the same approach described for crystalline amino acids, samples of purified proteins: bovine serum albumin (defatted), gelatin, and bovine Achilles tendon collagen (all from Sigma) were subjected to bomb calorimetry; whole Wistar rat protein was also obtained from male rat carcasses [11], defatted with acetone, dried and analysed. In all cases, water content was estimated by differential weighing after dessication. Since we had the amino acid composition for all four proteins (ref. [12] for albumin, Sigma catalogue data for gelatin, ref. [13] for collagen, and ref. [6] for rat protein), we were able to calculate both the heat and ATP equivalent yield using our theoretical approach, and compare them with direct bomb calorimeter measurements. Table 4 shows the calculated versus measured energy yields of these proteins. Direct combustion produced practically the same heat than calculated; only small differences (maximal 1.4% overestimation for collagen and 1.4% underestimation for gelatin) were observed. The actual heat produced by the proteins tested was very similar, in the range of 22.5–23.0 kJ/g. As expected, the range of differences in ATP-equivalent yield were wider: from 172 mmol

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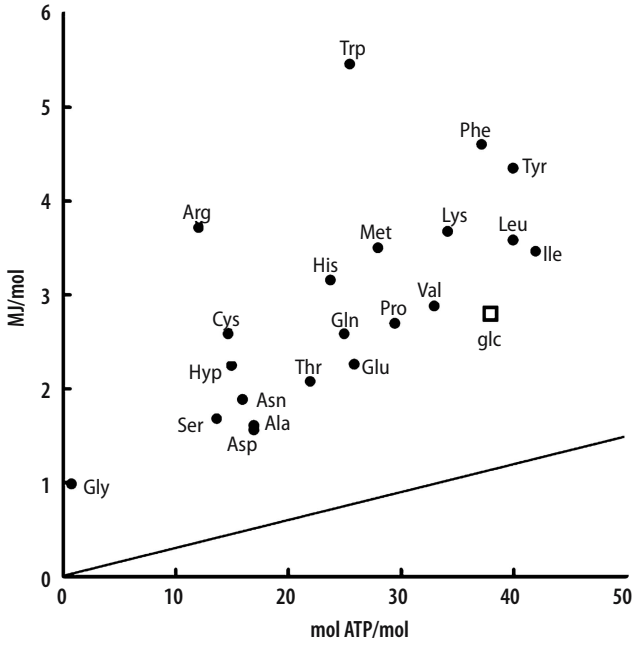

Fig. 1 Relationship between the bomb calorimeter-derived energy of amino acids and its metabolisable energy, expressed as moles of ATP equivalent per mol of the amino acid residue. The relationship for glucose has been included (square). The line shows the unity of relationship between the energy content and the energy contained in the distal phosphate bond of ATP using the standard values for that energy (i.e. 30.5 kJ/mol). These data are only an approximation, since the standard energy values for ATP depend on the conditions of the medium and this value refers to ideal conditions

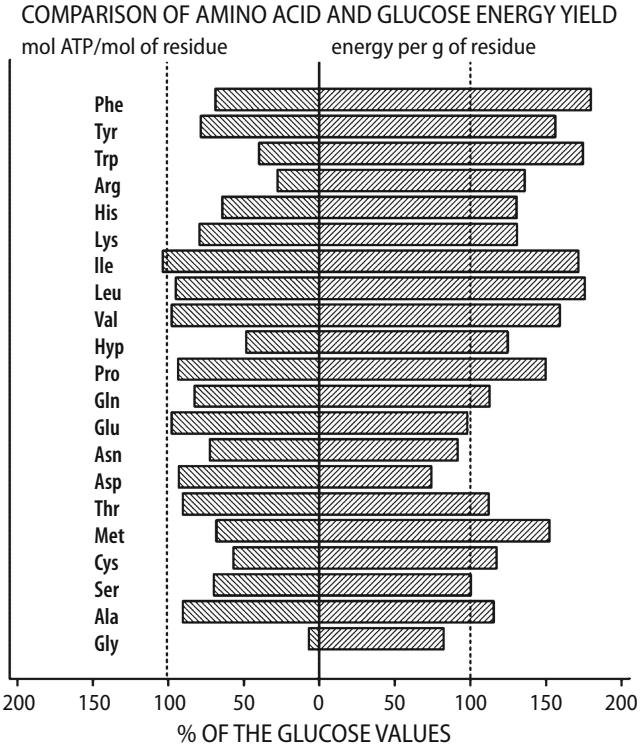

Fig. 2 Comparison of the energy yield of protein amino acid residues and that of the glycosyl residue. The left part of the graph compares the different aminoacyl residues with the glycosyl residue (100% dot line) in the moles of ATP yield per mol of residue; the right part compares the bomb calorimeter energy yield (kJ/g) of aminoacyl residues and the glycosyl residue (100% of glucose = dot line)

ATP/g in collagen to 220 mmol ATP/g in bovine serum albumin, i.e. about one fourth more. The ample difference between markedly different proteins, albumin and collagen or gelatin also translated into wide deviations from the correspondence between heat and ATP-equivalent usable energy.

Calculated calorimetry and energy yield of dietary proteins

We used bibliographic data corresponding to common foodstuffs (protein: cereals, pulses, meats, fish, egg and milk); when possible, the data were obtained from references using high numbers of replicates. All data were extracted from a FAO publication [14] summarizing the content of amino acids in food proteins as mg of amino acid per g of protein N. In any of the proteins selected, the transformation of these data into mg of amino acid residue per g of protein did not justify the whole protein weight; the reasons for this discrepancy may lie in the use of inadequate conversion factors, imprecision of the amino acid analyses, lack of measurement of some amino acids (Hyp, Trp) or amide groups (for Gln and Asn), etc. Since no complete matching sets of data were available, we decided to correct the bibliographic data to assume a 100% of the protein weight, by dividing the individual amino acid data by the fraction of protein weight justified by the amino acids listed. These data were then used for the calculation of the individual amino acids' contribution to total protein N, energy yield and ATP-equivalence metabolic energy yield (Table 5), using the factors presented in Table 2.

Nitrogen content and energy relationships

There was a relative uniformity in the nitrogen content of the widely different food proteins (Table 5), from 144 mg N/g (milk) to 174 mg N/g (cassava), i.e. a 21% (vs. milk values) span. The heat yield was more uniform, from 21.3 kJ/g (apple) to 24.0 kJ/g (cheese), i.e. a 12% (vs. apple) span. The ATP-equivalent yield ranged from 206 mmol ATP/g (apple, hazelnut) to 237 mmol ATP/g (cheese), i.e. a 15% (vs. apple and hazelnut) span. Finally, the metabolizable energy versus heat energy ratio ranged from 9.11 mmol ATP/kJ (hazelnut) to 9.88 mmol ATP/kJ (cheese), i.e. a 5%

Table 4 Direct bomb-calorimetry of proteins

	Units	Whole Wistar rat protein	Bovine serum albumin	Gelatin	Bovine collagen
Sample water content[a]	%	8.1	4.6	13.3	16.3
Measured energy[a]	kJ/g[c]	22.79 ± 0.44	22.74 ± 0.08	22.21 ± 0.10	22.26 ± 0.17
Calculated energy[b]	kJ/g[c]	22.57	22.95	22.52	21.94
Difference between measured vs. calculated energy[b]	% of measured	−0.97	0.92	1.40	−1.44
Estimated metabolic energy equivalence[b]	mmol ATP/g[c]	199.7	220.1	186.5	172.9
	molA TP/MJ	8.84	9.59	8.28	7.88
Estimated N content[b]	mg/g[c]	0.165	0.157	0.180	0.186

[a] Experimental data; heat yield data were corrected by sample water content; [b] calculated data; [c] dry weight

(vs. cheese) span. The differences were smaller than those of N content, fairly in the range of the method-related variability. When the mmol ATP/kJ data were correlated with protein N, a significant direct relationship was observed (Fig. 3); i.e. proteins with lower relative N content had a higher ATP-equivalence energy yield.

We compared the amino acid composition (in eight metabolic and structurally related families of amino acids) of the proteins with the proteins' energy or ATP-equivalent yields. Branched chain and aromatic amino acids, plus [Met+Thr] were the main elements responsible for higher gross energy yields; ATP-equivalent production was related to the content of most groups of amino acids (negatively to Gly and Arg), with a neutral effect for the most directly implicated in 3C [Ala+Ser+Cys] metabolism or Krebs Cycle intermediaries [Glu+Asp+Pro] (Table 6).

Table 5 Calculated nitrogen content, gross oxidative energy and metabolizable energy yield of food proteins

Protein source	kJ/g prot	mmol ATP/g prot	mmol ATP/kJ	Efficiency ratio	Corrected kJ/g prot	mg N/g prot
Cow's milk cheese protein (95%)	24.0	237	9.88	0.86	20.7	145
Corn protein (85%)	23.9	229	9.58	0.84	19.9	148
Spinach leaves' protein (87%)	23.7	225	9.48	0.83	19.6	156
Whole cow's milk protein (93%)	23.6	232	9.81	0.86	20.2	144
Whole hen's egg protein (87%)	23.5	224	9.54	0.83	19.5	154
Potato protein (66%)	23.5	222	9.44	0.82	19.4	159
Beef meat protein (85%)	23.3	219	9.38	0.82	19.1	162
Pilchard-like fish protein (92%)	23.2	223	9.61	0.84	19.4	158
Lamb flesh protein (84%)	23.2	217	9.37	0.82	18.9	162
Pork protein (99%)	23.1	224	9.70	0.85	19.5	159
Chicken meat protein (78%)	23.1	219	9.45	0.82	19.1	157
Tuna-like fish protein (80%)	23.0	220	9.58	0.84	19.2	159
Mollusk protein (85%)	23.0	217	9.46	0.83	19.0	160
Cabbage protein (60%)	23.0	210	9.16	0.80	18.3	173
Haddock-like fish protein (95%)	22.9	222	9.68	0.84	19.3	159
Chick-pea protein (83%)	22.9	216	9.41	0.82	18.8	167
Polished rice protein (83%)	22.8	215	9.41	0.82	18.7	156
Crustacean's protein (91%)	22.8	214	9.40	0.82	18.7	165
Pea protein (80%)	22.7	216	9.54	0.83	18.8	165
Soybean protein (85%)	22.7	216	9.50	0.83	18.8	156
Lentil protein (85%)	22.7	215	9.47	0.83	18.8	165
Sesame protein (80%)	22.7	208	9.19	0.80	18.2	167
Bean protein (77%)	22.6	217	9.59	0.84	18.9	156
Wheat protein (82%)	22.6	217	9.58	0.84	18.9	145
Hazelnut protein (88%)	22.6	206	9.11	0.79	18.0	173
Cassava protein (63%)	22.4	208	9.27	0.81	18.1	174
Carrot protein (66%)	21.6	209	9.68	0.84	18.2	150
Apple protein (71%)	21.3	206	9.65	0.84	17.9	150

These values were obtained by applying the factors of Table 2 to the amino acid composition of published food protein data (reference [14]). The figure in parenthesis indicates the percentage of total protein justified by the list of amino acids given in the references

"kJ/g prot" = calculated gross energy yield per unit of protein weight; "mmol ATP/g prot" = calculated metabolizable energy yield in ATP-equivalents per unit of protein weight; "mmol ATP/kJ" = ratio of calculated gross energy vs. metabolizable energy; "efficiency ratio" = (vs. glycosyl residue ATP-derived energy yield) see the text for further explanations; "corrected kJ/g prot" = gross energy yield multiplied by the efficiency ratio, yielding a "corrected" energy value in relation to the metabolizable energy derived from glycosyl residues; "mg N/g prot" = N content of the protein in a weight basis

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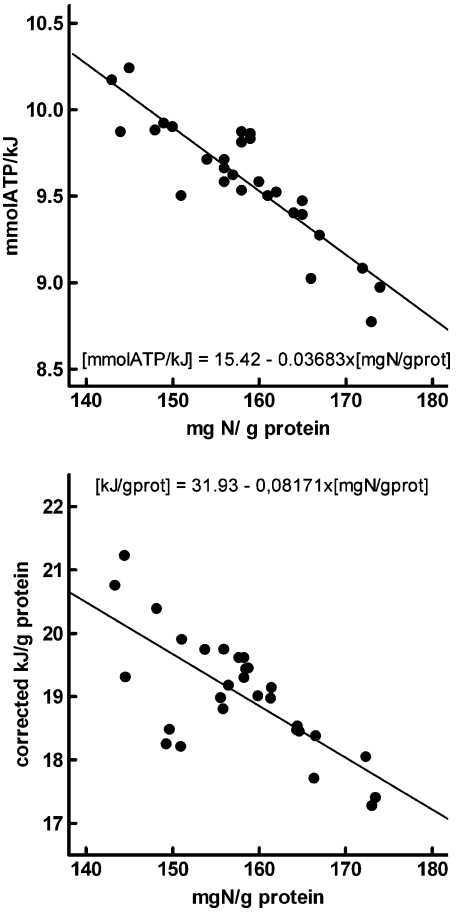

Fig. 3 Relationship between the metabolisable energy yield of dietary proteins and their N content. Upper panel: comparison of molATP/MJ and mg N/g protein; the line depicts the linear correlation equation, which formula is given; the data were fit with $r^2 = 0.659$, $P < 0.0001$. Lower panel: comparison of corrected kJ/g protein and mg N/g protein; the line depicts the linear correlation equation, which formula is given; the data were fit with $r^2 = 0.364$, $P = 0.0005$

Protein metabolizable energy equivalence

The remarkable similitude in the calculated data for widely different diet proteins favor the applicability of the mean values obtained from this set of proteins for additional comparisons between oxidizable substrates. Thus, the comparison of the experimental values for proteins with the values obtained with the glycosyl residues: 17.5 kJ/g and 234 mmol ATP/g values (i.e. 13.4 mmol ATP/kJ), show that dietary proteins yield more heat than starchy polysaccharides, but energy was much lower when only metabolizable energy is considered.

By taking the ATP-equivalent vs. total energy ratio of glucose as the "standard" metabolic relationship, we compared the equivalent values for the different proteins and derived the "efficiency ratio" values of protein metabolic use (Table 5). Applying this same approach, the calculated energy yield of the proteins can be "corrected" by the efficiency ratio to obtain a much more credible approach to the actual usable energy derived from these proteins, i.e. the "corrected kJ/g of protein" (Table 5). This figure is an inverse correlate of the N content of the protein and is influenced by amino acid composition of the protein (Fig. 3).

The mean value for protein energy content was 22.94 ± 0.11 kJ/g protein (i.e. 5.49 ± 0.03 kcal/g protein); when corrected for ATP-energy equivalence and compared with the glycosyl residues, the value was 19.00 ± 0.17 kJ/g protein (i.e. 4.55 ± 0.04 kcal/g protein), i.e. a metabolic efficiency of 0.828 ± 0.006.

Discussion

The results obtained in the calorimetry of glycine peptides, and the similarity of results obtained with

Table 6 Relationships between protein energy content and amino acid composition

Parameter		Relationship and *P* values for linear correlation					
		kJ/g prot		mmol ATP/g prot		mmol ATP/kJ	
% of N in the amino acid group	Phe + Tyr + Trp	+	<0.0001	+	0.0001	–	NS
	Met + Thr	+	0.0110	+	0.0022	+	0.0475
	Glu + Gln + Asp + Asn + Pro	–	NS	–	NS	+	0.0027
	Val + Leu + Ile	+	<0.0001	+	<0.0001	+	0.0010
	Ala + Ser + Cys	–	NS	–	NS	–	NS
	Lys + His	–	NS	+	0.0408	+	0.0136
	Gly	–	NS	–	0.0010	–	<0.0001
	Arg	–	NS	–	0.0001	–	<0.0001
mg N/g protein		–	NS	–	0.0005	–	<0.0001
kJ/g protein				+	<0.0001	–	NS

The data are the linear correlation *P* values for the comparison of the energy content of the proteins (i.e. data for bomb calorimeter measurements, ATP-equivalent yield calculations and their ratio) vs. the distribution of N in amino acid groups and the overall N content of the protein. A positive sign (+) indicates a direct relationship; a negative sign (–) indicates an inverse relationship. The data were derived from the composition of the proteins listed in Table 4

NS = $P > 0.05$

proteins through direct calorimetry or calculation confirm the applicability of the proposed algorithms.

The wide differences in metabolic usage of the hydrocarbon skeletons of amino acids and their ability to produce heat in the bomb calorimeter are the key issue in the evaluation of the biological usefulness of dietary protein. We have proposed here a slight improvement in the way to deal with the energy translation of protein, based on some of its chemical properties. Protein-derived energy is a source of trouble for the dietitian or nutritionist, since, when considering the content of protein in the diet, its eventual utilization by the organism is conditioned by its state of need/surplus of amino N, specific essential amino acid needs and overall energy availability [15, 16]. In addition, the concentration of protein in the diet determines the fate of a large part of absorbed protein in the intestine by increasing the operation of the intestine-liver split urea cycle, irrespective of the overall availability of N [17, 18], as well as changes in appetite/satiation affecting energy intake [19, 20]. To further compound the problem, the digestibility of the protein is often modified by heat transformations and other reactions affecting the integrity of amino acids [21]. The absorption of amino acids is also affected by the intestinal biota, which further transforms, uses, and often destroys, amino acids for nutritional purposes [22]. And, last but not least, the fate of the absorbed amino acids is highly dependent on the availability of energy substrates such as glucose [23].

It is difficult to establish reliable energy correlates for protein, since there is a shortage of complete amino acid analyses: most databases contain only partial protein amino acid analyses, lacking critical data such as amide, Pro and Trp content, and have been computed using widely different—and often conflicting—methodological approaches. This, and the complexity, diversity and largely unknown regulation of mammal amino acid metabolism, have severely limited the study of proteins as suppliers of energy other than as providers of essential amino acids. As a consequence, most nutritionists tend to essentially rely on the century-old seminal work of Atwater [5, 24, 25], which centred his corrections on the derivation of protein N to urea molecules, with its corresponding drainage of energy to be discounted from the direct bomb calorimeter data for protein. The heat of combustion for protein (essentially high quality animal protein) found by Atwater was 23.6 kJ/g (i.e. 5.65 kcal/g), to which he introduced two "corrections": (a) the loss during the digestive process of about 8%, and (b) 5.22 kJ/g protein as the cost of urinary urea N loss [5]. In the present approach, the difference between raw heat production and corrected energy was 3.9 ± 0.2 kJ/g protein (i.e. 0.94 kcal/g protein), 24.3% lower than the estimate of Atwater. The final corrected Atwater energy equivalence for protein was 4.05 kcal/g protein (i.e. 16.9 kJ/g protein).

Our data were derived from a wider range of proteins than Atwater's, including cereals, nuts, fruits and pulses, which resulted in a mean value of 22.9 kJ/g protein (ranging from 21.3 to 24.0 kJ/g protein, Table 5), i.e. a 3% lower bomb-calorimeter figure. This initial difference tends to buffer the impact of the differences observed in the estimation of the cost of nitrogen excretion; this way, the N excretion-corrected Atwater value of 18.4 kJ/g protein was only a 3.2% lower than our derived value, 19.0 kJ/g protein. However, if we apply our mean correction for N excretion to the Atwater's direct energy density of 23.6 kJ/g protein, we obtain a value of 19.7 kJ/g protein, i.e. 7.1% higher than the Atwater corresponding value.

We did not include the digestibility of protein as a specific fixed factor because of the wide variations in digestibility of proteins from different sources [26]. We found more appropriate to correct the protein energy data in a given study by the most suitable (optimally, by experimentally derived) digestibility factor, instead of using a single value for all proteins. However, if we applied to our data the 8% "digestibility" correction of Atwater, we would obtain an energy density of 17.5 kJ/g protein, (3.5% higher than Atwater's).

Indirect calorimetric analyses of protein energy [27] produced energy density values of 19.7 kJ/g, fairly close to our 19.0 kJ/g; this was obtained—as ours—not factoring the digestibility of protein. This result was thus comparable to an Atwater's "uncorrected" factor of 18.4 kJ/g, and was identical to the 19.7 kJ/g protein value we obtained by applying our calculation procedure to Atwater's direct heat data. The main difference in both sets of data lies on the evaluation of the energy cost of N excretion. Atwater's simplified the approach considering that all protein N was converted into urea, but we excrete protein N not only as urea, but also as ammonia and several other N-containing compounds; it has been even postulated that we produce nitrogen gas [28]. Our approach shows that the cost of disposal for an amido N is different from an amino N, and this further depends on the amino acid (i.e. Ser and Ala); other amino N is incorporated into nucleic acids, porphyrins, creatine, etc. and follow long, complex and often poor-energy profitable pathways. The individual amino acid computation of the fate of N allows for a more precise estimation of the costs of N excretion.

It is evident that we are dealing with mean values throughout, since we have found that the energy yield is dependent on protein amino acid composition and nitrogen content, obviously modified by digestibility.

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The proportion of aromatic and branched chain amino acids is the main factor affecting the energy yield of a given protein; conversely, the higher proportion of N, especially, but not exclusively, related to Arg and Gly content correlated with a lower metabolizable energy yield. The high correlation between "corrected" energy yield and the proportion of N allows for a fine tuning of the energy equivalent of dietary proteins of known N content. In any case, knowing the amino acid composition of the proteins may be used to better define the protein metabolizable energy by using the algorithms described.

The question of different handling of amino acids under different dietary protein loads or physiological (and pathological) situations is a further complication for this set of calculations (and for any other system of estimation of protein energy yield), since the multiplicity of amino acid degradative pathways and the options for amino N handling may slightly modify the overall energy yield of protein. The costs of absorption and transport may somewhat diminish our estimations of usable energy, but this may be partly counteracted by the fixed assumption of ATP yield of reducing power we used. The adjustments we introduced allow for a realistic estimation of the energy yield of protein amino acids, and, at least, help us to understand the sources of the energy derived from protein metabolism.

All in all, we believe that the introduction of metabolic parameters in addition to raw heat production analysis and overall chemical considerations on the fate of nitrogen should be addressed, even if the bank of data available is not sufficient yet to fine-tune the derivation of more precise correlations between dietary protein and energy availability from this substrate. Protein N content affected not only the metabolizable energy yield (which spurred the Atwater and others corrections for urea excretion) but also the direct heat produced in their bomb-calorimeter oxidation.

The modifications we postulate in the estimation of protein metabolizable energy yield may influence our estimations of energy balance when factoring proteins in. We found a slightly higher metabolizable energy proportion than that usually used for calculations. However, this is not the sole finding of our study, we consider necessary to introduce as many—known—factors as possible in the calculations in order to get a more precise estimation of protein energy yield. This starts with the question of digestibility, we can substitute gross estimations for data more adapted to the system we want to study, second, we can introduce the amino acid composition (or at least N content) if known, and thus the final data will be much more precise. In the absence of these additional information we can apply the factors we introduced here.

The application of the factors postulated here may result in higher estimations of protein energy content, and, especially, yield, in the calculations of energy balance. In any case it should be kept in mind that protein's structural and chemical complexity extends to its use as energy source, which contrasts with oversimplification of its energy yield by taking "protein" as a homogeneous energy source comparable to carbohydrate (starches) or lipid (fats).

The methodology we have presented here suggests that we can roughly and differentially calculate both the purported heat production of a protein (pure or mixture) for which we know its amino acid composition (and even get a good estimate if we only know its N proportion), and its metabolic energy equivalence or significance, a more contemporary and informative approach than the genial and lasting contribution of Atwater. We also propose the use of a new energy correlate of dietary protein: 19 kJ/g protein (i.e. 4.55 kcal/g protein), not including its digestibility; this can be further tuned if the N proportion in the protein is known and even further if its amino acid composition is known.

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