Comparison of phenylalanine tolerance in singleton and twin pregnancies in patients with phenylketonuria

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Abstract

Objectives: Empirical determination of phenylalanine (Phe) tolerance in patients with phenylketonuria (PKU) relies on frequent assessment of blood Phe concentrations in relation to Phe intake from detailed meal records. This study aimed to determine Phe tolerance in twin pregnancies.

Methods: The reviewed cases included three women with PKU who each had a singleton and twin pregnancy (i.e., they were pregnant twice). All patients were under regular supervision to maintain Phe concentrations in a steady state and determine safe Phe intake. Restriction of Phe in the patient's diet was determined depending on the amount of Phe intake, which allowed for stable blood Phe concentrations within the target range.

Results: In all three patients with PKU, the ratio of Phe tolerance during the course of the twin and singleton pregnancies was <1 for most of the pregnancy. The ratio of the increase in Phe tolerance between 29 and 34 weeks of gestation and that between 15 and 28 weeks of gestation was 0.66 and 1.17, 0.51 and 0.14, and 0.76 and 1.42 in the twin and singleton pairs of pregnancies, respectively.

Conclusions: Our study shows that Phe tolerance in a twin pregnancy is not greater than that in a singleton pregnancy.

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Introduction

Pregnant women with classic phenylketon-uria (PKU, OMIM 261600) need appropriate management to avoid the teratogenic properties of elevated phenylalanine (Phe) levels.[1] A low-Phe diet is challenging for female patients of childbearing age, and they need a lot of support and education on treatment of PKU in pregnancy. Phe tolerance (mg/day) is defined as the amount of Phe in the diet that maintains blood Phe levels within the acceptable range.[1,2] Clinical determination of Phe tolerance relies on frequent assessment of blood Phe levels in relation to Phe intake from detailed daily food intake records. For optimal pre-conception care, stable blood Phe control within the therapeutic range of 120 to 360 µmol/L should be maintained before women with PKU conceive.[1,2] Phe intake should provide the minimum requirement for this essential amino acid needed to maintain physiological protein turnover for the current body mass of the mother and fetus. Notably, the guidelines for predicting changes in Phe tolerance during the course of pregnancy are not clear.

Twin pregnancies are becoming increasingly common in Poland and other Western countries. The lower birth weight and gestational age of twins compared with singletons are major factors contributing to twins' relatively poorer early outcomes, as well as possible health complications in adult life.[3] Despite awareness of the possible coexistence of multiple gravidas and PKU in pregnant women, there have been few studies on twin pregnancies in women with PKU.[4,5] More precise data are required to document what may be considered as the "normal" range for Phe tolerance in twin and multiple pregnancies. Therefore, this study aimed to determine changes in Phe tolerance during the course of singleton and twin pregnancies in women with PKU.

Materials and methods

A retrospective review of singleton and twin pregnancies of patients with PKU was performed by examining dietary and medical records in the PKU Polyclinic in Warsaw. Inclusion criteria for the study were a medical diagnosis of classic PKU based on newborn screening, absence of comorbidities (e.g., hypertension, diabetes), and a full medical history of twin pregnancy on a low-Phe diet (see below) initiated before conception or during the first trimester. Gestational age is expressed as the number of weeks after the last menstrual period. Fasting blood Phe concentrations were measured from whole blood spots on filter paper cards by using tandem mass spectrometry (Sciex, Concord, Ontario, Canada). The blood target range for this amino acid was set at 120 to 360 µmol/L, according to national recommendations (Polish PKU Consensus, 2006). Phe concentrations, as well as written daily dietary records, were assessed at least twice weekly during pregnancy, with at least bimonthly clinic visits. Food records were analyzed to calculate the intake of Phe (mg/day). Body mass index (BMI, kg/m²) was defined as the individual's body weight divided by the square of their height.

We implemented a diet restricting natural protein and supplemented it with Phe-free amino acid substitutes (Milupa PKU3 Tempora/Advanta, XP Maxamum, and PKU Lophlex LQ/Nutricia North America, Gaithersburg, MD, USA; PKU Cooler/Vitaflo International, Alexandria, VA, USA; Phenyl-free 2HP/Mead-Johnson, Chicago, IL, USA).

For this study, the IRB issued a waiver because the study was retrospective, and only anonymized data were used. Some data regarding the patients in the current

study have been previously published in Polish.[6]

Results

Three women with PKU (1.Q383X/R408W, 2.EX3DEL/EX3DEL, 3.R281L/R408W) had one singleton and one (dizygotic) twin pregnancy each (i.e., they were pregnant twice) and fulfilled the inclusion criteria. All patients were Caucasians of Polish origin. Table 1 includes descriptive characteristics of the pregnancies, birth measurements, and Phe concentrations. The mean age at conception was 27 ± 3 years. Only half of the pregnancies had a low-Phe diet initiated pre-conceptionally. The course of the pregnancies was normal in all six and they lasted at least 35 weeks. Patient 3 had severe nausea in both pregnancies between weeks 6 and 10. Patient 1 suffered from acute urinary infection in week 26 of her singleton pregnancy and viral gastroenterocolitis in week 19 of her twin pregnancy. Neonates had a normal birth weight. Only one neonate from the singleton pregnancy of patient 2 in whom a low-Phe diet was initiated in the second trimester had a relatively small head circumference. One of the twins of patient 3 was affected by hypospadias. Generally, the frequency of fetal abnormality in dizygotic twins is comparable with that of singleton pregnancies, and hypospadias does not belong to the clinical picture of so-called maternal PKU syndrome.[1] None of the neonates had PKU or any degree of persistent hyperphenylalaninemia.

In five of the six pregnancies, there was high rate of Phe values above the recommended range during trimester 1 (Table 1). In all pregnancies, the mean Phe concentrations in the second and third trimesters were $<360 \,\mu mol/L$. In the second and third trimesters, the mean rate of undesirable low Phe concentrations ($<120 \,\mu mol/L$) was 44% and 17%

respectively. Phe concentrations were below the reference range for healthy pregnant women (9% of Phe values) only in the third trimester of the twin pregnancy in patient 1.[7]

All patients were under the regular supervision of the same metabolic dietitian (J.Ż.) to stabilize Phe levels and determine Phe tolerance. None of the pregnant patients was hospitalized in the first and second trimesters. General vitamins, including folic acid and mineral supplementation, were provided before and during pregnancy. A diet-restricting natural protein, which was supplemented with special low-protein foods and tyrosine-augmented Phe-free amino acid substitutes, was implemented. Synthetic protein was consumed at least three times daily because it is absorbed and oxidized more rapidly than intact protein sources. Fruit and vegetable consumption was restricted. The mean daily protein intake from amino acid supplement(s) and energy intake are shown in Table 2. High protein products of animal origin were introduced to the diet in singleton and twin pregnancies in weeks 13 and 12, 25 and 24, and 18 and 20 in patients 1, 2, and 3, respectively. When expanding the diet, the daily supply of Phe was calculated as 320 and 375 mg, 800 and 350 mg, and 540 and 420 mg, respectively. Whether energy requirements were met was assessed by monitoring weight gain.

Table 3 shows Phe tolerance in singleton and twin pregnancies. The highest mean Phe tolerance in singleton and twin pregnancies was 1245 ± 230 mg and 989 ± 294 mg, respectively. Figures 1–3 show Phe tolerance and energy intake plotted by gestational age in the three patients. In all three patients with PKU, the ratio of daily Phe tolerance during the course of twin to singleton pregnancies was <1 for most of pregnancy (Figure 4). In two mothers with PKU, who started on a low-Phe diet before conception or early

Table 1. Characteristics of pregnancies and offspring.

	Patient 1		Patient 2		Patient 3	
	Singleton pregnancy	Twin pregnancy	Singleton pregnancy	Twin pregnancy	Singleton pregnancy	Twin pregnancy
Patient's age at conception (years)	28	32	24	28	23	28
Pre-pregnancy weight (kg)	41	39	61	72	62	78
Weight gain during pregnancy (kg)	13.9	16.3	15.0	6.0	21.0	20.2
Pre-pregnancy BMI (kg/m²)	17.1	16.2	21.8	25.7	22.1	27.8
BMI in trimester 1 (kg/m²)	17.8	17.5	21.8	25.4	21.9	28.2
BMI in trimester 2 (kg/m²)	19.8	21	23.9	25.7	25.7	29.6
Pre-delivery BMI (kg/m²)	22.9	23.0	27.0	28.3	30.8	30.0
Gestational age when diet was initiated	0	0	16	1	0	4
Phe concentration, mean ± SD, µmol/L						
Trimester 1	233 ± 117	265 ± 184	No data	535 ± 87	266 ± 222	379 ± 105
Trimester 2	138 ± 58	118 ± 85	169 ± 202	211 ± 91	115 ± 42	159 ± 111
Trimester 3	240 ± 69	212 ± 126	254 ± 119	168 ± 93	213 ± 131	296 ± 129
% of Phe measurements >360 µmol/L						
Trimester 1	31	31	No data	100	15	50
Trimester 2	0	6	9	4	0	8
Trimester 3	0	17	11	43	17	33
% of Phe measurements <120 µmol/L						
Trimester 1	15	31	No data	0	54	0
Trimester 2	38	65	64	22	61	38
Trimester 3	6	33	38	35	30	0
% of Phe measurements <120 µmol/L and above the upper range for normal pregnant women[7]						
Trimester 1 (>68.4 µmol/L)	8	17	No data	0	39	0
Trimester 2 (>62.9 µmol/L)	22	46	46	22	56	31
Trimester 3 (>57 µmol/L)	6	28	0	30	33	0
% of Phe measurements below the lower range for normal pregnant women[7]						
Trimester 1 (<17.9 µmol/L)	0	0	No data	0	0	0
Trimester 2 (<22.5 µmol/L)	0	9	0	0	0	0
Trimester 3 (<22.6 µmol/L)	0	0	0	0	0	0
Gestational age at delivery (weeks)	38	35	40	38	40	37
Offspring's sex (F/M)	F	M/M	M	F/F	F	M/M
Birth weight (g)	3280	2290/1890	3600	3090/3030	3120	3580/2900
Neonatal length (cm)	55	46/46	50	53/54	55	57/53
Neonatal head circumference (cm)	33	30/30	31	33/34	35	37/34

BMI, body mass index; Phe, phenylalanine; SD, standard deviation; F, female; M, male.

post-conception, the ratio of the increase in Phe tolerance between 29 and 34 weeks of pregnancy to that in the second trimester was nearly two times higher in singleton pregnancies compared with twin pregnancies, despite the ratio of total birth weight of offspring between singleton and twin pregnancies ranging from 0.48 to 0.78

Table 2. Protein and energy intake.

Gestational age (weeks)	Patient 1 Singleton pregnancy	Patient 1 Twin pregnancy	Patient 2 Singleton pregnancy	Patient 2 Twin pregnancy	Patient 3 Singleton pregnancy	Patient 3 Twin pregnancy
Daily protein intake from a dietary formula* (g)						
8	49 XP Maxamum (Nutricia)	49 XP Maxamum (Nutricia)	no diet	74 PKU Cooler (Vitaflo)	60 PKU Lophlex LQ (Nutricia)	91 PKU Lophlex LQ (Nutricia)
14	59	49	78 XP Maxamum (Nutrcia)	88 Milupa PKU 3	70 LQ (Nutricia)**	99
20	59	58	98	101	80	102 Phenyl-free 2HP (Mead Johnson)
28	59	78	98	108 Tempora (Milupa)	90	102 Milupa PKU 3
31	68	78	98	108	100	102
34	78	88	98	115	100	109 Advanta (Milupa)
Pre-delivery week	78	88		115	100	109
Daily energy intake (kcal)						
8	1254–1361	1256–1687	No data	1565–2583	1500–1800	2000–2200
14	1638–1911	1725–2204	No data	2435–3017	1056–1178	2300–2500
20	1333–1970	1493–2118	2186–3121	1958–2441	1088–2253	2500–2800
28	1645–2238	1654–2428	2301–2501	1637–2563	1692–2250	2500–2800
31	1655–2313	1465–2499	2414–2690	1914–3339	2134–2456	2500–2800
34	1589–2489	1500–2300	2048–2614	2114–3076	2079–2282	2500–2800
Pre-delivery week	1738–2010	1500–2300	1819–2457	1996–2081	2432–2516	2500–2800

*Tyrosine-augmented phenylalanine-free amino acid substitutes.
**In the first weeks of pregnancy, treatment was based on Phenyl-free 2HP (Mead Johnson).

Table 3. Changes in phenylalanine tolerance.

	Patient 1		Patient 2		Patient 3	
	Singleton pregnancy	Twin pregnancy	Singleton pregnancy	Twin pregnancy	Singleton pregnancy	Twin pregnancy
Pre-conception Phe tolerance (mg)	230	250	no data	250	200	200
Increase in Phe tolerance during pregnancy (lowest to highest value) (mg)	1104	1050	984	467	1217	750
Mean phe tolerance per kg of the patient's body weight in early pregnancy* (mean ± SD, mg/kg)	10.9 ± 1.6	11.3 ± 0.7	4.1 ± 1.3	3.3 ± 0.5	5.7 ± 0.7	4.4 ± 1.0
Mean Phe tolerance per kg of the patient's body weight in late pregnancy** (mean ± SD, mg/kg)	26.3 ± 3.4	23.7 ± 0.7	13.2 ± 1.0	8.1 ± 0.7	13.3 ± 1.7	7.9 ± 0.6
Ratio of the increase in Phe tolerance to the patient's weight gain throughout pregnancy (mg/kg)	79	64	66	78	58	37
Ratio of the increase in Phe tolerance to the patient's weight gain in trimester 2 (mg/kg)	149	55	111	180	40	67
Ratio of the increase in Phe tolerance to the patient's weight gain in trimester 3 (mg/kg)	182	116	36	56	89	26
Ratio of the increase in Phe tolerance between 29–34 weeks' gestation to that between 15–28 weeks' gestation	1.17	0.66	0.14	0.51	1.42	0.76
Ratio of the increase in Phe tolerance in trimester 3 to the offspring's birth body weight (mg/g)	0.42	0.12	0.11	0.06	0.27	0.01

Phe, phenylalanine; SD, standard deviation.

*The mean value was from 13 to 19 weeks of pregnancy according to Ennis et al.[13] who proposed intervals called early and late pregnancy. We adopted these intervals because of late implementation of a low-Phe diet from 15 to 19 weeks in the singleton pregnancy of patient 2.

**The mean value was from 33 to 39 weeks of pregnancy according to Ennis et al.'s[13] pregnancy intervals. This range was chosen because of different gestational ages at delivery from 33 to 38 weeks and from 33 to 35 weeks in the singleton and twin pregnancies of patient 1, respectively, from 33 to 37 weeks in the twin pregnancy of patient 2, and from 33 to 38 weeks and from 33 to 34 weeks in the singleton and twin pregnancies of patient 3, respectively.

(a)

(b)
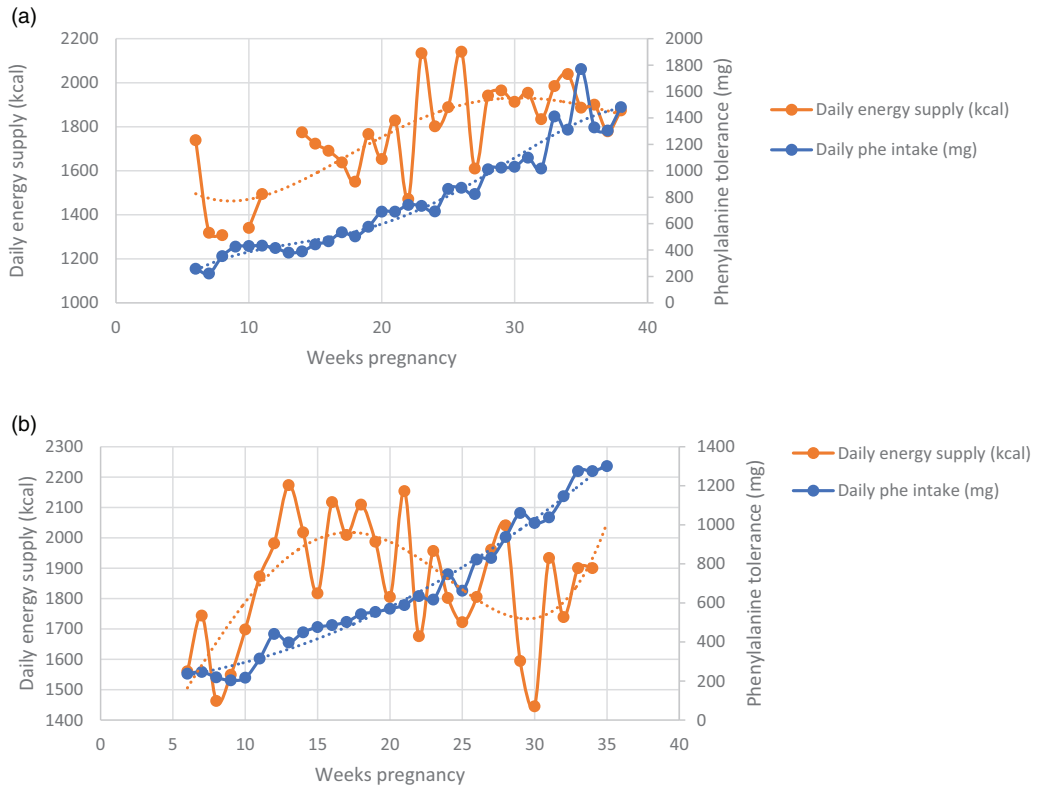
Figure 1. Phenylalanine tolerance and energy supply in patient 1. (a) Singleton pregnancy and (b) twin pregnancy.

(Table 1). In patient 3, the highest third trimester Phe tolerance in twin pregnancy reached only 67% of the highest value in the singleton pregnancy. However, pre-conception Phe tolerance was the same for the singleton and twin pregnancies in patient 3 (Table 3). The ratio of increase in Phe tolerance in the third trimester to the birth weight of offspring (mg/g) ranged from 0.11 to 0.42 in singleton pregnancies and from 0.01 to 0.12 in twin pregnancies (Table 3).

Discussion

In Caucasians, the highest population-based evaluation of the prevalence of

PKU is up to 3 per 1000 births.[8] Pregnancy in women with PKU can be viewed as a successful outcome of survival and transition into adulthood, achieving as normal a life as possible.[9]

Physiologically, in the first two trimesters of pregnancy, maternal anabolism predominates, while from approximately 30 weeks' gestation, maternal catabolism with an accelerated breakdown of fat deposits is favored because of placental hormones and adipocytokines.[9,10] Anabolism is associated with increased Phe requirements.[1] With the exception of one twin pregnancy (patient 2), the lowest mean Phe concentrations were observed in the second trimester. However, mean Phe
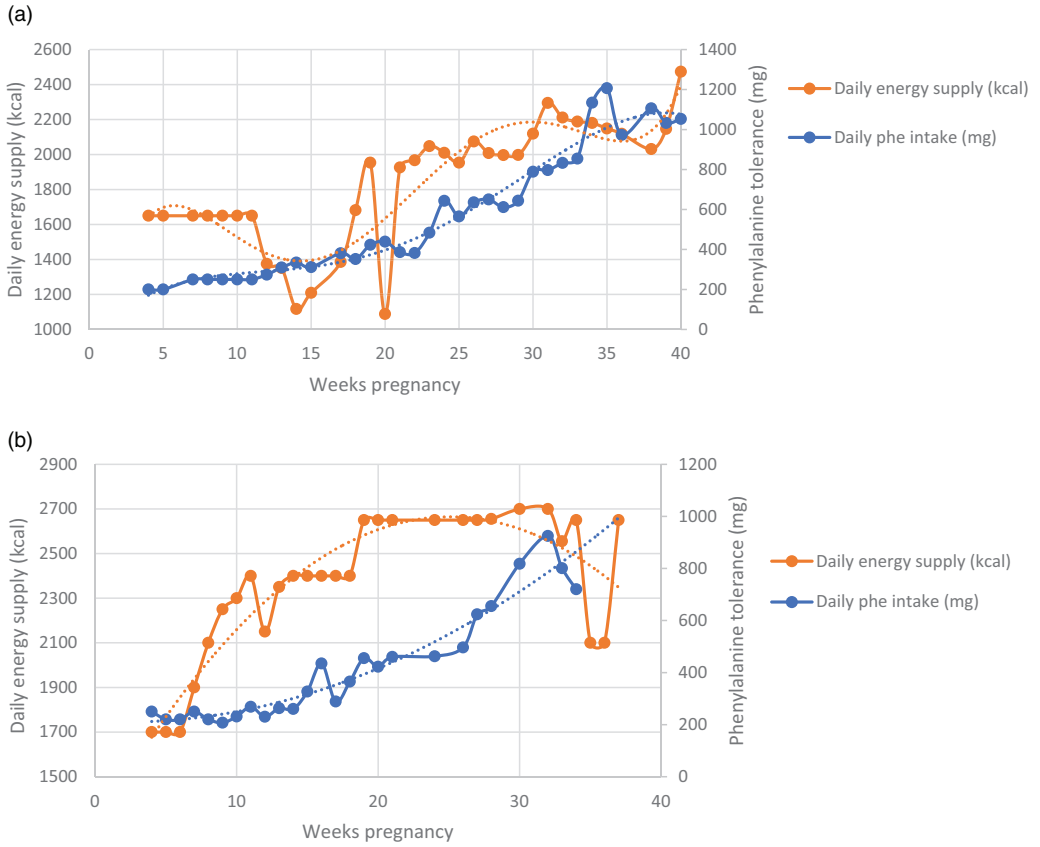
(a)

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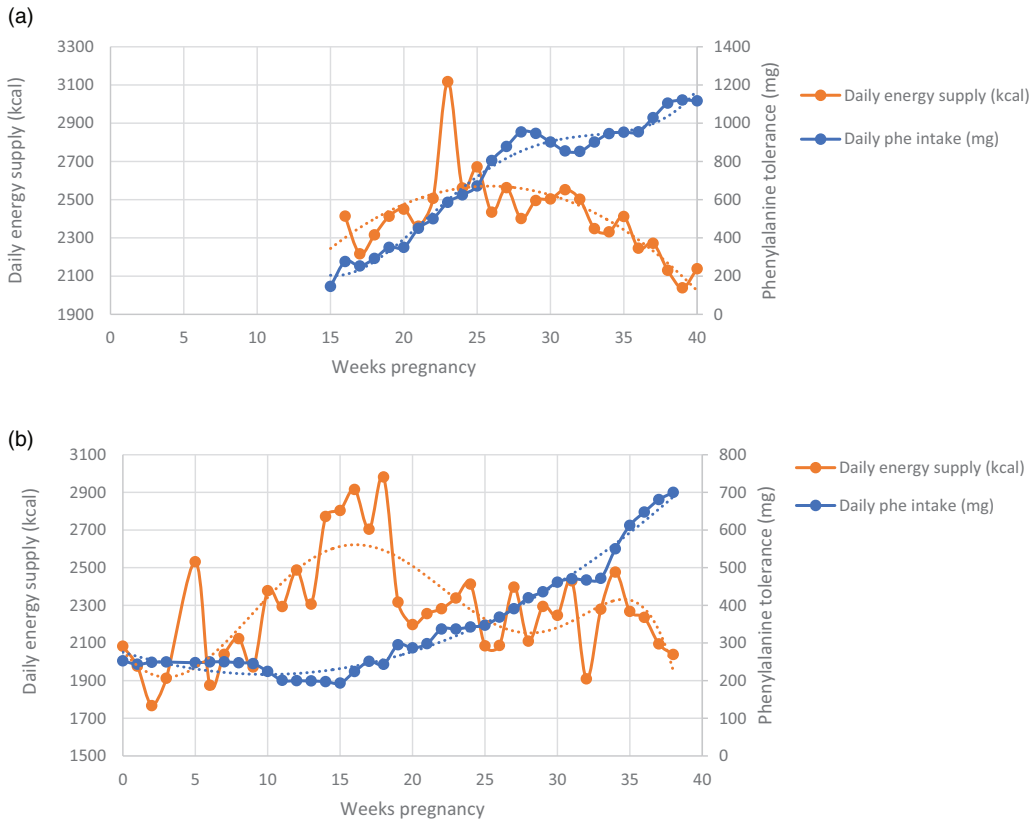
Figure 2. Phenylalanine tolerance and energy supply in patient 2. (a) Singleton pregnancy and (b) twin pregnancy.

concentrations in the second and third trimesters were acceptable. In one pregnancy, blood Phe concentrations never rose >360 µmol/L (singleton pregnancy of patient 1). Interestingly, most Phe values <120 µmol/L were above the gestational age-dependent upper reference limits of Phe in whole blood for singleton pregnancies in healthy women, as recently analyzed by tandem mass spectrometry.[7] McBride and colleagues[7] showed that Phe levels declined from the first to the second trimesters and then remained relatively stable to the end of the singleton pregnancy. The optimal range of Phe concentrations of 120 to 360 µmol/L remains unknown.[11,12] There is sufficient evidence to warrant

further investigation of phenylalanine concentrations and gestational age-specific phenylalanine reference ranges in twin pregnancies in healthy women to better understand homeostasis in multiple gestations in patients with PKU.

Recently, minimum daily Phe requirements per kg of body weight in the presence of excess tyrosine, which is in line with the treatment concept of our PKU cases, was experimentally determined using the direct amino acid oxidation technique in healthy women with singleton pregnancies by Ennis et al.[13] These authors showed that the minimum daily Phe requirement was 15 mg (95% confidence interval: 10.4–19.9 mg) in early pregnancy (13–19 weeks' gestation)

(a)

(b)

Figure 3. Phenylalanine tolerance and energy supply in patient 3. (a) Singleton pregnancy and (b) twin pregnancy.
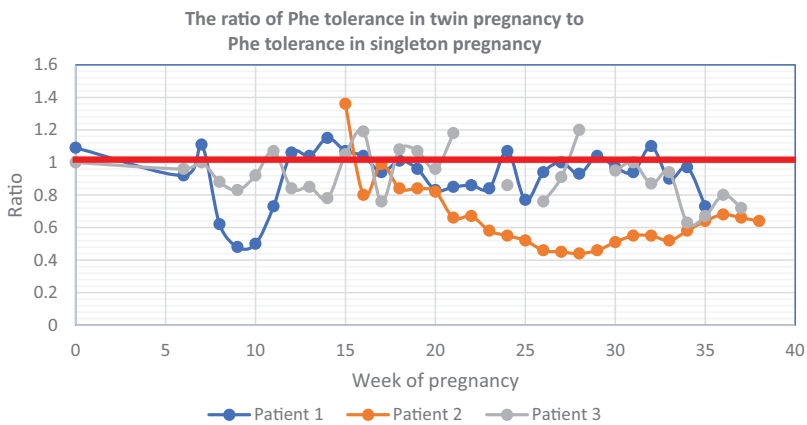
Figure 4. Ratio of phenylalanine tolerance in singleton to twin pregnancies. Phe, phenylalanine.

and 21 mg (95% confidence interval: 17.4–24.7 mg) in late pregnancy (33–39 weeks' gestation). In early pregnancy, as well as in late pregnancy, our Phe tolerance estimates are consistent with data by Ennis and colleagues[13] only in patient 1. In the other patients, the estimated Phe tolerance was less than the minimum demand as calculated by direct amino acid oxidation.

The dominant view on Phe tolerance in the third trimester in treated maternal PKU is that its increase parallels accelerated fetal growth and uptake of Phe, as well as an increase in phenylalanine hydroxylase activity in the fetal liver.[1,2,9] Intriguingly, there was an unexpected comparable Phe tolerance in singleton and twin pregnancies in our study. Our dizygotic twin pregnancies are a powerful clinical model because in each patient the fetuses had similar birth weights without intrauterine growth retardation, while each twin had a distinct placenta in a similar maternal environment. Physiologically, there is little discordance in biometry between singletons and twins in the second trimester, but in the third trimester, growth in twins is consistently slower than that in singletons.[3] Phe tolerance may vary significantly, even between singleton pregnancies in the same patient, possibly at least in association with currently unrecognized maternal anthropometric factors and diet modalities.[1,14,15] In our cases, this hinders interpretation of the comparability of Phe tolerance between the singleton and twin pregnancies. Pre-pregnancy BMIs were different in the singleton and twin pregnancies of patients 2 and 3.

The European PKU Guidelines,[1] as well as the previous American College of Medical Genetics guidelines on PKU,[2] state that treatment should commence at pre-conception for maternal PKU. These guidelines also emphasize that considerable effort should be made to avoid any unplanned pregnancies, with precautionary advice taking into account individual cultural and religious backgrounds. Ford and colleagues[16] showed that women with PKU of childbearing age had anxieties about sex and may have had problems with religious or moral beliefs. All patients need to feel comfortable discussing pregnancy and their choices regarding contraception.[16] In our study, patient 2 had decided not to use contraceptives. In a recent Italian study by Caletti and colleagues[14] on Phe tolerance in women with PKU, 6 of 10 conceptions were unplanned. Therefore, in the future, we will develop two algorithms of care in maternal PKU. One algorithm will be preferred and supported by the evidence-based recommendation of a low-Phe diet introduced pre-conceptionally.[1,2] The other algorithm will be optional, with rapid and restricted dietary treatment introduced in early unplanned pregnancies, and will use daily care in outpatient clinics or hospitalization. Homeostasis of Phe changes in a twin pregnancy, with a low Phe diet beginning after conception appearing to be, to some extent, different compared with twin pregnancies with a diet introduced pre-conceptionally. Notably, among our patients, the highest rate of Phe values exceeding the upper limit of the ideal target range in the first trimester was observed in a twin pregnancy with a low-Phe diet that was not initiated pre-conceptionally (patient 2). Medical professionals should attempt to close the gap between women with PKU of childbearing age of different religious beliefs, which affect pregnancy planning, and the programs of care that are available.[16]

The current study has notable strengths. The patients with PKU were ethnically homogeneous and under regular supervision of the same metabolic dietician. The study also has certain limitations that need to be taken into account. In twin pregnancies, the incidence of preterm delivery before 38 weeks can be up to 50%. In our study, the number of pregnancies was

limited and the length of pregnancy varied (35–40 weeks). A low-Phe diet was introduced pre-conceptionally in only half of the patients. The sex of the offspring in every pair of singleton and twin pregnancies was not concordant. A further limitation to be taken into account in this study is its retrospective design. Diet compliance in twin pregnancies may be affected by the need to care for the children from the preceding singleton pregnancies and the transition of support networks.[17,18] We also cannot rule out the effect of other factors, such as viral infections and other stressors. Moreover, birth data were used as a surrogate for the lack of placental data.

There are still many gaps in our knowledge regarding normal and abnormal physiological changes and effective interventions in multi-fetal pregnancies. The physiology of a twin pregnancy appears to be fundamentally different from that of a singleton pregnancy. Several reports have shown surprisingly different effects of disturbances in metabolism (e.g., gestational diabetes mellitus) on singleton and twin pregnancies.[3,19] A study by Wang and colleagues[20] showed disruption of the Phe and tyrosine biochemical pathways in twins with intrauterine growth retardation. Multiple gestations are increasing in frequency because of a number of factors, including assisted reproductive techniques and increasing maternal age. Therefore, medical providers dealing with PKU need to develop a detailed appreciation of the pathophysiological differences and subsequent challenges of managing maternal PKU in multi-fetal pregnancies.

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