

Nutritional factors affecting serum phenylalanine concentration during pregnancy for identical twin mothers with phenylketonuria

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Abstract:

The effect of energy, protein, fat, and phenylalanine on serum phenylalanine concentrations during pregnancy for a set of identical twins with phenylketonuria (PKU) was examined. Blood samples were collected one to two times per week. The subjects completed a 3-d food record prior to each blood collection. The effect of the factors on serum phenylalanine levels was evaluated statistically using time-series analysis. Dietary intakes of the nutrients evaluated were similar for the subjects. For one subject, there were highly significant effects of energy, protein, and fat on serum phenylalanine levels. In contrast, these nutrients had no significant effect on serum phenylalanine for the other subject. Dietary phenylalanine had no significant effect on serum phenylalanine for either twin.

Conclusions: There was no effect of phenylalanine intake and no consistent effect of energy, protein, or fat on serum phenylalanine. Other dietary or environmental factors or a combination of factors may impact serum phenylalanine levels of pregnant women with PKU.

Keywords: Genetics | maternal PKU | nutrition | phenylalanine

Article:

Maternal phenylketonuria (PKU) refers to fetal damage from PKU in the pregnant woman. If untreated, women with PKU are at risk for giving birth to infants who are mentally retarded, microcephalic, of low birthweight, and have anomalies such as heart disease (1–3). It is recommended that serum phenylalanine levels be maintained between 120 mmol/L and 360 mmol/L at conception and throughout pregnancy (4). The purpose of this study was to examine the impact of energy, protein, fat, and dietary phenylalanine intake on serum phenylalanine level during pregnancy for identical twin mothers with PKU. Both subjects were enrolled in the

National Collaborative Maternal PKU Study. Additional samples were collected to provide a more extensive profile of results for our subjects.

Patients and methods

NC and CC were diagnosed with PKU at birth. Mutation analysis revealed the twins have the genetic compound status for two phenylalanine hydroxylase mutations, IVS-12nt1/R408W, both associated with classical PKU (5). Serum phenylalanine levels for these twins have been high and difficult to control throughout childhood. Despite low average cognitive scores (NC-80, CC-74), the twins functioned well at home and in the community, planned their pregnancies together, and provided each other with emotional support to maintain the phenylalanine-restricted diet. These twins interacted several times a day, even during meals, and though one was of lower intellect, they functioned similarly.

Height and pre-pregnancy weight were as follows: 170 cm, 56.2 kg for NC and 170 cm, 57.7 kg for CC. NC and CC had been off diet for several years prior to pregnancy. The subjects were instructed to resume the phenylalanine-restricted diet prior to conception and to obtain a serum phenylalanine level below 360 mmol/L on three consecutive samples before attempting pregnancy. Dietary treatment consisted of a phenylalaninefree medical formula, Phenex II (Ross Products Division, Abbott Laboratories, Columbus, OH), low protein foods (no more than 200 mg phenylalanine per day), and fats and sugars for additional energy.

Blood samples were collected one to two times per week throughout pregnancy. Serum was analyzed for total phenylalanine concentration by the standard fluorimetric method using ninhydrin (6). Subjects recorded all food (including free foods) and fluids consumed for three consecutive days prior to each blood collection. Food records were analyzed for dietary phenylalanine, energy, protein, and fat using the Amino Acid Analyzer Software (Version 2.3, Ross Products Division, Abbott Laboratories, Columbus, OH). Nutrition counseling was provided when serum phenylalanine levels were elevated or when weight gain did not follow recommendations (7).

The effect of energy, protein, fat, and phenylalanine on serum phenylalanine during pregnancy was evaluated statistically using time-series analysis (8) (SPSS statistical software version 7.5). Analyses were completed separately for each subject and each nutrient. Data that are collected repeatedly over time from the same subject are usually correlated and thus violate the assumption of independence that underlies general linear model statistical techniques such as regression and analysis of variance. Time-series analysis is a statistical technique appropriate for analyzing data that are serially correlated. The analysis provides an estimate and a statistical test of the relation between the dependent variable and the independent variable that is similar in interpretation to the estimates obtained in multiple regression. However, it is a more accurate test because the correlation is taken into consideration and does not inflate the value of the test statistic.

To determine more specifically the nature of the time-series process, graphs of the autocorrelations between the data points are examined. Specific patterns in the graphs are indicative both of the type of process (autoregressive or moving average) and of the order of the process (whether the data points are correlated at time lag 1, time lag 2, etc.). We determined that the process was an autoregressive process in which a data point was related only to the immediately preceding data point (order 1), commonly referred to as a first-order autoregressive process or AR (1). A test of the autoregressive parameter indicated that the autocorrelation was statistically significant at the 0.05 level (another indication that the autoregressive model was appropriate).

Having appropriately modeled the correlation in the data, we next estimated the effect of individual nutrients on serum phenylalanine. An effect was considered to be statistically significant if the probability value was less than 0.05. The B0 represents the mean level serum phenylalanine when the nutrient has the value of zero; it is the intercept term in a regression analysis. The B1 is the regression coefficient for the nutrient, representing the amount by which the serum phenylalanine level changes for each one unit change in the nutrient level.

Results

Total pregnancy weight gain was 16.9 kg for NC and 15.2 kg for CC. VC and AC, female offspring, were delivered by normal spontaneous vaginal delivery. Genetic testing revealed VC had R408W and AC had IVS-12ntl phenylalanine hydroxylase mutations (5) but serum phenylalanine levels were normal (9). Results of the effect of dietary factors on serum phenylalanine levels are presented in Table 1 and Fig. 1. Serum phenylalanine fluctuated greatly, with serum phenylalanine levels for NC averaging 411 \pm 278 mmol/L and for CC averaging 744 \pm 387 mmol/L during pregnancy.

For NC, there were highly significant effects of energy, protein, and fat on serum phenylalanine levels, whereas for CC no significant effect was observed (Table 1). Dietary phenylalanine intake had no significant effect on serum phenylalanine for either subject. Intakes of energy, protein, and fat were similar, yet NC had serum phenylalanine levels that were one-half those of CC, suggesting NC had greater phenylalanine tolerance. As shown in Fig. 1, both subjects tended to have lower serum phenylalanine levels after about weeks 19–20 of pregnancy, although their concurrent phenylalanine intake was not markedly different than during earlier pregnancy. However, the presence of greater phenylalanine tolerance in later pregnancy could not be statistically evaluated due to the small number of data points.

Table 1. Contributions to serum phenylalanine (Phe) during pregnancy.

	Intake ¹	B ₀ ²	B ₁ ³	<i>p</i> value
NC				
Phe (mg)	254 ± 64	8.9	-0.01	NS
Energy (kcal)	1922 ± 409	19.9	-0.01	≤0.0001
Protein (gm)	91.7 ± 24	16.7	-0.11	≤0.0001
Fat (gm)	71.4 ± 16	18.6	-0.17	≤0.0001
CC				
Phe (mg)	202 ± 44	7.1	0.03	NS
Energy (kcal)	2163 ± 327	24.3	-0.01	NS
Protein (gm)	98.6 ± 12	15.3	-0.03	NS
Fat (gm)	76.5 ± 13	14.6	-0.03	NS

¹ Mean SD for entire pregnancy: *n* = 43 for NC and *n* = 47 for CC.

² B₀ is the theoretical serum Phe level without that nutrient contribution.

³ B₁ is the amount by which the theoretical serum Phe level would change due to that nutrient.

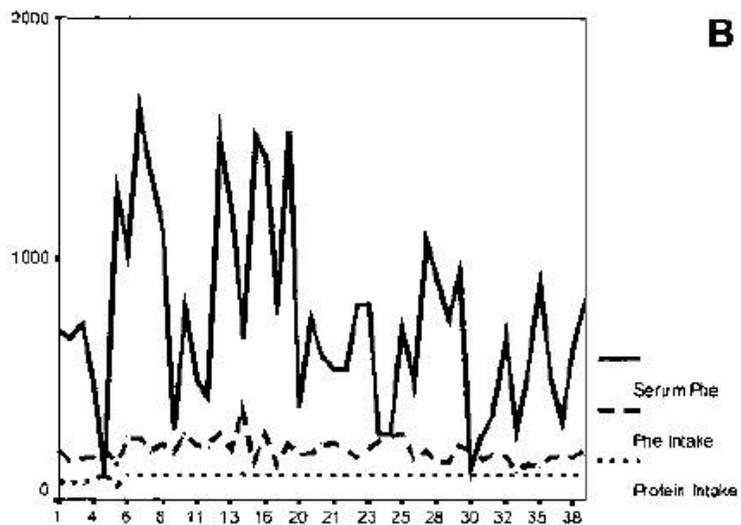
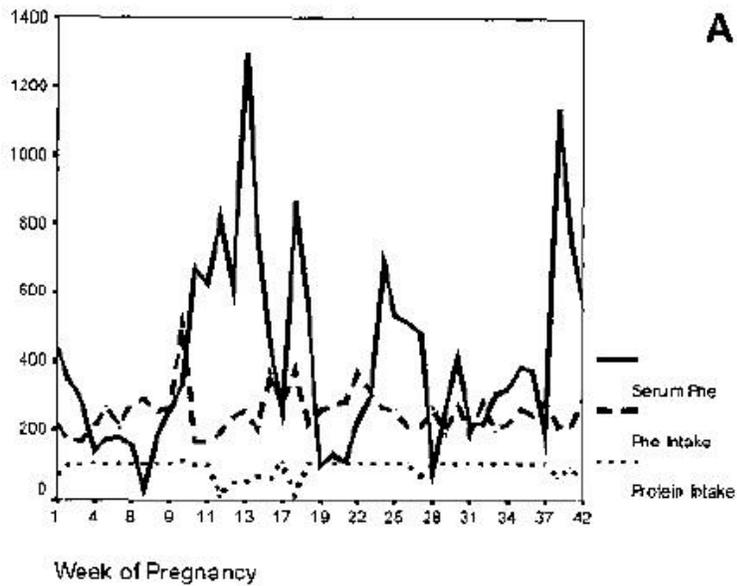


Fig. 1. Serum phenylalanine (Serum Phe, mmol/L), phenylalanine intake (Phe intake, gm/d), and protein intake (gm/d) throughout pregnancy for NC (A) and CC (B). Phenylalanine and protein intakes are the average of 3 d. Serum phenylalanine was determined on the third day of food intake records

Discussion

A simple explanation for the differing statistical results between the two subjects could be that the food reports for NC may be better reflections of her intake than for CC. However, our results provide evidence to suggest that other factors may be involved. The reasons are as follows: NC gained 16.9 kg during pregnancy while CC gained 15.2 kg. CC's child (VC) weighed 427 g less than NC's child, had a 1 cm lower head circumference, and 1 cm greater body length. The relatively small difference in pregnancy weight gain between the twins (1.7 kg) does not explain the greater difference in infant outcome, particularly because both subjects gained adequate overall weight during pregnancy. CC's poorer serum phenylalanine control during pregnancy may in part explain her child's lower birthweight and head circumference. Because the subjects were so close emotionally and interacted so frequently (many times in a day including at meals), similarly accurate food records for the two subjects are likely. These subjects were also very consistent in providing detailed food records throughout pregnancy.

The effect of individual nutrients and other factors on serum phenylalanine levels has been previously reported, although the literature citations are few. Dietary phenylalanine intake had no effect on serum phenylalanine level during pregnancy for either subject, which is consistent with a prior report of pregnant women with PKU (10). Consistent with our observation for NC, several investigators (10–12) reported a negative correlation between protein intake and plasma phenylalanine level in pregnant women with PKU. MacDonald and co-workers (13) reported that timing of medical formula consumption had a greater impact on serum phenylalanine than did dietary phenylalanine or energy intake. In our study, both subjects consumed equal volumes of their medical formula with each meal.

Matalon et al. (14) reported maternal blood phenylalanine, weight gain, and energy intake had significant interactive effects on birth measurements of offspring, suggesting several factors probably interact to impact serum phenylalanine levels. The total number of data points for our study was greater than 40 for both subjects, and is considered adequate for time-series analysis for individual nutritional factors. However, an analysis of multiple nutrients on the serum phenylalanine level would have required a larger number of data points, thus could not be completed. We were also unable to analyze the effects of nutrients during early pregnancy versus late pregnancy or among trimesters due to insufficient data points.

In conclusion, there was no effect of dietary phenylalanine intake and no consistent effect of energy, protein, and fat intake on serum phenylalanine for our subjects during pregnancy. Since our subjects were identical twins, one would assume that their serum phenylalanine

concentrations would respond similarly to a given set of circumstances such as similar intakes of energy, protein and fat and the even distribution of formula throughout the day. However, NC had serum phenylalanine levels that were about one-half that of CC. Although there is the possibility that NC's food records were more accurate than that of CC's, our results suggest that there may be other factors or interactions among factors that impact serum phenylalanine levels of pregnant women with PKU.

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References

1. Rouse B, Lockhart L, Matalon R, Azen C, Koch R, Hanley W, et al. Maternal phenylketonuria pregnancy outcome: a preliminary report of facial dysmorphism and major malformations. *J Inher Metab Dis* 1990; 13: 289–91
2. Smith I, Glossop J, Beasley M. Fetal damage due to maternal phenylketonuria: effect of dietary treatment and maternal phenylalanine concentrations around the time of conception. *J Inher Metab Dis* 1990; 13: 651–7
3. Levy HL, Waisbren SE. Effects of untreated maternal phenylketonuria and hyperphenylalaninemia on the fetus. *N Engl J Med* 1983; 309: 1269–74
4. Kock R, Wenz E, Azen C, Friedman G, Levy H, Rohr F, et al. The effect of nutrient intake on pregnancy outcome in maternal phenylketonuria. *Ann NY Acad Sci* 1993; 687: 348–9
5. Guldberg P, Levy HL, Hanley WB, Koch R, Matalon R, Rouse BM, et al. Phenylalanine hydroxylase gene mutations in the United States: Report from the Maternal PKU collaborative study. *Am J Hum Genet* 1996; 59: 84–94
6. McCaman MW, Robins E. Fluorimetric method for the determination of phenylalanine in serum. *J Lab Clin Med* 1962; 59: 885–90
7. Acosta PB, Yannicelli S. Nutrition Support Protocol. Ross Products Division, Abbott Laboratories, Columbus, OH; 1993:37–55
8. McCleary R, Hay JR, editors. *Applied Time Series Analysis*. Beverly Hills: Sage Publishing Co.; 1980
9. Fox-Bacon C, McCamman S, Therou L, Moore W, Kipp DE. Maternal PKU and breastfeeding: case report of identical twin mothers. *Clin Pediatr* 1997; 36: 539–42
10. Acosta PB, Michals-Matalon K, Austin V, Castiglioni L, Rohr F, Wenz E, Azen C. Nutrition findings and requirements in pregnant women with phenylketonuria. In: Platt LD, Koch R, de la

Cruz, F, editors. Genetic disorders and pregnancy outcome. London: McParthenon Publishing Co; 1977: 21–31

11. Michals K, Acosta PB, Austin V, Castiglioni L, Rohr F, Wenz E. Nutrition and reproductive outcome in maternal phenylketonuria. *Eur J Pediatr* 1996; 155 Suppl 1: 165–8

12. Rohr F, Matalon K, Acosta P, Austia V, Castiglioni L, Funk-Wentzel P, et al. Protein intake and plasma phenylalanine concentrations in maternal phenylketonuria. *J Am Diet Assoc* 1997; 97: Suppl A25

13. MacDonald A, Rylance G, Hall SK, Asplin D, Booth IW. Factors affecting the variation in plasma phenylalanine in patients with phenylketonuria on diet. *Arch Dis Child* 1996; 74: 412–7

14. Matalon R, Michals K, Azea C, Friedman EG, Koch R, Wenz E, et al. Maternal PKU collaborative study: the effect of nutrient intake on pregnancy outcome. *J Inher Metab Dis* 1991; 14: 371–4