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The Effect of Lowering Plasma Tryptophan on Food Selection in Normal Males

SIMON N. YOUNG,*†[1] SMADAR V. TOURJMAN,* KAREN L. TEFF,† ROBERT O. PIHL*‡ AND G. HARVEY ANDERSON§

**Department of Psychiatry, †The Division of Experimental Medicine, Department of Medicine and the ‡Department of Psychology, McGill University, Montreal, Canada and §the Department of Nutritional Sciences, University of Toronto, Toronto, Canada*

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YOUNG, S. N., S. V. TOURJMAN, K. L. TEFF, R. O. PIHL AND G. H. ANDERSON. *The effect of lowering plasma tryptophan on food selection in normal males*. PHARMACOL BIOCHEM BEHAV 31(1) 149–152, 1988.—The effects of a tryptophan deficient amino acid mixture on food selection were studied using a double-blind counterbalanced crossover design in normal male subjects. The subjects ingested tryptophan deficient or nutritionally balanced amino acid mixtures in the morning after an overnight fast. Five hours after the trypotphan deficient amino acid mixture plasma tryptophan was only 19% of the level found five hours after the nutritionally balanced amino acid mixture. After both mixtures subjects were allowed to select lunch from a buffet. The tryptophan-deficient mixture was associated with a modest but significant decline in protein selection with no significant alteration in selection of carbohydrate, fat or total kcal. Our results suggest that 5-hydroxytryptamine is involved in the control of protein selection in humans.

Tryptophan 5-Hydroxytryptamine Amino acids Protein Carbohydrate Fat

THERE is a large literature concerned with the effect of altered 5-hydroxytryptamine (5HT) function on feeding in experimental animals [1, 3, 12]. Results vary somewhat depending on the particular experimental paradigm, but in general 5HT acts to suppress food intake. Fluctuations in 5HT can also, in some circumstances, be involved in the modulation of macronutrient selection, with elevated 5HT increasing protein intake or decreasing the ratio of carbohydrate to protein in the diet.

The possible involvement of 5HT in macronutrient selection may help to explain why 5HT is altered by dietary intake [1, 3, 12]. The rate of synthesis of 5HT depends in part on the level in brain of its precursor tryptophan. The effect of acute dietary intake on brain tryptophan is not what might intuitively be expected. Thus, ingestion of protein, which contains tryptophan, lowers rat brain tryptophan and 5HT. This is because all the large neutral amino acids compete for transport across the blood-brain barrier. Protein ingestion increases plasma levels of the other large neutral amino acids more than tryptophan. This increased competition for the transport system results in a lowering of tryptophan in the brain. On the other hand carbohydrate, which contains no tryptophan, causes an increase in brain tryptophan and 5HT. This is because insulin enchances uptake of branched chain amino acids into muscle, thus decreasing their plasma level and competition at the blood-brain barrier. As protein and carbohydrate have opposite effects on brain 5HT, and 5HT may control relative intakes of protein and carbohydrate, 5HT may be part of a system ensuring that an animal takes in adequate supplies of both these macronutrients.

A possible role of 5HT in food selection in humans has been studied by giving tryptophan. Tryptophan was given over a 2-week period to obese subjects with a craving for carbohydrate snacks. It significantly diminished carbohydrate intake in three of the eight subjects, and increased it in one subject. It did not significantly modify snacking patterns in the group as a whole [11]. Tryptophan was also tested against placebo in a double-blind crossover study of eight refractory carbohydrate-craving obese subjects in a weight loss program. Weight loss during the six weeks on tryptophan was not significantly different from the six weeks on placebo [10]. Tryptophan was also tested against placebo in a double-blind crossover study in healthy lean men. Tryptophan in doses from 1 to 3 g, or placebo, was given 45 min

[1]Requests for reprints should be addressed to Dr. S. N. Young, Department of Psychiatry, McGill University, 1033 Pine Avenue West, Montreal, Canada, H3A 1A1.

TABLE 1
FOODS AVAILABLE FOR SELECTION AND THEIR MACRONUTRIENT CONTENT

Food	Amount Served	Approximate Weight Served (g)	kcal/g	Nutrients (g/g food) Protein	Carbo-hydrate	Fat
White bread	12 slices	350	2.71	0.09	0.50	0.03
Butter	2 tbls.	50	7.10	0.01	0.01	0.81
Ham	10 slices	400	2.33	0.19	0	0.17
Salami	10 slices	300	3.14	0.18	0.01	0.26
Chedder cheese	10 slices	200	3.98	0.25	0.02	0.32
Brick cheese	10 slices	200	3.71	0.22	0.02	0.30
Tomato	2 (sliced)	300	0.20	0.01	0.04	0
Apple	2 (quartered)	300	0.55	0	0.14	0.01
Coconut cookies	8	100	4.94	0.06	0.64	0.25
Chocolate chip cookies	8	125	4.71	0.06	0.70	0.21

before subjects selected from a buffet luncheon. Whereas 1 g had no effect, both 2 and 3 g reduced total calorie intake significantly by 13 to 20% [7]. In a double-blind comparison of the effects of tryptophan (0.5 g) or placebo on various measures in a questionnaire, tryptophan had no effect on the subjects' own rating of their hunger or carbohydrate/protein preference, even though they found tryptophan more sedating [8]. Only one study found an effect of tryptophan on selection of macronutrients in humans [4]. Tryptophan at a dose of 1 g or placebo was given with a high protein or high carbohydrate lunchtime meal. Food intake was measured at a free selection test meal three hours later. Tryptophan did not influence total food intake, but caused a significant decrease in carbohydrate selection. This effect was seen when the tryptophan was given with the high protein meal, but not when it was given with the high carbohydrate meal.

Recently we have developed a method for lowering tryptophan levels acutely in humans by giving a tryptophan deficient amino acid mixture orally. This causes a rapid lowering of mood [13], an effect consistent with a decrease in 5HT function. We have now looked at the effect of lowering tryptophan on food selection in normal male subjects.

METHOD

The subjects were 22 normal males between the ages of 18 and 30 years, who were recruited through newspaper advertisements. All had at least a high school education, no history of psychiatric disorder or food allergies and were within 10% of their ideal body weight for their height. They were not taking any prescription medication and found the foods used in the study acceptable.

A double-blind counterbalanced crossover design was used. Each subject came into the laboratory on two days, one week apart. On each occasion they ingested an amino acid mixture orally five hours before selecting lunch from a buffet. Half the subjects received a tryptophan-deficient (T−) amino acid mixture on the first visit and a nutritionally balanced (B) mixture on the second visit. The other half received the mixtures in reverse order. The tryptophan-deficient mixture consisted of L-alanine, 2.75 g; L-arginine, 2.45 g; L-cysteine, 1.35 g; glycine, 1.6 g; L-histidine, 1.6 g; L-isoleucine, 4.0 g; L-leucine, 6.75 g; L-lysine monohydrochloride, 5.5 g; L-methionine, 1.5 g; L-phenylalanine, 2.85 g; L-proline, 6.1 g; L-serine, 3.45 g; L-threonine, 3.25 g; L-tyrosine, 3.45 g; and L-valine, 4.45 g, for a total of 50 g of amino acids. The B mixture contained the same plus 1.15 g L-tryptophan. The amino acids were in the same proportion as in milk except that aspartic acid and glutamic acid were omitted because of concern about their toxicity at high doses. As they are not essential amino acids, omitting them should not have influenced the efficacy of the T− mixture in reducing tryptophan levels.

Subjects arrived in the laboratory at 8:00 a.m. after an overnight fast. They had the purpose of the study explained to them, signed a consent form, had a baseline blood sample taken and ingested an amino acid mixture orally as a suspension as described previously [13]. For the next five hours they were kept in a room and entertained by being shown a movie and allowed to read magazines. The 5 hour interval is necessary for plasma tryptophan to fall to its lowest level [13]. At the end of this waiting period a second blood sample was taken. Then they were led into a room and allowed to select their lunch from a buffet. The foods presented and their nutritional values taken from food tables are given in Table 1. The menu was identical on each visit. For each type of food, enough was given so that some always remained at the end. In this way all the types of food were always available for selection. All the food was preweighed. Food intake was determined by weighing it again at the end of the eating period. Tap water was supplied with the meal. Subjects were by themselves in the room and were allowed 30 minutes to eat. To diminish any effects of cognitive function on food selection, the subjects were told that the purpose of the study was to examine the effect of food intake on mood. To reinforce this they were given various paper and pencil tests to fill in before ingesting the amino acid mixtures and before and after lunch. They were unaware that their food intake was determined. At the end of the second visit they were told of the real purpose of the study and paid for their participation. The protocol was approved by the Ethics Committee of the Department of Psychiatry, McGill University.

The blood samples were used for the determination of plasma tryptophan, by the fluorometric method of Denckla and Dewey [5]. Large neutral amino acids in plasma were

TABLE 2

PLASMA LEVELS OF TRYPTOPHAN AND THE OTHER LARGE NEUTRAL AMINO ACIDS BEFORE AND FIVE HOURS AFTER INGESTION OF TRYPTOPHAN FREE OR NUTRITIONALLY BALANCED AMINO ACID MIXTURES

		Tryptophan Free Amino Acid Mixture		Balanced Amino Acid Mixture	
	n	Before	After	Before	After
Tryptophan	22	70.8 ± 11.6	20.8 ± 8.3	74.9 ± 13.6	112 ± 96
Histidine	6	114 ± 25	137 ± 36	120 ± 19	115 ± 26
Isoleucine	6	72.2 ± 7.4	189 ± 90	86.9 ± 12.2	143 ± 78
Leucine	6	146 ± 20	369 ± 83	150 ± 17	269 ± 129
Methionine	6	32.4 ± 4.4	46.0 ± 13.7	34.2 ± 8.2	47.4 ± 12.6
Phenylalanine	6	72.8 ± 7.5	95.5 ± 37	68.9 ± 12.1	81.2 ± 21.8
Valine	6	237 ± 20	397 ± 107	237 ± 47	416 ± 126

Results are given as mean ± SD in μmoles/l. A comparison of values after tryptophan free or balanced amino acid mixtures by the paired t-test revealed that values from tryptophan were significantly lower ($p<0.001$) after the tryptophan free mixture than after the balanced mixture. Values after the two amino acid mixtures were not significantly different for the other amino acids.

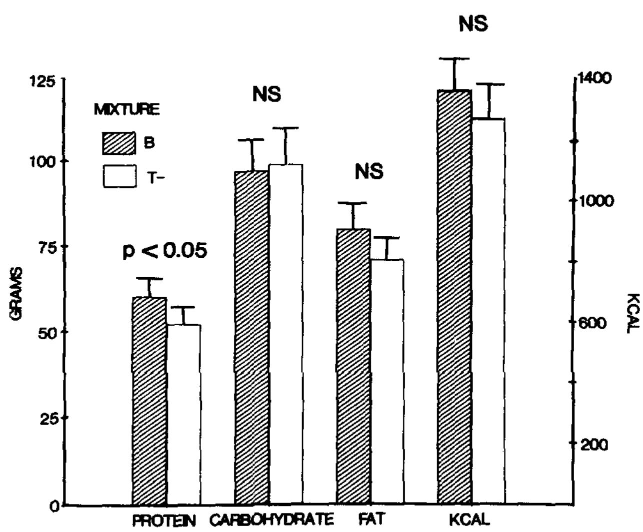

FIG. 1. Intake of protein, carbohydrate, fat and kcal after balanced (B) and tryptophan deficient (T−) mixtures. A two-tailed paired t-test showed that the T− mixture significantly decreased selection of protein, $t(21)=2.41$, $p<0.05$, but not of carbohydrate ($t=-0.55$), fat ($t=1.66$) or kcal ($t=1.44$) relative to the B mixture.

determined by high performance liquid chromatography from a subset of six patients chosen at random.

Differences between food selection and amino acid levels after ingestion of T− and B amino acid mixtures were analysed using a two-tailed paired t-test.

RESULTS

Table 2 gives plasma levels of tryptophan and the other large neutral amino acids both before and after T− and B mixtures. Values for plasma tryptophan after the T− amino acid mixture were only 19% of those after the B amino acid mixture. On the other hand a comparison of the other large neutral amino acid levels after the two mixtures revealed no significant differences.

Figure 1 shows the mean protein, carbohydrate, fat and kcal taken in by the subjects after the T− or B mixtures. There was a small (14%) but statistically significant decline in protein intake after the T− mixture compared to the control B mixture. There was no significant difference in carbohydrate, fat or kcal intake. There was also no significant change in the ratio of protein intake to carbohydrate intake, $t(21)=0.98$, NS.

DISCUSSION

The T− mixture caused a substantial decline in plasma tryptophan levels. In rats a decline in plasma tryptophan of the same order of magnitude as that seen in our subjects was associated with a decline in brain 5HT of about 50% [2]. The roles of different mechanisms in producing this decline in 5HT have been studied [6]. The decline in plasma tryptophan seems to occur because the tryptophan-deficient amino acid mixture, like any mixture of essential amino acids, promotes synthesis of new protein. The tryptophan that is incorporated into this protein comes from free tryptophan in blood and tissues, resulting in a decline in its level in blood and brain. Competition at the blood-brain barrier might contribute to the decline in the brain (but not blood) tryptophan. However, administration of a mixture of six amino acids which share a common transport system with tryptophan lowered rat brain tryptophan and 5-hydroxyindoleacetic acid about 20% without affecting brain 5HT. A mixture of nine of the ten essential amino acids (tryptophan is the tenth) caused larger declines in brain tryptophan and 5-hydroxyindoleacetic acid and a significant fall in brain 5HT [6]. Thus, competition for transport at the blood-brain barrier plays only a small role in regulating brain tryptophan and 5HT after administration of a T− amino acid mixture. In the present study, plasma levels of the other large neutral amino acids were not significantly different five hours after administration of either T− and B mixtures (Table 2). Therefore competition of the blood-brain barrier would have been the same for the two treatments, and plasma tryptophan levels, which were one fifth the value after T− mixtures compared to B mixtures, should have provided a good index of the decline in brain tryptophan.

The most likely mechanism for the decline in protein selection is a decline in brain 5HT function. Although it is not possible to demonstrate that the fall in plasma tryptophan that we saw is accompanied by a decrease in 5HT metabolism in human brain, it would be surprising if an effect of the magnitude that we saw in plasma did not influence brain 5HT. Also, the decline in protein selection is consistent with the known effects of decreasing 5HT function in animals [1, 3, 12]. None of the studies in which tryptophan was given to human subjects found any specific effect on protein selection [4, 7, 8, 10, 11] and the question arises as to why our results differ in this respect. Food selection is exquisitely sensitive to details of experimental design and can be influenced greatly by factors such as the degree of hunger of the subjects, the variety of food available for selection and its sensory qualities [3]. It is interesting from this point of view to compare our study with the one most similar in design, that of Hrboticky *et al.* [7]. In the latter study acute tryptophan administration decreased total calorie intake in normal male subjects. We found no increase in calorie intake with tryptophan depletion. However, this is not surprising as work with experimental animals has shown that an increase in food intake due to inhibition of 5HT metabolism is a much weaker phenomenon than the inhibition of food intake by increased 5HT function [3]. The lack of effect on food selection in the study of Hrboticky *et al.* [7] might be explained in part by the slightly smaller variety of foods available for selection than in the present study. Cognitive, social and mood factors might also have played some role. In the study of Hrboticky *et al.* [7] the subjects ate together and were aware that food intake was being measured. In the present study the subjects ate by themselves and were not aware that food selection was being measured. Furthermore, while tryptophan administration can in some circumstances elevate mood [14], tryptophan depletion causes a lowering of mood [13]. It is not known how mood might influence food selection.

While Blundell and Hill [4] found an effect of tryptophan administration on carbohydrate selection, we found that tryptophan depletion influences selection of protein. However, the importance of precise experimental conditions in determining results of food selection experiments is seen in the report of Blundell and Hill [4]. Thus, tryptophan decreased carbohydrate selection when it was given with a high protein meal, but not when it was given with a high carbohydrate meal. The design of the present experiment differs from that of Blundell and Hill [4] in several respects. Furthermore, there is no discrepancy between the findings that tryptophan supplementation can decrease carbohydrate selection while tryptophan depletion decreases protein intake, if tryptophan availability influences the relative intakes of protein and carbohydrate rather than affecting selection of either macronutrient separately. In our study the type of meal given to the subjects might have influenced the results. Thus, for many of the subjects a significant portion of their carbohydrate intake was in the form of bread. Bread is often eaten as a sandwich with high protein fillings such as ham or cheese. It is impossible to say how factors such as this could have influenced our results. Nonetheless it is quite possible that with a different series of foods to select from we might have seen an increase in carbohydrate selection rather than a decrease in protein selection after tryptophan depletion. Thus, our study does not contradict the other studies on the effect of altered tryptophan levels on food selection in humans. It merely adds to the overall picture of the types of effects that can be seen under different circumstances.

Intake of a balanced meal is associated with a modest decline in CSF tryptophan and 5-hydroxyindoleacetic acid in humans. This has been taken as evidence that a balanced meal lowers CNS 5HT in humans [9]. Our results raise the question of whether this decline in 5HT would be enough to alter food selection at the next meal. At the moment there are insufficient data to answer this question. Thus, although our results indicate that lowered 5HT can decrease protein selection in humans it remains to be seen whether this mechanism operates in physiological circumstances.

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