Bioavailability of Nutrients of Public Health Concern and their Association with the Animal/Plant Ratio in Diets of Female Residential Students of Bangladesh

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Abstract

Students, particularly female students, living in university dormitories are susceptible to various nutritional deficiencies due to their improper dietary practice and poor diet quality. Whether food originates from animal or plant sources contributes greatly to the quality of their diets in terms of nutritional adequacy. This study made a bioavailability assessment of four nutrients (vitamin A, iron, protein, and zinc) from students' diets followed by examining whether bioavailability relates to the animal/plant ratio (A/P) in the diets of female residential students (FRS) of Bangladesh. Previously published dietary algorithms or conversion factors specific to different food groups were used to assess nutrient bioavailability in the diets of 180 (60 for iron) FRS of the University of Dhaka. Nutrient inadequacy was evaluated using i) an individual diet approach (estimating bioavailability from individual diets), and ii) a dietary pattern approach (presuming average bioavailability for mixed diets). The mean absorptions of iron and zinc, quality of protein, and conversion factor for β-carotene were 15.5%, 35.1%, 66.1%, and 17:1, respectively. Among the individual diets, a large range in bioavailability was observed, which was explained inadequately by their A/P. There was a significant difference in inadequacy prevalence when iron and protein bioavailability from individual diets were compared with bioavailability estimates calculated applying average conversion factors for mixed diets. The A/P could not necessarily predict the nutrient bioavailability in FRS diets. Hence, it is important to consider the diet composition when evaluating nutrient adequacy in the diets of FRS and other students.

Keywords: Bioavailability, Nutrients, Nutritional adequacy, Animal to plant ratio, University students, Bangladesh

Introduction

Parental supervision and control tend to diminish when students transition from school/college to university. Consequently, many students, particularly those who reside in a university dormitory, must adapt their dietary behaviour to their new environment and thus, face challenges in maintaining proper health and nutritional status (Murashima et al., 2012). Besides, recommended dietary guidelines may not be followed owing to a change in diet quality (Demory-Luce et al., 2004). However, these students, especially females, have exceptional nutritional requirements due to their eminent productive and reproductive roles in society. Inadequate dietary intake resulting from faulty/imbalanced dietary composition may have adverse effects on their nutritional condition and, in the future, their offspring's health. Many previous studies found that female residential students tend to avoid micronutrient-rich animal foods. They are prone to consume more plant food-based diets comprising of cereals, legumes, seeds, vegetables (both leafy and non-leafy), etc. (Fayet-Moore et al., 2014). Animal foods are the principal source of many essential nutrients, e.g., vitamin B_{12}, protein, iron, and zinc; thus, nutritional deficiencies or inadequacies among female residential students (FRS) may result from reduced consumption of animal foods (Millward & Garnett, 2010). Nutrient adequacy depends on both the amount of nutrients consumed and their bioavailability on the basis of meal composition. Bioavailability can be defined as the proportion of a consumed nutrient that is available for absorption and utilized through normal metabolic pathways (Gibson, 2007). A number of factors can affect the bioavailability of a nutrient, including host-related determinants such as sex, age, genotype and phenotype, health and nutritional status, dietary and culinary determinants i.e., the nutrient's chemical structure, food matrix, food preparation practices, the interaction of dietary components that augment or inhibit its absorption (Gibson, 2007). The

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animal-to-plant ratio (A/P) of the diet was found to influence the above-mentioned dietary factors. Several studies found that the bioavailability of many essential nutrients such as iron, zinc, protein, and vitamin A was strongly dependent on their food source (plant or animal-sourced foods) (Gibson, 2007; WHO/FAO, 2004; WHO/FAO/UNU, 2007). These key nutrients are more readily bioavailable from animal-sourced foods than from plant-sourced foods (Fayet-Moore et al., 2014). Consequently, the bioavailability of these nutrients with public health problems may be greatly reduced with the plant food-based diet of the FRS.

This study aimed to make a bioavailability assessment of four nutrients of public health importance (vitamin A, iron, protein, and zinc) in each individual diet of the FRS included in this study followed by examining if such bioavailability factors relate to the A/P in the diets of FRS.

Materials and methods

Study participants

A cross-sectional study was conducted among 180 FRS (18-26 years of age) of three selected dormitories (Ruqayyah Hall, Shamsun Nahar Hall, and Kabi Sufia Kamal Hall) of the University of Dhaka, Bangladesh from January to March 2016. Sixty subjects were drawn randomly from the lists provided by each dormitory authority for each dormitory. The only inclusion/exclusion criteria were that the participants had to be a current student of the University of Dhaka and reside in any of the three dormitories. The first author explained the study objectives to the students at each of the three dormitories. The students who were willing to participate gave their oral and written consent. The socio-demographic and other details of the study participants are described elsewhere (Sultana et al., 2019a, 2019b).

From 180 respondents, 60 were selected (20 respondents from each dormitory) as a sub-sample for biochemical analysis of the serum ferritin level. The provision of blood was voluntary and willing participants signed a consent form. A total of 5 mL of venous blood was collected from each respondent and serum ferritin concentrations were analysed using enzyme-linked immunosorbent assay with the Pathozyme-Ferritin kit (Omega Diagnostics Limited, Scotland and UK). The bioavailability of zinc, VA, and protein was estimated for 180 subjects, while iron absorption was assessed for 60 subjects.

Food consumption data

Dietary information was recorded by using multiple pass 24-hour recall method for three consecutive days (two weekdays and one weekend) and the mean 24-h dietary intake was calculated for each respondent. Mean food consumption data were converted into weights in grams using standard reference tables (Shaheen et al., 2013). The intakes of protein, phytate, iron, zinc, and β-carotene were calculated using the mean of the 24-hour dietary data and the "Food Composition Table for Bangladesh (FCTB)" (Shaheen et al., 2013). The mean intakes of α- carotene and β-cryptoxanthin were estimated using the "Indian Food Composition Tables (IFCT)" (Longvah et al., 2017). The IFCT was also used to calculate phytate and β-carotene intakes for foods that were unavailable in the FCTB. The values of α-carotene and β-cryptoxanthin were taken from the USDA National Nutrient Database for Standard Reference (SR28) for foods that were unavailable in the IFCT (USDA, 2018).

Assessment of the bioavailability factors

The present study used several definitions to express the term "Bioavailability factors" based on the nutrients assessed. These bioavailability factors include absorption efficiency for iron and zinc, the pro-VA carotenoids (i.e., β-carotene, α-carotene, and β-cryptoxanthin) conversion factor (considering absorption and bioconversion), and protein quality (considering digestibility and biological value) (Perignon et al., 2018). From each individual diet, the bioavailability factors were estimated and expressed as means ± standard deviation (SD). Total iron absorption from an individual's diet was taken as a sum of heme and non-heme iron absorption in that diet after calculating non-heme iron absorption following the equation by Armah et al. (Armah et al., 2013) and heme iron absorption by the equation developed by Hallberg and Hulthen (Hallberg & Hulthen, 2000). The total amount of absorbed zinc, calculated using the equation described by Miller et al. (Miller et al., 2007), was divided by total intake of dietary zinc to estimate zinc absorption. For each individual diet, retinol equivalent (RE) was estimated by using food and food-group specific conversion factors of pro-VA (β-carotene, α- carotene, and β- cryptoxanthin) obtained from the literature (Perignon et al., 2018). The following conversion factors (on a weight basis) were used for β-carotene: 21:1 for leafy vegetables, and 14:1 for carrot (Khan et al., 2007; Tang et al., 2005), 12:1 for fruits (Khan et al., 2007; Perignon et al., 2018), 27:1 for other vegetables (Haskell, 2012), 3.8:1 for rice (Tang et al., 2009) and 9:1 for fats (Tang et al., 2003; Wang et al., 2004). The conversion factor for fats was also applied for animal-origin foods, given that β-carotene is a fat-soluble vitamin (Perignon et al., 2018). The conversion factors for α-carotene and β-cryptoxanthin were estimated at half that of β-carotene, as they possess half the retinol activity of β-carotene (Perignon et al., 2018). The protein digestibility corrected amino acid score (PDCAAS), described in the report of a Joint FAO/WHO/UNU expert consultation (WHO/FAO/UNU, 2007) was used as an indicator of protein quality. Table 1 shows the reference amino acid requirements for adults and the factors of digestibility used for the calculation of PDCAAS (WHO/FAO/UNU, 2007).

Table 1. Requirements of nutrients and protein digestibility factors

Estimated average requirements[a]	
Iron (mg absorbed/day)	1.46
Protein (g/kg/day)	0.66
Vitamin A (g RE/kg/day)	6.7
Zinc (g absorbed/kg/day)	17.7*
Amino acid requirements of adults (mg/g protein)	
Cystine	6
Histidine	15
Isoleucine	30
Leucine	59
Lysine	45
Methionine	16
Methionine + cysteine	22
Phenylalanine + tyrosine	30
Threonine	23
Tryptophan	6
Valine	39
Protein digestibility by food source (%)	
Bread, white	97
Bread, whole	92
Egg	97
Flour	96
Legumes	85
Meat, poultry, fish	94
Milk, cheese	95
Ready to eat cereals	75
Rice, refined	89
Wheat refined	96
Whole grain cereals	86

RE: Retinol equivalents. [a]Estimated average requirements for adult women. *EAR for an assumed bioavailability of 30% (59 g/kg/day for women) converted to requirements for absorbed zinc.

Indicators of animal/plant food ratio

Two indicators were used to determine the A/P: (i) the animal/plant protein ratio, calculated as the ratio of protein intake from foods of animal origin to that from foods of plant origin; and (ii) the share of animal energy, calculated as the ratio of energy attained through consumption of animal-origin foods to that from the total intake of food for each individual diet. Meat (poultry, red meat, organ meat, etc.) and meat products, fish and fish products, seafood, eggs, milk, and dairy products (butter, cream, cheese, yogurt, etc.) were considered animal-sourced foods.

Assessment of nutrient inadequacy

The following two approaches were used to assess the prevalence of inadequate intake of iron, zinc, pro-VA, and protein among the respondents: i) an "individual diet (ID) approach" in which bioavailability factors were estimated for each individual diet, and used to ascertain nutrient intake, and ii) a "dietary pattern (DP) approach" in which average bioavailability factors, calculated for so-called 'mixed' diets, were employed to determine nutrient intake (Perignon et al., 2018). An average bioavailability of about 12% or 15% for iron and a moderate bioavailability of 30% for zinc were used in the dietary pattern approach based on the WHO suggestion for populations consuming a mixed diet (WHO/FAO, 2004). In the dietary pattern approach, two conversion factors were used to assess VA: (i) 6:1 for β-carotenes and 12:1 for other pro-VA carotenoids, as recommended by EFSA (EFSA, 2015), or (ii) 14:1 for β-carotenes and 28:1 for other pro-VA carotenoids, as recommended by WHO (WHO/FAO, 2004). For protein estimation, total protein intake was used in the dietary pattern approach without adjusting for the quality of protein (Perignon et al., 2018).

For both approaches, the estimated average requirement (EAR) cut-off values were applied to determine the prevalence of inadequate intake of zinc, iron, VA, and protein (Carriquiry, 1999). The WHO/FAO recommended EAR values for females above 18 years of age were used and are shown in Table 1 (WHO/FAO, 2004).

Statistical analysis

Data were analysed using the Statistical Package for the Social Sciences (SPSS) software version 25 for Windows (SPSS Inc., Chicago, IL, USA). For each nutrient, McNemar's test was used to compare the prevalence estimates of dietary inadequacy found using the ID and DP approaches. A linear regression model was applied to identify the association of bioavailability factors for each of the nutrients concerned with animal/plant protein ratio or share of animal energy. For all tests, a P-value of less than 0.05 was considered statistically significant.

Results

Estimation of bioavailability factors

For each nutrient, the distributions of the bioavailability factors from each individual diet are presented in Table 2. For FRS, iron absorption ranged from 6.9% to 25.9%, with an estimated mean of 15.5%. The mean zinc absorption was 35.1%, ranging from 25.2% to 42.2%. The -carotene conversion factor ranged from 9.2:1 to 25.7:1, with an estimated mean of 17.2:1. The mean (range) PDCAAS value in each individual diet was 66.1% (34.2% - 93.8%).

Table 2. The distribution of iron absorption, zinc absorption, β-carotene conversion factor, protein quality, animal-to-plant protein ratio, and share of animal energy of diets of female residential students

	Mean	Standard deviation
Iron absorption (%) (n=60)	15.5	4.8
Zinc absorption (%) (n=180)	35.1	3.2
-carotene conversion factor (n=180)	17.2	4.4
PDCAAS (%) (n=180)	66.1	16.8
Animal-to-plant protein ratio (n=180)	4.2	1.5
Share of animal energy (%) (n=180)	8.7	4.9

PDCAAS: Protein digestibility corrected amino acid score.

Prevalence estimates of dietary inadequacy: comparison between ID and DP approaches

The percentages of individuals with inadequate intakes of iron, VA, and protein were estimated to be 58.3%, 0%, and 86.7%, respectively, in the ID approach. In the DP approach, the prevalence of inadequate intake was estimated to be 66.7% (assuming 15% bioavailability) and 93.3% (assuming 12% bioavailability) for iron, 7.2% (using conversion factors of 6:1 for β-carotene and 12:1 for other pro-VA carotenoids) and 0% (using conversion factors of 14:1 for β-carotenes and 28:1 for other pro-VA carotenoids) for VA, and 44.4% for protein. None of the participants were found to have inadequate zinc intake using either the ID or the DP approach (Table 3).

Compared with the ID approach, the prevalence of inadequate iron intake was significantly higher using the DP approach with an average conversion factor for bioavailability of 12% (P<0.001), while no significant difference was found with an average conversion factor for bioavailability of 15% (P=0.267). No respondents were found to have inadequate VA intake using the average conversion factor of 6:1 for β-carotenes and 12:1 for other pro-VA carotenoids as recommended by EFSA and ANSES, or 14:1 for β-carotenes and 28:1 for other pro-VA carotenoids according to the WHO/FAO recommendation. The prevalence estimate of inadequate protein intake was significantly (P<0.001) lower using the DP approach.

Association between bioavailability factors and the animal/plant ratio

The mean animal/plant protein ratio among female students was 4.2 (ranging from 0.8 to 8.9), while the ratio of animal proteins to total protein intakes ranged from 59% to 93%. The share of animal energy ranged from 4.5% to 47.8% with an estimated mean of 8.7% (Table 2). The animal/plant protein ratio was significantly associated with iron absorption,

Table 3. Estimation of iron, zinc, vitamin A, and protein inadequacy prevalence by the "individual-diet approach" (ID) and by the "dietary pattern approach" (DP) for female residential students

	Prevalence of inadequate intake (%)	p-value[a]
Iron (n=60)		
ID approach	58.3	
DP approach		
i) 15% bioavailability	66.7	0.267
DP approach		
ii) 12% bioavailability	93.3	0.001*
Zinc (n=180)		
ID approach	0.0	
DP approach	0.0	--
Vitamin A (n=180)		
ID approach	0.0	
DP approach		
i) factors 6:1 and 12:1	7.2	--
DP approach		
ii) factors 14:1 and 28:1	0.0	--
Protein (n=180)		
ID approach	86.7	
DP approach	44.4	<0.001*

[a]p-value by McNemar's test when prevalence compared with the individual diet approach. *P<0.05.

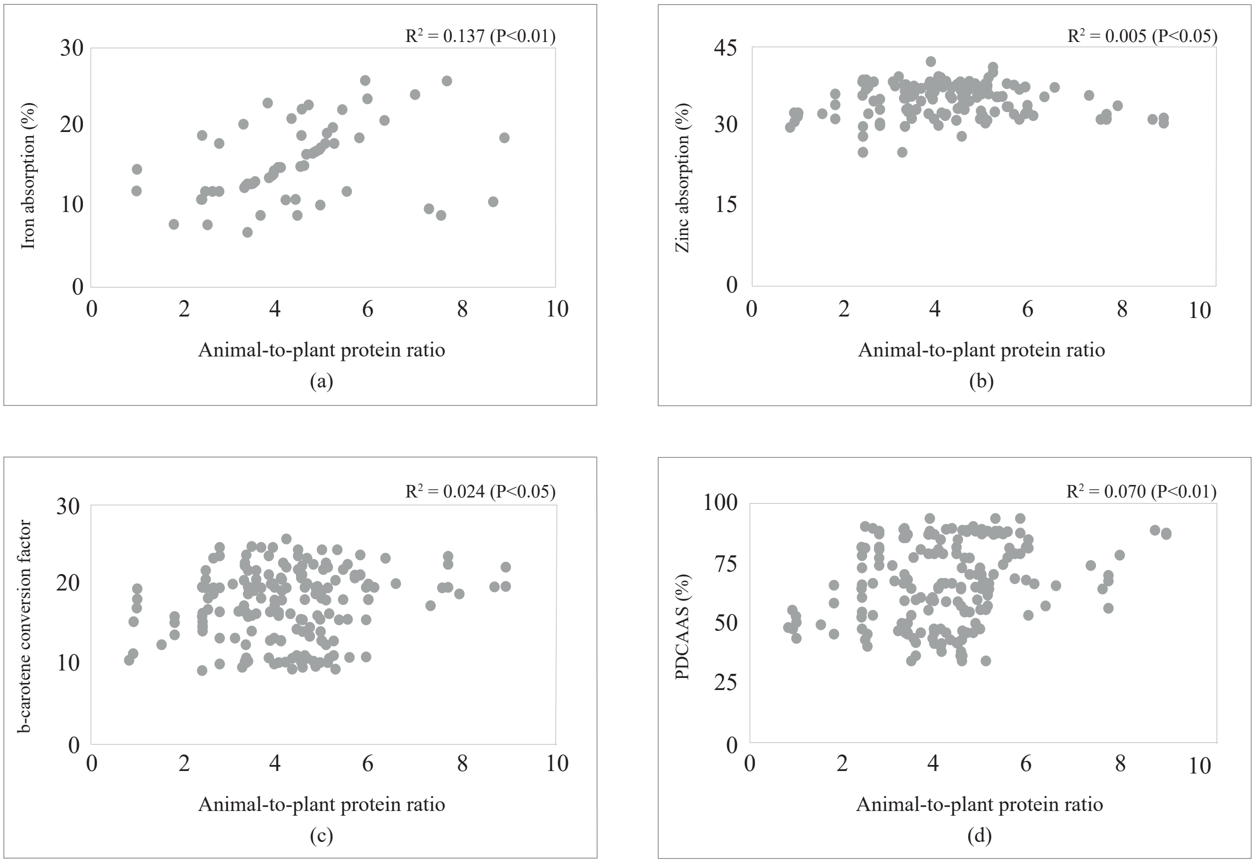

Figure 1. Association between animal-to-plant protein ratio and iron absorption (a), zinc absorption (b), β-carotene conversion factor (c) and protein quality (d) in the diet of female residential students

carotene conversion factor, and protein quality, but no statistically significant association was observed with zinc absorption (Fig. 1). The percentage of variation that could be explained by the animal/plant protein ratio was 13.7% for iron absorption, 2.4% for β-carotene conversion factor, and 7.0% for protein quality. None of the bioavailability factors (i.e., absorption zinc, iron absorption, protein quality, and β-carotene conversion factor) were significantly associated with the share of animal energy (data not shown).

Discussion

The present study evaluated the bioavailability of four key nutrients (iron, zinc, protein, and VA) among FRS. Individual diets showed a high variation in bioavailability factors, and differences in the prevalence of nutrient inadequacies between the ID and DP approaches were observed. The A/P of individual diets, expressed in either protein or energy, could only poorly explain the observed variation in bioavailability factors. To the best of our knowledge, this was the first time that the bioavailability of iron, zinc, VA, and protein and their association with A/P were assessed among FRS considering the actually available nutrient composition of their diets.

Previous studies have identified various diet and host-related factors that exert an influence on the bioavailability of iron. Haemoglobin and myoglobin from animal-origin foods provide heme iron which is absorbed more readily than non-heme iron, which is found in both plant and animal-based foods (Hurrell & Egli, 2010). The absorption of non-heme iron depends on the iron status of an individual, and the presence of dietary promoters (ascorbic acid and other organic acids, proteins, etc.) and inhibitors (phytate, oxalate, calcium, tannins, phenolic compounds, etc.) (Hurrell & Egli, 2010). The mean bioavailability of iron in the present study was about 15.5%, which exceeds the WHO assumed average bioavailability of 12% or 15% for populations consuming mixed-type diets. Nonetheless, iron absorption in individual respondents' diets ranged from 6.9% to 25.9%, and over half of the respondents

(58.3%) were at risk of inadequate iron intake when the bioavailability factor was estimated using the ID approach. The prevalence of iron intake inadequacy differed significantly between the ID and DP approaches when an average bioavailability factor of 12% was used, but no significant difference was found between the two approaches using an average bioavailability factor of 15%. This indicates a probability of miscalculation when an average factor of 12% is used for assessing the prevalence of inadequate iron intake. So, if an individual dietary assessment cannot be accomplished for FRS, an average bioavailability factor of 15% will give the most precise result.

Previous research revealed that cereals and legumes along with some other plants inhibit dietary zinc absorption owing to the presence of phytates, whereas animal proteins enhanced zinc absorption (WHO/FAO, 2004). Among FRS in the present study, the mean zinc absorption was 35.1%, and the range, from 25.2% to 42.2%, encompassed two of the three levels of WHO-presumed bioavailability (15%, 30%, and 50%) to attain the recommended zinc intake. This implies that the FRS consumed diets of medium bioavailability (30%) according to the WHO categorization. When the bioavailability of zinc was calculated using both the ID and DP approaches, assuming a moderate bioavailability level (30%), no respondents were found to be at risk of inadequate zinc intake. Therefore, in contrast to iron, a relatively accurate estimate of the risk of zinc inadequacy can be obtained using the moderate bioavailability (30%) level for mixed diets without considering the variation of bioavailability in individual diets.

The quality of a protein is determined by its digestibility, which is the amount available following digestion and absorption by an organism, and biological value, defined as the proportion of absorbed protein that becomes incorporated into the proteins of the organism's body (WHO/FAO/UNU, 2007). Animal proteins are assumed to be of good quality given that all indispensable amino acids are contained in proper proportions. In contrast, many plant-origin proteins are of lower quality owing to a suboptimal amino acid profile, and lower digestibility than animal proteins (Day, 2013). In the diet of FRS, the mean protein quality (assessed by PDCAAS in the ID approach) was 66.1%, giving a significantly higher protein inadequacy prevalence (86.7%) than that (44.4%) measured by total protein intake. We suggest that a significant underestimation of the prevalence of inadequate protein intake might occur if protein quality is not adjusted.

Dietary VA can only be obtained as retinol from animal foods (i.e., meat, poultry, fish, and dairy products) and/or from pro-VA carotenoids obtained from plant-based foods (i.e., fruits, vegetables, and other plant-based products). Almost all (~90%) preformed vitamin A (retinol and its esterified form retinyl ester) is absorbed by the human body, whereas the human intestinal cells must convert pro-VA carotenoids to retinol prior to absorption (Perignon et al., 2018). A number of food and diet-related factors, viz., type of food and its matrix, food processing and preparation, fat content and composition of the food, and content of a meal, are attributed to the efficacy of bioconversion of pro-VA carotenoids to retinol and its absorption (Haskell, 2012; Tang, 2010). Previous studies have reported that the β-carotene conversion factor varies within foods from 3.6:1 to 28:1 (on a weight basis) (Tang, 2010). In the present study, the mean conversion factors were estimated at 9.2:1 for β-carotene, and 25.7:1 for other pro-VA carotenoids. For a typical mixed diet, these estimates closely resemble the WHO recommended conversion factors of 14:1 for β-carotene and 28:1 for other pro-VA carotenoids. However, our study findings differed from the EFSA- and ANSES- recommended conversion factors for European diets (6:1 for β-carotene and 12:1 for other pro-VA carotenoids) (EFSA, 2015). Using the EFSA- recommended conversion factors, about 7.2% of the study participants were at risk of inadequate VA intake with the DP approach, which was higher than the percentage estimated with the ID approach. Using the WHO-recommended conversion factors (14:1 for β-carotene, and 28:1 for other pro-VA carotenoids), the DP approach produced more similar results to the ID approach. Hence, when individual diet assessment is not possible for FRS diets, the WHO conversion factors provide a more accurate estimate of inadequate VA intake than those of the EFSA.

In a typical diet, the level of animal foods usually determines the degree of bioavailability, and the appropriate recommended nutrient intake (RNI). For the Bangladeshi diet, the RNI is generally adapted from the WHO/FAO recommendation (Nahar et al., 2013; WHO/FAO, 2004). The WHO/FAO set three categories of zinc bioavailability: high (50%), moderate (30%), and low (15%), according to the characteristics of the diet. For iron, the WHO/FAO assumes four levels of absorption as 15%, 12%, 10%, and 5% to estimate the RNI, depending mostly on the level of animal-sourced food (i.e., meat/fish intake) (WHO/FAO, 2004). Conversely, in the current study, factors of bioavailability were assessed in each respondent's diet and their association with the contribution of animal-sourced foods to total protein and energy intakes was evaluated.

Mostly, in FRS diets, animal-origin foods provided approximately 9% (4.5% to 47.8%) of the total energy intake and about 83% (59% to 93%) of the total protein intake. For the four studied nutrients, the A/P could only poorly explain the variation in bioavailability factors, when expressed in terms of protein, and no relation was found when expressed in terms of energy. The proportion of animal foods in the diet could explain ≤1% of the variation in the β-carotene bioavailability factor. This association might be weak because this pro-VA carotenoid can be found only in a few animal products (dairy products). The bioavailability of iron showed the strongest correlation, among the four nutrients studied, with the amount of animal foods present in the diet. The animal/plant protein ratio explained about 13.7% of the variation in iron absorption. Nevertheless, similar A/P values, showed a large range of iron absorption, e.g., for A/P values around 4, iron absorption, ranged from 10% to 22% within the diets of FRS. This may be due to differences in the subtypes of animal-sourced foods in different individuals' diets, e.g., a diet high in dairy products would have lower iron absorption than a diet high in meat. Hence, it is imperative to consider the subcategories of dietary animal-origin foods (e.g. dairy items vs. meat products) when estimating iron absorption. For protein, a lower PDCAAS value was not always associated with lower amounts of animal products in the diet. However, a large variation in protein quality was observed for a given animal/plant protein ration, e.g., an animal/plant protein ratio of 4 represented both the lowest and highest protein quality in the diets. Thus, whilst a reduction in the proportion of animal products may jeopardize the quality of protein, an increase in plant proteins may contribute significantly to the level of high-quality proteins in the diet.

There are several limitations to the present study that should be considered. Firstly, the study recruited only subjects living in university dormitories, and hence, the subjects did not represent all students of the University of Dhaka. Secondly, the study included only a small number (n=60 for iron, and n=180 for zinc, VA, and protein) of individual diets which resulted in a wide range of bioavailability factors in diets, even within a similar animal/plant ratio. Further research with a larger number of subjects is needed to evaluate the bioavailability of these and other nutrients; for example, some plant foods containing high-oxalate can impair calcium absorption. Finally, the conversion factors used to calculate bioavailability were taken from published literature and might not be accurate for the studied populations.

Conclusion

It can be concluded that the A/P, expressed in terms of either protein or energy, could only poorly explain the high variation in bioavailability factors of iron, zinc, β-carotene, and protein in the diets of FRS. High levels of zinc and iron absorption, the conversion factor for β-carotene, and protein quality were observed even for diets with a low A/P. However, high A/P was not necessarily reflected in the favoured levels of bioavailability factors. Our findings highlight that, when assessing nutrient bioavailability, it is important to consider other dietary characteristics in addition to the content of animal-origin foods in the FRS diet. In order to properly evaluate the nutritional quality of FRS diets and recommend more sustainable food choices for them, nutrient bioavailability should be given primary importance. Lastly, the methods of the present study could be applied to many national dietary surveys to analyse the diversity of individual diets and the role of the A/P.

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