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DAILY RHYTHMS IN THE CONCENTRATIONS OF VARIOUS AMINO ACIDS IN HUMAN PLASMA*

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Abstract Blood was collected at intervals during a 24-hour period from 23 healthy male volunteers on diets containing various amounts of protein; the plasma was assayed for 16 amino acids. Among subjects receiving 0.71 or 1.5 gm of protein per kilogram of body weight, the concentrations of tyrosine, phenylalanine and tryptophan tended to be lowest at 2:00 and highest at or after 10:30 a.m. Volunteers given a diet containing less than 0.04 gm of protein per kilogram showed similar fluctuations in the concentrations of the above amino acids and methionine, but peak plasma levels were observed somewhat earlier in the morning. All the amino acids studied showed some tendency to vary with time of day. Tyrosine, tryptophan, phenylalanine, methionine, cysteine and isoleucine underwent the greatest per cent changes; the amino acids whose plasma concentrations were highest (that is, alanine, glycine and glutamic acid) showed the least tendency to vary.

THE concentration of the amino acid tyrosine in human plasma is not constant, but varies predictably as a function of time of day.[1,2] When blood is taken at intervals from subjects maintained on a "house diet" and normal schedule of activity, its tyrosine concentration is observed to be lower between 1:30 and 4:30 a.m. than it is later in the day. The rhythm in plasma tyrosine concentration is not generated by the postprandial escape of large amounts of dietary amino acids into the blood, since it persists in subjects on a "low-protein formula diet" (that is, less than 0.04 gm of protein per kilogram of body weight) for two weeks.[1] It also does not appear to result from changes in tyrosine metabolism that might result from cyclic physical activity.[1]

The total concentration of amino acids in human blood also varies rhythmically during the course of the 24-hour day.[3] This biochemical cycle is similar in phase to the plasma tyrosine rhythm: its minimum also occurs at 4 a.m. However, its amplitude is four or five times greater than that of the tyrosine rhythm alone, indicating that it must result from cyclic changes in several amino acids. We have now studied 15 additional amino acids in human plasma to determine whether diurnal rhythmicity is limited to a few circulating amino acids (for example, tyrosine[1] and tryptophan[4]), or is a general property of all such substances. All the amino acids examined showed some diurnal rhythmicity; however, some exhibited large daily shifts in concentration, and others varied only slightly.

MATERIALS AND METHODS

Twenty-three healthy male undergraduate students, ranging from 18 to 25 years of age, were housed in the Massachusetts Institute of Technology Clinical Center for at least 24 hours before blood was sampled, and for the duration of the experiment. Unless otherwise noted, they were given one of the following diets starting with the time of their admission: the "house diet," containing approximately 1.5 gm of protein per kilogram of body weight and presented as three regular meals daily; a "formula diet," containing 0.71 gm of egg protein per kilogram; and a "low-protein formula diet," described previously,[1] which included less than 0.04 gm of protein per kilogram. All diets supplied sufficient calories to prevent weight loss; the approxi-

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mate net protein utilization of each was 70 ("house diet"), 90-100 ("formula diet") and 0-10 ("low-protein formula diet"). The "formula diet" and "low-protein formula diet" were presented in four equal portions at 8:00 a.m. and at 12:30, 5:30 and 10:00 p.m. The subjects were asked to remain in bed between midnight and 7:00 a.m.; however, no other restrictions were placed on their physical activity. Starting at 7:30 a.m. seven 5-ml samples of venous blood were taken at intervals of three to six hours during a period of 24 hours. The blood was mixed with heparin, and the plasma was separated by centrifugation. Portions were assayed for tyrosine,[5] tryptophan[6] and phenylalanine[7] with the use of spectrophotofluorometric methods. Two samples from each subject (those with the highest and lowest tyrosine concentrations) were assayed for threonine, serine, glutamic acid, glycine, alanine, valine, cysteine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine and arginine by means of a Technicon amino acid Auto-Analyzer. The methionine contents of samples from two subjects on the "low-protein formula diet" were kindly determined by Dr. Sanford Miller and Miss Anna F. Woo, of the Massachusetts Institute of Technology, who used a microbiologic assay.[8]

Results

Tyrosine

A plasma tyrosine rhythm similar to that described previously[1] was observed in all but one of the 23 volunteers studied. This subject had received the "formula diet." The various dietary regimens were associated with slight differences in the temporal characteristics of the tyrosine rhythm: among the six subjects on the "house diet" (Fig. 1) or the nine given the "formula diet" (Fig. 2), plasma tyrosine was lowest at 2:00 a.m.; however, the former group attained peak levels of the amino acid between 1:30 and 9:30 p.m., whereas among the latter, plasma tyrosine was highest at 10:30 a.m. In both groups, the decline in plasma tyrosine level between 5:00 p.m. and 2:00 a.m. was interrupted by a small rise between 5:00 and 9:00 p.m. This observation raises the suspicion that the concentration of the amino acid may oscillate with additional frequencies besides the dominant 24-hour rhythm. Among the eight volunteers on the "low-protein formula diet" plasma tyrosine peaked sharply at 8:30 a.m. (Fig. 3), or several hours earlier than among subjects given diets containing normal amounts of protein (Fig. 1 and 2).

To determine the extent to which the tyrosine rhythm could be perturbed by dietary factors, a single volunteer was fed small amounts of protein every three hours for 36 hours. Blood samples collected during a previous control day demonstrated the expected fluctuations in tyrosine concentration (Table 1). However, this rhythm was effectively dampened by three-hourly feedings.

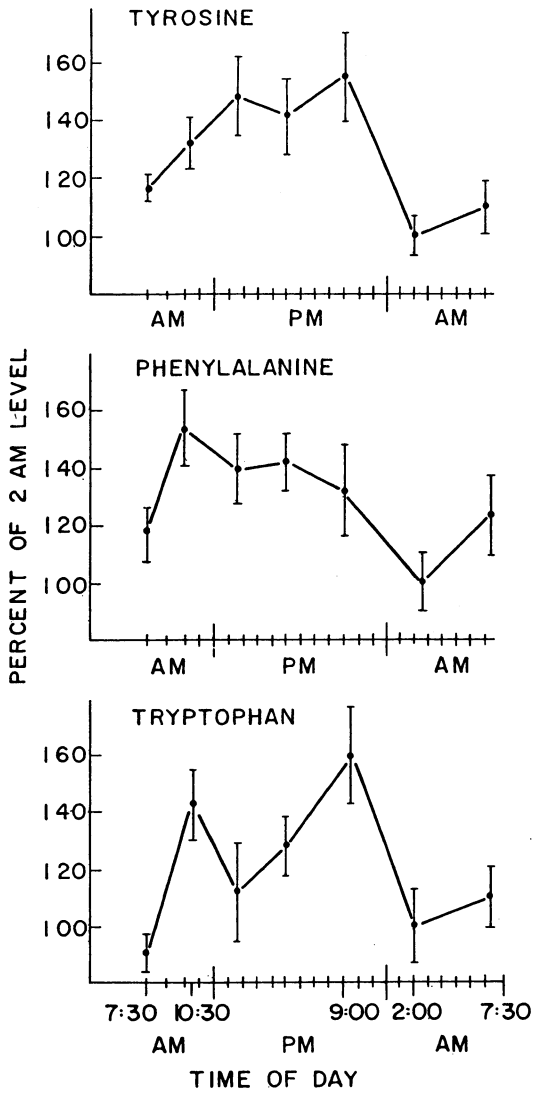

FIGURE 1. *Changes in Plasma Concentrations of Tyrosine, Phenylalanine and Tryptophan with Time of Day among Six Subjects Given the "House Diet."*

Blood was taken at 7:30 and 10:30 a.m., at 1:30, 5:00 and 9:00 p.m. and at 2:00 and 7:30 a.m. Data are presented as mean ± standard error. The 2:00 a.m. mean concentrations of tyrosine, phenylalanine and tryptophan were 12.20 ± 0.94, 8.14 ± 0.85, and 9.13 ± 1.22 μg per milliliter respectively.

Phenylalanine

The concentration of phenylalanine in the plasma showed temporal variations that were similar to those of tyrosine, both among the six subjects given the "house diet" (Fig. 1) and the eight receiving the "low-protein formula diet" (Fig. 3). In the former, the concentration of the amino acid was lowest at 2:00 and highest at 10:30 a.m.; among the latter phenylalanine levels peaked sharply at 8:30 a.m. The amplitudes of the tyrosine and phenylalanine rhythms were also similar.

Tryptophan

The plasma tryptophan concentration tended to be

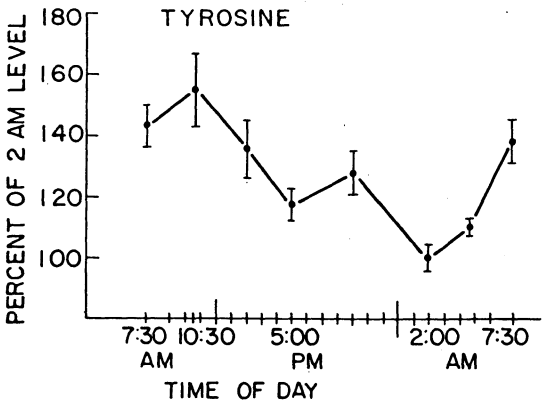

FIGURE 2. *Change in Plasma Tyrosine Concentration with Time of Day in Nine Subjects on the "Formula Diet."*

Data are presented as mean ± standard error. The 2:00 a.m. mean concentration of the amino acid was 10.04 ± 0.39 μg per milliliter.

somewhat more variable than that of tyrosine or phenylalanine; this tendency was reflected in higher standard errors around the mean values. Among the six subjects on the "house diet" the tryptophan level was low between 2:00 and 7:30 a.m. and rose to peaks at both 10:30 a.m. and 9:00 p.m. (Fig. 1). The plasma tryptophan rhythm in the eight subjects given the "low-protein formula diet" was similar to the tyrosine and phenylalanine rhythms (Fig. 3).

Methionine

Methionine concentrations were assayed in plasmas from two subjects on the "low-protein formula diet." In both, the amino acid rose almost twofold between 10:00 p.m. and 6:30 a.m. (Table 2).

Other Amino Acids

Fourteen amino acids were measured by autoanalysis in plasmas obtained from six subjects on the "house diet" and from three who received the "low-protein formula diet." Two plasma samples were assayed from among the seven taken from each subject — that is, those containing the highest and lowest concentrations of tyrosine. The "low-tyrosine" sample was the 2:00 a.m. plasma in all the subjects given the "house diet"; the "high tyrosine" specimen was the 8:30 a.m. plasma in all the subjects receiving the "low-protein formula diet."

All the amino acids showed some tendency to rise and fall as a function of time of day (Table 3). The extent to which the concentration of a given amino

TABLE 1. *Plasma Tyrosine Concentration in Subject Given Small Protein Meals at Three-Hour Intervals.*

DIET	TIME BLOOD COLLECTED	TYROSINE CONCENTRATION (μG/ML)
"Formula diet":		
	7:30 a.m.	14.4
	10:00 a.m.	16.9
	2:00 p.m.	14.6
	5:00 p.m.	12.8
	9:00 p.m.	15.1
3-hr. feeding*		
	2:00 a.m.	9.9
	7:30 a.m.	12.5
	10:00 a.m.	14.4
	1:00 p.m.	14.5
	5:00 p.m.	13.5
	9:00 p.m.	14.3
	2:00 a.m.	12.4
	7:30 a.m.	12.5

*3-hr meals of "formula diet" started at 10 p.m.

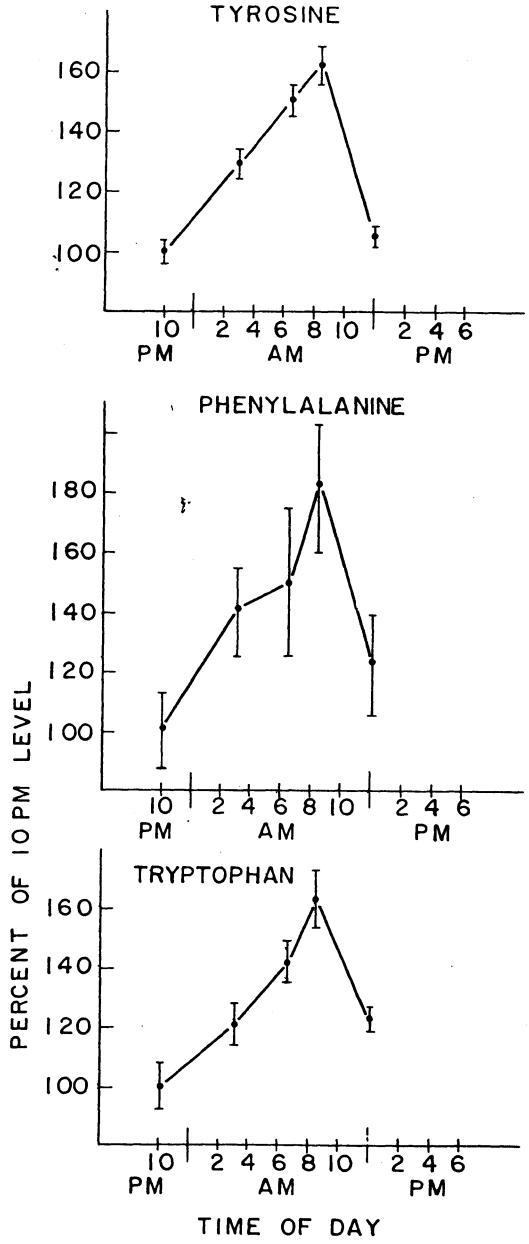

FIGURE 3. *Changes in Plasma Concentrations of Tyrosine, Phenylalanine and Tryptophan with Time of Day among Eight Subjects on the "Low-Protein Formula Diet" (2.7 gm per day).*

Blood was taken at 10:00 p.m., at 3:00, 6:30 and 8:30 a.m. and at noon. Data are presented as mean ± standard error. The 10:00 p.m. mean concentrations of the amino acids were 7.80 ± 0.31, 6.46 ± 0.86 and 6.30 ± 0.49 μg per milliliter respectively.

TABLE 2. *Plasma Methionine Concentrations in Subjects Given the "Low-Protein Formula Diet."*

TIME BLOOD COLLECTED	METHIONINE CONCENTRATION (μg/ML)	
	SUBJECT 1	SUBJECT 2
10:00 p.m.	2.5	2.6
3:00 a.m.	3.4	4.6
6:30 a.m.	4.9	5.0
8:30 a.m.	4.8	4.3
12:00 m.	2.6	2.6

acid changed was similar among subjects receiving either the "house diet" or the "low-protein formula diet." Amino acids with low plasma concentrations (such as tyrosine, cysteine, phenylalanine and isoleucine) tended to show greater percentage variations than alanine, glycine or glutamic acid, which are present in blood in larger quantities. These three compounds, together with glutamine, account for about 80 per cent of the total pool of free amino acids in the body.[9] Hence, it should not be surprising that their plasma concentrations were less subject to diurnal fluctuations. In general, amino acids showing the greatest percentage change during the day also tended to have lower plasma concentrations at both times tested among subjects on the "low-protein formula diet" (Table 3).

Because the levels of some amino acids in blood changed more than others, the amino acid pattern among our subjects was not constant throughout the day. For example, the ratio of tyrosine to glycine in plasma decreased by about 40 per cent when the tyrosine levels fell (Table 3).

It was not possible to submit to amino acid autoanalysis more than two of the plasma samples collected daily from any subject. Hence, it cannot now be stated whether the diurnal variations exhibited by all the amino acids are synchronous. However this possibility seems likely on the basis of the previous observation[3] that the total concentration of amino acids in plasma varies with a rhythm whose phasing is similar to that of the tyrosine rhythm.

DISCUSSION

The demonstration that most or all of the amino acids in plasma vary normally as a function of time of day provides further evidence that the "normal values" for the concentration of a given circulating substance more often than not describe its normal range at a particular part of the day or night, but not at other times. "Normal" plasma tyrosine levels at 2 a.m. may be only half as great as those observed at 10 a.m. If, as seems likely, the concentrations of amino acids in the blood ever limit the availability of these substances in peripheral organs, future studies might be expected to demonstrate daily rhythms in the rates at which brain, muscle and other organs synthesize proteins.

The biochemical mechanisms responsible for these amino acid rhythms are not clear, and are probably complex. It was initially suggested that the tyrosine rhythm might result from diurnal variations in the activity of the tyrosine-metabolizing enzyme, tyrosine transaminase.[1,10,11] However, studies described in this report have shown that the tyrosine rhythm persists under experimental conditions in which the rhythm in enzyme activity would be expected to disappear (that is, the "low-protein formula diet"), and that many additional amino acids that are not metabolized by this enzyme also show diurnal rhythmicity. The persistence of the amino acid rhythms among subjects given the "low-protein formula diet" confirms the previous suggestion[1] that plasma amino acid rhythms are not the consequence of a postprandial overflow of dietary amino acids from the portal system. It does not, however, rule out the possibility that the rhythms are related to changes in the total pool size of the free amino acids caused by dietary and hormonal factors. Indeed, such a relation is suggested by the high degree of correlation between individual

TABLE 3. *Plasma Amino Acid Concentrations at Times of Day when Tyrosine Level Was Highest or Lowest.**

AMINO ACID	"HOUSE DIET"			"LOW-PROTEIN FORMULA DIET"		
	AMINO ACID CONCENTRATION (μMOLE/ML)			AMINO ACID CONCENTRATION (μMOLE/ML)		
	high	*low*	*ratio (high/low)*	*high*	*low*	*ratio (high/low)*
Threonine	0.17 ± .02	0.14 ± .05	1.45 ± .27	0.21 ± .10	0.15 ± .07	1.39 ± .03
Serine	0.22 ± .07	0.17 ± .05	1.39 ± .39	0.21 ± .09	0.15 ± .03	1.43 ± .19
Glutamic acid	0.28 ± .12	0.24 ± .15	1.29 ± .54	0.32 ± .10	0.26 ± .12	1.26 ± .23
Glycine	0.27 ± .05	0.21 ± .02	1.25 ± .17	0.41 ± .09	0.32 ± .05	1.29 ± .05
Alanine	0.46 ± .10	0.30 ± .07	1.59 ± .37	0.58 ± .29	0.52 ± .21	1.12 ± .33
Valine	0.36 ± .07	0.28 ± .05	1.31 ± .32	0.17 ± .03	0.11 ± .02	1.50 ± .16
Cysteine	0.08 ± .12	0.04 ± .02	1.83 ± .61	0.05 ± .02	0.03 ± .02	1.89 ± .55
Isoleucine	0.13 ± .02	0.09 ± .02	1.51 ± .42	0.07 ± .02	0.03 ± .01	2.16 ± .02
Leucine	0.23 ± .05	0.15 ± .02	1.63 ± .54	0.13 ± .03	0.06 ± .01	2.02 ± .36
Tyrosine	0.09 ± .02	0.05 ± .02	2.01 ± 1.00	0.05 ± .01	0.02 ± .02	2.13 ± .21
Phenylalanine	0.08 ± .02	0.05 ± .02	1.60 ± .44	0.06 ± .02	0.03 ± .02	1.74 ± .30
Lysine	0.27 ± .05	0.18 ± .05	1.58 ± .34	0.16 ± .03	0.12 ± .03	1.40 ± .09
Histidine	0.14 ± .02	0.11 ± .02	1.39 ± .34	0.14 ± .02	0.09 ± .02	1.60 ± .47
Arginine	0.13 ± .05	0.11 ± .02	1.46 ± .24	0.12 ± .02	0.08 ± .03	1.43 ± .30

*Data presented as mean ± standard deviation.

amino acids whose plasma concentrations show the greatest percentage changes during the day and those that decline in subjects fed the "low-protein formula diet" and have the smallest pool sizes (Table 3).

The phasing of the plasma tyrosine rhythm can be shifted by treatment of human subjects with dexamethasone, a synthetic steroid hormone.[2] Moreover, carbohydrate-active steroids are known to depress plasma amino acid levels,[12] possibly by changing their steady-state ratios to the free amino acid pools in certain tissues. Hence, it is possible that one factor that generates diurnal amino acid rhythmicity is the daily rhythm in the concentration of hydrocortisone in the blood.[13,14] The levels of immunoreactive insulin in the blood of healthy young fasted males has also been shown to vary diurnally: activity is consistently higher at 7 to 8 than at 3 to 4 p.m.[15] Since insulin alters the rates at which amino acids are transferred across cellular membranes,[16] it is also possible that the plasma amino acid rhythms are related to insulin secretion.

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