Diurnal variations in plasma concentrations of tryptophan, tyrosine, and other neutral amino acids: effect of dietary protein intake[1, 2]

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ABSTRACT The effect of dietary protein content on the diurnal variations in plasma neutral amino acid levels was studied in normal human subjects. For three consecutive 5-day periods, subjects consumed diets containing 0, 75, or 150 g of egg protein per day. Blood samples were drawn at 4-hr intervals on the 4th and 5th days of each period. Consumption of the protein-free diet caused plasma concentrations of all amino acids studied to fall in the late morning and afternoon, while the 150-g protein diet elicited increases in these levels during the daytime. Ingestion of the diet containing 75 g of egg protein tended to diminish the amplitudes of the daily rhythms in plasma amino acid levels, but most amino acids still exhibited small but significant elevations late in the evening. At all times of day, plasma concentrations of the large neutral amino acids studied (i.e., aromatic and branched-chain amino acids, and methionine) varied directly with the protein content of the diet. In contrast, the relationships between dietary protein content and the plasma concentrations of glycine and alanine, two small neutral amino acids, were inverse. The ratios of plasma tryptophan, tyrosine, and phenylalanine levels to the sum of the concentrations of other large neutral amino acids tended to fall as the protein content of the diet was increased. The corresponding ratio for valine increased as protein was added to the diet, while the leucine and isoleucine ratios were not correlated with dietary protein content. Since diet-induced changes in plasma tryptophan and tyrosine ratios in animals are known to cause parallel alterations in brain tryptophan and tyrosine levels, and thus in the rates of brain serotonin and catecholamine synthesis, our data suggest that the ingestion of carbohydrates and protein may also normally affect brain monoamine synthesis in humans. *Am. J. Clin. Nutr.* 32: 1912–1922, 1979.

Plasma amino acid concentrations in humans (1, 2) and laboratory rodents (3–5) exhibit characteristic diurnal fluctuations that are generated largely by the ingestion of food (1, 6). The composition of each meal can affect plasma amino acid levels by at least two mechanisms: by directly contributing a portion of the amino acids present in the dietary protein, and by stimulating the secretion of insulin, which facilitates the passage of amino acids from the blood into peripheral tissues such as skeletal muscle (7). Amino acids that exhibit the greatest diurnal fluctuations in plasma concentration tend also to be those whose plasma concentrations decline most markedly in response to insulin (1).

Although abundant data are available indicating that dietary protein content is a major factor imparting rhythmicity to plasma amino acid concentrations, there has been no systematic study to date in which the protein content of the diet has been directly correlated with the levels of individual amino acids in plasma samples collected at various times of day. Such data could contribute to our understanding of central nervous system function, inasmuch as the ratios of the plasma concentrations of certain amino acids are known to affect the rates at which brain neurons synthesize neurotransmitters such as serotonin (8) and catecholamines (9–11).

Serotonin synthesis in rat brain varies as a function of the concentration in brain of its

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amino acid precursor, tryptophan (12–14); this in turn depends on the rate at which tryptophan is taken up into the brain, which varies with the ratio of plasma tryptophan levels to the sum of the plasma concentration of other large neutral amino acids that compete with it for a common transport mechanism (8). Thus, treatments (e.g., consumption of carbohydrates (15)) that increase plasma tryptophan levels and/or decrease the plasma concentrations of the other aromatic amino acids or the branched-chain amino acids (and possibly methionine and histidine), increase brain tryptophan levels and accelerate serotonin synthesis in the rat. In contrast, when a meal rich in protein is consumed, the contribution of branched-chain amino acids to the blood far exceeds the increase in serum tryptophan since, unlike tryptophan, the branched-chain amino acids are abundant in protein and are not catabolized by the liver (16). Consequently, brain tryptophan levels (and serotonin synthesis) do not increase and may even decrease (8, 17). Available information concerning amino acid uptake and the characteristics of the tryptophan-hydroxylating enzyme in human brain suggests that, if plasma amino acid ratios were to change postprandially in humans as they do in the rat, similar changes in brain tryptophan levels and serotonin synthesis would ensue (e.g., see Reference 18).

Similar to the above relationship for tryptophan and serotonin, the ratio of plasma tyrosine to the other neutral amino acids controls brain tyrosine levels in rats (9, 19), and in turn influences the rates at which catecholamine neurons synthesize dopamine and norepinephrine (9, 10). Hence, information about diurnal rhythms in these ratios, and about the relationships between these rhythms and food consumption, might suggest ways in which nutritional and metabolic factors affect the functions of catecholamine neurons in humans.

This article describes changes in the concentrations of certain plasma amino acids (the large and small neutral amino acids) and in their ratios that occur in human subjects as a function of time of day and of dietary protein content. It will be shown that the changes in these ratios caused by adding protein to the diet are quite similar to changes noted previously in the rat (8, 19, 20).

Materials and methods

Subjects

Seven healthy male undergraduates at the Massachusetts Institute of Technology (MIT), ranging in age from 18 to 24 years old and weighing 62 to 83 kg, were studied. Each subject signed an advised consent form that stated the purpose of the study, the nature of the experiment, and the sampling to be done. The protocol had prior approval of departmental and MIT Committees on the Use of Humans as Experimental Subjects.

Experimental procedure

The subjects were inpatients at the MIT Clinical Research Center for the duration of the study. After consuming a standard "house diet" for 5 days, the subjects were given three special diets, one during each of three consecutive 5-day periods, which provided 0, 75, and 150 g protein per day, respectively. Blood samples were drawn on the 4th and 5th days of each period, beginning at 7 AM on day 4, and thereafter at eleven 4-hr intervals. The samples were collected into heparinized tubes and immediately centrifuged; plasma was frozen until assayed. At the end of the third experimental period, all subjects consumed the house diet for several days before they were discharged.

Diets

All meals were prepared in the kitchen of the Clinical Research Center under the supervision of the research dietitian. Each patient's diet was designed to suit his individual caloric needs; daily caloric intakes ranged between 2700 to 3800 kcal/day. Subjects consumed one-third of their daily caloric intake at each of three identical meals, served at 8 AM, 12 noon, and 5 PM. The staff ensured that each subject consumed all of the food provided at each meal.

The diet contained protein as dried egg yolk or egg white and fat as corn oil, Crisco, or butter. Carbohydrate sources included ginger ale, applesauce, Junket danish dessert, oatmeal, and sucrose and dextromaltose in Kool-Aid and specially formulated cookies.

Total protein, carbohydrate, and fat contents of each meal were calculated from standard food composition tables. As protein was added to the diet, carbohydrate was removed. The fat content was thus consistently maintained at about 35% of total daily caloric intake. (Two subjects were exceptions; the fat content of their diets was 25% of the protein-free diet, but 35% of the 75- and 150-g protein diets.)

Analytical methods

Plasma was prepared for amino acid analysis by adding 50 mg of 5-sulfosalicylic acid to 1-ml aliquots of plasma; the samples were left in an ice bath for 5 min, and then centrifuged in a Sorvall RC2-B refrigerated centrifuge for 20 min at 10,000 rpm. The resulting supernatant was filtered through a Swinex Millipore filter apparatus (filter #HAWP 01300, Millipore Corp., Bedford, Mass.). The final supernatants were clear, and were stored frozen until assayed for large and small neutral amino acids on a Beckman 121 amino acid analyzer (Beckman Instruments, Palo Alto, Calif.). Since tryptophan could not be detected reliably using the analyzer,

plasma tryptophan concentrations were determined fluorometrically on 50-μl aliquots of plasma (21–23).

Data were analyzed by 3-way analysis of variance; the factors compared were diet (0, 75, or 150 g protein per day), day (1st or 2nd day of blood sampling), and time of day (3 AM, 7 AM, 11 AM, 3 PM, 7 PM, 11 PM). The least significant difference was calculated for making comparisons among selected means ($P < 0.05$ or $P < 0.01$) (24).

Results

Large neutral amino acids

Branched-chain amino acids (Fig. 1). Plasma concentrations of leucine, isoleucine, and valine varied directly with the protein content of the diet at all times of day ($P < 0.05$), and exhibited characteristic daily fluctuations: levels were lowest between 11 AM and 7 PM when subjects consumed protein-free meals, but were highest during these hours when meals contained protein. These rhythms were significant ($P < 0.05$) at all levels of protein consumption.

Methionine (Fig. 2). The effects of dietary protein content and time of day on plasma methionine levels were quite similar to those described above for the branched-chain amino acids, i.e., methionine levels were increased at all times of day when protein was added to the diet ($P < 0.05$), and the daily rhythms, which were significant at all levels of dietary protein ($P < 0.05$), changed from nocturnal peaks with the protein-free diet to a pattern of marked daytime elevations with the 150-g protein diet.

Aromatic amino acids (Fig. 3). When the protein-free diet was consumed, plasma con-

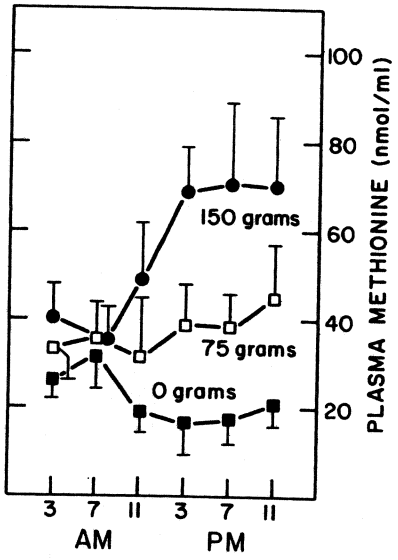

FIG. 2. Diurnal variations in plasma methionine levels in normal human subjects ingesting different levels of dietary protein. *Vertical bars* represent SD. Symbols are as in Figure 1.

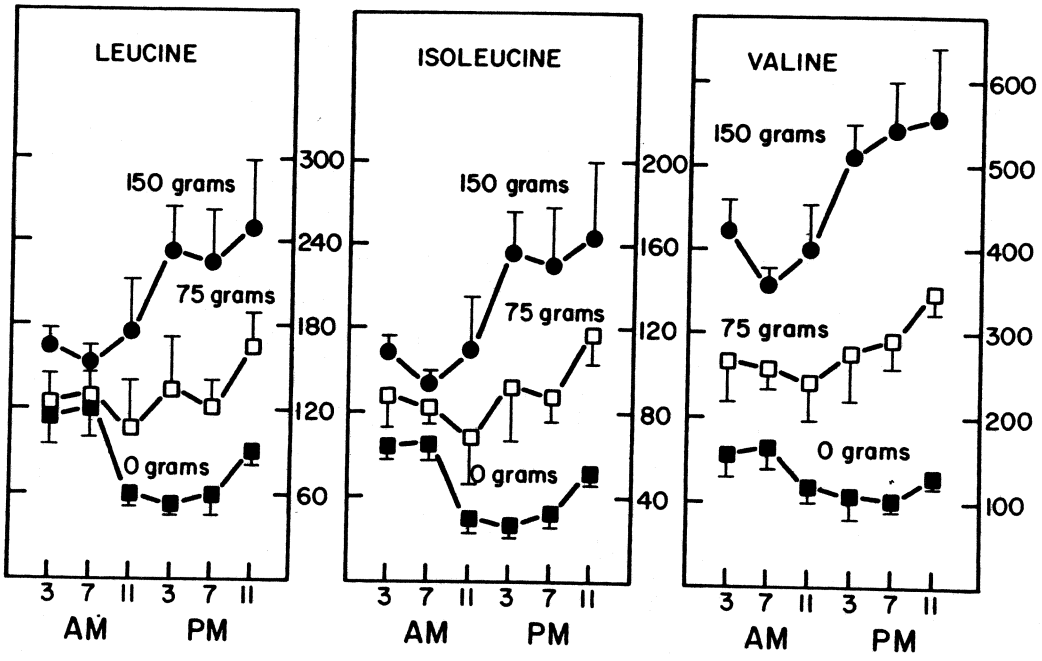

FIG. 1. Diurnal variations in plasma branched-chain amino acid levels in normal human subjects ingesting different levels of dietary protein. Plasma amino acid levels are expressed in nanomoles per milliliter; *vertical bars* represent SD. In this and all other figures: ■ = protein-free diet; □ = 75-g protein diet; ● = 150-g protein diet. Identical meals were served at 8 AM, 12 noon, and 5 PM. Analysis of variance revealed no significant differences in the 24-hr patterns of variation for any of the amino acids or ratios between days 4 and 5. Hence, the data from the 2 days were combined, and are so presented in all of the figures.

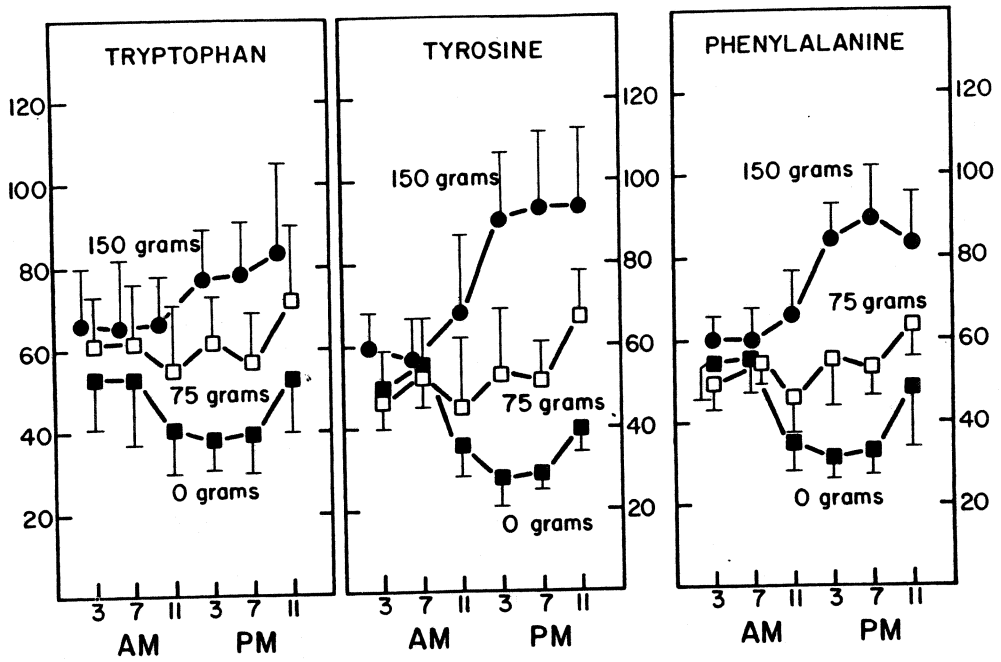

FIG. 3. Diurnal variations in plasma aromatic amino acid levels in normal human subjects consuming different levels of dietary protein. Plasma amino acid levels are expressed in nanomoles per milliliter; *vertical bars* represent SD. Symbols are as in Figure 1.

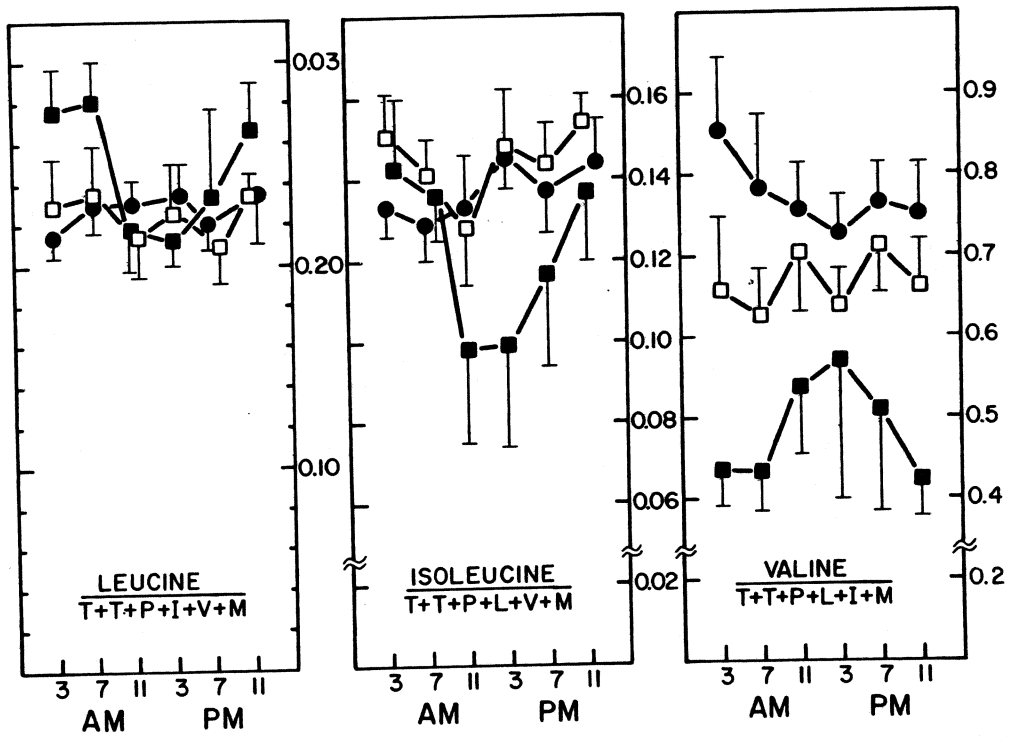

FIG. 4. Diurnal variations in plasma branched-chain amino acid ratios in normal humans ingesting different levels of dietary protein. *Vertical bars* represent SD; symbols are as in Figure 1. Abbreviations: T + T = tryptophan + tyrosine; P = phenylalanine; L = leucine; I = isoleucine; V = valine; M = methionine.

centrations of tryptophan, tyrosine, and phenylalanine, like those of the neutral amino acids described above, were significantly lower during the daytime hours ($P < 0.05$) than at 3 AM or 7 AM. They were high during the day when 150 g protein per day was consumed ($P < 0.05$). With the ingestion of the 75-g protein diet, the patterns of variation were significant ($P < 0.05$), but quite small, over the 24-hr period.

Neutral amino acid ratios (Figs. 4–6). The ratio of the plasma concentration of valine to

the sum of the plasma concentrations of the other neutral amino acids increased significantly at all times of day ($P < 0.05$) as protein was added to the diet (Fig. 4). When subjects consumed the protein-free diet, the valine ratio was significantly elevated ($P < 0.01$) during the daylight hours; with the high-protein diet, it was depressed ($P < 0.01$) during the day (Fig. 4).

The relationships between dietary protein content and the ratios of the plasma concentrations of leucine and isoleucine to those of the other neutral amino acids did not follow the pattern characteristic for valine. The leucine/competitor ratio was highest during the night and fell significantly ($P < 0.01$) during the day when the protein-free diet was consumed. The nocturnal elevations were absent when 75 or 150 g of protein were consumed. The isoleucine/competitor ratio was the same for all three levels of protein intake during the night, but when subjects consumed the protein-free diet, it was significantly depressed during the day ($P < 0.01$) (Fig. 4).

The ratio of methionine to its competitors appeared to be roughly inverse to the dietary protein content, but this effect was not statistically significant. Consumption of the 150-g protein diet was associated with low ratios during the night, and daytime values that were significantly higher ($P < 0.01$), but this was the only effect noted (Fig. 5).

The ratios of tryptophan, tyrosine, and phenylalanine to the other neutral amino acids varied inversely with dietary protein

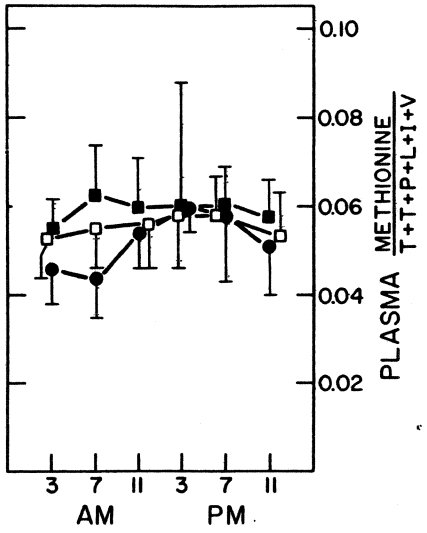

FIG. 5. Diurnal variations in plasma methionine ratio in normal human subjects ingesting different levels of dietary protein. *Vertical bars* represent SD; symbols are as in Figure 1; abbreviations are as in Figure 4.

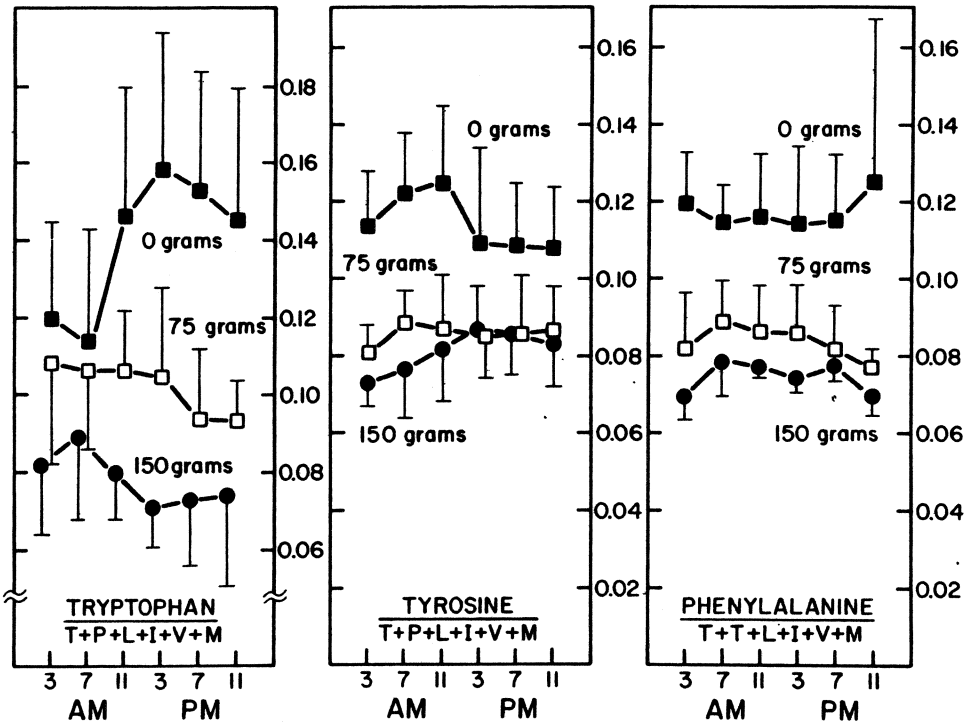

FIG. 6. Diurnal variations in plasma aromatic amino acid ratios in normal human subjects ingesting different levels of dietary protein. *Vertical bars* represent SD; symbols are as in Figure 1. Abbreviations: P, L, I, V, and M are defined in the legend to Figure 4; in the *left panel*, T = tyrosine; in the *middle panel*, T = tryptophan; in the *right panel*, T + T = tryptophan + tyrosine.

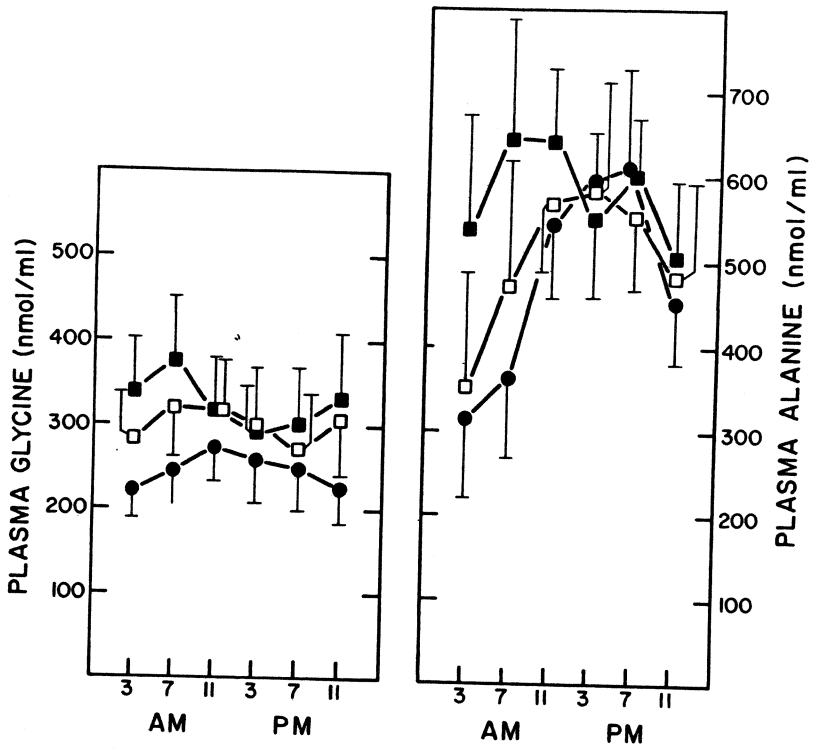

FIG. 7. Diurnal variations in plasma glycine and alanine levels in normal humans ingesting different levels of dietary protein. *Vertical bars* represent SD; symbols are as in Figure 1.

content ($P < 0.05$) (Fig. 6). A significant diurnal variation was noted in the tryptophan ratio at each level of protein intake ($P < 0.05$). The plasma tryptophan ratio in subjects consuming the protein-free diet was lowest at 3 AM and 7 AM, and increased significantly during the day ($P < 0.05$). It did not increase during the day when the 75-g protein diet was ingested, and the daytime values fell significantly below 3 AM and 7 AM values in subjects ingesting the 150-g protein diet ($P < 0.05$) (Fig. 6).

The plasma tyrosine ratio underwent small but significant ($P < 0.05$) diurnal variations at each level of protein intake. The ratio associated with the protein-free diet was higher ($P < 0.05$) at all times of day than those with the 75- or 150-g diets, which were statistically indistinguishable.

The plasma phenylalanine ratio exhibited a statistically significant daily rhythm when the protein-free diet was ingested ($P < 0.05$), but not when the protein-containing diets were consumed. At all times of day, the plasma phenylalanine ratio varied inversely with dietary protein content ($P < 0.05$).

Glycine and alanine

Significant daily rhythms in plasma alanine and glycine ($P < 0.05$) were noted at each level of protein intake. Plasma glycine and alanine levels tended to vary inversely with dietary protein content ($P < 0.05$); this effect was most apparent between 11 PM and 7 AM (Fig. 7). The differences between plasma alanine levels of subjects consuming 0 or 150 g of protein per day were considerable at 3 AM (320 versus 540 nmole/ml), but practically nonexistent 12 hr later.

Threonine, serine, and proline

The effects of time of day and dietary protein content on plasma concentrations of threonine, serine, and proline were similar, and resembled those described above for the branched-chain amino acids and methionine. Plasma levels rose at all times of day as protein was added to the diet ($P < 0.05$). Significant rhythms ($P < 0.05$) were very marked when the 0- or 150-g protein diets were consumed, and less so with the 75-g protein diet. The daily rhythms peaked between 3 and 7 AM when subjects consumed the protein-free diet, and between 3 and 11 PM when they ate 150 g of protein per day ($P < 0.05$) (Fig. 8).

Discussion

These data show that the plasma concentrations of all the large neutral amino acids

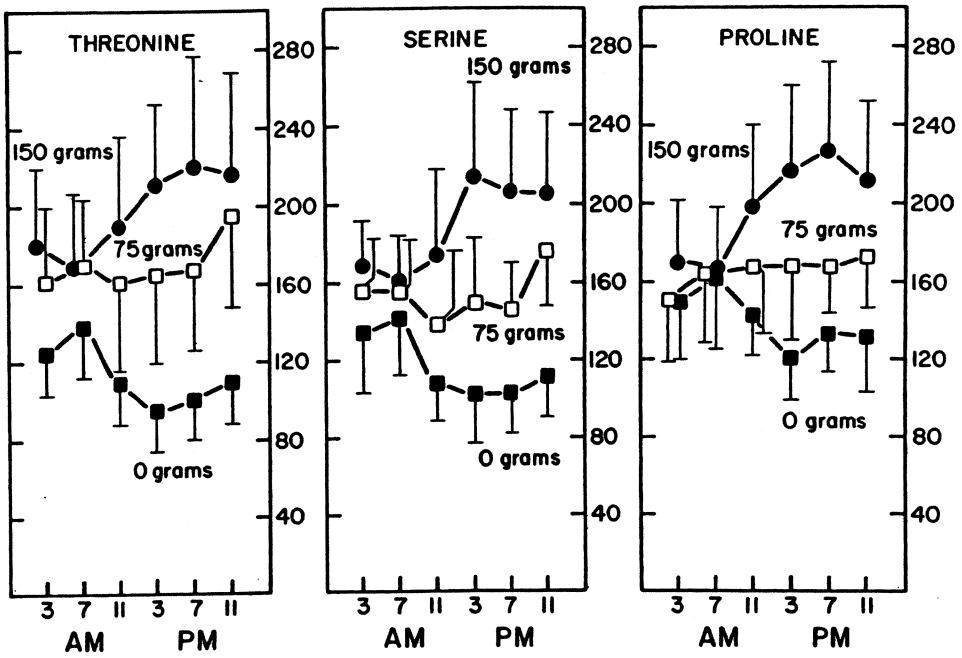

FIG. 8. Diurnal variations in plasma threonine, serine, and proline concentrations in normal human subjects ingesting different levels of dietary protein. *Vertical bars* represent SD; symbols as in Figure 1. Plasma amino acid concentrations are expressed in nanomoles per milliliter.

vary directly with the protein content of the diet. The relationship between dietary protein content and the plasma concentrations of the small neutral amino acids, glycine, and alanine, was inverse. The consumption of protein-free meals caused the plasma concentrations of all amino acids studied to fall in the afternoon, while ingesting 150 g of protein per day elicited an increase in these levels between 11 AM and 11 PM. The consumption of a diet containing 75 g of protein per day tended to moderate the daily rhythms in the plasma concentrations of the neutral amino acids, but most still attained a significant peak around 11 PM. The ratios of plasma levels of tryptophan, tyrosine, and phenylalanine to the sum of those of the other large neutral amino acids tended to fall as dietary protein content increased, an effect which was most pronounced for tryptophan (Fig. 6). The opposite was true for the relationship between dietary protein content and the ratio between plasma concentrations of valine and its competitors. No clear relationships emerged for plasma methionine, leucine, or isoleucine ratios (Figs. 4 and 5).

Plasma neutral amino acid levels

The plasma levels of each of the amino acids studied varied significantly over the 24-hr period in a manner consistent with earlier findings (25, 26). In the earlier experiments, subjects consuming about 100 g/day of mixed proteins had plasma amino acid concentrations that were generally highest during the evening and lowest in the early morning; those consuming protein-free diets had plasma amino acid levels that were usually higher in the early morning and lower in the evening. In the present study, in which many more timepoints were analyzed, we observed that the ingestion of the protein-free diet led to reductions in the plasma concentration of each amino acid after 7 AM. Coincident with these reductions were increases in plasma insulin levels, which then remained elevated throughout the day (27). Plasma insulin concentrations were similar in subjects eating all three of the test diets, that is, the peak and plateau levels attained after eating 0, 75, or 150 g of protein per day did not differ significantly (27). The daytime reductions (11 AM to 7 PM) in plasma amino acid levels were probably the result of the insulin secretion, the ultimate effect of which is to facilitate the passage of amino acids from the plasma into peripheral tissues (7). Wurtman (1) adduced further evidence of the importance of insulin secretion in generating plasma amino acid rhythms in subjects con-

suming protein-free diets by correlating the amplitude of the 24-hr variation for each amino acid in plasma with the percentage reduction in its plasma concentration normally obtained after an oral dose of glucose. A highly significant positive correlation was observed: the plasma concentrations of the aromatic and branched-chain amino acids exhibited the largest 24-hr variations and the biggest reductions after glucose; threonine and serine were intermediate, and glycine and alanine showed the smallest 24-hr variations and changes in response to glucose. The data from the present study tend to affirm this view: when the protein-free diet was consumed, tyrosine, leucine, and isoleucine levels fell in plasma during the day by 50 to 55% of early morning values; phenylalanine and valine fell by about 40%, threonine and serine by about 30%, and glycine and alanine by about 20%.

When subjects ingested the 75- or 150-g protein diets, the patterns of the daily rhythms in plasma concentrations of almost all the amino acids studied changed markedly from those observed when the protein-free diet was consumed. Whereas plasma amino acid levels tended to fall during the day when subjects ingested the protein-free diet, they rose during the day with consumption of the protein-containing meals. The most likely interpretation of this finding is that the amounts of dietary amino acids entering the circulation several hours after protein-containing meals were consumed more than offset the insulin-induced facilitation of amino acid efflux from the circulation. The largest increases occurring between 3 and 11 PM in subjects consuming the high-protein diet were in the concentrations of the branched-chain amino acids leucine, isoleucine, and valine. This is probably because these amino acids are not catabolized by the liver, and thus pass unchanged from the intestine into the circulation (16, 28). The increases in aromatic amino acid and methionine concentrations were very small, most likely reflecting significant hepatic catabolism (29–31). Tryptophan and methionine are often the most limiting amino acids for hepatic protein synthesis; thus, the smaller increments in their plasma concentrations after protein consumption could also reflect their proportionately greater utilization for hepatic protein synthesis (31).

The changes in plasma glycine and alanine levels after ingestion of the different diets contrasted with those of the other amino acids (Fig. 7). During the day (11 AM to 7 PM), dietary protein content bore no relationship to plasma glycine or alanine levels, but at 3 and 7 AM these levels decreased as dietary protein content increased. We lack an adequate explanation for these findings. The daytime increments in plasma alanine that occurred with ingestion of the 75- or 150-g protein diet might reflect in part the input from dietary protein during this time period. The high nocturnal levels observed in subjects ingesting the protein-free diet could, of course, reflect increased production or decreased utilization of the amino acid; however, the biosynthetic or catabolic pathway involved awaits identification.

Plasma neutral amino acid ratios

The uptake into the brain of the large neutral amino acids is mediated by a single competitive transport mechanism distributed throughout brain capillaries (32). Thus, for example, an increase in tryptophan uptake into brain can be caused either by elevating plasma tryptophan concentrations (12) or by reducing the plasma levels of one or more of the other neutral amino acids that compete with tryptophan for uptake into the brain (8). A reduction in brain tryptophan concentrations can similarly be produced either by decreasing plasma tryptophan or by raising the plasma concentrations of its competitors (17). We have developed a reasonably economic expression of the above relationship, i.e., that brain tryptophan varies directly with the plasma ratio of the tryptophan concentration to the sum of the concentrations of the other large neutral amino acids (8).

The plasma tryptophan ratio has been varied experimentally in laboratory animals using a variety of manipulations, and found to predict accurately the ensuing changes in brain tryptophan levels (17, 19, 20, 33) and the rate of influx of tryptophan into brain (19). In the present experiments, we have sought to determine whether some of the dietary influences on the plasma tryptophan ratio in animals also operate in humans. If so,

then it might be anticipated that the ingestion of particular foods might have a similar effect on brain tryptophan levels in rats and in humans. The results indicate that the plasma tryptophan ratio after the ingestion of proteins and carbohydrates does respond similarly in humans and in rats. Thus, carbohydrate ingestion (protein-free diet) elevated the plasma tryptophan ratio during the period of food intake normal for humans (Fig. 6) as it does in rats (15). Moreover, the addition of protein to the diet depressed the plasma tryptophan ratio, again similarly to the effect seen in rats (8, 20). In experimental animals, diet-induced alterations in brain tryptophan directly modify the rate of serotonin synthesis (12, 14). If the observed effects of the diet on the plasma tryptophan ratio were to modify brain tryptophan uptake in humans, then brain serotonin synthesis might very well be affected. Dunner and Goodwin (18) showed several years ago that the human brain responds to a dose of tryptophan by synthesizing increased amounts of serotonin, as indicated by increased accumulation of 5-hydroxyindoleacetic acid in the cerebrospinal fluid. Such alterations in serotonin formation might well modify brain functions that are at least in part under the control of serotonin-containing neurons.

Recently, we showed that the effect of diet on rat brain tyrosine levels, or for that matter, on the brain concentration of any of the aromatic and branched-chain amino acids, could be predicted surprisingly well by its effect on the plasma ratio of the corresponding large neutral amino acids (19). Thus, in rats, carbohydrate ingestion can increase the plasma tyrosine ratio and brain tyrosine concentrations. Similar relationships were noted for the plasma phenylalanine ratio and brain phenylalanine levels after the consumption of protein or carbohydrates. In contrast, carbohydrate ingestion lowers the plasma ratio and brain concentration of each of the branched-chain amino acids, while the addition of protein to the diet tends to moderate these reductions (19).

In the present clinical study, certain similarities between humans and rats were noted in the response of the plasma tyrosine and phenylalanine ratios to the ingestion of protein and carbohydrates, i.e., these ratios were highest after subjects consumed the protein-free diet and lowest when the high-protein diet was ingested (Fig. 6). For the branched-chain amino acid ratios, the effect of diet was most clear on the plasma valine ratio (Fig. 4), and was similar to the responses seen in rats. The valine ratio was lowest when carbohydrates alone were consumed, and increased as protein was added to the diet. No clear influence of diet on plasma leucine or isoleucine ratios emerged from the present data, except that during the day carbohydrate ingestion seemed to lower both ratios, compared to nocturnal values.

The importance of the effects of diet on plasma tyrosine and phenylalanine ratios in humans lies in the fact that brain catecholamine synthesis and probably release are influenced by variations in brain tyrosine (and phenylalanine) levels. Injections of tyrosine, which elevate brain tyrosine, or of tryptophan or leucine, which lower brain tyrosine, have been shown to increase and decrease, respectively, the rate of hydroxylation of tyrosine to dopa (9, 10); brain tyrosine levels can also influence the release of dopamine (34) and of norepinephrine (11). Moreover, the ingestion of meals that modify plasma tyrosine ratios and brain tyrosine levels also directly influence the formation and release of catecholamines (10, 11). Finally, since phenylalanine has been shown to be a competitive inhibitor of tyrosine hydroxylase in brain (35), increases in the plasma phenylalanine ratio and in brain phenylalanine levels should, and do, inhibit the formation of catecholamines in the brain (9, 10). If diet-induced alterations in the plasma tyrosine and phenylalanine ratios of humans also affect brain tyrosine and phenylalanine levels (as they do in rats), then the ingestion by humans of particular foods should also modify brain catecholamine synthesis.

At the present time, few data are available on the functions of branched-chain amino acids in brain, other than as substrates for brain protein synthesis. However, the brain possesses significant capacity for the enzymatic transamination of these amino acids (36, 37), and the greatest arteriovenous differences across the brain for any of the amino acids are for the branched-chain amino acids, in particular valine (38). The rate of trans-

amination of these amino acids varies directly with the availability of substrates (39); thus it is possible that diet-induced changes in the plasma ratios and brain levels of valine in particular, and perhaps also of leucine and isoleucine, modify their rates of transamination in the brain. The ultimate significance of such a relationship, however, remains unknown at present.

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