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Short Communication

The ingestion of different dietary proteins by humans induces large changes in the plasma tryptophan ratio, a predictor of brain tryptophan uptake and serotonin synthesis

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SUMMARY

Background & aims: The ingestion by rats of different proteins causes large differences in the plasma ratio of tryptophan to other large neutral amino acids, which predicts brain tryptophan uptake and serotonin synthesis. We evaluated in humans whether ingesting these proteins also produces large excursions in the tryptophan ratio.

Methods: Fasting males ($n = 6$) ingested V-8 Juice containing 40 g of α-lactalbumin, gluten, zein or starch. Blood was drawn before and at 30 min intervals after ingestion for 4 h; tryptophan and other large neutral amino acids were quantitated.

Results: Pre-meal plasma tryptophan was ~50 nmol/ml; the tryptophan ratio was ~0.010. α-Lactalbumin increased plasma tryptophan (3-fold) and the tryptophan ratio (50%); starch did not change either tryptophan variable, while gluten caused a modest (25%) and zein a large reduction (50%) in plasma tryptophan. Gluten and zein reduced the tryptophan ratio. The maximal difference in the tryptophan ratio occurred between α-lactalbumin and zein and was large (~3-fold).

Conclusion: Since the plasma tryptophan ratio predicts brain tryptophan uptake and serotonin synthesis in rats, the differences in the ratio produced in humans by these proteins may modify serotonin synthesis, and perhaps elicit serotonin-linked changes in behavior.

1. Introduction

The brain neurotransmitter serotonin (5-hydroxytryptamine; 5HT) is synthesized from the essential amino acid tryptophan. The rate of 5HT synthesis is sensitive to tryptophan concentrations in brain.[1] Brain tryptophan concentrations are influenced by tryptophan uptake from the circulation, mediated by a competitive transporter located at the blood–brain barrier (BBB) and shared among several large neutral amino acids (LNAA), including tryptophan, tyrosine, phenylalanine, leucine, isoleucine and valine.[1] Brain tryptophan uptake and 5HT synthesis are therefore influenced by changes in the plasma concentrations of tryptophan and the other LNAA. Because foods containing carbohydrates and proteins modify plasma concentrations of the LNAA, their ingestion by rats alters brain tryptophan uptake and 5HT synthesis. Inasmuch as changes in 5HT synthesis modify neuronal 5HT release, a potential link is made between diet and brain functions involving 5HT neurons.[1–3]

Initial studies in rats connecting food intake and brain tryptophan defined the difference between ingesting carbohydrates alone or carbohydrates with protein. Carbohydrate ingestion increased tryptophan uptake into brain (and 5HT synthesis) by lowering plasma concentrations of the LNAA and raising plasma tryptophan. The combination of protein with carbohydrates blocked the rise in brain tryptophan because the rise in plasma tryptophan was counterbalanced by increases in the plasma concentrations of other LNAA (hence, no net change in competition for transport).[1] The influence of meals on plasma LNAA and the BBB uptake of tryptophan was summarized as a plasma ratio of tryptophan to the sum of its LNAA competitors. When carbohydrates are ingested, plasma tryptophan rises while plasma concentrations of the other LNAA

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decline, resulting in an increase in the plasma tryptophan ratio. When protein is ingested with carbohydrates, both plasma tryptophan and the other LNAA rise, by proportionally-similar amounts, and the ratio does not change.[1]

This basic set of observations, that carbohydrate ingestion raised the plasma tryptophan ratio, while added protein (*any* protein) blocked the effect, began to change when Markus and associates reported that the ingestion of either of two mammalian proteins (casein, α-lactalbumin) produced *different* effects on the plasma tryptophan ratio and on 5HT-mediated behaviors in stress-vulnerable human subjects.[4] Subsequent rat studies confirmed the finding, showing that ingesting α-lactalbumin raised the plasma tryptophan ratio, brain tryptophan and neuronal 5HT release, compared to casein consumption.[5,6] Such effects were then found to be much larger, when a range of animal and plant proteins was examined.[7] Notably, the differences observed between rats ingesting a meal containing α-lactalbumin vs. those ingesting zein were remarkable. Soon after ingesting α-lactalbumin- or zein-containing meals, the plasma tryptophan ratio, brain tryptophan concentrations and 5HT synthesis rate differed by several-fold, effects substantially greater than those seen between casein- and α-lactalbumin-containing meals.[7]

These findings suggest in humans that ingesting different proteins should produce much greater changes in the plasma tryptophan ratio, and ultimately 5HT-linked behaviors, than those previously observed.[4] In the present study, we have begun to explore this possibility by examining the effects of a meal containing each of three dietary proteins or carbohydrates on the plasma tryptophan ratio in normal humans. The proteins selected elicited the largest increase (α-lactalbumin), no change (wheat gluten), or the largest decrease (zein) in the plasma tryptophan ratio in rats.[7]

2. Methods and materials

2.1. Subjects

Male subjects were recruited by advertisements at local universities in San Diego. Respondents were screened briefly over the phone, and following completion of a signed consent form, were interviewed in person and examined by a study physician. Subjects were included who were 18–30 years of age and had a body mass index (mass(kg)/height(m)^2) of 23–27. Subjects were excluded who had (a) an allergy to gluten and/or celiac disease, (b) any metabolic disease (e.g., diabetes (verified by fasting plasma glucose measurement), liver or kidney disease) or digestive disease, (c) a history of an Axis I disorder, including major depression, evidence of current depression, and/or (d) been taking a psychoactive medication.

2.2. Study design

The final study group consisted of six male subjects, 27 ± 2 years old with a body mass index of 24 ± 1 (mean ± SEM). They reported to the lab at 7–8 AM on four occasions, separated by 3–5 days, having consumed no food or beverages (except water) since 10 PM the night before. On admission, an indwelling venous catheter was inserted, and a blood sample taken just prior to beverage ingestion (0 min). The beverage was then ingested in less than 15 min, and blood samples were obtained at 30 min intervals for 4 h. During this time, subjects could read, watch television, or play video games. Water was available for consumption. At the end of the study, the catheter was removed, subjects were offered lunch, and then released. This protocol was conducted by the Eating Disorders Program at the Center for Clinical Research, University of California, San Diego, and was approved by the Human Research Protections Program of the University of California, San Diego.

2.3. Test beverage composition

The base was spicy hot V-8 juice (16 oz; Campbell's Soup Co., Camden NJ; 102 kcal; 4.0 g protein, 20.0 g carbohydrates, 0 g fat), to which was added 40 g protein (α-lactalbumin, Davisco Foods International, Le Sueur MN; wheat gluten, MGP Ingredients Inc., Atchison KS; zein, Freeman Industries, Tuckahoe, NY) or cornstarch (Argo, ACH Food Companies, Oakbrook IL). The total caloric content of each beverage was 262 kcal.

2.4. Biochemical analyses

Blood samples were collected in heparin, and centrifuged to obtain plasma. Plasma samples were assayed for large neutral amino acids by HPLC and electrochemical detection.[7] The plasma tryptophan ratio was calculated as (molar ratio) (tryptophan)/(tyrosine + phenylalanine + leucine + isoleucine + valine), and the plasma tyrosine ratio as (tyrosine)/(tryptophan + phenylalanine + leucine + isoleucine + valine).[1] Plasma insulin concentrations were

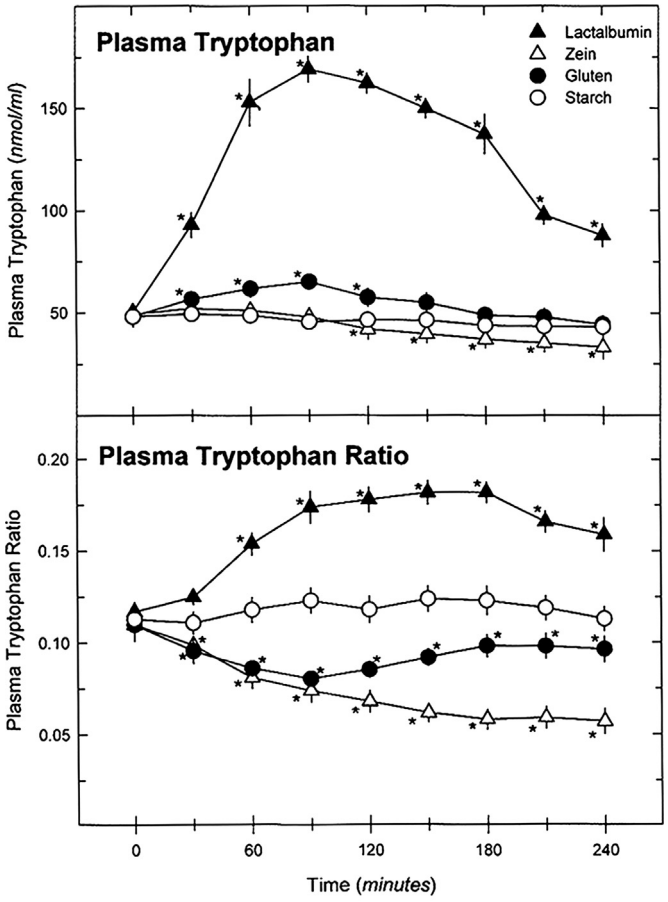

Fig. 1. Effect of protein ingestion by human subjects on plasma tryptophan concentrations and the plasma tryptophan ratio. Fasting male subjects ($n = 6$) ingested a V-8-based drink (16 oz) containing 40 g of the indicated protein on four separate occasions. Blood samples were collected at 0 min and at 30 min intervals thereafter for 240 min. The plasma tryptophan ratio is the molar ratio of the tryptophan concentration divided by the sum of the concentrations of tyrosine, phenylalanine, leucine, isoleucine and valine. Data are presented as the means ± sem. Data were analyzed by 2-way, repeated measures ANOVA. For plasma tryptophan, the effect of protein ($F = 80.36$, df = 3) and of time ($F = 30.49$, df = 8) were significant ($P < 0.001$); the interaction ($F = 26.21$, df = 24) was also significant ($P < 0.001$). For the plasma tryptophan ratio, the effect of protein ($F = 81.95$, df = 3; $P < 0.001$) and of time ($F = 2.48$, df = 8, $P < 0.05$) were significant; the interaction ($F = 46.17$, df = 24) was also significant ($P < 0.01$). *$P < 0.05$ vs. 0-time value (Newman–Keuls test).

quantitated by ELISA (C/N EZHI-14K, Millipore Corp, Billerica MA) on 0, 30, 60 and 90 min samples.

2.5. Data analysis

Data were analyzed by two-way analysis of variance (repeated-measures); post-hoc testing utilized the Neuman–Keuls test (SigmaPlot 11, Systat Software, San Jose CA). Data are presented as means ± SEM.

3. Results

Ingestion of the α-lactalbumin-containing beverage produced a notable rise in plasma tryptophan concentrations, peaking about 3-fold over fasting values within 90 min, and then declining markedly by 240 min (Fig. 1, top panel). Ingestion of the gluten-containing drink raised plasma tryptophan modestly, peaking at 90 min. Ingestion of the drink containing cornstarch did not change plasma tryptophan, while ingestion of the zein-containing drink lowered plasma tryptophan to values about 33% below fasting values at 240 min (0 min: 49 ± 1 nmol/ml; 240 min: 33 ± 6 nmol/ml). Following α-lactalbumin ingestion, the plasma tryptophan ratio rose from 0.117 ± 0.005 at 0 min to 0.182 ± 0.006 at 150 min (Fig. 1, bottom panel). Ingestion of the cornstarch beverage did not change the plasma tryptophan ratio, while ingestion of wheat gluten elicited a modest reduction in the plasma tryptophan ratio (from 0.110 ± 0.009 at 0 min to a nadir of 0.080 ± 0.004 at 90 min). Ingestion of the zein-containing drink reduced the plasma tryptophan ratio from 0.110 ± 0.004 to a nadir of 0.057 ± 0.007 at 240 min. The maximal difference in the plasma tryptophan ratio between the day α-lactalbumin was ingested, compared to that when zein was consumed, was thus about 3-fold.

The plasma concentrations of the other aromatic and branched-chain amino acids were also modified by the ingestion of the meals. For each amino acid, significant effects of treatment (a protein or starch) and time occurred (see Table 1 legend). Plasma tyrosine rose about two-fold after α-lactalbumin (peak at 60 min) and gluten ingestion (peak at 90 min), about 50% following zein ingestion (peak at 90–120 min), but not after cornstarch ingestion. Plasma phenylalanine concentrations did not change following starch ingestion, and rose less than 2-fold after consumption of the protein-containing drinks (peaks at 90 min; Table 1). Plasma leucine rose about three-fold following ingestion of α-lactalbumin (peak at 60 min), two-fold after gluten (peak at 90 min) and zein (peak at 150 min) consumption, and declined modestly after cornstarch ingestion (maximal decline at 180 min). Plasma isoleucine concentrations showed a modest decline after starch ingestion, large (3-fold) and intermediate (2-fold) increases after ingestion of α-lactalbumin and gluten, respectively, and no significant change after zein ingestion. Plasma valine concentrations showed no changes after starch or zein ingestion, and modest increments after α-lactalbumin (peak at 60 min) and gluten (peak at 90 min) ingestion.

Plasma insulin concentrations peaked 30 min following the ingestion of all beverages (Supplemental Fig. 1). Analysis of variance revealed a significant effect of time, but not treatment (protein or starch).

4. Discussion

These results show in humans that the ingestion of small meals differing in protein source produces different effects on the plasma tryptophan ratio. The amount of protein ingested is not large (about 40% of the average daily protein intake of Americans[8]). Ingestion of α-lactalbumin raised the plasma tryptophan ratio about 55%, gluten produced a modest reduction of about 25%, and zein induced a larger reduction of about 50%. The maximum difference in plasma tryptophan ratio between ingesting the α-lactalbumin and zein meals was large (about 3-fold; Fig. 1). Such changes in the tryptophan ratio occur in rats ingesting these same proteins, and produce marked differences in brain tryptophan concentrations and 5HT synthesis.[7] The animal findings thus raise the possibility that the plasma tryptophan ratio changes observed in this study may also influence brain tryptophan concentrations and 5HT function in

Table 1
Effect of protein ingestion by human subjects on the plasma concentrations of PHE, ILE and VAL.

Amino acid	Meal	Time after meal ingestion (minutes)								
		0	30	60	90	120	150	180	210	240
TYR	Starch	49 ± 3	51 ± 3	48 ± 2	44 ± 2	47 ± 4	44 ± 2	40 ± 1*	41 ± 2	43 ± 2
	α-Lactalbumin	49 ± 3	82 ± 4*	116 ± 9*	111 ± 9*	104 ± 8*	97 ± 5*	91 ± 8*	73 ± 6*	69 ± 5*
	Zein	54 ± 4	62 ± 3*	70 ± 4*	78 ± 5*	78 ± 6*	77 ± 3*	77 ± 4*	74 ± 5*	70 ± 4*
	Gluten	52 ± 4*	69 ± 2*	85 ± 6*	99 ± 7*	87 ± 6*	80 ± 6*	69 ± 4*	66 ± 4*	61 ± 5*
PHE	Starch	46 ± 4	48 ± 3	46 ± 2	43 ± 2	47 ± 3	45 ± 3	43 ± 1	44 ± 2	45 ± 2
	α-Lactalbumin	45 ± 3	72 ± 3*	82 ± 6*	75 ± 6*	68 ± 5*	62 ± 3*	58 ± 4*	48 ± 2*	46 ± 1
	Zein	49 ± 3	57 ± 3*	66 ± 2*	70 ± 5*	67 ± 4*	66 ± 2*	66 ± 3*	62 ± 3*	61 ± 3*
	Gluten	48 ± 2	65 ± 3*	74 ± 2*	84 ± 3*	72 ± 3*	67 ± 4*	58 ± 4	57 ± 3	53 ± 2
LEU	Starch	123 ± 10	127 ± 11	111 ± 9	98 ± 9*	105 ± 8*	97 ± 8*	90 ± 7*	94 ± 4*	98 ± 5*
	α-Lactalbumin	116 ± 5	243 ± 11*	361 ± 20*	348 ± 24*	318 ± 18*	279 ± 10*	251 ± 26*	178 ± 12*	170 ± 10*
	Zein	115 ± 4	154 ± 8*	200 ± 11*	241 ± 13*	252 ± 12*	262 ± 11*	262 ± 13*	253 ± 14*	243 ± 15*
	Gluten	116 ± 6	173 ± 6*	218 ± 10*	243 ± 11*	197 ± 16*	167 ± 16*	139 ± 8*	130 ± 9*	124 ± 9*
ILE	Starch	56 ± 7	58 ± 7	50 ± 6	43 ± 5*	45 ± 5*	41 ± 5*	38 ± 4*	39 ± 3*	42 ± 3*
	α-Lactalbumin	54 ± 4	112 ± 4*	153 ± 9*	172 ± 6*	159 ± 7*	136 ± 8*	121 ± 15*	86 ± 7*	77 ± 7*
	Zein	55 ± 2	64 ± 2	68 ± 2	66 ± 2	60 ± 2	58 ± 2	56 ± 3	49 ± 1	47 ± 3
	Gluten	54 ± 4	86 ± 8*	109 ± 2*	121 ± 4*	92 ± 8*	75 ± 7*	59 ± 6	57 ± 5	52 ± 4
VAL	Starch	165 ± 18	170 ± 16	164 ± 11	150 ± 10	155 ± 9	155 ± 14	149 ± 9	149 ± 9	157 ± 11
	α-Lactalbumin	169 ± 12	233 ± 7*	280 ± 17*	278 ± 12*	270 ± 15*	255 ± 14*	240 ± 14*	207 ± 9*	194 ± 10*
	Zein	176 ± 9	189 ± 10	196 ± 10	194 ± 10	183 ± 13	175 ± 8	175 ± 10	157 ± 8	154 ± 11
	Gluten	166 ± 10	206 ± 14*	229 ± 4*	265 ± 10*	227 ± 15*	210 ± 15*	181 ± 17	181 ± 11	171 ± 9

For plasma TYR, the effect of protein ($F = 42.69$, df = 3) and time ($F = 25.25$, df = 8) were significant ($P < 0.001$); the interaction ($F = 15.74$, df = 24) was also significant ($P < 0.001$). For plasma PHE, the effect of protein ($F = 24.85$, df = 3) and time ($F = 17.30$, df = 8) were significant ($P < 0.001$); the interaction ($F = 9.21$, df = 24) was also significant ($P < 0.001$). For plasma LEU, the effect of protein ($F = 60.86$, df = 3) and time ($F = 36.21$, df = 8) were significant ($P < 0.001$); the interaction ($F = 36.99$, df = 24) was also significant ($P < 0.001$). For plasma ILE, the effect of protein ($F = 58.08$, df = 3) and time ($F = 31.66$, df = 8) were significant ($P < 0.001$); the interaction ($F = 21.14$, df = 24) was also significant ($P < 0.001$). For plasma VAL, the effect of protein ($F = 16.84$, df = 3) and time ($F = 18.96$, df = 8) were significant ($P < 0.001$); the interaction ($F = 8.09$, df = 24) was also significant ($P < 0.001$). *$P < 0.05$ vs. 0-time value.

human subjects, a notion consistent with other behavioral and neurochemical findings.[4,9]

The present study expands on the work of Markus and colleagues, who fed humans beverages containing casein or α-lactalbumin.[4] They examined the test proteins in a breakfast–snack–lunch format, and made only a single measure of the plasma tryptophan ratio. As a result, neither the maximal effects of the proteins on the plasma tryptophan ratio, nor their duration, were assessed. And, the full effects of the proteins themselves may have been obscured by their inclusion in multiple meals. We therefore studied the ingestion of proteins in a simple food vehicle given as a single load, and followed the plasma tryptophan ratio over 4 h. In addition, we selected proteins expected to produce larger differences in the tryptophan ratio than those observed previously.[4,7] The protein drinks produced clear effects on the tryptophan ratio that appeared within 60 min and persisted for at least 4 h (Fig. 1). Moreover, the difference in the tryptophan ratio between zein and α-lactalbumin was notably larger than that observed previously between α-lactalbumin and casein.[4] Conceivably, therefore, the behavioral differences noted comparing α-lactalbumin and casein might be greater, if α-lactalbumin were tested against zein.

Several other points should be noted. First, the plasma ratio of tyrosine to the sum of its LNAA competitors was also calculated (see Supplemental Fig. 2), because this ratio determines tyrosine uptake into brain, and as a result influences its rate of conversion to catecholamine neurotransmitters.[10] In contrast to the observed effects of protein ingestion on the plasma tryptophan ratio, the effects on the plasma tyrosine ratio were too small to be expected to influence brain tyrosine levels or catecholamine synthesis.[7] Second, the rise in plasma leucine after zein ingestion reached and maintained a plateau for the entire 240 min period, while leucine concentrations following α-lactalbumin or gluten ingestion peaked and declined soon after meal ingestion (Table 1). This effect of zein probably reflects its relatively poor digestibility,[11] leading to slower amino acid absorption from the gut during the study. This effect was most apparent for leucine, since the leucine content of zein is quite high, while that of the other LNAA is much lower.[11] The finding suggests that the observed decline in the plasma tryptophan ratio may persist for a much longer time than for the other proteins (Fig. 1). Third, peak changes in the plasma tryptophan ratio (Fig. 1) reflected roughly both the molar percent tryptophan content and the tryptophan ratio of the proteins examined.[11] Though this reflection is based on a very limited number of proteins, it may suggest that the tryptophan content and/or the tryptophan ratio of individual dietary proteins, where available, can serve as a rough predictor of their effect on the plasma tryptophan ratio. This possibility depends on the plasma insulin response to each protein meal being similar, since insulin-mediated amino acid uptake into muscle is the major post-prandial sink for plasma amino acids.[12] The main effect of the meal on plasma insulin was not significant in the present study, consistent with this requirement. Nonetheless, this notion should be viewed as tentative, pending further study with additional proteins. And fourth, the plasma tryptophan ratio did not rise following carbohydrate ingestion (Fig. 1). This finding is typical of humans; rats and humans are known to differ in this regard.[13]

Conflict of interest statement

The authors declare that they have no conflict of interest.

Author contributions

JDF, KAL, MHF, WHK designed and conducted the study; JDF, KAL, LMM, ZLEI participated in data collection and analysis; JDF, MHF, WHK, ZLEI participated in data interpretation and manuscript writing. All authors read and approved the final manuscript.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.clnu.2012.11.027.

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