

SERUM TRYPTOPHAN LEVEL AFTER CARBOHYDRATE INGESTION:
SELECTIVE DECLINE IN NON-ALBUMIN-BOUND TRYPTOPHAN COINCIDENT
WITH REDUCTION IN SERUM FREE FATTY ACIDS*

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Summary

Plasma free tryptophan exists in two forms, bound to albumin and unbound. Ninety to 180 min after adult males received 75 g glucose, the plasma tryptophan not bound to albumin had decreased maximally by 35%, without significant change in the albumin-bound fraction. The maximal fall in non-bound tryptophan occurred later than the maximal rise in blood glucose and coincided with the fall in non-esterified fatty acids. Addition of oleic acid to serum obtained at this time raised the proportion of free tryptophan to that found in serum from fasting subjects. It is suggested that physiological changes in plasma non-esterified fatty acids levels can affect the availability of free tryptophan to the tissues by altering the binding properties of plasma albumin.

Introduction

Availability of free tryptophan can be a limiting factor in hepatic protein biosynthesis (1) and also in brain serotonin formation, the latter being affected directly by the level of tryptophan in plasma (2). It has been established (3) that a major fraction of "free" tryptophan like bilirubin, free fatty acids and some drugs, circulates in plasma combined with albumin.

It has also been shown *in vitro* that the binding properties of albumin for tryptophan and other small molecules can be modified
*Some of these observations were previously noted in: Fernstrom, J. and Wurtman, R.J., Science 178: 414-416 (1972).

by competing ligands (4). In the present communication, we show that carbohydrate ingestion causes a selective decline in non-albumin-bound serum tryptophan coincident with the reduction in serum free fatty acids.

Methods

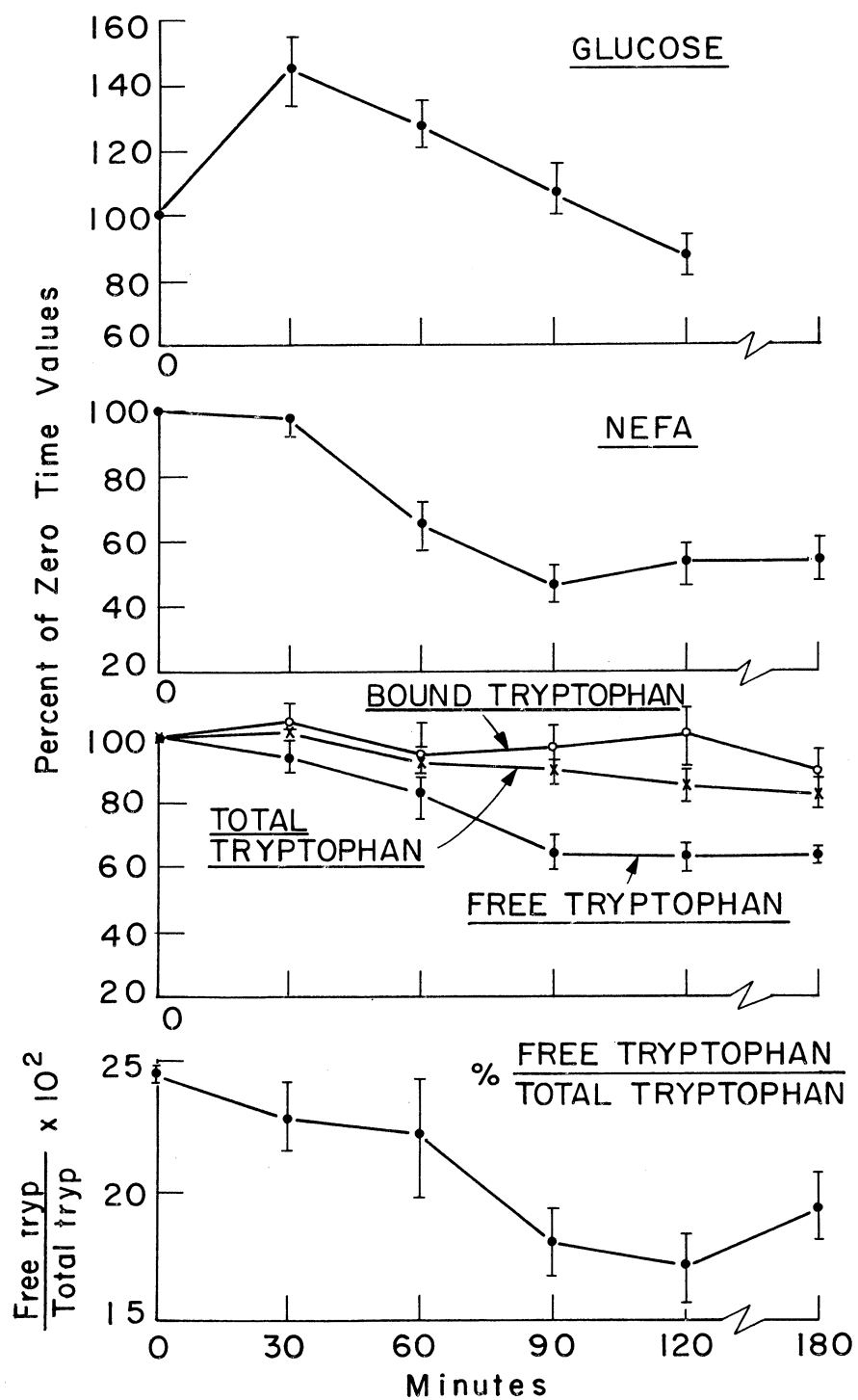
Seven healthy males, aged 21-27 years, fasted overnight from 8 p.m. to 8 a.m. At 8 a.m., a blood sample was taken; the subjects were then given 75 g glucose to drink (as "Glucola," Ames Company, Elkhart, Indiana). Blood samples (20 ml) were collected in "Vacutainers," after 30, 60, 90, 120, and 180 min, and allowed to clot. The serum was harvested by centrifugation at 2000 rpm (I.E.C. International Centrifuge head #279, 15-cm radius) for 10 min and samples were set aside for determinations of glucose and of free fatty acids (5). Total serum tryptophan and the tryptophan not bound to serum proteins were measured using a fluorescence assay (6). The unbound tryptophan was obtained by equilibrium dialysis: Dialysis bags were made by cutting Visking tubing (1 cm flat width) into 15-cm lengths; these were cleaned by boiling in 0.0002 M EDTA, and then in distilled water. One ml of Krebs-improved Ringers bicarbonate buffered salts solution, with amino acids as recommended by McMenamy (3), was placed in each bag; the bag was then folded into a pyrex tube containing 4 ml of the serum. Each tube was flushed through with $\text{CO}_2\text{-N}_2$ (5%-95%) and stoppered tightly. Dialysis was carried out at 37°C for 3.5 hr to achieve equilibrium (3). The $\text{CO}_2\text{-N}_2$ mixture was renewed half-way through dialysis; this allowed the pH of the serum, measured before and immediately after dialysis, to remain at 7.5-7.6. Added tryptophan does not bind appreciably to the dialysis membrane, and is fully recovered at

the end of dialysis; this indicates that no loss occurs under our conditions. The fractions of serum tryptophan that are bound and free do not vary over a wide range of serum to buffer ratios.

Results

Blood glucose levels rose to their maximum at 30 min after glucose consumption and started to decline within 60 min (Fig. 1). The serum concentration of NEFA (non-esterified fatty acids) fell progressively, reaching its lowest level 90 min after glucose; it remained depressed for the remaining 90 min of the study (Fig. 1). After glucose consumption, the total concentration of tryptophan in the serum decreased gradually to a maximum fall of 17% at 180 min (13.9 $\mu\text{g/ml}$ to 11.6 $\mu\text{g/ml}$ after 180 min; $p < 0.02$). In contrast, non-bound tryptophan, which was 3.4 $\mu\text{g/ml}$ prior to glucose consumption, fell by 35% between 90 and 180 min after glucose ($p < 0.01$ at all these times). The concentration of tryptophan bound to serum protein did not change significantly from the initial value of 10.5 $\mu\text{g/ml}$. In consequence, the proportion of bound to total tryptophan rose significantly from 75% to 81-83%, and that of the unbound amino acid fell from 25% to 17-19% during the interval 90-180 min after glucose. The course of the reduction in non-bound tryptophan was coincident with that of NEFA (Fig. 1).

In a separate study (Table 1), serum was obtained from a subject at 0, 90 and 120 min after glucose ingestion. To a portion of each sample was added oleic acid to increase the NEFA content by 0.4 meq/liter. The free and bound tryptophan levels were measured in the control and supplemented samples. Table 1 shows that glucose administration produced the expected fall in NEFA level; this was accompanied by a small reduction in total



tryptophan level which was entirely due to a fall in free tryptophan concentration. Addition of oleic acid to each sample caused an increase in the free and a reduction in the bound tryptophan. The amount of oleic acid added was sufficient to raise the NEFA level of the 120 min sample to the same concentration as that of the untreated zero time sample. The proportion of free to total tryptophan rose from 22% in the unsupplemented serum to 35% after addition of oleic acid. This is essentially the same proportion (33%) found for the untreated zero time serum with a similar NEFA level. This adds strength to the conclusion that a given NEFA level is associated with a given distribution ratio of free to total tryptophan.

Discussion

This study confirms earlier evidence that dietary carbohydrate causes only a small decrease in the total serum tryptophan level of man compared with the larger reductions in most other amino acids (7). This decrease occurs in the fraction of tryptophan not bound to serum albumin, so that the ratio of free to total tryptophan falls significantly after glucose administration. *In vitro* studies (4) show that, in a solution containing serum albumin, tryptophan is distributed in the same ratio between its free and albumin-bound forms over a wide range of total concentrations. Consequently, the altered distribution observed after

FIGURE 1 LEGEND

Effect of Glucose Administration on Serum Glucose and NEFA Levels, and on Tryptophan Levels and Distribution Between Albumin-Bound and Free Forms.

The data are mean values $\pm$ standard errors for 7 adult male subjects, and are expressed as deviations from the initial fasting level taken as 100 (initial glucose 84.8 ± 2.8 mg/100 ml, NEFA 0.73 ± 0.087 meq/liter, total tryptophan 13.9 ± 0.3 μ g/ml, free tryptophan 3.4 ± 0.06 μ g/ml, bound tryptophan 10.5 ± 0.3 μ g/ml).

TABLE 1

Changes in free and bound tryptophan distribution following *in vitro* addition of oleic acid to serum obtained at various times after glucose administration

	Time after glucose		
	0 min	90 min	120 min
NEFA (meq/liter)	0.83	0.68	0.36
Total tryptophan ($\mu\text{g/ml}$)	15.2	14.5	13.9
Bound tryptophan ($\mu\text{g/ml}$)			
Untreated	10.1	10.9	10.8
+ Oleic acid	7.1	8.1	9.1
Free tryptophan ($\mu\text{g/ml}$)			
Untreated	5.1	3.6	3.1
+ Oleic acid	8.1	6.4	4.8
Percent free/total tryptophan			
Untreated	33	29	22
+ Oleic acid	54	44	35

A fasting subject received 75 g glucose and serum was obtained at 0, 90 and 120 min thereafter. To part of each sample, oleic acid was added in 10 μl ethanol to increase the serum NEFA content by 0.4 meq/liter. Ethanol, 10 μl , was added to the untreated sample. After 15 min at 25°C, equilibrium dialysis was carried out.

glucose administration cannot be due to the small reduction in total serum tryptophan content, and must represent a change in the affinity of albumin for tryptophan. The most likely basis for such an alteration is the decline in blood NEFA content, inasmuch as NEFA is known to affect the albumin-binding properties

for ligands including tryptophan (4,8). The reduction in NEFA would be expected to increase the affinity of albumin for tryptophan. This interpretation is confirmed by the change in tryptophan distribution following oleic acid addition. When oleic acid was added to serum obtained 120 min after glucose in an amount sufficient to raise the NEFA level to that of the fasting sample, the percentage of free tryptophan was restored to the fasting proportion.

It thus seems that carbohydrate administration has two actions on the availability of tryptophan to the tissues: First, there is the well-established, insulin-mediated increase in the transport of the free amino acids into some tissues notably muscle (9). Second, the reduction in NEFA following carbohydrate ingestion reduces the availability of free tryptophan by increasing its binding to albumin. This suggests that a number of dietary and physiological factors known to affect blood NEFA levels may result in changes in tryptophan availability. Consequently, total serum tryptophan levels may not adequately reflect the amount of the amino acid that is actually accessible to the tissues. This effect may be especially important in the case of the brain, where serotonin synthesis is influenced by the tryptophan level in the blood (2).

Acknowledgment

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