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Assessment of protein adequacy in developing countries: quality matters

Shibani Ghosh[1,2], Devika Suri[2] and Ricardo Uauy[2,3]*

[1]*Friedman School of Nutrition Science and Policy, Tufts University, 150 Harrison Avenue, Boston, MA 02111, USA*
[2]*Nevin Scrimshaw International Nutrition Foundation, 150 Harrison Avenue, Room 232, Boston, MA 02111, USA*
[3]*Institute of Nutrition and Food Technology (INTA), University of Chile, Santiago, Chile*

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Abstract

Dietary protein and amino acid requirement recommendations for normal "healthy" children and adults have varied considerably with 2007 FAO/WHO protein requirement estimates for children lower, but dietary essential AA requirements for adults more than doubled. Requirement estimates as presented do not account for common living conditions, which are prevalent in developing countries such as energy deficit, infection burden and added functional demands for protein and AAs. This study examined the effect of adjusting total dietary protein for quality and digestibility (PDCAAS) and of correcting current protein and AA requirements for the effect of infection and a mild energy deficit to estimate utilizable protein (total protein corrected for biological value and digestibility) and the risk/prevalence of protein inadequacy. The relationship between utilizable protein/prevalence of protein inadequacy and stunting across regions and countries was examined. Data sources ($n = 116$ countries) included FAO FBS (food supply), UNICEF (stunting prevalence), UNDP (GDP) and UNSTATS (IMR) and USDA nutrient tables. Statistical analyses included Pearson correlations, paired-sample/non-parametric t-tests and linear regression. Statistically significant differences were observed in risk/prevalence estimates of protein inadequacy using total protein and the current protein requirements versus utilizable protein and the adjusted protein requirements for all regions ($p < 0{\cdot}05$). Total protein, utilizable protein, GDP per capita and total energy were each highly correlated with the prevalence of stunting. Energy, protein and utilizable protein availability were independently and negatively associated with stunting ($p < 0{\cdot}001$), explaining 41 %, 34 % and 40 % of variation respectively. Controlling for energy, total protein was not a statistically significant factor but utilizable protein remained significant explaining ~ 45 % of the variance ($p = 0{\cdot}017$). Dietary utilizable protein provides a better index of population impact of risk/prevalence of protein inadequacy than crude protein intake. We conclude that the increased demand for protein due to infections and mild to moderate energy deficits, should be appropriately considered in assessing needs of populations where those conditions still prevail.

Key words: **Protein quality: protein inadequacy: stunting: PDCAAS**

Introduction

Protein and amino acid requirement recommendations for "healthy" infants, children and adults have varied considerably over the years(1). The most recent FAO/WHO report provides estimates of protein requirements that are lower than previously established for adults and children. However the relative importance of protein quality is greater since estimates of the dietary essential amino acid requirements are twice the previous recommendations with lysine requirement estimates having increased 2·5 times from 12 mg/kg body weight to 30 mg/kg body weight in adults(1). In children, the estimated dietary essential amino acid requirements are only slightly lower (94 % of previous estimate for lysine).

In environments where individuals have persistent or repeated infections and impaired intestinal absorptive capacity, there is an increased demand for protein, despite the absence of overt clinical symptoms(1,2). Thus in vulnerable populations such as women and children commonly affected by acute and chronic infections, protein and amino acid needs are likely to be greater. Growth rates (linear and ponderal) are likely to be affected by repeated infections (bacterial and parasitic) with long-term functional deficits compromising learning and adult productivity(2,3).

Interactions between energy deficit and protein needs have been documented by determining nitrogen balance and these studies have been previously reported(4). Energy imbalance below and above energy needs affects body nitrogen equilibrium. At a protein intake of 0·57 g/kg body weight, N equilibrium is achieved if energy intake is ~10 % above that required for balance(5,6). Conversely people in energy deficit will need additional protein, considering that even a modest energy deficit of 5 % increases protein needs by about 10 %(7). Thus, beyond the role of protein in the maintenance of nitrogen equilibrium, commonly accepted as the criterion

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* **Corresponding author:** Dr Ricardo Uauy, fax +1 617 636 3771, email Shibani.ghosh@tufts.edu

for sufficiency, mild energy deficit and infections place additional demands on body nitrogen.

The operational definition of what is a sufficient amount of dietary protein has up to now been based mostly on body N balance; thus this is the method used to define requirements and establish recommendations. However there are multiple other roles that amino acids and protein play which impact health and wellbeing. Other possible roles such as the role of amino acids in up-regulating growth hormone and IGF-1 secretion thus driving anabolism and linear growth could lead to estimates of protein requirements that differ from those established based on the need for N equilibrium and the fractional daily N accretion related to body growth. There is now evidence indicating that both protein quantity and quality affect growth hormone release and thus have the potential for modulating linear growth. Protein intake in infancy has been shown to stimulate early growth and is also associated with body weight and length at later stages of life(8). Protein intake at 9 months of age has also been positively associated with height and weight but not percentage of body fat at 10 years of age(8). Currently, there is no consideration given to the potential role of specific dietary essential and non-essential amino acids in defining hormonal responses associated with linear growth, nor are other organ-specific functional needs related to protein and amino acids, reflected in the estimation of protein requirements.

The evaluation of protein quality has been discussed extensively in several key FAO/WHO policy documents(1,9). While different methods exist for evaluating protein quality, the currently accepted method is the protein digestibility-corrected amino acid score (PDCAAS). The PDCAAS method assesses the quality of protein of mixed diets and/or mixed formulations based on the digestibility of the protein sources and the essential amino acid composition thus giving an estimate of "utilizable protein". Diets that have the right amino acid composition (matching the reference protein) have a high score and if the source(s) are well digested the protein is considered of high quality. Foods of animal origin are likely to have total protein and utilizable protein values higher and closer to each other compared to diets that have a low content of essential amino acids and that are poorly digested, such as cereals and other grains(1,9). The PDCAAS method can be used for evaluating food products (e.g. cereal-legume mixtures), for evaluating overall protein quality of mixed diets (individual) and evaluating the per capita availability and accessibility of good quality protein at the country/global level. While amino acid scoring methods have been used for evaluating protein quality at country and global level, the use of PDCAAS to determine the effect of digestibility and the amino acid composition of per capita availability of protein quality has not been examined extensively. The evaluation of protein quality and quantity for growth (specifically linear growth) has been examined mainly in developed country populations, but not sufficiently in developing country settings. Thus the aim of our analysis was the evaluation of the impact of applying the PDCAAS method correcting for protein quality to assess adequacy at the level of the national food supply, examining its relationship with the corresponding national data on linear growth. The underlying hypothesis proposed by our analysis was that linear growth retardation (stunting) would be more strongly related with available protein corrected for quality by PDCAAS, and also better related to the estimated risk/prevalence of protein inadequacy if protein requirements were adjusted for mild energy deficit and for the prevalence of infections, factors prevalent in developing countries that are known to increase protein needs. The latest WHO report on protein and amino acid recommendations(1) noted that there was very little work assessing the protein requirements in children and adult populations with high disease burden. Furthermore, given the high levels of stunting and the early onset of stunting, such an analysis would also allow for the development of testable hypotheses on the potential impact of protein quality on linear growth of young children in developing countries.

Experimental methods

Data sources

National level data from 180 countries, used to estimate total protein and utilizable protein supply and to estimate the risk of protein inadequacy, were obtained from food balance sheets (FBS) from the Food and Agriculture Organization of the United Nations (FAO) for the year 2005 using methods previously described(10–12). The FBS provide estimates of per capita supplies of specific food items available for human consumption in a given year and a given country. Per capita supplies of each listed commodity available for food consumption (i.e. supply) are the sum of domestic production, stocks, and imports minus exports and amounts used for feed, seed, processing, and other purposes.

To assess the relationship between protein quality and linear growth, national level data were acquired for moderate to severe stunting ($n = 116$) from UNICEF(13). The indicator "Moderate to Severe Stunting" (referred to as "stunting") is defined by UNICEF as the percentage of children under five years of age in a particular country who fall below -2 and -3 standard deviations for height-for-age z-score. Gross domestic product (GDP) data were extracted for the same 116 countries from the United Nations Development Programme (UNDP) Human Development Report(14). The regional breakdown included 17 countries in East and Southern Africa, 23 in West and Central Africa, 2 in Oceania, 6 in South East Asia, 7 in South Asia, 23 in Latin America and the Caribbean, 3 in Eastern Asia, 16 in North Africa and the Middle East, 12 in Eastern Europe, 5 in Central Asia and 2 in North America (with prevalence estimates of stunting and wasting). Data on Infant Mortality Rates (IMR) were obtained from the United Nations Statistics division that compiles data on such indicators(15).

Nutrient database development

The nutrient database was developed for FAO FBS food commodity categories using USDA food composition tables(16). Protein digestibility values for each food item were obtained from FAO/WHO guidelines on protein quality evaluation as well as specific digestibility studies(9,17–22).

Data calculations and analysis

Utilizable protein and Prevalence of Protein Inadequacy: Based on Current Requirements. Utilizable protein was calculated using the Protein Digestibility Corrected Amino Acid Score (PDCAAS) method described by WHO(1) and compared with WHO (2007) protein requirements to allow estimation of the adequacy of available dietary protein in each country or region. For the FAO FBS data, requirement estimates pertaining to the adult for total energy, total protein and amino acids (total requirement and the amino acid reference pattern) were assumed and related to the entire population. The requirement values used were 0·66 g/kg body weight for utilizable protein, 2525 kcal total energy calculated for a moderately active adult (PAL 1·75, average for moderately active adult male and female) and the amino acid reference pattern for lysine (30 mg/kg body weight/day), tryptophan (4 mg/kg body weight/day), sulphur amino acids (SAA) (15 mg/kg body weight/day) and threonine (15 mg/kg body weight/day)(1,23). Risk of protein inadequacy at the country level was computed using the protein requirement of a 60 kg adult, considering a 25 % coefficient of variation in "intake" (supply) and assuming that quality protein would be uniformly distributed per person(24,25).

Utilizable Protein and Prevalence of Protein Inadequacy: Corrected Requirements. Given the high rates of disease and infections in most developing countries, we examined the effect of correcting current estimates of protein requirements to account for energy deficit as well as for the increased need for protein for infectious episodes as well as for the period of recovery post-infection. On the basis of data taken from the studies of Garza *et al.*(5) and Kishi *et al.*(7) in adults, a 10 % increase per day was added for energy deficit (assuming a moderate deficit in energy). This was applied across all countries.

To calculate an additional dietary protein need due to infection, countries were ranked in tertiles of infant mortality rates (IMR), as a proxy for the burden of infection in early life(26–29). Infections in early life were assumed to be primarily respiratory and diarrhoeal in nature as these are the main causes of death and disability in developing country children(30). Daily protein requirements are augmented a further 10 % during each day of illness(31,32), assuming 7 days per episode (with 5 days ill and 2 days of recovery) and 8 episodes per year for the highest IMR tertile(33), 5·3 for the middle and 2·7 for the lowest tertile. To find the increase in daily protein needs due to infection over one year, a weighted average of the protein requirements on total days ill and not ill over one year was calculated. Combined with the 10 % increased protein needs for moderate energy deficit, the final figure for each IMR tertile represented the new daily protein needs accounting for both infection and energy deficit. Estimates of the above calculation and resulting adjusted requirements are presented in Table 1. These new requirement estimates based on adjustments for energy deficit and infection were used in place of the standard adult protein requirement estimate of 0·66 g/kg, in the calculation of prevalence of protein inadequacy for each country.

Statistical analysis

All data calculation and analyses were conducted in Statistical Analysis Systems statistical software package version 9.2 (SAS Institute, Cary, NC, USA), SPSS version 15.0 (SPSS, Inc, Chicago, IL, USA) and Microsoft Excel 2007 (Microsoft Corp, Redmond, WA, USA). Nutrient calculation and estimation of PDCAAS was conducted in SAS while prevalence of inadequacy calculations in MS Excel. All statistical analyses were conducted in SPSS. Statistical analyses and tests included descriptive statistics, frequency analyses, non-parametric Chi-square, independent sample tests and linear regression analyses. Significance was set at the 0·05 level.

Results

Estimates of per capita dietary energy, utilizable protein, percentage of stunting and prevalence of protein inadequacy are presented in Table 2 by region. Total energy supply per capita per day ranged from 2323 ± 398 kcal in East and South Africa to as high as 4017 ± 686 kcal in North America. Total protein values ranged from 60 ± 14 g/capita/day to over 120 ± 23 g/capita/day. While there was a general trend of increasing total energy and total protein, in the case of Eastern Asia despite lower energy availability (2360 ± 279 kcal), total protein levels were 84 ± 17·5 g/capita/day. Stunting prevalence ranged from 36·5 ± 10·6 % in Oceania (countries of Comoros and Sao Tome and Principe) to 9·5 ± 12·0 % in North America while wasting ranged from 12·6 ± 2·4 % in South Asia to

Table 1. Calculation of increased protein needs due to infection and moderate energy deficit, by IMR tertile (infant mortality rate per 1000 births) for 115 countries

	Tertiles of IMR		
	Lowest (n = 39)	Middle (n = 38)	Highest (n = 38)
IMR (mean ± SD)	16·1 ± 5·5	42·1 ± 12·5	90·3 ± 19·4
Normal adult daily protein requirement g/kg	0·66	0·66	0·66
10 % additional protein needs from moderate energy deficiency	0·066	0·066	0·066
Estimated episodes of infection per year	2·7	5·3	8
Total days ill per year (Duration of illness assumes 5 days infection, 2 days recovery)	18·9	37·1	56
Weighted average increased daily protein needs due to infection (10 % increase per day ill) g/kg	0·004	0·007	0·011
New adult daily protein requirement estimate g/kg, accounting for energy deficit and infection	0·73	0·73	0·74
Total percent increased daily protein needs due to infection and moderate energy deficiency	10·6 %	11·1 %	11·7 %

Table 2. Supply per capita per day of energy, protein and utilizable protein, prevalence of stunting and wasting, Gross Domestic Product (GDP) by regions

Region	N	Energy (kcal/capita/ day) *Mean* ± *SD*	Total protein (g/capita/day) *Mean* ± *SD*	Utilizable protein (g/capita/day) *Mean* ± *SD*	Prevalence of Stunting[1] (%) *Mean* ± *SD*	Prevalence of Wasting[1] (%) *Mean* ± *SD*	Gross Domestic Product (US $ per capita/yr) *Mean* ± *SD*
East and Southern Africa	17	2323 ± 398	60·6 ± 14·0	50·1 ± 13·8	34·7 ± 12·1	7·0 ± 4·7	3449 ± 4233
West and Central Africa	23	2431 ± 410	62·1 ± 19·4	51·3 ± 16·3	32·7 ± 8·5	9·3 ± 3·7	2113 ± 3125
Oceania	2	2523 ± 721	59·0 ± 15·2	52·2 ± 13·4	36·5 ± 10·6	6·0 ± 2·8	1391 ± 350
South East Asia	6	2491 ± 183	64·6 ± 5·5	57·9 ± 5·1	35·0 ± 13·2	10·2 ± 4·4	3138 ± 2605
South Asia	7	2590 ± 261	73·6 ± 7·0	64·9 ± 35·3	35·4 ± 12·9	12·6 ± 2·4	2555 ± 1663
Latin America and the Caribbean	23	2809 ± 430	77·0 ± 17·2	68·8 ± 15·8	15·0 ± 11·1	2·5 ± 2·5	8107 ± 4881
Eastern Asia	3	2360 ± 279	84·0 ± 17·5	74·9 ± 16·9	23·7 ± 11·9	5·0 ± 2·8	4310 ± 1518
North Africa and Middle East	16	3124 ± 493	95·3 ± 15·6	82·9 ± 13·5	19·6 ± 11·9	6·9 ± 4·8	14154 ± 16044
Eastern Europe	12	3162 ± 395	99·0 ± 12·8	88·1 ± 11·8	10·8 ± 8·4	3·8 ± 2·9	8742 ± 4003
Central Asia	5	3084 ± 410	106·5 ± 15·1	91·3 ± 18·8	22·8 ± 9·3	4·6 ± 2·1	4400 ± 3831
North America	2	4017 ± 686	121·9 ± 23·2	110·0 ± 22·7	9·5 ± 12·0	4·0 ± 2·8	29848 ± 22265

[1] Defined as the percentage of children under five years of age in a particular country who fall below −2 standard deviations for height-for-age (stunting) or weight-for-height(wasting) z-score

4·0 ± 2·8 % in North America. Protein requirements (g/kg body weight) were adjusted as described above. Figure 1 depicts the extra needs imposed by infection and moderate energy deficit as aggregated by IMR (Infant Mortality rate) tertile ($n = 115$ countries). The adjusted protein need for an adult in a given country was 0·73 (both low and middle IMR) and 0·74 g/kg (for countries with high IMR), respectively (Fig. 1).

Risk of Protein Inadequacy

Risk of protein inadequacy was calculated using the estimates of total protein and utilizable protein (Fig. 2) both compared to the current estimates of requirements (Fig. 2 lines a, b) and utilizable protein compared to the revised requirements by country (Fig. 2 line c). Prevalence of inadequacy ranged from 0·7 ± 0·8 % in North America to 37·2 ± 23 % in East and Southern Africa. It was the highest in East and Southern Africa (irrespective of type of protein and requirement used) and the lowest in North America. Correction of prevalence using utilizable protein (line b) increased the inadequacy estimates for East and Southern Africa, West and Central Africa, Oceania, South Asia and South East Asia and to a lesser extent in Latin America and the Caribbean. As can be seen from Fig. 2 (line c), inadequacy estimates based on utilizable protein and requirements adjusted for infection and energy deficit were significantly higher than the estimates based on total protein and utilizable protein that were compared to the current requirements ($p < 0{\cdot}05$).

Examining changes in estimates at the country level, countries with less than 2000 kcal/capita/day of energy supply, show a change in estimates in risk of protein inadequacy from 20–80 % (Eritrea) to 40–90 % (Guinea Bissau) (Fig. 3) (going from total protein and current requirements to utilizable protein and revised requirements). Estimates in the Congo (Democratic Republic) are high to begin with and signify very low levels of total protein in the diet. In countries with 2000–2500 kcal energy supply/capita/day (Fig. 4 a and b), risk estimates of protein inadequacy using total protein and current estimates of requirement range from 60 % (Liberia) to 10 % (Djibouti) in Sub Saharan Africa and 10 % (India) to 5 % (Thailand) in South Asia and South East Asia. On the basis of total protein and the current requirement estimates alone, countries in South Asia and South East Asia have relatively low risk estimates of protein inadequacy. Correcting for quality (utilizable protein) and needs (adjusted requirements) changes the prevalence in Sub Saharan Africa to 90 % (Liberia) and almost 40 % (Djibouti) while in South Asia and South East Asia, risk of protein inadequacy changes to 45 % (India) to about 15 % (Thailand). Examining countries with total energy intakes ranging from 2500–3000 kcal/capita/day, similar changes are observed. In countries like Burkina Faso and Niger, a very low prevalence (less than 2 %) changes to 20 % (Burkina Faso) and about 15 % (Niger) on using the figures for utilizable protein and adjusted requirements (Fig. 5a). This is also observed in South Asia in the case of Nepal, and Sri Lanka, Myanmar and the Philippines (Fig. 5b).

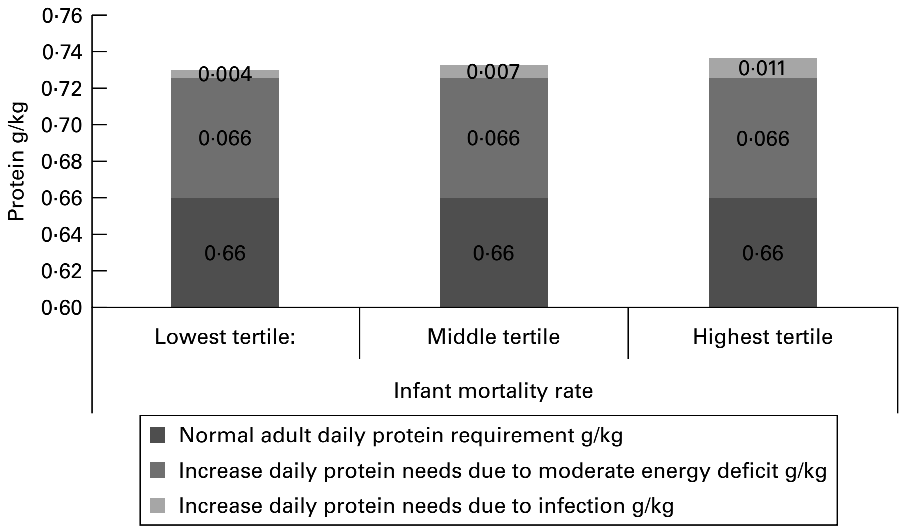

Fig. 1. Estimates of adult daily protein requirement, with added needs for infection and moderate energy deficit, by country-level IMR tertile, for 115 countries.

Growth status and Protein Adequacy

As noted in Table 1, most regions with a high prevalence of protein inadequacy also have high rates of stunting (ranging from 34·7 ± 12·1 % to 35·4 ± 12·9 % in Sub-Saharan Africa and South Asia). An examination of the relationship between levels of stunting, utilizable protein (total) and prevalence of protein inadequacy are presented in Tables 3, 4 and 5. Using Pearson correlations, stunting was significantly and negatively correlated with total energy, protein, utilizable protein and GDP per capita, (Table 3) ($p < 0.001$ for all variables).

Linear regression analyses show that total energy, total protein and total utilizable protein estimates are all independently and significantly negatively associated with prevalence of stunting (Table 4) with $p < 0.001$. Total energy explained 41 % of the variation in stunting, (r-square of 0·406), total protein explained 34 % of the variation and utilizable protein explained 40 % of the variation in prevalence of stunting (separately). When total energy and total protein were incorporated into the model together, only total energy was significant explaining about 41 % of the variation in prevalence of stunting ($p < 0.001$, r-square = 0·407); however when total energy and utilizable protein were incorporated in the model together, both were significant together explaining 43 % of the variation in stunting ($p = 0.006$ and $p = 0.017$, respectively, r-square = 0·430). GDP per capita was a significant predictor of stunting ($p < 0.001$) by itself and remained significant when energy was controlled ($p = 0.048$) (data not shown). When GDP, utilizable protein and total energy variables were included in the model, GDP remained significant ($p < 0.001$), the utilizable protein factor was not significant and total energy as a factor remained significant ($p = 0.022$) (Table 4).

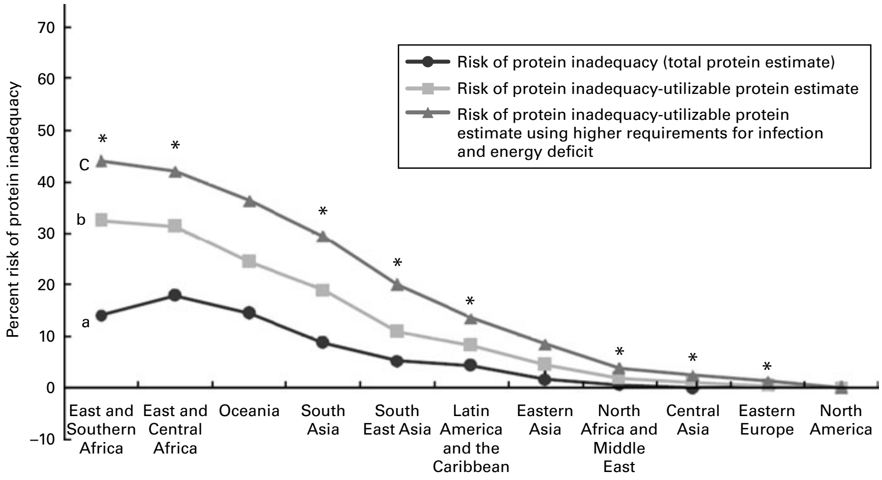

Fig. 2. Differences in risk estimates of protein inadequacy, calculated using total protein, utilizable protein (UP), and UP plus higher requirements for infection and moderate energy deficit, by regions of the world. * Significant difference between risk estimates of protein inadequacy using total protein (line a) and utilizable protein (line b) compared current requirements versus inadequacy estimates using utilizable protein (line c) compared current requirements that have been adjusted for infection and energy deficit. Analysis conducted using paired t-tests or non-parametric tests ($p < 0.05$).

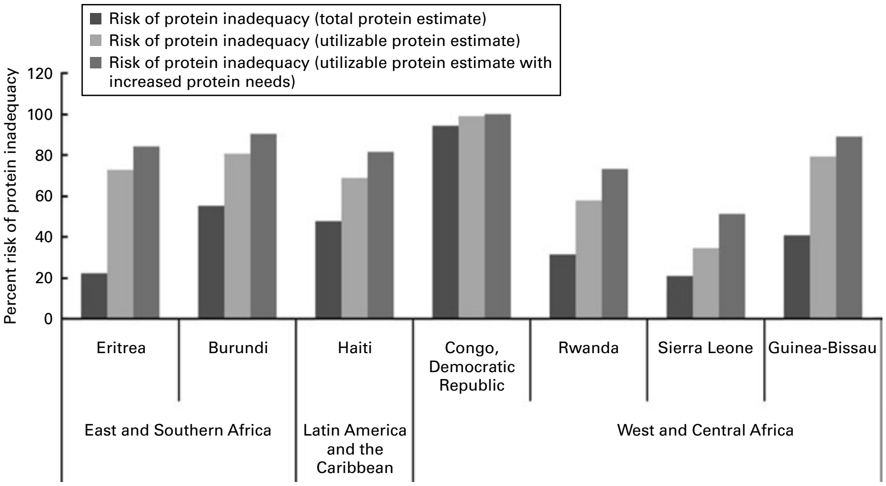

Fig. 3. Risk of protein inadequacy as determined by protein needs adjusted for infection and moderate energy deficiency, compared with energy supply and risk of protein inadequacy determined by total and utilizable protein, in countries with less than 2000 kcal/capita/day energy supply.

Similar to the estimates using total protein and utilizable protein, prevalence of protein inadequacy that was estimated using utilizable protein and the adjusted requirements (adjusted for infection and moderate energy deficit) was significantly associated with stunting ($p = 0{\cdot}003$) when controlling for energy (which was also significant, $p < 0{\cdot}001$) (Table 5). Prevalence of protein inadequacy calculated from total protein and the current requirements was not significantly associated with stunting once energy was incorporated into the model (Table 5).

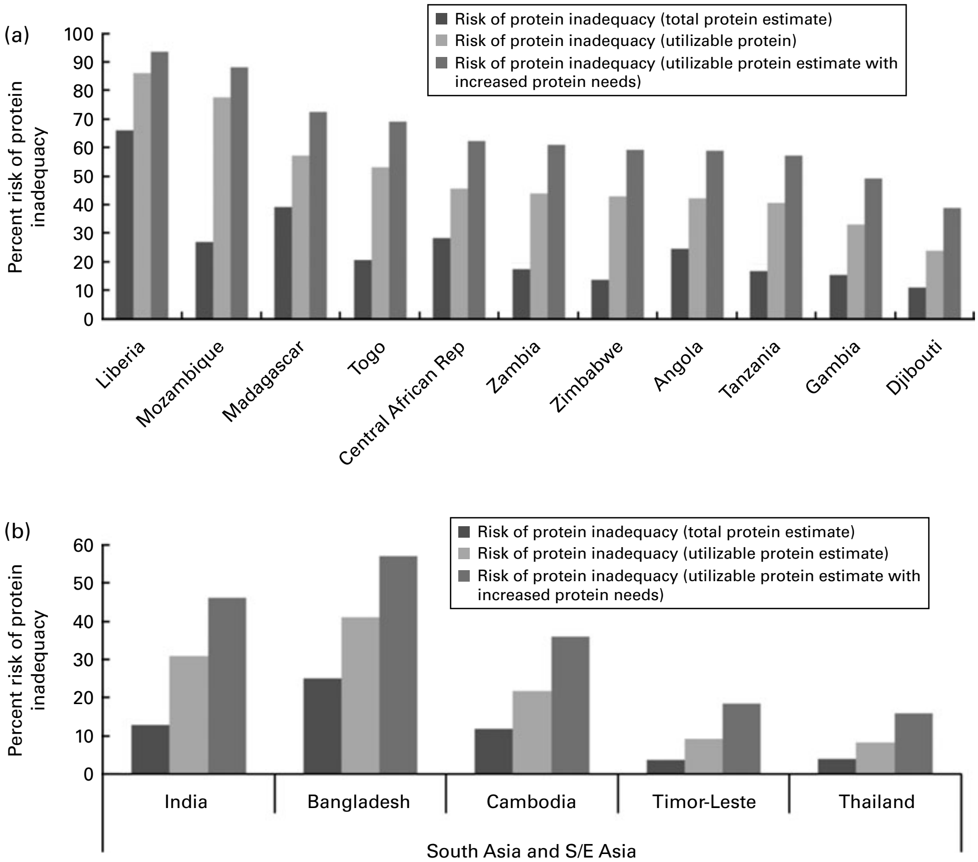

Fig. 4. (a) Risk of protein inadequacy as determined by protein needs adjusted for infection and moderate energy deficiency, compared with energy supply and risk of protein inadequacy determined by total and utilizable protein, in Sub Saharan African countries with 2000–2500 kcal/capita/day energy supply. (b) Risk of protein inadequacy as determined by protein needs adjusted for infection and moderate energy deficiency, compared with energy supply and risk of protein inadequacy determined by total and utilizable protein, in South and South-East Asian countries with 2000–2500 kcal/capita/day energy supply.

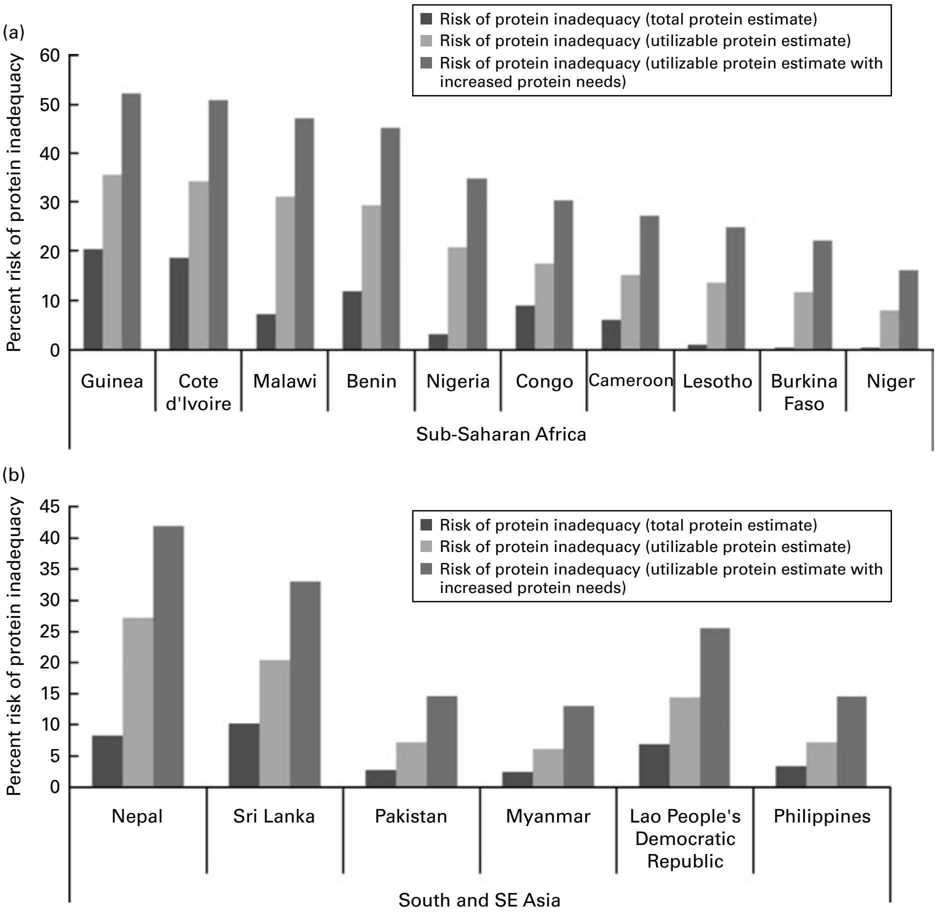

Fig. 5. (a) Risk of protein inadequacy as determined by protein needs adjusted for infection and moderate energy deficiency, compared with energy supply and risk of protein inadequacy determined by total and utilizable protein, in Sub Saharan African countries with 2500–3000 kcal/capita/day energy supply. (b) Risk of protein inadequacy as determined by protein needs adjusted for infection and moderate energy deficiency, compared with energy supply and risk of protein inadequacy determined by total and utilizable protein, in South and South-East Asian countries with 2500–3000 kcal/capita/day energy supply.

Discussion

The aim of the analysis reported here was to examine the effect of applying PDCAAS to total protein values from national food supply data (FAO FBS) and examining the difference in estimates of risk/prevalence of protein inadequacy in relation to total protein versus utilizable protein. We also aimed to calculate the difference in protein requirement estimates accounting for increased needs from infection and moderate dietary energy deficit. National food supply data for the year 2005 were used to calculate total energy, protein and essential amino acid intakes and these intakes corrected for protein quality and digestibility estimates to obtain utilizable protein values. The requirement estimates for protein (adults, 0·66 g/kg body weight) were corrected for moderate energy deficit (10 % correction) and increased demand due to infections using IMR as a proxy for the burden of infections to estimate an exact additional need by country IMR. Based on methods defined by WHO(34), prevalence estimates for protein inadequacy were calculated using total protein and the current requirement estimates as well as utilizable protein and adjusted requirements. For all analyses an estimated intake CV of 25 % was used.

Our findings on total protein and energy availability as well as levels of lysine in the supply are in line with Pellett(11) with developing country regions specifically in Sub-Saharan Africa

Table 3. Correlation coefficients for relationships between country-level nutrient supply, prevalence of stunting and GDP per capita, for 115 countries

	Total protein (g/capita/day)	Utilizable Protein (g/capita/day)	Stunting prevalence[1]	GDP (US $ per capita/yr)
Energy[2] (kcal/capita/day)	0·848	0·841	−0·644	0·525
Total protein[2] (g/capita/day)	1·000	0·983	−0·585	0·515
Utilizable Protein[2] g/capita/day		1·000	−0·631	0·549
Stunting prevalence[2]			1·000	−0·465
GDP per capita[2]				1·000

[1] Defined as the percentage of children under five years of age in a particular country who fall below −2 standard deviations for height-for-age z-score

[2] All coefficients are significant at $p < 0{\cdot}001$

Table 4. The association (linear regression) between prevalence of stunting and total and utilizable protein supply (g/capita/day) for 115 countries

	Regression Coefficient						
Dependent variable	Energy (kcal/capita /day)	Total protein (g/capita/day)	Utilizable protein (g/capita/day)	LnGDP (US $ per capita/yr)	Constant	Adjusted R square	F
Stunting[1]	−0·017 $p < 0·001$				70·77	0·406	78·18
		−0·347 $p < 0·001$			51·77	0·338	58·79
			−0·400 $p < 0·001$		51·81	0·395	74·76
	−0·013 $p < 0·001$	−0·089 NS			68·47	0·407	39·76
	−0·010 $p = 0·006$		−0·202 $p = 0·017$		65·08	0·430	43·70
			−0·133 $p < 0·029$	−7·13 $p < 0·000$	92·24	0·543	67·04
	−0·007 $p = 0·022$		−0·008 NS	−6·580 $p < 0·000$	99·16	0·561	48·33

[1] Defined as the percentage of children under five years of age in a particular country who fall below −2 standard deviations for height-for-age z-score

and South Asia being the lowest in total energy and protein availability. Correcting total protein for quality and digestibility further increases the gap between developing country regions and developed regions indicating the differences in protein sources and quality. Prevalence of protein inadequacy after correction for quality and digestibility ranges from 5–50 % irrespective of the energy availability by region and/or by country. Almost all the countries with total energy availability less than 2000 kcal/capita/day had a high prevalence of protein inadequacy which further increased when requirements were adjusted for need due to energy deficit and increased need during infections (during the infection and post recovery). All countries had lysine as the primary limiting amino acid. (data not shown). This is confirmed in other examinations of dietary data that have also found lysine as the first limiting amino acids in developing country diets. Prior work in the area also demonstrates that for diets providing over 50 % of protein from cereal sources, protein quality is relatively poor thus affecting biological value and limiting protein utilization(9,17–21).

The increase in prevalence of protein inadequacy is incremental following the respective corrections for quality, digestibility, energy deficit and infections. This was as expected since these variables play an important role in defining protein needs within the context of developing countries. We justify the use of adjusted protein requirements above and beyond quality and digestibility on the following basis: firstly, protein requirements are known to be higher in the context of chronic and acute infections(34). For example in the case of acute bacterial infections such as pneumonia and diarrhoea, requirements increase by 20–30 %(1). Requirements (using the indicator amino acid oxidation method (IAAO)) of essential amino acids such as lysine are up to 50 % higher in chronically undernourished adults living in India compared to well-nourished controls (high socio-economic levels and clean environments). Furthermore, following successful treatment for parasites, the requirement for amino acids such as lysine return to their usual level, supporting the interpretation that the increased requirement was attributable to the presence of intestinal parasites(31,32).

Secondly, interactions between dietary protein and energy are well established(4) and changes in food energy (both below and above energy needs) affect body nitrogen balance. Thirty-three percent of total variation can be explained by

Table 5. Associations (linear regression) between prevalence of stunting and prevalence of protein inadequacy (total protein and utilizable protein with adjusted protein requirements) and energy supply, for 115 countries

	Regression Coefficient					
Dependent variable	Energy (kcal/capita /day)	Prevalence of Protein Inadequacy[1] (%)	Adjusted Prevalence of Protein Inadequacy[2] (%)	Constant	Adjusted R square	F
Stunting[3]			0·330 $p < 0·000$	17·55	0·379	70·52
	−0·011 $p < 0·001$		0·170 $p = 0·003$	49·83	0·446	46·92
		0·437 $p < 0·000$		21·47	0·198	29·11
	−0·015 $p < 0·001$	0·102 NS		65·31	0·407	40·18

[1] Prevalence of protein inadequacy calculated using estimates of total protein (g/capita/day) and current protein requirements of 0·66 g/kg for 60 kg adult

[2] Prevalence of protein inadequacy calculated using estimates of utilizable protein (g/capita/day) and protein requirements of 0·66 g/kg for 60 kg adult adjusted for energy deficit and infection needs

[3] Defined as the percentage of children under five years of age in a particular country who fall below −2 standard deviations for height-for-age z-score

nitrogen intake while 36 % can be explained by variation in energy intake with both energy intake and nitrogen intake levels individually effective in improving nitrogen balance. Data from studies at MIT indicate that maintenance of N equilibrium at a low protein intake of 0·57 g/kg body weight requires additional energy ranging from 9–20 %. Furthermore, while other substrates are preferentially utilized when energy intakes are slightly below requirements (protein sparing effect), additional protein metabolized accounts for about 10 % of the energy deficit(5,6). Work done in Japan also found an impact of energy on maintaining nitrogen equilibrium with a reduction of 20 % in energy availability increasing nitrogen requirement by almost 50 %(7).

An examination of other indicators within the country and region indicate that not surprisingly countries with high prevalence rates of protein inadequacy and/or low utilizable protein levels are also those with high rates of stunting and lower GDP per capita. The linear correlation analysis clearly shows an association between the levels of protein available (when corrected for quality and digestibility and using adjusted requirements) and prevalence of stunting, irrespective of energy supply. Quality of dietary protein will affect linear growth especially at intakes close to maintenance or when energy intake is potentially insufficient. The mechanisms for protein effects on growth are multiple including if protein amounts and digestibility are marginal, net retention will be affected thus potentially compromising growth. In addition, specific dietary essential and non essential amino acids play a role in defining hormonal responses to food including effects on GH release(35).

Evidence indicates that protein restriction leads to low levels of IGF-1 in healthy children(36). When older children in the study underwent energy restriction (50 % reduction in intake) or protein restriction (reducing protein from 1·0 to 0·66 g/kg body weight per day) with both forms of restriction led to a significant decrease in nitrogen balance and decline in IGF-1 concentrations as well as concentrations of specific IGF binding proteins. IGFBP-2 was responsive to re-feeding only in the children that were protein restricted. There is also a significant impact of the type and quality of protein on gene expression especially genes associated with insulin like growth factor I and insulin like growth factor binding protein I, both of which play an important role in whole body protein synthesis and growth promotion and body composition(37–40).

Type of dietary protein seems to have a specific stimulating effect on weight and length gain. Milk intake is positively associated with serum IGF-I concentrations and height suggesting a stimulating effect of milk on insulin like growth factor and subsequently on growth(52). Eight year old boys receiving a high milk intake had higher IGF-1 levels compared to boys receiving protein from meat(41,42). It is postulated that amino acids, peptides specific to milk and/or other milk components (e.g. β-lactoglobulin, α-lactalbumin, immunoglobulins, lactoferrin) are likely to be the active components.

An association between intake of dietary protein and linear growth has been observed in pre-pubertal girls over a 3 year follow up period(35) and a 6 year follow up period(43). High arginine and lysine intakes (ranging from 3·8-4·6 g/day) were inversely associated with fat mass index in pre-pubertal lean girls in both studies(35,43). In an analysis of dietary and anthropometric data collected on Ghanaian children aged 2-13 years, an association has been found between utilizable protein and the risk of being stunted(44). While energy intake was low in the population, it was not significantly associated to stunting levels(44). The finding of the combined effect of improved linear growth and reduced fat mass index is especially interesting in growth promotion practice since stunted children in a setting of rapid diet and nutrition transition are increasingly becoming overweight or obese(45).

Oral ingestion of dietary protein, amino acid mixtures to resemble soya protein and an arginine-lysine test drink have been shown to increase growth hormone release in normal women(46). Dietary restriction of single essential amino acids including leucine, lysine, methionine and threonine have been shown to decrease plasma IGF-I production but not affect plasma insulin like growth factor binding protein 1 (IGFBP-1 production)(38). However it does decrease IGFBP-I production in hepatocyte cultures(47–50).

While we did not examine individual amino acid effects in this study, evidence exists for the specific roles of amino acids (including arginine and lysine) within the context of growth hormone release mechanisms via an effect on the somatotropic axis(46). It is postulated that arginine and lysine could have individual or combined effects within this mechanism via the somatotropic axis(46). Soya protein ingestion has also been found to increase growth hormone secretion (an effect similar to direct arginine supplementation) however this effect is reduced when soya protein is ingested as part of a meal(51,52). Ingestion of soya proteins with a carbohydrate or fat alone increased secretion at the same level as soya and/or arginine alone however when soya was combined with both carbohydrate and fat, the effect was reduced. Peak plasma arginine concentrations were higher indicating a role for arginine in the somatotropic activity of protein(52).

In conclusion, the findings of this analysis indicate the need to reconsider the adequacy of dietary protein intakes in relation to protein requirement estimates after adjusting for quality, digestibility, burden of infection and energy deficit. There is clearly an association between the quality of protein available at the national level and the prevalence of stunting. Whether this association exists at the individual level needs to be explored by examining cross sectional and cohort data specifically in infants 6 months and older to determine if there is a true effect of protein quality on linear growth pattern. Such a finding would have significant public health and policy implications from the perspective of targeting linear growth and the prevention of stunting, a major cause of disease and disability in the developing world(53).

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