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Co-ingestion of black tea reduces the indispensable amino acid digestibility of hens' egg in Indian adults

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Abstract

Background—Tea, a commonly consumed beverage, contains high amounts of polyphenols that could impair protein digestibility, as demonstrated *in vitro*. There are no human studies examining the inhibitory influence of tea polyphenols (TPP) on high-quality protein digestibility.

Objective—The study aimed to determine the effect of black tea on the true indispensable amino acid (IAA) digestibility of whole boiled egg protein, in healthy adult humans, using a dual isotope tracer approach.

Methods—The effect of black tea polyphenols (TPP, 4.6 mg/ ml, ingested as a beverage with the meal) on ^{2}H-labeled whole boiled egg protein, administered with ghee rice and tomato curry, was measured with reference to ^{13}C-spirulina protein in healthy Indian adults aged 20-27y of both sexes with BMI of 22.0 ± 2.8 kg/m^{2}. The results were then compared to previously determined whole egg mean IAA digestibility measured by the same method, without black tea, in the same subjects (n=5). To correct for any independent effect of TPP on spirulina protein (used as a standard protein), the true IAA digestibility of ^{13}C-spirulina protein was independently measured with reference to a ^{2}H-AA mixture, with and without co-ingestion of black tea, in 3 of the same subjects.

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Author contribution

The authors' responsibilities were as follows - AVK, SK, NS, SD and TP designed the research. SK and NS conducted research, while SK, AV and SD contributed to sample analysis. AVK, SK and NS were involved with interpretation of results. AVK, SK, NS wrote the paper. AVK had primary responsibility for final content. All the authors read and approved the final manuscript. The authors declare no competing financial interests.

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Results—The true IAA digestibility of whole boiled egg protein significantly decreased by 17% when co-ingested with black tea. However, there was no significant reduction in the true IAA digestibility of spirulina protein when co-ingested with black tea.

Conclusion—TPP-protein interactions reduced whole egg digestibility in healthy Indian adults but had minimal effect on spirulina protein digestibility. In populations who are at risk of dietary quality protein inadequacy, the consumption of tea during or after a meal can further increase the risk of inadequacy. This study is registered in the Clinical Trials Registry of India with registration number: CTRI/2018/03/012265 (http://ctri.nic.in)

Keywords

Black tea; tea polyphenol; intrinsically labeled egg; true indispensable amino acid digestibility; dual isotope tracer technique

Introduction

Habitually consumed diets of food-insecure populations, particularly those from low- and middle-income countries, are predominantly plant based, with only a modest increase in animal source foods (ASF) such as eggs and milk over time (1). In India, diets are mainly cereals, whose proteins are relatively low-quality due to their low lysine content, and whose digestibility is poor (78% in children) when compared to ASF (87% in children or 92% in adults) (2–4). The proportion of the adult population at risk of quality protein inadequacy is about 24%, nearly doubling in a poor environment due to additional demand and possible adverse effects of impaired intestinal function (2, 5, 6). While plant food matrices reduce protein digestibility owing to anti-nutritional factors (7) and a robust cell wall structure, the co-ingestion of beverages such as tea with meals could also show a similar effect on plant or animal source proteins. Tea polyphenol (TPP)-protein interactions have been implicated in reducing protein bioavailability *in vitro* and in rodents (8–11). This inhibitory effect of TPP on protein digestibility could be relevant in populations that consume sub-optimal quality protein intakes, and in whom tea is a popular beverage that is consumed with meals (12).

The *in vivo* determination of protein digestion and absorption (digestibility) is now possible in humans through the use of the dual stable isotope tracer technique, which was developed recently and applied in adults and children to determine the true Indispensable Amino Acid (IAA) digestibility of different protein sources, such as ASF and plant proteins, relative to a standard protein (spirulina) of known digestibility (3, 4, 13).

This study aimed to determine the effect of TPP from black tea (fully fermented tea leaves) on the digestibility of whole hens' egg protein, a high-quality protein with no intrinsic polyphenols, using the dual stable isotope tracer technique. However, in this method, the digestibity of the test protein (in this study, ^{2}H-labeled whole hens' egg protein) is measured in comparison to a standard protein of known digestibility (^{13}C-labeled spirulina protein); it is possible that TPP from co-ingested black tea could affect the digestibility of both the test and the standard protein, thereby yielding no relative measurable effect. Therefore, two experiments were conducted. First, using the dual-tracer method, the effect of co-ingestion of tea on the IAA digestibility of whole hens' egg protein was measured against a standard

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protein (^{13}C-labeled spirulina protein), in subjects in whom whole hens' egg protein digestibility, without tea, had earlier been measured (4) by the same method. Second, since the true IAA digestibility of the standard protein used in the first experiment (^{13}C-labeled spirulina protein) could also be affected by TPP, the digestibility of ^{13}C-spirulina protein was measured with and without the same amount of co-ingested black tea (as in the first experiment), in same subjects, relative to a standard of a ^{2}H-labeled crystalline IAA mixture.

Methods

Subjects in whom whole boiled egg protein digestibility had earlier been determined with reference to a spirulina protein standard (4) were approached, since their data of true IAA digestibility of egg protein without tea could be used as control for this study. The present study was conducted 2 months later, and the subjects reported no gastrointestinal symptoms or illnesses during the intervening period. Five subjects (n=3 female and n=2 male) consented to be studied again for determining whole egg protein digestibility against the same standard spirulina protein, when ingested with black tea. Since the independent effect of black tea on spirulina protein (used as a standard protein in the egg protein digestibility assessment) also needed to be characterized, 3 (n=1 female and n=2 male) of the 5 subjects consented to additional measurements of spirulina protein digestibility referenced to a standard crystalline ^{2}H-AA mixture, with and without the ingestion of black tea.

The subjects were within the normal BMI range (18.5 to 25 kg/m^{2}), aged between 20-27 years, had no food allergies, and did not smoke. They had no serious illness three months prior to the study, not on antibiotics within four weeks before the study, and had not consumed alcohol in the last 24 hours. Details of subject screening and enrolment are provided in Supplemental Figure 1. The study was approved by Institutional Ethical Review Board and all the subjects provided informed written consent.

For determining the effect of black tea polyphenols on spirulina protein digestibility, two sequential test meals that contained [U-^{13}C]-labeled spirulina protein (test protein, Cambridge Isotope Laboratories, Andover, MA, USA) and a [U-^{2}H]-labeled AA mixture (standard protein, Cambridge Isotope Laboratories, Andover, MA, USA) were administered with and without black tea. The composition of the [U-^{2}H]-labeled AA mixture is given in Supplemental Table 1. For the whole egg protein digestibility measurement, [U-^{2}H]-labeled whole boiled egg protein (test protein) and [U-^{13}C]-spirulina (standard protein, whose digestibility was measured as above, with and without black tea), were administered with and without the same quantity of black tea. The ^{2}H intrinsically labeled hen's eggs were produced as described earlier (4). Briefly, 2 layer hens' were orally dosed with 12 mg/day of [U-^{2}H] labeled crystalline AA mixture and eggs were collected. The labeled eggs were then boiled, shelled and stored immediately at -80°C. For the experiment, the eggs were thawed at 4°C overnight, minced and added to the curry.

A culturally acceptable meal consisting of ghee-rice and tomato curry was used as the base meal for all experiments. The protein content of the meals that had no egg protein (as in the spirulina digestibility experiment) was kept constant by adding unlabeled chickpea as the protein source. The unlabeled chickpea was soaked overnight for 12 hours, pressure cooked

on the morning of the experiment, and added to the curry as needed. The test meals provided one-third of the daily energy and protein requirement for adults, of which the egg or chickpea contributed two-thirds of the protein in the meal. The nutrient composition of the test meals are provided in Table 1. The black tea was prepared by adding 15 g of black tea powder (Brooke Bond, Red Label, Mumbai, India) to 240 g of boiling water for 10 minutes. The test meal and black tea were divided into aliquots (see below) for plateau feeding and were warmed in a microwave for 10 seconds before they were administered to the subjects.

On the day of the digestibility measurement, subjects reported at 0630 h to the metabolic unit after an overnight fast of 10 hours. The experiment started at 0700 h and continued for the next 8 hours. The subjects were restricted to minimal physical activity during the experiment. A primed plateau feeding protocol was initiated, in which the whole meal with the isotopes was divided into mini-meals and fed at hourly intervals. The cooked test meal was portioned into 11 parts, each part constituting one mini-meal and the black tea was portioned into 10 parts, each part consisting of one mini-cup, which on an average was 13 g. A priming meal was fed (consisting of 3 mini-meals) along with ^{13}C-bicarbonate as a priming dose for the bicarbonate pool (4 mg/kg, Cambridge Isotope Laboratories, Andover, MA, USA, >99% purity), with or without tea (priming dose of 3 mini-cups). This was followed by hourly single mini-meals, with or without a mini-cup of tea, depending on the experiment, for the next 7 hours. One of the mini-meal portions was retained for isotopic analysis. The test meals and black tea were tested for their total polyphenol content (TUV SUD, South Asia) using the Folin Ciocalteau (14) method.

A basal blood sample was collected after securing an indwelling venous catheter (Jelco 22 G, Medex Medical Ltd, Lancashire, UK) followed by half hourly samples from the 5th to 8th hour (a total of 8 blood samples were collected), representing the plateau fed-state period (13). At each time point 4 mL blood was withdrawn. Whole blood was transferred into EDTA coated evacuated tubes (Becton Dickenson, NJ, USA) to separate plasma in a refrigerated centrifuge, which was then aliquoted and stored at -80°C until analysis. Breath samples were collected using 10 mL evacuated plain glass tubes (Becton Dickenson, NJ, USA) at baseline, followed by hourly collections for the experimental duration. These samples were stored at room temperature until analysis.

Plasma samples were deproteinized, and amino acids were collected by cation exchange and derivatized to their ethoxycarbonyl ethyl esters. The [^{13}C] and [^{2}H] isotopic enrichments of the IAA were analysed by LC–MS/MS (Liquid Chromatography with Tandem Mass Spectrometry, 6495 QQQ with i-Funnel technology; Agilent, CA, USA), as explained in detail earlier (13). Whole meal samples underwent gas phase acid hydrolysis prior to measurement of [^{13}C] and [^{2}H] isotopic abundance of the IAA (except for tryptophan) in the meal. Baseline meal isotopic abundance were measured in exactly similar test meals containing unlabeled whole boiled egg and spirulina. The [^{13}C] and [^{2}H] enrichments of IAA were expressed as parts per million excess (ppme) over the baseline. Breath samples were analysed for $^{13}CO_2$ abundance using the isotope ratio mass spectrometry (IRMS, Delta V Advantage, Thermo Fisher Scientific Inc., Bremen, Germany).

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The true IAA digestibility (%) of spirulina protein with or without black tea was calculated as:

[Plasma ^{13}C IAA (ppme)/Meal ^{13}C IAA (ppme)] / [Plasma ^{2}H IAA (ppme)/Meal ^{2}H IAA (ppme)] x 100

The true IAA digestibility (%) of whole boiled egg protein without black tea was taken from the previous study (4), and was re-calculated as:

[Plasma ^{2}H-IAA (ppme)/Meal ^{2}H-IAA (ppme)] / [Plasma ^{13}C-IAA (ppme)/Meal ^{13}C-IAA (ppme)] x 100 x (Dig_{Std}/100)

where, Dig_{Std}: mean true IAA digestibility of spirulina protein determined in the present study with reference to crystalline IAA (as above), without the ingestion of black tea. Since these paired spirulina IAA digestibility values were available only for 3 subjects and digestibility values of spirulina matched with that of an earlier study in subjects with similar characteristics (13), the mean of these values was used for the other 2 subjects.

The true IAA digestibility (%) of whole boiled egg protein co-ingested with black tea was calculated as:

[Plasma ^{2}H-IAA (ppme)/Meal ^{2}H-IAA (ppme)] / [Plasma ^{13}C-IAA (ppme)/Meal ^{13}C-IAA (ppme)] x 100 x ($Dig_{Std\text{-}TPP}$/100)

where, $Dig_{Std\text{-}TPP}$: mean true IAA digestibility of spirulina protein determined in the present study with reference to crystalline AA mixture, when black tea was co-ingested (as above). As noted earlier, since paired $Dig_{Std\text{-}TPP}$ values were available only for 3 consenting subjects, the mean $Dig_{Std\text{-}TPP}$ was used for the other 2 subjects.

The number of subjects needed to evaluate differences in egg protein digestibility between the black tea conditions was calculated using an observed difference of 6% in mean digestibility between chicken meat and lyophilized egg-white protein from an earlier study (4). Five subjects were required to detect a 12% significant difference (doubling the difference previously observed) in digestibility between egg protein with and without tea, at a 5% level of significance, with a power of 80%. Since the mean and median of the mean true digestibility and the individual IAA digestibility values were similar and the SD's were low (less than ½ mean) the data were assumed to be normally distributed. Paired t-tests were performed to evaluate the difference between the mean and individual IAA true digestibilities of the egg with and without black tea (n=5); and spirulina with and without black tea (n=3). For all the comparisons, P<0.05 was considered significant. All calculations were performed on SPPS Statistics, version 17.0 (IBM, USA).

Results

The demographics and clinical assessment of the subjects in both experiments is provided in Table 2. The TPP content of the test meals approximately doubled when black tea was added; for the spirulina protein digestibility meal, these were 0.22% and 0.12% (W/W) with and without black tea respectively, and for the whole boiled egg protein digestibility meal,

these were 0.29% and 0.15% respectively. The ^{2}H and ^{13}C IAA enrichments (ppme) of the test meals are given in Supplemental Table 2. The ^{2}H and ^{13}C plasma enrichments of each IAA at plateau (from 5th to 8th hour of the experimental protocol) are shown in Supplemental Figure 2 and the mean ^{2}H and ^{13}C IAA plasma enrichments at plateau are given in Supplemental Table 3. The mean inter-individual coefficient of variation (CV) of the ^{2}H and ^{13}C plasma IAA enrichments at plateau was 24% and 19% respectively. These values ranged from 19% for methionine to 33% for lysine for specific ^{2}H plasma IAA enrichment, and from 17% for phenylalanine and 23% for valine for specific ^{13}C plasma IAA enrichments. The breath $^{13}CO_2$ enrichment reached a similar plateau value after 5 h in all the four experiments (two for egg protein and two for spirulina protein digestibility, with and without black tea) indicating a similar oxidative disposal of [U-^{13}C] labeled spirulina (Supplemental Figure 3) (4).

The true mean or individual IAA digestibility of spirulina protein ingested without and with black tea did not differ significantly (P=0.803); the differences in individual IAA digestibility were minimal, in the range of 0.3% (valine) to 7.4% (methionine) (Table 3). However, there was a significant effect of black tea on the mean true IAA digestibility of whole boiled egg when co-ingested without and with black tea, resulting in a significant reduction of 17% (P<0.001, Figure 1). A similar significant reduction was noted for the individual true IAA digestibility of whole boiled egg protein, when ingested with black tea (P<0.05 for all IAA, Table 3). This difference in IAA digestibility ranged from 12% lower for leucine to 22% lower for lysine. Since the calculation of digestibility involved a two-step measurement, meaning that the digestibility of the standard was measured separate to the measurement of egg digestibility, the overall variance was estimated as the variance of the product of egg and standard protein digestibilities. The variability remained the same for egg without tea (2.7 %) whereas it increased to 5% for egg with tea.

Discussion

This study determined the inhibitory effect of black tea polyphenols on the true IAA digestibility of whole boiled egg protein, using the dual isotope tracer technique. The approach involves the comparison of two differentially labeled proteins, a test (whole egg) and a standard (spirulina) protein, which are ingested together. However, in this method, if the digestibility of both proteins are equally affected by black tea, the specific TPP effect on egg protein digestibility will not be discernible. Therefore, the present study independently determined the effect of black tea on spirulina protein, in comparison to a standard of labeled crystalline AA mixture that do not require digestion. As observed, the co-ingestion of black tea resulted in a significant decrease of 17% in the mean true IAA digestibility of whole egg protein in comparison to spirulina protein, but there was no specific significant effect of black tea on spirulina protein.

The effect of TPP on protein digestibility appears to vary depending on the source of the protein. This has been studied extensively *in vitro* for beverages such as black tea, green tea, and coffee on varying protein sources; however, there are limited studies in animal models and none in humans (15,11). Among the *in vitro* studies of the effect of black TPP on egg protein, one found a dose-dependent decrease in the digestibility of spray dried egg yolk

protein. While the details of the assay were not available, it appeared that at a similar black tea content as the present study, the reduction in digestibility of egg yolk protein was 32% (8). In another *in vitro* study, the addition of quercetin, a pure extract of black TPP (at 2μM, which compares to four times the concentration given through black tea in the present study), decreased the enzymatic (trypsin and chymotrypsin) digestion of egg white and yolk proteins by 6 and 10% respectively (9). Methodological differences, the accessibility of the proteins within the food matrix, the type and concentration of digestive proteases and substrates used, and the duration of the reaction, could explain the variation in the reduction observed between the studies.

TPP-protein interactions have been studied in great detail *in vitro*, and depend on the polyphenol structure and molecular size, the amino acid composition and secondary or tertiary structure of the protein and the pH (which influences protein folding); these may be either covalent or non-covalent interactions (hydrogen bonds or hydrophobic interactions) (16,10). TPP interacts both with the substrates (dietary protein) and the proteases that hydrolyse them (15, 17). The binding of TPP to proteases can alter the active configuration of the enzyme by destabilizing the orientation of the active site, or by allosteric inhibition (15). Black TPP extracts, which are rich in high MW polyphenols, strongly inhibited trypsin activity at 0.01 mg/ ml, a lower concentration than used in the present study (4.6 mg/ ml of black TPPs) (18). Among the amino acids, the aromatic, amino, and thiol R-groups are prone to interactions with TPP (16,19), rendering them inaccessible to protease activity. In particular, TPP interactions with specific egg proteins (ovalbumin and lysozyme) were related to secondary structural changes of the proteins that promoted their digestion by pepsin in acidic pH but inhibited their digestion by bovine pancreatic extract in alkaline conditions (10).

In this study, spirulina protein digestibility was not significantly affected by TPP. This could be because of intrinsic polyphenols or molecules that bind to proteins and reduce the effect of extrinsic polyphenols; an example is the presence of phycobilisomes (phycobiliprotein-chromophore complexes). Multiple chromophores are known to bind to phycobiliproteins mainly through sulfhydryl groups of cysteine and through non-covalent interactions with surrounding amino acids. Since, the amino acid side chains are already engaged, the TPP associations with spirulina proteins may be minimal (20). Similarly, in other protein foods, the presence of varied amounts of intrinsic polyphenols or molecules that bind to proteins might influence the effect on extrinsic polyphenols on protein digestibility. For example, the fecal IAA digestibility of barley and soya in rats, determined by fecal nitrogen balance, merely reduced by about 5 % and 3 % when co-administered with a black tea extract (11).

The matrix in which the protein is consumed affects its digestibility. Anti-nutritional factors such as protease inhibitors, polyphenols, tannins, and phytic acid in the food matrix lower protein digestibility (7), affecting plant sources more than ASF(4,13). However, as this study shows, the concomitant consumption of an ASF (egg) with tea, reduces its protein digestibility substantially. Beverages rich in polyphenols, such as tea and coffee, are generally taken after meals, especially following breakfast (12). In India, the mean per capita daily intake of tea is 11 g, ranging from 2 to 25 g between different Indian states (21). The consumption of tea has been increasing over time, with a predicted increase of 3% per

annum (22). Owing to its affordability, there is no distinction in the consumption of tea between socioeconomic groups. While multiple health benefits are ascribed to the anti-oxidant and anti-inflammatory properties of TPP, deleterious effects on nutrient bioavailability also exist. The amount of TPP given in this study during the plateau feeding protocol was equivalent to 100 ml of 6 cups of black tea, 2 cups of coffee and 10 cups of green tea (23). However, this can vary, as the TPP content of black tea infusions has been reported to range from 13 to 100 mg Gallic-Acid Equivalents per g of dry matter, depending on the type of black tea used and the infusion or brewing time (24, 25).

In the Indian population, where diets are cereal-based, there is 24% (urban and rural combined) risk of dietary quality protein inadequacy (2). With an additional demand due to the consumption of tea or coffee (assuming a 5% reduction in digestibility if one cup of tea or coffee were consumed with one of the major meals), along with the extra demand of a poor environment, the risk, based on survey data (21) and assumptions reported in Minocha et al (2), could increase to 43%. This is particularly important in vulnerable populations (growing children, women of reproductive age and the elderly) with the potential to adversely impact growth and functionality. Equally, the effect may be lower in plant protein sources such as legumes, that are otherwise rich in polyphenols, but still may be significant when legumes are the principal source of high quality protein.

The strength of this study is the design that allowed for paired individual determination of the effect of TPP on egg protein digestibility *in vivo* and the use of a relatively minimally invasive method to determine IAA digestibility when compared to naso-gastric intubation or ileostomy. However, since the dual isotope tracer technique has been recently developed, there is still need for validations against standard methods that directly measure IAA digestibility at the ileum. Limitations of this method include the assumption that amino acids are completely absorbed and amino acids from both test and the standard protein undergo identical splanchnic extraction. A limitation of the design is the use of control data on whole boiled egg (without black tea) digestibility from an earlier experiment, even though the same subjects were studied, and the repeat measurement with the co-ingestion of tea was made within a period of 2 months from the earlier experiment. In addition, the subjects were healthy to begin with, and remained apparently healthy in the intervening period, with no gastrointestinal symptoms. Another limitation was that since the variability of individual IAA digestibility increased on co-ingestion of TPP with spirulina, the possibility of an inhibitory effect of TPP on spirulina cannot be completely ruled out. Further, it was desirable that in this set of successive experiments on the same subjects, a subject- specific spirulina digestibility (without tea) would be used as the standard protein digestibility in the first experiment, in which egg protein digestibility was measured relative to the spirulina protein as the standard. However, spirulina protein digestibility was not measured in 2 of the 5 subjects owing to their unavailability; therefore, the mean measured spirulina IAA digestibility was used as the standard protein digestibility value for these 2 subjects. This was reasonable, since earlier measurements of spirulina digestibility against a standard of crystalline ^{2}H IAA had shown low interindividual variability, and in the present study as well, the interindividual variation in IAA digestibility in the 3 subjects was low (ranging from 1.2% for lysine to 3.4% for methionine). Finally, the measured effect of tea needs to be further investigated when it is ingested as an infusion with milk, because tea is culturally

drunk with milk in India (12). Milk protein has also been shown to interact with chocolate polyphenols, reducing their bioavailability *in vivo* (26). Therefore, the reductive effect of TPP on the digestibility of co-ingested dietary protein may be different when consumed as tea with milk, but this needs to be tested with labeled milk protein.

In conclusion, the co-ingestion of high-quality egg protein with black tea is detrimental for its true IAA digestibility. In populations subsisting on cereal-diet with small amounts of high-quality protein foods, this effect could further contribute to the risk of protein inadequacy, but the complex effects of a mixed food matrix, including milk, needs to be evaluated. In addition, the detrimental effect is not uniform, and could be minimal with foods that have other molecules inherently binding to their proteins.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AA	Amino Acids
ASF	Animal Source Foods
BMI	Body Mass Index
IAA	Indispensable Amino Acids
LC-MS/MS	Liquid Chromatography with Tandem Mass Spectrometry
IRMS	Isotope Ratio Mass Spectrometry
PE ratio	Protein energy ratio
PPME	Parts Per Million Excess
TPP	Tea Polyphenols

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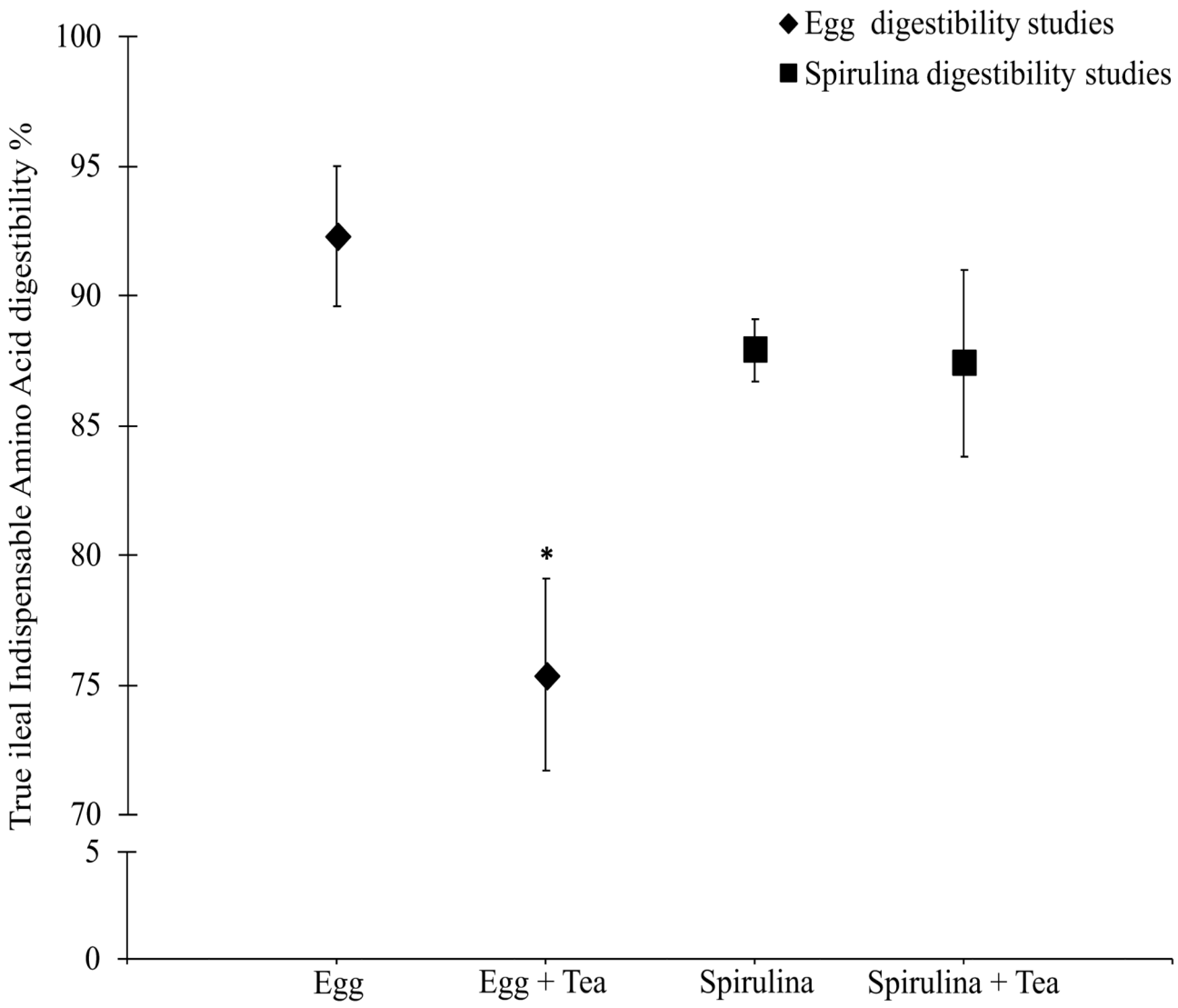

Figure 1.

True IAA digestibility of whole egg and spirulina protein consumed without and with black tea in Indian adults. Values are mean ± SD, n= 5 (egg) or 3 (sprirulina). Paired t tests were performed within egg and spirulina studies. * Different from egg, $P<0.001$. IAA, indispensable amino acids.

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Table 1

Nutrient composition of the standardized egg and spirulina test meals consumed by Indian adults with and without black tea [1,2]

	Egg	Egg + Tea	Spirulina	Spirulina + Tea
Energy, kcal/meal	691 ± 44.6	697 ± 47.7	766 ± 44.5	767 ± 45.7
Protein, g/meal	21.5 ± 0.5	21.7 ± 0.9	19.8 ± 0.6	19.9 ± 0.6
Fat, g/meal	29.7 ± 0.9	29.95 ± 1.1	24.6 ± 0.2	24.6 ± 0.3
Carbohydrate, g/meal	83.7 ± 8.7	84.4 ± 8.9	115.9 ± 9.8	116.1 ± 10.0
PE ratio[3]	12.5 ± 0.6	12.5 ± 0.6	10.4 ± 0.3	10.4 ± 0.3

[1] Values are mean ± SD, n=5 for Egg ± Tea meal, n=3 for Spirulina ± Tea meal, paired studies in subjects egg and egg + tea; and spirulina and spirulina + tea. The subjects in spirulina ± tea were subset from egg ± tea study. The nutrient composition of the egg meal is an mean ± SD of n=5 subjects from a previously published whole boiled egg study (4).

[2] Standardized meal consisted of a culturally acceptable tomato curry and ghee rice including the test protein.

[3] Protein energy ratio

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Table 2

Demographic characteristics and anthropometry of Indian adults who participated in egg and spirulina with and without black tea experiments[1]

Variables	Egg ± Tea	Spirulina ± Tea[2]
Age, y	23.6 ± 2.5	23.7 ± 3.5
Weight, kg	55.3 ± 3.6	55.3 ± 2.2
Height, m	1.6 ± 0.1	1.6 ± 0.1
BMI[3], kg/m^2	22.0 ± 2.8	21.0 ± 1.8
Hemoglobin, g/dl	12.7 ± 1.4	13.2 ± 1.6

[1] Values are mean ± SD, n=5 for Egg ± Tea (*n*=3 female and *n*=2 male) and n=3 for Spirulina ± Tea experiment (*n*=1 female and *n*=2 male), paired studies in subjects for egg and egg + tea; and spirulina and spirulina + tea.

[2] The subjects in spirulina ± tea were subset from egg ± tea study

[3] BMI, Body Mass Index

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Table 3

True IAA digestibility values for hens' whole boiled egg and spirulina protein with and without black tea in Indian adults[1,2]

	True IAA digestibility, %					
	Egg *n*=5	**Egg + Tea[3] *n*=5**	***P*-value**	**Spirulina *n*=3**	**Spirulina + Tea[3] *n*=3**	***P*-value**
Methionine	87.3 ± 7.3	71.9 ± 10.3	0.024	86.2 ± 3.4	93.6 ± 8.6	0.169
Phenylalanine	93.3 ± 2.4	76.8 ± 10.9	0.041	93.1 ± 2.6	88.2 ± 18.0	0.718
Threonine	95.8 ± 1.4	75.0 ± 9.6	0.007	82.3 ± 2.7	83.5 ± 14.2	0.890
Lysine	102.2 ± 6.7	80.6 ± 6.0	<0.001	89.2 ± 1.2	87.7 ± 6.2	0.654
Leucine	88.5 ± 3.0	76.6 ± 2.4	0.001	86.7 ± 1.5	85.0 ± 3.2	0.549
Iso-leucine	87.2 ± 3.3	70.2 ± 3.9	0.003	85.4 ± 3.1	82.0 ± 5.2	0.531
Valine	92.1 ± 6.3	74.0 ± 3.3	0.010	92.4 ± 3.0	92.1 ± 5.5	0.858

[1] Values are mean ± SD; paired studies. The subjects in spirulina ± tea were a subset from egg ± tea study.

[2] IAA, indispensable amino acids

[3] Paired t tests were performed within egg and spirulina studies.