Large daily fluctuations in plasma tyrosine in treated patients with phenylketonuria

Francjan J van Sponser, Theo van Dijk, G Peter A Smit, Margreet van Rijn, Dirk-Jan Reijnoud, Ruud Berger, and Hugo SA Heymans

ABSTRACT In patients with phenylketonuria (PKU), extra tyrosine supplementation is advocated in addition to tyrosine-enriched amino acid mixtures. PKU patients have low fasting plasma tyrosine concentrations, but little is known about tyrosine fluctuations during the day. Plasma tyrosine concentrations were studied in 12 PKU patients in response to a test without breakfast and to three tests with different tyrosine contents in breakfast and lunch: 0%/30%, 25%/30%, 50%/10%, and 75%/10% tests, reflecting the protein consumption at breakfast and lunch, respectively. Prolonged fasting resulted in a small decrease in the already low overnight fasting plasma tyrosine concentrations. Breakfast and lunch with 25% and 30% of the daily tyrosine intake resulted in both lower than normal and higher than normal tyrosine concentrations. The 50%/10% and 75%/10% tests resulted in excessively high plasma tyrosine concentrations in most patients. Therefore, both lower than normal and higher than normal postprandial plasma tyrosine concentrations were found in treated PKU patients, even if the daily tyrosine intake was distributed evenly. When there was a large fractional tyrosine intake from one meal, very high plasma tyrosine concentrations were found. Therefore, strict control of plasma tyrosine is necessary if tyrosine supplementation is considered in addition to the tyrosine-enriched amino acid mixtures. *Am J Clin Nutr* 1996;64:916-21.

KEY WORDS Phenylketonuria, dietary tyrosine, daily distribution of intake

INTRODUCTION

Patients with phenylketonuria (PKU) cannot convert phenylalanine into tyrosine. Thus, tyrosine is an essential amino acid for them. If untreated, these patients show extremely high plasma phenylalanine and low to normal plasma tyrosine concentrations and become at risk for severe mental retardation (1, 2). Tyrosine supplementation as a single treatment is not sufficient (3), and high phenylalanine concentrations are generally accepted as the primary cause of mental retardation in PKU patients (4, 5). Treatment aims to lower plasma phenylalanine and to keep plasma concentrations of tyrosine and other amino acids within the normal range. Treatment consists of restriction of natural protein with supplementation of other amino acids by means of a tyrosine-enriched amino acid mixture.

Despite use of tyrosine-enriched amino acid mixtures, very low plasma tyrosine concentrations have been reported in treated PKU patients, especially after an overnight fast (6-11). Extra tyrosine supplementation in addition to the tyrosine-enriched amino acid supplements has been advocated to prevent low plasma tyrosine concentrations (7), especially during pregnancy (5, 12-17), and to improve neuropsychologic functioning (10, 18, 19), presuming that low plasma tyrosine concentrations may damage growth and development. Clinical experience with treated PKU patients, however, indicates that they not only have low overnight fasting and postprandial plasma tyrosine concentrations, but also high postprandial plasma tyrosine concentrations. Little is known about the daily fluctuations of tyrosine concentrations in PKU patients (6, 11); therefore, additional tyrosine supplementation is not based on data about the occurrence of both low and high plasma tyrosine concentrations in treated PKU patients. We investigated the response of plasma tyrosine to various proportional fractions of the total daily tyrosine intake to study daily fluctuations and to learn about the occurrence of both low and high plasma tyrosine concentrations in treated PKU patients. On the basis of these results, the necessity of additional tyrosine supplementation should be reevaluated.

SUBJECTS AND METHODS

The study was conducted according to the guidelines of the medical ethical committee of the University Hospital of Groningen.

Plasma tyrosine responses were studied one to four times in 12 PKU patients between 1 and 20 y of age. Informed consent was obtained from all subjects and/or their parents. The height of the patients was between the 50th and 97th percentile except for three patients (3rd to 10th percentile) (20). The weight-for-height was between the 50th and 90th percentile, except for one patient (3rd to 10th percentile) (20). At the time of the tests, the

1 From Beatrix Children's Hospital, University of Groningen, Netherlands, and "Wilhelmina Kinderziekenhuis", University Hospital of Utrecht, Netherlands.

2 Supported by a grant from the "Nederlands Praeventiefonds" (Dutch Foundation for the Prevention of Disease).

3 Address reprint requests to FJ van Sponser, Department of Pediatrics, Martini Hospital Groningen, van Swietenlaan 4, 9728 NZ Groningen, Netherlands.

Received September 28, 1995.
Accepted for publication June 20, 1996.
patients were free from intercurrent disease. After the classification and the therapeutic guidelines of Güttler and Lou (21), most patients—except for two who had a milder form—had the serious form of PKU and all but one adhered to the diet adequately.

The investigations were carried out in our metabolic ward between 1988 and 1994 in patients at rest and after a 10–12-h overnight fast. In the first test, patients fasted until lunch whereas the other three tests consisted of breakfast and lunch. In the latter three tests, breakfast contained 25%, 50%, and 75% of the daily, individually tailored natural protein allowance and age-adjusted total protein intake, respectively (22). For lunch, these figures were 30%, 30%, 10%, and 10% for the first, second, third, and fourth tests, respectively. The composition of the meals chosen for use in this study resembled that of meals usually consumed by PKU patients, who daily may consume some 25%, 30%, 50%, or even 75% of their daily tyrosine intake at one time. The tests are referred to as follows: 0%/30%, 25%/30%, 50%/10%, and 75%/10%, reflecting the protein consumption at breakfast and lunch, respectively. For example, the 25%/30% test means that the patient received 25% of his daily, individually tailored, natural protein allowance and age-adjusted total protein intake at breakfast, and 30% of his daily, individually tailored natural protein allowance and age-adjusted total protein intake at lunch. The rest of the daily natural protein allowance was consumed at home. The 0%/30% test was performed in three patients, the 25%/30% test in nine and repeated in two, the 50%/10% test in five, and the 75%/10% test in seven and repeated in two.

The composition of each meal was determined by a dietician, who also supervised the preparation. Breakfast and lunch each contained 25–35% of the daily, age-adjusted, energy intake according to Dutch guidelines (22). Fat did not exceed 35% of the energy intake. Meals contained bread with low and normal amounts of protein, macaroni with low and normal amounts of protein, dairy products, jam, applesauce, fruits, adapted formulas, and maltodextrin (Nutricia, Zoetermeer, Netherlands). The amino acid mixtures were PKU1, PKU2, PKU3 (Milupa, Zoetermeer, Netherlands), and Phenylod AM (Nutricia), with tyrosine comprising 8.4%, 10.8%, 8.0%, and 10.1% of the protein, respectively. Breakfast and lunch were served at 0835 and 1135, respectively. Meals were consumed within 15–30 min.

Before the test, an indwelling venous catheter was inserted and kept open by infusion of isotonic saline. Venous blood samples were drawn at 0830 (time 0) and after 15, 30, 60, 90, 120, 135, 150, 165, 180, 210, 240, 270, and 300 min in the tests with breakfast and after 60, 120, 180, 240, and 300 min in the tests without breakfast. Blood samples were collected into heparinized tubes and centrifuged at 2000 × g for 10 min at 18 °C within 2 h after sampling. The resulting plasma samples were stored at −70 °C until analyzed. Plasma tyrosine concentrations were determined in plasma exclusively by using the same ion-exchange liquid-chromatography amino acid analyzer (Carlo Erba 3A30; Interscience, Breda, Netherlands). In our laboratory the CV of the method was 2%.

Plasma tyrosine concentrations were expressed as either absolute values or as percentage changes related to 0 time (baseline values). The overnight fasting plasma tyrosine concentrations were compared with reference values of healthy children (45–89 μmol/L), adult males (42–102 μmol/L), and adult females (35–87 μmol/L) (23). Postprandial plasma tyrosine concentrations were compared with data from healthy adults because no information was available in children older than 1 year. In adults, a breakfast or evening meal, containing either a normal or high amount of protein, resulted in postprandial plasma tyrosine concentrations between 61 and 109 μmol/L (24–26). Changes in plasma tyrosine from baseline were analyzed by using the two-tailed paired t test with an α level of 0.01 as adjustment for numerous comparisons. If a test was performed twice in the same individual, only the first value was taken for statistical analysis. The mean maximum percentage increases above baseline were compared by using the one-tailed paired t test with a P value < 0.01. This value was chosen as adjustment for numerous comparisons.

RESULTS

The individual overnight fasting plasma tyrosine concentration varied between 17 and 56 μmol/L (x ± SD: 34 ± 9.9 μmol/L), showing a small decrease until lunch if breakfast were omitted (results not shown), and a clear postprandial rise with considerable interindividual variation if breakfast were consumed (Figures 1, 2, and 3). During the 25%/30% test, the maximum individual increase above baseline varied between 61% and 448% in the nine patients. Postprandial plasma tyrosine concentrations remained below normal in two and came just into the normal range in one patient, but reached above normal values in 44% of the patients (Figure 1). During the 50%/10% test, the maximum individual increase above baseline varied between 137% and 913% in the five patients, resulting in above-normal values in 80% of the patients (Figure 2). During the 75%/10% test, the maximum individual increase above baseline varied between 282% and 817% in the seven patients, resulting in above-normal values in 86% of the patients (Figure 3).

The mean postprandial tyrosine response showed a significant rise above baseline in the 25%/30% and 75%/10% test, but not in the 50%/10% test. If the test was performed twice in the same patient, the plasma tyrosine response showed considerable intraindividual variation. Increasing the tyrosine intake at breakfast resulted in a rise in maximum increments in all patients but one. When the means of the maximum tyrosine responses of the different tests were compared, the mean increment tended to enlarge when the tyrosine intake was increased (Table 1). The mean of the maximum increases after the 75%/10% test was significantly different from that after the 25%/30% test (P < 0.005), whereas there were no significant differences between the 75%/10% and the 50%/10% tests (P < 0.25) or between the 50%/10% and the 25%/30% tests (P < 0.1).

DISCUSSION

We measured the plasma tyrosine response to both a prolonged overnight fast until lunch, and to breakfast and lunch containing different percentages of the daily tyrosine intake to determine the occurrence of lower than normal and higher than normal plasma tyrosine concentrations and daily fluctuations in treated PKU patients. Lower than normal
plasma tyrosine concentrations occurred at least once in all tested individuals in the overnight fasting condition, and postprandially in 100% and 33% of the individuals after the 0%/30% and 25%/30% tests, respectively, but not after the other two tests. Abnormally high plasma tyrosine concentrations occurred postprandially in 44%, 80%, and 86% of the patients given the 25%/30%, 50%/10%, and 75%/10% tests, respectively, especially when a high fraction of the daily tyrosine intake was given. Thus, the aim of keeping plasma tyrosine within the normal range may often not be achieved. This study showed that the dietary treatment of PKU in fact may result in both very low and very high plasma tyrosine concentrations, even in the same individual on a single day. Two questions arise: what is the explanation

**FIGURE 1.** Plasma tyrosine concentrations in patients with phenylketonuria before (time 0) and after breakfast (time 5 min) and lunch (time 185 min), which contained 25% and 30%, respectively, of the individual daily phenylalanine allowance and total daily protein intake. The reference ranges of the fasting and postprandial tyrosine concentrations are presented as a shaded area.

**FIGURE 2.** Plasma tyrosine concentrations in patients with phenylketonuria before (time 0 min) and after breakfast (time 5 min) and lunch (time 185 min), which contained 50% and 10%, respectively, of the individual daily phenylalanine allowance and total daily protein intake. The reference ranges of the fasting and postprandial tyrosine concentrations are presented as a shaded area.
PKU: LARGE DAILY TYROSINE FLUCTUATIONS

FIGURE 3. Plasma tyrosine concentrations in patients with phenylketonuria before (time 0) and after breakfast (time 5 min) and lunch (time 185 min), which contained 75% and 10%, respectively, of the individual daily phenylalanine allowance and total daily protein intake. The reference ranges of the fasting and postprandial tyrosine concentrations are presented as a shaded area.

for the abnormal plasma tyrosine concentrations and what is its clinical significance?

Because tyrosine becomes an essential amino acid in PKU patients, plasma tyrosine concentrations largely depend on the nutritional intake of tyrosine. As a consequence, plasma tyrosine concentrations can be very low after an overnight fast (11), a finding that agrees with our preliminary study showing a decrease of plasma tyrosine during the night (27). Even if the

TABLE 1
Clinical characteristics and metabolic results in patients after lunch and breakfast with different tyrosine contents

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Phenylalanine tolerance
(mg · kg body wt⁻¹ · d⁻¹)²
At 5 y                     | 21.0 ± 2.0       | 16.1 ± 0.6       | 15.0 ± 0.4       | 14.1 ± 0.5   |
On day of test             | 22.0 ± 0.3       | 15.8 ± 0.6       | 15.2 ± 0.4       | 13.4 ± 0.5   |
Body weight (kg)           | 12.7 ± 0.4       | 28.3 ± 2.1       | 19.2 ± 0.8       | 31.4 ± 2.5   |
Age at time of test (y)    | 1.7 ± 0.4        | 8.2 ± 0.8        | 4.4 ± 0.3        | 9.1 ± 0.9    |
Fasting plasma phenylalanine (μmol/L)  | 180 ± 16         | 343 ± 29         | 456 ± 48         | 453 ± 27     |
Estimated tyrosine intake (mg)
Breakfast                  | 0                | 936 ± 5          | 1373 ± 93        | 2704 ± 188   |
Lunch                      | 662 ± 39         | 1088 ± 67        | 255 ± 15         | 287 ± 38     |
Plasma tyrosine (μmol/L)
Fasting                    | 33 ± 3           | 35 ± 1           | 32 ± 2           | 36 ± 1       |
Maximum⁴                   | 25 ± 2           | 91 ± 4           | 146 ± 11         | 211 ± 14     |
After breakfast            | 48 ± 3           | 101 ± 6          | 144 ± 16⁵        | 170 ± 10     |
Maximum increment above baseline | 49 ± 6           | 200 ± 14         | 444 ± 60         | 516 ± 30⁶,⁷  |

¹ ± SEM.
² Percentage of individually tailored daily protein allowance in breakfast and lunch, respectively.
³ Tolerance was intended to keep phenylalanine concentrations below 600 μmol/L at 5 y of age and at the day of the test according to the guidelines of Güttler and Lou (21).
⁴ “Maximum” refers to the maximum concentration after breakfast or lunch. In the 0%/30% test, “maximum” refers to the concentration just before lunch.
⁵ Different from 25%/30% test, P < 0.25.
⁶ Significantly different from 25%/30% test, P < 0.005.
⁷ Different from 50%/10% test, P < 0.1.
intake were distributed evenly, the plasma tyrosine concentration varied widely. The low postprandial plasma tyrosine concentrations may simply have been caused by the fact that the tyrosine intake was too small for the patient on that day. The large postprandial increase in tyrosine concentration may have been due to a faster intestinal absorption of free amino acids in comparison with whole protein and/or the tyrosine enrichment of the amino acid mixtures. The studies by Gropper et al (28, 29) showed no significant differences in plasma tyrosine and total amino acid responses when the ingestion of free amino acids, whole protein, and a combination of both were compared in healthy adults (28, 29). These findings suggest that the large postprandial increase in tyrosine concentrations is not due to a faster intestinal absorption of free amino acids in comparison with whole protein. The tyrosine content of the amino acid mixtures used exceeded the usual 3–4% tyrosine content of most food products, to compensate for insufficient conversion of phenylalanine into tyrosine (30). Consequently, the tyrosine-enriched amino acid mixtures used resulted in unphysiologically high doses of tyrosine in a single meal, which may result in transient hypertyrosinemia as has been shown for other amino acids (31). Therefore, we consider this factor to be the primary reason for the large postprandial rise of tyrosine. Because of the small number of patients using either PKU1, PKU2, or PKU3 (Milupa), however, this hypothesis could not be tested by comparing the tyrosine responses to different amino acid mixtures.

The clinical significance of the inappropriate plasma tyrosine concentrations and the large daily fluctuations of plasma tyrosine in PKU patients, as found in our study, is difficult to determine. On the one hand, both low and high tyrosine concentrations have been considered a cause of impairment of brain function (4, 5, 10, 18, 19, 32–35). In addition, low tyrosine concentrations may be hypothesized to cause growth retardation as described by different authors (36–38), and concomitant high phenylalanine and tyrosine concentrations have been suggested to cause fetal damage in both experimental animal and human maternal PKU studies (39, 40). On the other hand, our group of patients had a wide range of postprandial plasma tyrosine concentrations without manifesting growth retardation or mental impairment. Therefore, the clinical significance of inappropriate tyrosine concentrations in PKU patients remains a matter of debate, whereas the unsteadiness of the individual plasma tyrosine concentrations hampers further investigations of the possible role of inappropriate tyrosine concentrations and the large daily fluctuations in these patients.

Tyrosine supplementation in addition to the tyrosine-enriched amino acid mixtures increases the plasma tyrosine concentration in PKU patients, although not in all (10, 13). The present study shows that the usual dietary treatment in PKU patients may already result in very high plasma tyrosine values. Because of the large number of patients with high plasma tyrosine concentrations, additional tyrosine supplementation seems controversial and should not be applied without strict control. The inappropriately low and high tyrosine concentrations in the same individual on the same day, and the finding that an extra amount of the amino acid mixture late in the evening resulted in higher overnight fasting plasma tyrosine concentrations (6), suggest that an alternative distribution of the daily individual tyrosine intake may be more effective in preventing inappropriate plasma tyrosine concentrations than would additional tyrosine supplementation.

We thank J Fernandes and WG Zijlstra for their critical review of the manuscript, and J van der Molen, A Gerding, and T Dijkstra for technical and dietary assistance.

REFERENCES


