

Phenylketonuria: tyrosine supplementation in phenylalanine-restricted diets^{1,2}

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ABSTRACT Treatment of phenylketonuria (PKU) consists of restriction of natural protein and provision of a protein substitute that lacks phenylalanine but is enriched in tyrosine. Large and unexplained differences exist, however, in the tyrosine enrichment of the protein substitutes. Furthermore, some investigators advise providing extra free tyrosine in addition to the tyrosine-enriched protein substitute, especially in the treatment of maternal PKU. In this article, we discuss tyrosine concentrations in blood during low-phenylalanine, tyrosine-enriched diets and the implications of these blood tyrosine concentrations for supplementation with tyrosine. We conclude that the present method of tyrosine supplementation during the day is far from optimal because it does not prevent low blood tyrosine concentrations, especially after an overnight fast, and may result in largely increased blood tyrosine concentrations during the rest of the day. Both high tyrosine enrichment of protein substitutes and extra free tyrosine supplementation may not be as safe as considered at present, especially to the fetus of a woman with PKU. The development of dietary compounds that release tyrosine more slowly could be beneficial. We advocate decreasing the tyrosine content of protein substitutes to $\approx 6\%$ by wt (6 g/100 g protein equivalent) at most and not giving extra free tyrosine without knowing the diurnal variations in the blood tyrosine concentration and having biochemical evidence of a tyrosine deficiency. We further advocate that a better daily distribution of the protein substitute be achieved by improving the palatability of these products. *Am J Clin Nutr* 2001;73:153–7.

KEY WORDS Phenylketonuria, PKU, tyrosine, protein substitute, amino acid supplementation, tyrosine supplementation, maternal PKU

INTRODUCTION

Normally, tyrosine is a nonessential amino acid synthesized from phenylalanine. Tyrosine is incorporated into all proteins and is a precursor of thyroxine, melanin, and the neurotransmitters dopamine and norepinephrine. Persons with phenylketonuria (PKU) cannot synthesize tyrosine from phenylalanine because of a severe deficiency of the hepatic enzyme phenylalanine hydroxylase (phenylalanine 4-monooxygenase). Therefore, in these persons, tyrosine is an essential amino acid. Left untreated, PKU results in low to normal tyrosine concentrations in blood (1). The mainstay of PKU treatment is a low-phenylalanine diet. This is achieved by

restriction of natural protein to an amount that meets the phenylalanine need for protein synthesis and supplementation with a protein substitute to meet the total protein need. The protein substitute consists of an amino acid mixture that lacks phenylalanine or a protein hydrolysate that contains very little phenylalanine.

From the start of PKU treatment in 1954, tyrosine was added to the protein substitutes to a content equal to that found in human milk (2). Tyrosine enrichment of the protein substitutes to compensate for the phenylalanine normally converted into tyrosine was reported as early as 1961 (3). At present, the tyrosine enrichment of the different protein substitutes varies greatly, resulting in a tyrosine content of 4.6–14.7% by wt (4.6–14.7 g/100 g protein equivalent; **Table 1**).

Recently at our center, a pregnant woman with PKU had plasma tyrosine concentrations that were clearly increased on various occasions, sometimes even $>200 \mu\text{mol/L}$, even though she consumed her total daily amount of protein substitute in 3–4 equal parts. Note that reported reference values of tyrosine are always between 35 and $102 \mu\text{mol/L}$ (7–10). This finding urged us to address the following question: is such a large tyrosine enrichment in the protein substitute for the treatment of PKU and maternal PKU really needed?

Especially in the treatment of maternal PKU, some authors advise extra free tyrosine in addition to the use of tyrosine-enriched protein substitutes (5, 11–18). Again, we question whether this is needed, and even safe, especially for fetuses of women with PKU.

In this article, we address these questions about tyrosine supplementation in PKU and maternal PKU. We first discuss tyrosine metabolism in healthy individuals. Second, we address blood tyrosine concentrations in PKU patients during low-phenylalanine, tyrosine-enriched diets. Third, we discuss the implications of these tyrosine concentrations in blood for possible tyrosine deficiency and toxicity, and, as a consequence, for tyrosine supplementation in (maternal) PKU. We conclude with theoretical and practical considerations for achieving better tyrosine supplementation.

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TABLE 1

Tyrosine content of protein substitutes commonly used in the treatment of phenylketonuria

Company, reference, and protein substitute	Tyrosine content
	% by wt ¹
Mead Johnson, Evansville, IN (4)	
Lofenolac	5.4
Phenylfree	4.6
Milupa, Friedrichsdorf, Germany (5)	
PKU-1	6.8
PKU-2	7.4
PKU-3	14.7
PKU-1-mix	9.1
Nutricia, Zoetermeer, Netherlands (5)	
Phenylidon AM	12.5
Phenylidon-1 mix	12.3
Phenylidon-2 mix	12.3
Phenylidon-3 mix	12.3
Phenylidon-Formula	12.6
Ross Laboratories, Columbus, OH (6)	
Phenex-1	10
Phenex-2	10
Scientific Hospital Supplies (SHS), Liverpool, United Kingdom (5)	
PKU-Aid XP	11.7
Albumaid XP	12.6
Maxamaid XP	10.8
Maxamum XP	10.8
Flexy-10	11.6
Analog	11.0

¹g/100 g protein equivalent.

NORMAL TYROSINE METABOLISM

In vivo metabolic studies that focused on tyrosine metabolism in healthy humans are scarce (19–21) and data on recommended intakes and deficiency in humans concern the combination of phenylalanine and tyrosine (22–24). Gleaser et al (19) showed that plasma tyrosine concentrations increased to a mean of 203 $\mu\text{mol/L}$ in response to large doses (up to ≈ 10 g) of extra tyrosine in healthy individuals. The results of a pilot study with [¹³C]tyrosine conducted by Cortiella et al (20) showed that tyrosine oxidation doubles the rates of phenylalanine oxidation and hydroxylation, but no data were presented on the relation between tyrosine oxidation and the subjects' nutritional condition (fasting or nonfasting). The studies of Basile-Filho et al (24) made clear that both nutritional condition and phenylalanine intake (generous, intermediate, or low) influence the rates of phenylalanine hydroxylation and tyrosine oxidation as well as plasma phenylalanine and tyrosine concentrations. Basile-Filho et al's studies were hampered, however, by the fact that all individuals had low tyrosine intakes. Thus, it is still unclear what the metabolic system does with tyrosine from dietary sources. The results of in vitro studies in rat liver and in vivo studies in healthy adult humans suggest that the metabolic fates of tyrosine from phenylalanine and dietary sources might be different (21, 25).

Reference values of tyrosine concentrations in plasma vary between 35 and 102 $\mu\text{mol/L}$ for different age groups and sexes when measured after an overnight fast (7). In the nonfasting condition in healthy young adults, plasma tyrosine concentrations vary between 61 and 99 $\mu\text{mol/L}$ (8–10). Studies of diurnal variations in amino acid concentrations in healthy adults with normal intakes of dietary protein showed that plasma tyrosine concen-

trations decrease during the evening and early nighttime. Concentrations are lowest between 0200 and 0400 and highest in the nonfasting state (26, 27), but this pattern changes in cases of deficient phenylalanine and tyrosine intakes.

Obligate heterozygotes for phenylalanine hydroxylase deficiency (eg, parents of children with PKU) have normal plasma tyrosine concentrations both after an overnight fast and postprandially (28), suggesting that these individuals do not carry a risk of tyrosine deficiency. The phenylalanine hydroxylating system is active from early in fetal life (29, 30), although the importance of this system for the fetus may be questioned (31).

TYROSINE CONCENTRATIONS IN TREATED PKU PATIENTS

Studies of treated PKU patients conducted by Güttler et al (F Güttler, ES Olesen, E Wamberg, unpublished observations, 1968) and Brouwer et al (32) showed that overnight, fasting serum tyrosine concentrations can be clearly below reference values. Koepf and Held (33) showed that serum tyrosine concentrations in treated PKU patients are normal at noon compared with those in healthy persons. More recent studies in treated PKU patients underlined this decrease in plasma tyrosine concentrations after an overnight fast (34–37). It was further shown that plasma tyrosine concentrations decrease even more when breakfast is skipped (36). This may be caused by the lack of tyrosine intake over the previous hours combined with the decreased hydroxylation rate of phenylalanine into tyrosine in PKU patients (38).

Two studies reported individual daily fluctuations in plasma tyrosine concentrations in patients in whom the individually tailored intake of natural protein and tyrosine-enriched protein substitute was distributed either equally or unequally throughout the day (36, 37). A third study presented data on plasma tyrosine concentrations after extra free tyrosine was given in addition to the tyrosine-enriched protein substitute (14). The first study showed that an equal distribution of the protein substitute resulted in both intraindividual and interindividual variations in tyrosine concentration (36). Tyrosine values remained low in 3 of the 9 patients studied, became normal in 2 of 9, and were clearly elevated in 4 of 9. Additionally, postprandial plasma tyrosine concentrations were always higher than preprandial concentrations (36). The second study showed that unequal distributions of the total daily natural protein intake and protein substitute ($n = 12$) resulted in even larger intraindividual fluctuations in tyrosine concentrations (37). In that study, postprandial plasma tyrosine concentrations above the reference range were observed in >80% of the patients, the highest being 340 $\mu\text{mol/L}$. The third study showed that giving extra tyrosine to 5 women with PKU in addition to an amino mixture with either a low or a much larger tyrosine content resulted in plasma tyrosine concentrations up to 200 $\mu\text{mol/L}$ (14). In summary, this implies that not only very low but also high to very high plasma tyrosine concentrations can occur frequently in PKU patients.

IMPLICATIONS OF TYROSINE CONCENTRATIONS FOR DEFICIENCY, TOXICITY, AND TYROSINE SUPPLEMENTATION

In a discussion of tyrosine supplementation, knowledge of the problems that may be related to both tyrosine deficiency and tyrosine toxicity is needed. In both deficiency and toxicity, a

distinction should be made between the consequences for a patient with PKU and those for a fetus carried by a woman with PKU.

With regard to possible tyrosine deficiency, a low tyrosine intake is suggested to result in impaired growth in patients with PKU (39). Bessman (40) proposed that an absolute tyrosine deficiency causes mental retardation, but this hypothesis has never been proven. At present, in addition to the classic model of phenylalanine intoxication, 2 hypotheses to explain the mental retardation that occurs in untreated PKU are under investigation. These hypotheses are based on a dopamine deficiency in the brain due to either a decreased availability of tyrosine or a decreased flux through tyrosine hydroxylase in the brain (41–43). According to these hypotheses, raising tyrosine concentrations in blood may increase the amount of tyrosine and decrease the amount of phenylalanine transported across the blood-brain barrier. This would increase both the tyrosine concentration and the flux through tyrosine hydroxylase in the brain and, as a consequence, the synthesis of dopamine (41–44). So far, treatment with tyrosine alone, however, has not shown clear positive results.

Apart from the question of whether tyrosine deficiency causes problems, it must be questioned whether there is biochemical evidence of tyrosine deficiency in treated PKU patients. To answer this question, we must take into account studies of plasma concentrations of essential amino acids in healthy individuals. Such studies showed that a deficient intake of an essential amino acid does not result in a decrease in the overnight fasting concentration but results in a lower postprandial concentration of that specific amino acid compared with the overnight fasting concentration (45, 46). These studies also showed that in the case of sufficient intake, the postprandial concentration is higher than the overnight fasting concentration. Therefore, although the clinical importance of a possible tyrosine deficiency has yet to be proven, the results in PKU patients discussed above do not support the notion of a clear biochemical tyrosine deficiency in these patients.

No detrimental effects of transiently elevated blood tyrosine concentrations in PKU patients have been documented. Hypertyrosinemia is observed particularly in tyrosinemia type II and type III, and in type I when treated with 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione (NTBC). Treatment of tyrosinemia type I with NTBC results in consistently elevated plasma tyrosine concentrations. An international survey of NTBC treatment of tyrosinemia type I reported eye problems but no mental retardation and no relation between the eye problems and the plasma tyrosine concentration (47). In tyrosinemia type II, which causes mental retardation, corneal lesions, and skin disease, it is recommended that plasma tyrosine concentrations be kept $<600 \mu\text{mol/L}$ (48). In tyrosinemia type III, which has been associated with ataxia, convulsions, and cerebral atrophy, there is no clear relation with the clearly increased plasma tyrosine concentration (49). With this lack of a clear toxic effect of high plasma tyrosine concentrations in PKU patients, and the hypotheses concerning dopamine deficiency in the brain, it could be hypothesized that increased plasma tyrosine concentrations may be beneficial to the brain of PKU patients. If one of these hypotheses on dopamine deficiency in the brain is accepted, normalizing the ratio between phenylalanine and tyrosine to ≈ 1 (and between phenylalanine and other amino acids) becomes an additional aim of treatment.

With regard to the fetus, Bessman (40) suggested many years ago that low fetal tyrosine concentrations in blood cause the mental retardation observed in both PKU fetuses and fetuses of moth-

ers with PKU. However, this hypothesis has not been proven in any trial with a treatment based on tyrosine supplementation without phenylalanine restriction. Because of the expected risk of fetal tyrosine deficiency, tyrosine supplementation is often advised in maternal PKU (5, 11–18), but application varies largely. As the result of several factors, fetal plasma tyrosine concentrations in blood may become lower than normal. The first factor is an already low maternal tyrosine concentration. Second, because of the active transport of tyrosine by the placenta, fetal tyrosine concentrations in blood will be some 1.8 to 3.3 times higher than in the maternal blood (31). This active transport will increase the difference between the fetal blood tyrosine concentration of a healthy mother and that of a mother with PKU. Third, because of the competitive inhibition of the placental transport of tyrosine by high maternal plasma phenylalanine concentrations (50), the fetal concentration may become even lower.

In a discussion of the occurrence and possible effect of greatly increased plasma tyrosine concentrations, we should take into account that the present practice of tyrosine supplementation results in increased maternal plasma tyrosine concentrations. Combined with the active placental transport of tyrosine, without evident adaptation to abnormal maternal concentrations as shown for phenylalanine (11, 31), this may result in fetal plasma tyrosine concentrations clearly $>600 \mu\text{mol/L}$. So far, no studies have investigated a toxic effect of tyrosine to the human fetus carried by a mother with PKU. However, a toxic effect of a combination of mildly increased phenylalanine and tyrosine was shown experimentally in rats (51). Therefore, the data on tyrosinemia type II patients and the data on the placental transport of tyrosine suggest that tyrosine concentrations in fetal blood should be kept $\leq 600 \mu\text{mol/L}$. To achieve this, maternal plasma concentrations should be kept $\leq 200 \mu\text{mol/L}$, or even $<150 \mu\text{mol/L}$ as advised for the treatment of maternal tyrosinemia type II (52).

Apart from the fact that fetal blood tyrosine concentrations may become too high, the present mode of tyrosine supplementation results in large diurnal variations and clearly unphysiologic values of the tyrosine-phenylalanine ratio. Plasma tyrosine concentrations are low during the night (when plasma phenylalanine concentrations are the highest) and show large fluctuations during the day (when plasma phenylalanine concentrations decrease, sometimes even dropping below normal plasma concentrations) (53). Consequently, especially in the treatment of maternal PKU, we should not only keep plasma phenylalanine concentrations as normal as possible and prevent low plasma tyrosine concentrations, but also prevent maternal plasma tyrosine concentrations $>200 \mu\text{mol/L}$ and aim at more or less normal phenylalanine-tyrosine ratios. Therefore, it must be questioned whether the present practice of advising large amounts of tyrosine within the tyrosine-enriched protein substitute or as free tyrosine is safe, especially to pregnant women with PKU. Various approaches for improving tyrosine supplementation are discussed in the next section.

IS THERE AN OPTIMAL MODE OF TYROSINE SUPPLEMENTATION? THEORETICAL AND PRACTICAL CONSIDERATIONS

Natural proteins contain $\approx 4\%$ tyrosine and $4.5\text{--}5\%$ phenylalanine, of which $67\text{--}90\%$ is converted into tyrosine in healthy individuals according to the results of *in vitro* liver studies (11). According to these calculations, protein substitutes should con-




tain some 7–8.5% tyrosine by wt (ie, 7–8.5 g tyrosine/100 g protein equivalent) if we aim at tyrosine enrichment, which covers the lack of tyrosine, the reduced amount of natural protein, and the lack of conversion of phenylalanine into tyrosine in persons with PKU. More recent *in vivo* studies of whole-body phenylalanine-tyrosine fluxes, however, showed that only 27–41% of dietary phenylalanine is converted into tyrosine during the first 5–8 h after a generous dietary phenylalanine intake (24, 54). This suggests that a tyrosine content in the protein substitute of $\approx 6\%$ by wt at most may be more adequate. Theoretically, it is possible that larger amounts of phenylalanine are converted into tyrosine over a longer period, suggesting a greater need for tyrosine, but the results of a recent study by Bross et al (55) suggest that the need for tyrosine in PKU patients is $<6\%$ of the total protein intake. One of the reasons for a lower need for tyrosine may be that tyrosine is not only used to synthesize melanin, neuroactive compounds, and proteins including thyroxine, but also oxidized. Another source for establishing the tyrosine content of protein substitutes might be the recommended dietary allowances as published by the National Research Council (22). These, however, concern the combined intake of phenylalanine and tyrosine and therefore are of no help in establishing the optimum tyrosine content of the protein substitutes used in PKU.

In addition to these considerations, Herrmann et al (56) showed that too much of a protein substitute at one time results in an ineffective use of the amino acids. Thus, provision of both a tyrosine-enriched protein substitute and extra free tyrosine may not be optimal because it results in very high plasma tyrosine concentrations and increased oxidation rates without preventing low overnight fasting plasma tyrosine concentrations. Therefore, the ideal tyrosine supplement should be based on 2 kinds of tyrosine: the amount of tyrosine normally ingested as natural protein and a slow-release tyrosine compound. This slow-release compound should cover the amount of tyrosine normally synthesized from dietary phenylalanine and should resemble the natural conversion of phenylalanine into tyrosine, especially during the night.

Future studies in patients with PKU should focus on various aspects of tyrosine metabolism, including the effects of different means of supplying the protein substitute on the plasma concentrations of tyrosine and other amino acids and the rates of protein synthesis and oxidation during various nutritional conditions. Other methods of supplementation should be considered, including distribution of the total daily amount of protein substitute consumed over >4 times/d. Such a regular intake of the protein substitute necessitates good compliance, however, which may not be achieved in PKU patients (57) and may be even lower in maternal PKU because of emesis gravidarum. One of the major causes of noncompliance by patients with PKU is the poor palatability of the protein substitutes, which is caused by a combination of various compounds, including tyrosine (58). This may be another reason to decrease the tyrosine content of the protein substitutes to $\approx 6\%$ by wt at most.

In conclusion, the present mode of tyrosine supplementation in persons with PKU is far from optimal. The tyrosine enrichment of the protein substitutes is often greater than is necessary. Both the large tyrosine enrichment and the extra free tyrosine may not be as safe as presently believed, especially to the fetus of a woman with PKU, because these do not prevent low plasma tyrosine concentrations and result in largely increased plasma tyrosine concentrations. Therefore, it is worth trying to decrease

the tyrosine content of the protein substitutes to ≈ 6 by wt (6 g/100 g protein equivalent) at most, part of the tyrosine being a compound from which tyrosine is released slowly. We do not advocate use of extra free tyrosine without knowledge of the diurnal variations in plasma tyrosine concentrations and biochemical evidence of a tyrosine deficiency. Improving the palatability of the protein substitutes may be necessary to achieve a better daily distribution of these products. 

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