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Meal Composition and Plasma Amino Acid Ratios: Effect of Various Proteins or Carbohydrates, and of Various Protein Concentrations

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We examined the effects of meals containing various proteins and carbohydrates, and of those containing various proportions of protein (0% to 20% of a meal, by weight) or of carbohydrate (0% to 75%), on plasma levels of certain large neutral amino acids (LNAA) in rats previously fasted for 19 hours. We also calculated the plasma tryptophan ratios (the ratio of the plasma trytophan concentration to the summed concentrations of the other large neutral amino acids) and other plasma amino acid ratios. (The plasma tryptophan ratio has been shown to determine brain tryptophan levels and, thereby, to affect the synthesis and release of the neurotransmitter serotonin). A meal containing 70% to 75% of an insulin-secreting carbohydrate (dextrose or dextrin) increased plasma insulin levels and the tryptophan ratio; those containing 0% or 25% carbohydrate failed to do so. Addition of as little as 5% casein to a 70% carbohydrate meal fully blocked the increase in the plasma tryptophan ratio without affecting the secretion of insulin—probably by contributing much larger quantities of the other LNAA than of tryptophan to the blood. Dietary proteins differed in their ability to suppress the carbohydrate-induced rise in the plasma tryptophan ratio. Addition of 10% casein, peanut meal, or gelatin fully blocked this increase, but lactalbumin failed to do so, and egg white did so only partially. (Consumption of the 10% gelatin meal also produced a major reduction in the plasma tyrosine ratio, and may thereby have affected brain tyrosine levels and catecholamine synthesis.) These observations suggest that serotonin-releasing neurons in brains of fasted rats are capable of distinguishing (by their metabolic effects) between meals poor in protein but rich in carbohydrates that elicit insulin secretion, and all other meals. The changes in brain serotonin caused by carbohydrate-rich, protein-poor meals may affect subsequent food choice and various serotonin-mediated behaviors.

CONSUMPTION of a carbohydrate-rich, protein-free meal by rats presumed to have an empty stomach increases brain tryptophan levels and the rates at which neurons produce and release the neurotransmitter serotonin.[1–3] In contrast, consumption of a meal containing 18% casein fails to increase brain tryptophan or serotonin, and higher protein concentrations may even decrease brain levels of these indoles.[4,5] The effects of such meals on the brain are mediated by the changes that they produce in plasma amino acid patterns. Carbohydrates elicit insulin secretion, and the hormone markedly lowers plasma levels of most large, neutral amino acids (LNAA), without depressing those of tryptophan[6–11]; as a consequence, tryptophan is better able to compete for attachment to transport macromolecules in the capillary endothelia of the blood-brain barrier,[12,13] and thereby to enter the brain. Dietary proteins contribute some tryptophan molecules to the circulation, thereby raising plasma tryptophan levels, however, they also contribute far larger amounts of the other, more abundant LNAA, thereby lowering the plasma tryptophan ratio.[4,5] The ability of serotoninergic neurons to produce and release more or less serotonin, depending primarily on what the individual happens to be digesting, allows these neurons to function as "sensors" of nutritional and metabolic state, providing the rest of the brain with information that can influence such serotonin-dependent functions as control of appetite,[14] sleep,[15] mood,[16] and performance.[17]

Apparently no data are available on the proportions of proteins that must be present in a meal in order to block the increase that its carbohydrate content would otherwise produce in the plasma tryptophan ratio, nor in the proportion of carbohydrates needed to cause the increase. Information is also lacking on the relative efficacies of various natural proteins, especially those the rat might be likely to eat. We have thus examined the changes in the plasma tryptophan ratio produced after rats fasted for 19 hours consume single meals containing 0% to 20% casein in the presence of various amounts (0% to 75%) of carbohydrate, or those containing fixed proportions of a carbohydrate (70%) and one of the following proteins: casein, lactalbumin, egg white, peanut meal, and gelatin. We have also calculated the effects of the meals on other plasma amino acid ratios, ie, those known to determine brain levels of the other LNAA.[9] It will be shown that adding as little as 5% of a high-quality protein (casein) to a carbohydrate-rich meal can completely block its ability to increase the plasma tryptophan ratio, but that individual dietary proteins differ considerably in their ability to produce this effect.

MATERIALS AND METHODS

Adult male Sprague-Dawley rats weighing about 170 g (Charles River Breeding Laboratories, Wilmington, Mass) were exposed to light (Vita-Lite, Duro-Test Corp, North Bergen, NJ) between 9 PM and 9 AM daily, kept at a room temperature of 22 °C, and meal-fed a commercial stock diet (Charles River Rat, Mouse and Hamster Maintenance Formula) for a five-hour period (9 AM to 2 PM) once a day for two weeks. On the day of an experiment, groups of five were given access to one of several test diets, starting at 9 AM, and killed after two hours by decapitation. Plasma samples were frozen (−20 °C) until assay, and their amino acid contents (including total

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Table 1. Composition of Test Diets* and Radioimmunoreactive Total Insulin Contents in Plasma

Group	Carbohydrate† (% by wt)	Protein‡ (% by wt)	Fat§ (% by wt)	Cellulose (% by wt)	Caloric Density (calorie/g)	Radioimmunoreactive Insulin (μU/mL)
Experiment 1						
1.1 (Fasting)	—	—	—	—	—	13.7 ± 1.4 (5)¶^{a}
1.2	0 dextrin	0 casein	44	50	3.94	15.2 ± 0.7 (4)^{ab}
1.3	25 dextrin	0 casein	33	36	3.94	27.2 ± 3.9 (5)^{ab}
1.4	75 dextrin	0 casein	10	8	3.94	70.8 ± 5.3 (4)^{c}
1.5	0 dextrin	10 casein	39	45	3.94	20.0 ± 1.9 (4)^{ab}
1.6	25 dextrin	10 casein	28	31	3.94	28.1 ± 4.0 (5)^{ab}
1.7	75 dextrin	10 casein	6	3	3.94	75.5 ± 5.7 (4)^{c}
1.8	0 dextrin	20 casein	35	39	3.94	23.0 ± 1.8 (4)^{ab}
1.9	25 dextrin	20 casein	24	25	3.94	33.0 ± 3.1 (5)^{b}
1.10‖	75 dextrin	20 casein	2	0	3.94	72.5 ± 16.8 (3)^{c}
Experiment 2						
2.1 (Fasting)	—	—	—	—	—	15.0 ± 0.9 (5)^{a}
2.2	70 dextrin	0 casein	13	11	3.94	39.4 ± 4.2 (5)^{b}
2.3	70 dextrin	10 casein	8	6	3.94	65.8 ± 8.1 (5)^{c}
2.4	70 dextrin	20 casein	4	0	3.94	70.8 ± 5.5 (5)^{c}
2.5	70 dextrose	0 casein	13	11	3.94	41.0 ± 3.7 (4)^{b}
2.6	70 dextrose	10 casein	8	6	3.94	68.2 ± 9.5 (5)^{c}
2.7	70 dextrose	20 casein	4	0	3.94	65.8 ± 6.0 (5)^{c}
Experiment 3						
3.1 (Fasting)	—	—	—	—	—	16.5 ± 1.2 (5)^{a}
3.2	70 dextrose	0 casein	10	14	3.70	63.9 ± 7.9 (5)^{b}
3.3	70 dextrose	2.5 casein	10	11	3.80	74.2 ± 6.4 (5)^{b}
3.4	70 dextrose	5 casein	10	9	3.90	60.8 ± 5.4 (5)^{b}
3.5	70 dextrose	10 casein	10	4	4.10	74.1 ± 14.6 (5)^{b}
3.6	70 dextrose	10 lactalbumin	10	4	4.10	—
3.7	70 dextrose	10 egg white	10	4	4.10	—
3.8	70 dextrose	10 peanut	10	4	4.10	—
3.9	70 dextrose	10 gelatin	10	4	4.10	—

Values in parentheses are number of test animals.

*All diets contain 4% salt mixture (Rogers QR, Harper AE: J Nutr 87:267–273, 1965) and 2.2% vitamin mixture, (ICN Nutritional Biochemicals, Cleveland).

†Amalzo PFP starch, Rothstein Company, Woburn, Mass.

‡Teklad diets. A Harlan Sprague-Dawley Inc, Co, Madison, Wis.

§Crisco, Procter & Gamble, Cincinnati.

‖This diet contains approximately 73% dextrin because of its minerals and vitamins.

¶Means within a column not followed (for each experiment) by the same letter are significantly different ($P < 0.05$).

Table 2. Effect of Changes in Dietary Casein and Dextrin of Plasma Amino Acid Concentrations (Experiment 1)*

Group	Diet	Food Intake for 2 h (g)	Tyrosine (nmol/mL)	Tryptophan (nmol/mL)	Phenylalanine (nmol/mL)	Methionine (nmol/mL)	Valine (nmol/mL)	Leucine (nmol/mL)	Isoleucine (nmol/mL)
1	Fasting	—	37.3 ± 3.7^{a}	70.8 ± 4.8^{b}	36.7 ± 2.0^{b}	23.4 ± 1.9^{b}	84.7 ± 6.9^{b}	72.5 ± 5.3^{b}	41.1 ± 3.9^{c}
2	0% Casein/ 0% Dextrin	2.9 ± 0.4	39.4 ± 5.5^{a}	76.7 ± 10.5^{bc}	36.8 ± 0.7^{b}	22.1 ± 0.8^{b}	82.8 ± 6.6^{b}	66.7 ± 4.7^{b}	37.9 ± 2.9^{bc}
3	0% Casein/ 25% Dextrin	3.9 ± 0.5	31.9 ± 5.7^{a}	73.2 ± 0.9^{b}	37.3 ± 2.6^{b}	23.4 ± 1.4^{b}	78.1 ± 6.0^{b}	59.4 ± 4.2^{b}	32.1 ± 2.2^{b}
4	0% Casein/ 75% Dextrin	6.5 ± 1.3	22.4 ± 4.5^{a}	51.3 ± 5.5^{a}	25.8 ± 2.0^{a}	14.2 ± 1.4^{a}	41.4 ± 6.4^{a}	33.3 ± 4.8^{a}	15.9 ± 3.2^{a}
5	10% Casein/ 0% Dextrin	5.8 ± 1.2	65.2 ± 9.4^{b}	84.0 ± 10.3^{bc}	49.3 ± 1.5^{c}	42.9 ± 2.0^{c}	151.7 ± 5.7^{c}	115.9 ± 4.8^{c}	67.4 ± 2.1^{e}
6	10% Casein/ 25% Dextrin	7.1 ± 0.5	66.8 ± 5.0^{b}	95.7 ± 1.5^{c}	56.6 ± 1.5^{d}	54.0 ± 3.8^{d}	167.6 ± 5.9^{c}	127.3 ± 4.7^{c}	66.4 ± 2.0^{e}
7	10% Casein/ 75% Dextrin	8.7 ± 0.9	90.0 ± 10.2^{c}	81.1 ± 2.8^{bc}	59.2 ± 2.3^{d}	57.3 ± 3.7^{d}	152.6 ± 7.3^{c}	111.6 ± 6.3^{c}	56.6 ± 4.5^{d}
8	20% Casein/ 0% Dextrin	5.4 ± 0.5	91.3 ± 8.5^{c}	128.2 ± 4.9^{de}	66.4 ± 3.0^{e}	67.1 ± 3.1^{e}	252.1 ± 7.9^{d}	187.4 ± 6.5^{d}	101.5 ± 2.4^{f}
9	20% Casein/ 25% Dextrin	7.5 ± 0.8	172.8 ± 9.9^{e}	136.9 ± 3.4^{e}	84.9 ± 3.5^{f}	121.4 ± 2.9^{g}	308.2 ± 11.4^{e}	223.7 ± 10.0^{e}	114.8 ± 4.5^{g}
10	20% Casein/ 75% Dextrin	7.8 ± 0.4	144.7 ± 9.5^{d}	115.6 ± 7.0^{d}	73.5 ± 3.8^{e}	107.0 ± 4.9^{f}	303.4 ± 6.1^{e}	211.0 ± 4.7^{e}	110.2 ± 2.2^{fg}

Means ± SEM of five rats per group. Means within a column not followed by the same letter are significantly different ($P < 0.05$).

*Initial body weight of rats, 165 ± 6 g.

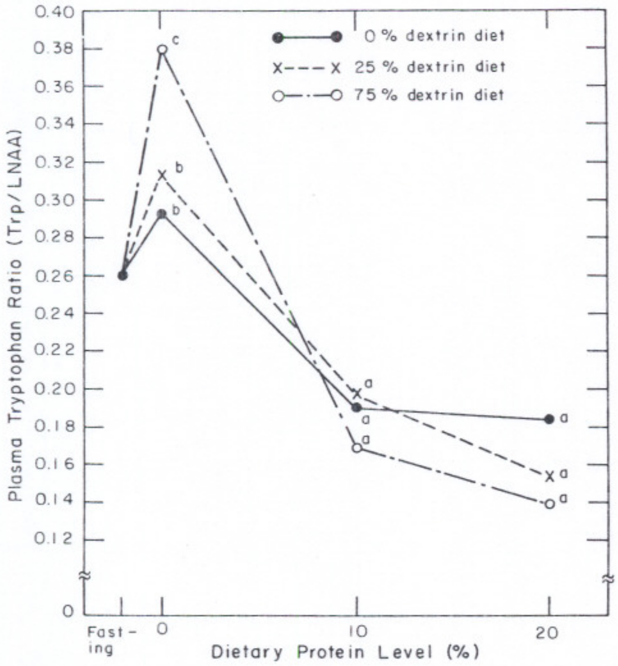

Fig 1. Effect of dietary protein level on plasma tryptophan ratios of rats fed 0%, 25%, or 75% carbohydrate diets. Significant differences ($P < 0.05$) are noted by a, b, and c (experiment 1).

plasma tryptophans) were then measured by high-performance liquid chromatography[18] (Waters Associates, Milford, Mass).

Three types of experiments were performed. In the first, dietary carbohydrate and protein contents were varied systematically, and caloric density was kept constant (at 3.94 calorie/g) by manipulating the fat content (Table 1). In the second, we compared the effects of two carbohydrates, dextrin and dextrose, on plasma amino acid responses to dietary casein. Caloric density was again kept constant by changing the fat contents of the diet. In the third, we examined the effects of relatively low amounts of several proteins on the changes in plasma amino acid levels caused by a high-carbohydrate meal; fat contents were kept constant, so that caloric densities varied among the diets (Table 1). (In these experiments, brains were also assayed for serotonin and its metabolite 5-hydroxyindole acetic acid (5-HIAA) as described previously.[19]) All test diets were mixed with the same weight of a 5% agar solution. Plasma insulin concentrations were measured by radioimmunoassay using a double antibody method (Immuno Nuclear Co, Stillwater, Minn).

The statistical significance of differences between values was determined by analysis of variance and 95% confidence intervals based on pooled standard deviation (minitab-program).

RESULTS

Effect of Dietary Protein Content on Plasma Amino Acid Responses to Dietary Carbohydrate

As shown previously,[1,2,9,11] consumption of a protein-free, carbohydrate-containing meal decreased plasma levels of all of the LNAA, including tryptophan (Table 2); this effect was dose-related, such that it required a 75% carbohydrate meal to produce significant reductions. The decreases in branched-chain amino acids tended to be greater than the decreases in the aromatic LNAA, so that the high-carbohydrate, protein-free meals also increased the plasma tryptophan ratio. Consumption of a meal lacking both protein and carbohydrate failed to change the plasma levels or ratios of any of the amino acids (Table 2).

Addition of protein to the diets caused dose-related increases in plasma levels of all of the LNAA, with greatest increases in methionine, valine, and leucine, and smallest increases in tryptophan and phenylalanine. As little as 10% protein fully blocked an increase in tryptophan-LNAA ratios caused by eating the 75% carbohydrate meal (Fig 1).

Effect of Dietary Protein Content on Plasma Amino Acid Responses to Meals Rich in Dextrin or Dextrose

Consumption of protein-free meals rich in dextrin (70%) caused significant reductions in plasma levels of methionine and the branched-chain amino acids; similar meals containing dextrose (70%) instead of dextrin also significantly reduced plasma levels of tyrosine, tryptophan, and phenylalanine (Table 3). Both carbohydrates elevated the plasma tryptophan ratio when consumed without protein (Table 4).

Addition of 10% or 20% casein to the test diet blocked the decreases in plasma amino acid levels, and the increases in the plasma tryptophan ratio associated with eating the dextrin or the dextrose without protein (Table 4). Addition of

Table 3. Effect of Casein Content on Plasma Amino Acid Concentrations of Rats Given High Dextrin or Dextrose Diets (Experiment 2)*

Group	Diet	Food Intake for 2 h (g)	Tyrosine (nmol/mL)	Tryptophan (nmol/mL)	Phenylalanine (nmol/mL)	Methionine (nmol/mL)	Valine (nmol/mL)	Leucine (nmol/mL)	Isoleucine (nmol/mL)
1	Fasting	—	38.0 ± 2.8^{b}	60.9 ± 2.2^{b}	35.3 ± 2.9^{b}	25.8 ± 2.3^{c}	85.5 ± 4.3^{c}	66.0 ± 4.8^{b}	36.1 ± 2.7^{c}
2	0% Casein/ 70% Dextrin	4.8 ± 0.7	29.3 ± 2.7^{ab}	55.8 ± 3.5^{b}	26.0 ± 1.5^{ab}	15.7 ± 1.6^{b}	54.1 ± 4.4^{b}	37.5 ± 3.8^{a}	19.1 ± 1.8^{b}
3	10% Casein/ 70% Dextrin	7.4 ± 0.9	86.2 ± 8.3^{c}	68.4 ± 5.4^{b}	50.2 ± 4.6^{b}	58.9 ± 1.7^{d}	158.8 ± 7.6^{d}	115.4 ± 9.5^{c}	57.2 ± 2.9^{d}
4	20% Casein/ 70% Dextrin	9.0 ± 1.2	140.7 ± 4.3^{d}	104.4 ± 3.6^{d}	63.0 ± 3.9^{cd}	111.1 ± 2.0^{e}	272.2 ± 2.4^{e}	215.2 ± 7.8^{d}	94.8 ± 1.9^{e}
5	0% Casein/ 70% Dextrose	5.0 ± 0.5	16.8 ± 2.3^{a}	39.6 ± 4.4^{a}	17.1 ± 1.2^{a}	9.8 ± 1.0^{a}	32.7 ± 2.1^{a}	27.6 ± 3.4^{a}	12.9 ± 1.5^{a}
6	10% Casein/ 70% Dextrose	9.2 ± 1.5	94.3 ± 4.2^{c}	87.0 ± 7.1^{c}	52.3 ± 4.1^{c}	61.0 ± 3.2^{d}	163.5 ± 2.9^{d}	119.6 ± 5.3^{c}	60.0 ± 2.8^{d}
7	20% Casein/ 70% Dextrose	10.5 ± 0.9	197.1 ± 10.7^{e}	121.0 ± 4.9^{e}	73.1 ± 4.5^{d}	125.7 ± 2.0^{f}	303.0 ± 11.1^{f}	223.2 ± 11.7^{d}	105.1 ± 3.3^{f}

Means $\pm$ SEM of five rats per group. Means within a column not followed by the same letter are significantly different ($P < 0.05$).

*Initial body weight of rats, 168 ± 5 g.

Table 4. Effect of Casein Content on Plasma Amino Acid Ratios of Rats Given High Dextrin or Dextrose Diets (Experiment 2)

Group	Diet	Tyrosine/LNAA* ($\times 10^{-3}$)	Tryptophan/LNAA ($\times 10^{-3}$)	Phenylalanine/LNAA ($\times 10^{-3}$)	Valine/LNAA ($\times 10^{-3}$)	Leucine/LNAA ($\times 10^{-3}$)	Isoleucine/LNAA ($\times 10^{-3}$)
1	Fasting	134 ± 10^{a}	235 ± 12^{b}	123 ± 5^{bc}	362 ± 12^{b}	257 ± 9^{ab}	127 ± 9^{b}
2	0% Casein/ 70% Dextrin	152 ± 9^{ab}	341 ± 25^{c}	135 ± 11^{c}	321 ± 11^{ab}	202 ± 13^{a}	94 ± 6^{a}
3	10% Casein/ 70% Dextrin	194 ± 20^{b}	146 ± 5^{a}	102 ± 4^{b}	425 ± 19^{c}	272 ± 10^{ab}	120 ± 3^{b}
4	20% Casein/ 70% Dextrin	188 ± 7^{b}	133 ± 6^{a}	76 ± 4^{a}	442 ± 8^{c}	319 ± 11^{b}	119 ± 2^{b}
5	0% Casein/ 70% Dextrose	130 ± 17^{a}	371 ± 40^{c}	132 ± 6^{c}	286 ± 7^{a}	246 ± 47^{ab}	95 ± 8^{ab}
6	10% Casein/ 70% Dextrose	196 ± 10^{bc}	177 ± 11^{a}	100 ± 10^{b}	397 ± 13^{bc}	261 ± 8^{ab}	116 ± 5^{b}
7	20% Casein/ 70% Dextrose	239 ± 14^{c}	135 ± 9^{a}	77 ± 3^{ab}	423 ± 22^{bc}	279 ± 11^{ab}	114 ± 2^{ab}

Means ± SEM of five rats per group. Means within a column not followed by the same letter are significantly different ($P < 0.05$).
*Sum of five LNAA of the following six: tyrosine, tryptophan, pheylalanine, valine, leucine, isoleucine.

20% casein to the diets also caused the plasma ratios of tryosine and valine to rise significantly, and lowered that of phenylalanine.

Effects of Various Proteins or of Low Concentrations of Casein on Plasma Amino Acid Responses to High-Carbohydrate Meal

Most of our previous studies on meal-induced changes in the plasma tryptophan ratio[4-6,9] had utilized casein as the protein source. To determine whether the amino acid composition of a dietary protein influenced its effect on plasma amino acid ratios, we compared the effects of relatively small amounts (10% by weight) of five different proteins on the increase in the plasma tryptophan ratio that would otherwise be produced by a dextrose-rich meal. When rats ate the 10% casein diet, greatest proportionate increases in plasma concentrations were seen in tyrosine, methionine and isoleucine (Table 5). The lactalbumin-containing diet had the greatest effects on tryptophan, but failed to elevate tyrosine or valine, and actually lowered phenylalanine. The egg white diet selectively elevated plasma tryptophan, methionine, valine, and isoleucine; peanut meal raised only plasma tyrosine and phenylalanine; and gelatin raised only valine, significantly lowering phenylalanine and tyrosine. In general, the effects of each protein on the plasma amino acid pattern tended to be correlated with its own amino acid composition.[20] Hence lactalbumin, which is rich in tryptophan, raised plasma tryptophan levels while gelatin, which is poor in this amino acid, failed to do so. And egg white, which is rich in methionine, raised plasma methionine levels while peanut meal, which is poor in this amino acid, failed to do so. Our data, however, cannot be construed as providing definitive documentation of this relationship. Addition of 10% casein, peanut meal, or gelatin to the 70% carbohydrate meal blocked the expected increase in the plasma tryptophan ratio, and casein or peanut meal increased the plasma tyrosine ratio (Table 6). In contrast, lactalbumin failed to block the rise in the tryptophan ratio or to increase that of tyrosine, and egg white only partially suppressed the rise in the tryptophan ratio.

As low a casein concentration as 2.5% partially blocked the rise in the plasma tryptophan ratio caused by a carbohydrate-rich meal; a 5% casein concentration fully blocked this rise and also significantly elevated the plasma tyrosine ratio (Table 6). Consumption of the 70% dextrose meal elevated brain 5-HIAA levels (serotonin plus 5-HIAA) significantly, from $0.86 + 0.03$ to $1.02 + 0.05$ $\mu g/g$ ($P < 0.05$). Addition of 5% casein to the meal fully blocked this response ($0.74 + 0.04$ $\mu g/g$).

Diet Composition and Plasma Insulin Levels

Radioimmunoreactive total plasma insulin levels were elevated about fivefold after consumption of diets containing

Table 5. Effect of Dietary Proteins on Plasma Amino Acid Concentrations of Rats Given High Dextrose Diets (experiment 3)*

Group	Diet	Food Intake for 2 h (g)	Tyrosine (nmol/mL)	Tryptophan (nmol/mL)	Phenylalanine (nmol/mL)	Methionine (nmol/mL)	Valine (nmol/mL)	Leucine (nmol/mL)	Isoleucine (nmol/mL)
1	Fasting		32.0 ± 2.7^{ab}	53.3 ± 6.2^{ab}	32.7 ± 2.0^{b}	26.8 ± 1.7^{b}	89.5 ± 7.6^{bc}	78.9 ± 5.1^{c}	36.6 ± 3.2^{b}
2	0% Casein	8.5 ± 0.9	23.8 ± 2.9^{ab}	82.1 ± 6.8^{c}	26.7 ± 1.8^{ab}	23.8 ± 1.5^{b}	63.1 ± 2.1^{a}	36.4 ± 2.6^{a}	23.0 ± 0.9^{a}
3	2.5% Casein	10.0 ± 2.0	39.7 ± 3.9^{bc}	74.7 ± 4.9^{bc}	31.8 ± 2.4^{b}	30.3 ± 2.6^{bc}	75.2 ± 6.1^{ab}	51.0 ± 3.8^{ab}	29.0 ± 2.4^{ab}
4	5% Casein	11.9 ± 0.9	58.1 ± 8.8^{c}	73.7 ± 2.4^{bc}	35.5 ± 2.1^{b}	39.2 ± 2.0^{c}	102.4 ± 4.5^{c}	73.4 ± 3.4^{bc}	37.0 ± 2.5^{b}
5	10% Casein	10.9 ± 1.0	88.4 ± 4.6^{d}	89.6 ± 8.4^{c}	49.3 ± 1.9^{d}	58.9 ± 3.6^{d}	161.4 ± 4.5^{d}	119.6 ± 2.8^{d}	64.4 ± 1.3^{d}
6	10% Lactalbumin	10.5 ± 1.0	36.5 ± 2.5^{b}	165.0 ± 7.1^{d}	22.1 ± 0.7^{a}	43.1 ± 1.8^{c}	78.1 ± 4.9^{ab}	132.3 ± 11.2^{d}	48.4 ± 4.0^{c}
7	10% Egg white	9.4 ± 0.7	53.0 ± 5.0^{c}	109.9 ± 3.3^{d}	51.6 ± 3.4^{d}	72.4 ± 7.4^{a}	160.4 ± 4.3^{d}	70.9 ± 3.0^{bc}	61.1 ± 1.9^{d}
8	10% Peanut meal	11.6 ± 1.9	50.2 ± 1.9^{bc}	60.2 ± 1.9^{b}	42.5 ± 2.4^{c}	13.9 ± 1.4^{a}	83.5 ± 6.9^{b}	61.3 ± 3.0^{b}	32.1 ± 2.9^{b}
9	10% Gelatin	8.1 ± 0.8	21.0 ± 0.8^{a}	40.2 ± 5.2^{a}	37.3 ± 1.1^{bc}	19.0 ± 1.0^{ab}	118.2 ± 8.5^{c}	71.1 ± 3.6^{bc}	30.4 ± 2.9^{ab}

Means ± SEM of five rats per group. Means within a column not followed by the same letter are significantly different ($P < 0.05$).
*Initial body weight of rats, 166 ± 5 g.

Table 6. Effect of Dietary Proteins on Plasma Amino Acid Ratios of Rats Given High Dextrose Diets (Experiment 3)

Group	Diet	Tyrosine/LNAA* ($\times 10^{-3}$)	Tryptophan/LNAA ($\times 10^{-3}$)	Phenylalanine/LNAA ($\times 10^{-3}$)	Valine/LNAA ($\times 10^{-3}$)	Leucine/LNAA ($\times 10^{-3}$)	Isoleucine/LNAA ($\times 10^{-3}$)
1	Fasting	111 ± 9^{ab}	197 ± 15^{ab}	113 ± 4^{c}	382 ± 8^{c}	325 ± 15^{e}	127 ± 2^{b}
2	0% Casein	102 ± 11^{a}	475 ± 32^{d}	117 ± 8^{c}	333 ± 23^{b}	166 ± 11^{a}	100 ± 3^{a}
3	2.5% Casein	155 ± 19^{b}	330 ± 16^{c}	118 ± 5^{c}	331 ± 14^{b}	204 ± 10^{b}	106 ± 5^{a}
4	5% Casein	178 ± 22^{b}	244 ± 17^{b}	103 ± 3^{bc}	370 ± 8^{bc}	240 ± 6^{c}	108 ± 3^{a}
5	10% Casein	184 ± 14^{b}	185 ± 17^{ab}	94 ± 3^{b}	393 ± 15^{c}	264 ± 3^{cd}	127 ± 2^{b}
6	10% Lactalbumin	94 ± 12^{a}	369 ± 19^{c}	54 ± 3^{a}	219 ± 8^{a}	436 ± 19^{f}	125 ± 4^{b}
7	10% Egg white	116 ± 9^{ab}	278 ± 11^{b}	113 ± 6^{c}	464 ± 9^{d}	162 ± 3^{a}	137 ± 2^{b}
8	10% Peanut meal	183 ± 16^{b}	225 ± 10^{b}	148 ± 5^{d}	337 ± 19^{bc}	228 ± 4^{bc}	107 ± 6^{a}
9	10% Gelatin	71 ± 4^{a}	143 ± 13^{a}	135 ± 8^{d}	590 ± 18^{e}	290 ± 13^{d}	105 ± 4^{a}

Means ± SEM of five rats per group. Means within a column not followed by the same letter are significantly different ($P < 0.05$).

*Sum of five LNAA of the following six: tyrosine, tryptophan, phenylalanine, valine, leucine, isoleucine.

70% to 75% of either dextrin or dextrose. They failed to rise significantly after meals containing 25% dextrin (Table 1). Consumption of meals containing 10% or 20% casein without carbohydrate produced small, statistically insignificant increases in plasma insulin, and addition of casein to a meal containing a given amount of dextrin or dextrose failed to modify the rise in insulin elicited by the carbohydrate.

Because animals tended to consume smaller amounts of certain diets [(eg, those lacking protein (Tables 3 and 5), or those lacking both protein and carbohydrates (Table 2)] than of others, we performed additional pair-feeding studies to affirm that, under our experimental conditions, the effect of any diet on the plasma amino acid pattern was independent of the quantity that had been consumed prior to autopsy. In each pair-feeding study, four diets were tested: the diet whose consumption was expected to be least, the diet with greatest expected consumption, and two intermediate diets. In no case were the plasma amino acid patterns associated with the pair-feeding paradigm different from those observed after two hours of ad libitum feeding (and described in Tables 2, 3, and 5). These observations are compatible with the fact that all of our animals had food in their stomachs at autopsy, regardless of how much food they had eaten in the prior two hours. Similarly, preliminary studies were done using the most extreme diets (those having the lowest and highest percentages of protein and carbohydrate) to affirm that the amino acid patterns observed after two hours of food exposure would also have been seen at other times. In these latter studies, groups of three animals were killed one or two hours after being given access to the diets. In no case were the plasma amino acid patterns observed after one hour of feeding significantly different from those seen after two hours.

DISCUSSION

These observations show that various dietary proteins differ in their effects on the plasma tryptophan ratio (and plasma LNAA levels) (Table 6); that only very small concentrations of some proteins (as little as 5% casein, Table 6) are needed to block the ability of dietary carbohydrate to elevate the plasma tryptophan ratio; that the effects of the added proteins are not due to suppression of insulin secretion (Table 1); and that high-carbohydrate meals containing dextrin or dextrose are alike in their ability to raise the plasma tryptophan ratio (Table 4) and to elicit insulin secretion (Table 1). Since a substantial increase in the plasma tryptophan ratio, as occurs after consumption of a carbohydrate-rich meal (Tables 4 and 6), invariably causes parallel increases in brain tryptophan levels[1,2] and in the synthesis and levels of the neurotransmitter serotonin,[21,22] our data provide additional support for the hypothesis that serotoninergic neurons have a special role as sensors of food-induced changes in plasma composition, ie, that they produce more or less of their signal, serotonin, depending on the composition of the food that the rat is currently digesting.[23] Other studies support the view that this information can be used by the rest of the brain in making choices about the proportions of carbohydrate to protein in the next meal,[24,25] and that it can also affect other serotonin-mediated behaviors like sleepiness, pain sensitivity, performance, and mood.[14–17]

Based on our earlier studies, we had anticipated observing a linear inverse relationship between the proportion of protein in a carbohydrate-rich meal and its effect on the plasma tryptophan ratio. Our present observations suggest that in rats this is not the case, and that proteins like casein exert differential effects on this ratio only within the relatively narrow range of 0% to 5%, beyond which they fully block the increase in the ratio that would otherwise be caused by the carbohydrates). This suggests that the main use of the serotonin-releasing neuron may be to distinguish between two classes of foods or meals, ie, those containing very little protein and large amounts of carbohydrate which raise the plasma tryptophan ratio (for example, rice or bread), and all other foods. (As shown in Fig 1, a meal that is not rich in carbohydrate will also fail to elevate the plasma tryptophan ratio, whatever its protein content.) Another use for the serotonin neuron in rats may be to distinguish among proteins. A meal of lactalbumin plus carbohydrate would probably be "read" by the brain as one lacking significant amounts of protein, while one containing 5% or more casein or peanut meal would be interpreted by serotoninergic neurons as protein-rich (Table 6). (Of course, lactalbumin is normally consumed along with casein). Gelatin, like casein, would also generate a signal (decreased serotonin release) characteristic of dietary proteins; however, it seems likely that the major reduction that this protein also causes in the plasma tyrosine ratio would affect brain catecholamine release,[26,27] perhaps with additional behavioral and physiologic consequences.

The fact that an insulin-releasing nonsweet carbohydrate (dextrin) elicits the same changes in plasma insulin (Table 1) and the plasma tryptophan ratio (Table 4) as a sweet, refined sugar (dextrose) supports the hypothesis that the known actions of carbohydrates on brain composition are independent of their gustatory effects, and result from their ability to elicit the secretion of insulin, which acts indirectly by changing the plasma amino acid pattern. It would now be useful to conduct studies like the present one on human subjects, to determine whether in man (as in the rat) the plasma tryptophan ratio distinguishes only two types of foods (carbohydrate-rich, protein-poor *v* all others), or responds linearly to variations in dietary protein and carbohydrate contents.

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