Improved nutritional management of phenylketonuria by using a diet containing glycomacropeptide compared with amino acids[1–4]

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ABSTRACT

Background: Phenylketonuria (PKU) requires a lifelong low-phenylalanine diet that provides the majority of protein from a phenylalanine-free amino acid (AA) formula. Glycomacropeptide (GMP), an intact protein formed during cheese production, contains minimal phenylalanine.

Objective: The objective was to investigate the effects of substituting GMP food products for the AA formula on acceptability, safety, plasma AA concentrations, and measures of protein utilization in subjects with PKU.

Design: Eleven subjects participated in an inpatient metabolic study with two 4-d treatments: a current AA diet (AA diet) followed by a diet that replaced the AA formula with GMP (GMP diet) supplemented with limiting AAs. Plasma concentrations of AAs, blood chemistries, and insulin were measured and compared in AA (day 4) and GMP diets (day 8).

Results: The GMP diet was preferred to the AA diet in 10 of 11 subjects with PKU, and there were no adverse reactions to GMP. There was no significant difference in phenylalanine concentration in postprandial plasma with the GMP diet compared with the AA diet. When comparing fasting with postprandial plasma, plasma phenalyalanine concentration increased significantly with the AA but not with the GMP diet. Blood urea nitrogen was significantly lower, which suggests decreased ureagenesis, and plasma insulin was higher with the GMP diet than with the AA diet.

Conclusions: GMP, when supplemented with limiting AAs, is a safe and highly acceptable alternative to synthetic AAs as the primary protein source in the nutritional management of PKU. As an intact protein source, GMP improves protein retention and phenylalanine utilization compared with AAs.   *Am J Clin Nutr* 2009;89:1068–77.

INTRODUCTION

Phenylketonuria (PKU) is an inborn error of metabolism caused by a defect in phenylalanine hydroxylase (*PAH*; EC 1.14.16.1), which metabolizes the indispensable amino acid (AA) phenylalanine to tyrosine. The resulting elevated phenylalanine concentrations adversely affect the developing central nervous system, which causes profound mental retardation and neurologic impairment (1, 2). Lifelong treatment with a low-phenylalanine diet results in reversal of this devastating phenotype (3–5). The PKU diet includes 2 major modalities. First, an AA-based medical food (formula) provides the major source of phenylalanine-free protein

equivalents, energy, and micronutrients in the diet. Second, intake of intact protein is restricted to naturally low-protein foods and specialty low-protein bread and pasta products made from starch. This allows for an adequate, but not excessive, supply of phenylalanine for growth and protein turnover (1). Dietary products for treatment of PKU have improved; however, poor compliance remains a problem, especially in adolescents and young adults (6–9). New treatment modalities are needed to improve the palatability, variety, and convenience of this diet.

Glycomacropeptide (GMP) provides an alternative to AAs as a source of low-phenylalanine protein for the PKU diet. GMP is a 64-AA glycophosphopeptide formed during cheese production when bovine κ-casein is cleaved by chymosin into para-κ-casein, which remains with the curd, and GMP, which remains with the whey (10). Pure GMP contains no aromatic AAs (phenylalanine, tryptophan, and tyrosine) and no arginine, cysteine, and histidine. However, GMP contains a 2- to 3-fold greater concentration of the large neutral AAs (LNAAs) threonine and isoleucine than do reference proteins (10). Isolation of GMP from cheese whey results in contamination with other whey proteins that contain phenylalanine and other AAs not found in pure GMP. Commercially available GMP contains 2.5–5.0 mg phenylalanine/g of protein (11, 12). GMP requires supplementation with limiting AAs to provide a complete source of protein for individuals with PKU (13, 14).

Studies in the PKU mouse model indicate that intake of GMP supplemented with limiting AAs provides a nutritionally ade-

quate source of protein and significantly reduces concentrations of phenylalanine in plasma and brain compared with an AA diet (15). Similar reductions in phenylalanine concentration in plasma or brain have been reported in subjects with PKU given supplementation with threonine (16) or mixtures of LNAAs (17–19). Competition among LNAAs, including threonine and isoleucine, with phenylalanine for intestinal absorption and transport across the blood-brain barrier may explain the observed reductions in phenylalanine (19, 20).

To further evaluate the potential benefits of GMP in the PKU diet, an 8-d clinical investigation was conducted in individuals with PKU. The objective was to investigate the effects of substituting GMP food products for the AA formula on acceptability, safety, plasma AA concentrations, and measures of protein utilization in subjects with PKU.

SUBJECTS AND METHODS

Subjects

Twelve subjects with PKU who were routinely monitored at the Biochemical Genetics Program, the Waisman Center, the University of Wisconsin-Madison, participated in this study between March 2006 and June 2008. One subject (age: 10 y) withdrew from the study because she was unable to complete the protocol. Thus, data from 11 subjects (age range: 11–31 y; 7 males and 4 females) are reported (**Table 1**). The University of Wisconsin-Madison Health Sciences Institutional Review Board approved this study.

Criteria for participation included a diagnosis of classical or variant PKU and a willingness to consume ≥50% of the prescribed volume of the AA formula. Optimal control of plasma phenylalanine concentrations, however, was not a prerequisite for participation. Optimal control includes maintenance of phenylalanine concentrations between 120 and 360 μmol/L for neonates through age 12 y, between 120 and 600 μmol/L for adolescents, and <900 μmol/L for adults (5). The diagnosis of PKU was based on concentration of phenylalanine measured before initiation of dietary treatment during infancy; those with classical PKU show phenylalanine concentrations ≥1200 μmol/L (Table 1) (1). All subjects in this study were diagnosed with classical PKU, except for one subject who was determined to have a variant form of PKU (subject 1).

Mutation analysis was completed for each subject by DNA sequencing of the *PAH* gene (Laboratory Service Section, Texas Department of State Health Services, Austin, TX) using primers designed by Guldberg et al (21). All subjects were compound heterozygous for *PAH* mutations (Table 1). Five subjects showed 2 copies of mutations considered to express primarily a classical phenotype and 6 subjects showed a classical mutation and a mutation observed in PKU patients with variant and/or non-PKU hyperphenylalaninemia mutations (http://www.pahdb.mcgill.ca/).

Because a formal evaluation of each subject's dietary prescription had not been completed within 2 y of study recruitment, a phenylalanine allowance for all subjects was determined before study initiation. For this study, phenylalanine allowance was defined as the amount of dietary phenylalanine intake that allowed for a constant plasma phenylalanine concentration (±5%

TABLE 1
Individual characteristics of 11 subjects with phenylketonuria

Subject no./sex	Age at study initiation	Height/ weight	Phenylalanine concentration at diagnosis[1]	Age at diagnosis[1]	Mutation	Baseline plasma phenylalanine[2]	Dietary phenylalanine allowance per day	Dietary phenylalanine allowance per kg
	y	cm/kg	μmol/L	d		μmol/L	mg	mg
1/M	27	173/73	1270	35	R408W IVS12nt1g→a	640	1151	15.8
2/M	29	170/67	2208	15	R408W R261Q	1011	1793	26.7
3/M	14	164/52	1210	8	R408W IVS10nt-11g→a	1009	673	13.0
4/M	11	137/35	2051	7	IVS4nt5g→t IVS12nt1g→a	767	372	10.7
5/M	12	148/45	2154	7	R408W Y356N	690	979	21.6
6/F	23	94/51	1488	10	R408W IVS12nt1g→a	536	545	10.6
7/F	28	159/64	2632	11	R408W L242F	603	545	8.3
8/M	27	170/76	1924	11	R261Q E280K	810	971	12.8
9/F	20	93/64	1016	10	L48S F299C	331	378	5.9
10/F	31	157/70	1876	15	R408W F299C	192	408	5.8
11/M	28	180/91	3122	13	IVS1nt5g→t IVS12nt1g→a	392	711	7.8

[1] Age and phenylalanine concentrations at diagnosis represent values when diet treatment was initiated during infancy.
[2] Values represent concentrations of phenylalanine in plasma 2.5 h after eating breakfast on day 3 while consuming the prescribed amino acid diet.

variance) as determined by sequential increases in phenylalanine intake with frequent monitoring of blood phenylalanine concentrations in blood spots. Each subject's dietary phenylalanine allowance was verified by completing one or more "dry runs" in which all food, beverages, and formula were provided for a 5-d period with measurement of phenylalanine concentrations in blood spots before and at the end of each dry run. Plasma phenylalanine concentrations at the initiation of the study ranged from 192 μmol/L (subject 10) to 1011 μmol/L (subject 2; Table 1). To maintain these plasma phenylalanine concentrations, the dietary phenylalanine allowance for the subjects ranged from 5.8 mg/kg (subject 10) to 26.7 mg/kg (subject 2).

Study protocol

Each subject served as his or her own control in this metabolic study, which included 2 dietary treatments of 4 d each: the AA diet (days 1–4) and the GMP diet (days 5–8). One 24-h menu was designed for the AA diet and another for the GMP diet; the same menu was repeated on all days of each diet treatment (**Table 2**). In each diet, the AA formula or GMP products were divided equally in each of 3 meals during the day. Distribution of protein equivalents throughout the day improves protein utilization and can lower plasma phenylalanine concentrations (22). During the study, all food, beverages, snacks, formula, and GMP products were weighed in grams by trained dietary staff at the Waisman Center or the University of Wisconsin Clinical and Translational Research Core (UW-CTRC). To ensure identical intake on all days of the study, subjects were encouraged to consume all foods and beverages. No subject failed to do this.

Each subject was provided with all food and formula to consume at home for 2 d before initiation of the study and for days 1 and 2 of the AA diet. Before dinner on day 2, each subject

was admitted to the UW-CTRC for continuation of the AA diet (days 3 and 4) and for 4 d of the GMP diet (days 5–8). A physical exam was completed on all days of the UW-CTRC admission. All subjects were required to walk or complete physical activity 2–3 times/d to allow for an activity level consistent with their usual routine. Timing of meals and snacks was also similar to each subject's usual routine.

On days 1 and 2, each subject collected a blood spot on filter paper for phenylalanine and tyrosine analysis. During the UW-CTRC admission, blood was drawn daily for plasma AA and automated chemistry panel analysis to measure serum concentrations of prealbumin, albumin, total protein, electrolytes, glucose, blood urea nitrogen (BUN), creatinine, calcium, magnesium, phosphate, uric acid, total and direct bilirubin, alkaline phosphatase, and liver enzymes (γ-glutamyltranspeptidase, alanine aminotransferase, aspartate aminotransferase, and lactic dehydrogenase). All postprandial blood samples were drawn daily 3 h after the start of breakfast or 2.5 h after eating breakfast (days 3–8).

After the first 5 subjects had completed the protocol, the Data Safety and Monitoring Board evaluated the protocol and study progress. As a result of the board's suggestions, blood draws for chemistry panels were eliminated on the first 2 d of the GMP diet (days 5 and 6), and an additional fasting blood sample was added before breakfast on the last 2 d of the AA diet (days 3 and 4) and on the last 2 d of the GMP diet (days 7 and 8) for the remaining 6 subjects. All fasting samples were analyzed for plasma AAs. The mean age of the 6 subjects for whom both fasting and postprandial blood samples were obtained was 26 ± 2 y and included 4 females and 2 males (subjects 6–11; Table 1).

Because the GMP food products were not supplemented with vitamins and minerals, all subjects were given a complete multivitamin with mineral supplement (Phlexy-Vits; Nutritia North America, Gaithersburg, MD) or a combination of Theragran M

TABLE 2
Comparison of typical menus for the amino acid (AA) and the glycomacropeptide (GMP) diets[1]

AA diet	GMP diet
Breakfast (103 mg phenylalanine, 14 g protein)	Breakfast (102 mg phenylalanine, 14 g protein)
177 mL PKU formula[2] (0 mg phenylalanine)	296 mL GMP chocolate beverage (51 mg phenylalanine)
30 g Cold cereal (51 mg phenylalanine)	30 g Cold cereal (51 mg phenylalanine)
11 g Pretzels (52 mg phenylalanine)	
Lunch (124 mg phenylalanine, 15 g protein)	Lunch (124 mg phenylalanine, 13 g protein)
177 mL PKU formula[2] (0 mg phenylalanine)	148 mL GMP chocolate beverage (24 mg phenylalanine)
12.5 g Cinnamon toast (62 mg phenylalanine)	113 g (1/2 cup) GMP chocolate pudding (38 mg phenylalanine)
Cheese sandwich (45 mg phenylalanine; 64 g low-protein bread,	Cheese sandwich (45 mg phenylalanine; 64 g low-protein bread,
1 Slice low-protein cheese, 8.7 g butter)	1 Slice low-protein cheese, 8.7 g butter)
125 g Peaches (17 mg phenylalanine)	125 g Peaches (17 mg phenylalanine)
Dinner (220 mg phenylalanine, 18 g protein)	Dinner (226 mg phenylalanine, 18 g protein)
177 mL PKU formula[2] (0 mg phenylalanine)	1 GMP bar (33 mg phenylalanine)
9 g Bowtie pasta (53 mg phenylalanine)	237 mL GMP sports beverage (19 mg phenylalanine)
Pasta Alfredo (61 mg phenylalanine; 60 g low-protein pasta,	Pasta Alfredo (61 mg phenylalanine; 60 g low-protein pasta,
5 g Regular bowtie pasta, 88 g low-protein Alfredo sauce)	5 g Regular bowtie pasta, 88 g low-protein Alfredo sauce)
92 g Broccoli, 50 g carrots and 14 g butter (105 mg phenylalanine)	92 g Broccoli, 50 g carrots and 14 g butter (105 mg phenylalanine)
140 g Pears (7 mg phenylalanine)	140 g Pears (7 mg phenylalanine)
237 mL Lemonade (0 mg phenylalanine)	237 mL Lemonade (0 mg phenylalanine)

[1] All foods were measured on a gram scale by trained staff. Since this study was completed, improved recipes have been developed to further lower the phenylalanine content of all of the GMP products shown in this typical menu (12). For example, a GMP bar can now be produced with only 14 mg phenylalanine compared with 33 mg phenylalanine, and the GMP chocolate pudding now contains 21 mg phenylalanine compared with 38 mg phenylalanine in the original formulation used for this research. PKU, phenylketonuria.

[2] The PKU formula used in this menu is 40 g Phenex 2 (Abbott Laboratories, Columbus, OH).

(Walgreen Co, Deerfield, IL) and Target-Mins (Country Life, Hauppauge, NY) during the GMP diet. Any subject consuming a formula or formulas that did not contain vitamins and minerals was given the same supplements provided for the GMP diet during the AA diet. Additional calcium was given, if needed, to meet Dietary Reference Intake (DRI) recommendations for age (23).

Study diets

GMP (Bio-Pure GMP; Davisco, Le Sueur, MN) was analyzed for AA content at the University of Missouri Experimental Station Chemical Laboratory (24). The phenylalanine content for the commercial GMP was 0.4 g phenylalanine/100 g GMP with a protein content of 86.0 g/100 g GMP. This GMP was used for 3 subjects with a higher phenylalanine tolerance. For 9 subjects with a lower phenylalanine tolerance, the original stock of GMP was further purified to reduce the phenylalanine content to an average of 0.21 ± 0.01 g phenylalanine/100 g GMP with an average protein content of 75.0 ± 0.7 g/100 g GMP (25). Purification of the GMP decreased only the phenylalanine content; the proportion of the other AAs remained unchanged in the purified GMP compared with the commercial GMP.

The GMP was supplemented with 4 limiting AAs, expressed as the final concentration in milligrams AA per gram GMP protein: histidine, 23; leucine, 72; methionine, 28; and tryptophan, 9. This is equivalent to 130% of estimated needs on the basis of the 2002 DRIs (13). Because tyrosine is an indispensable AA in PKU, tyrosine was supplemented at 150% of estimated needs for a final concentration of 71 mg/g GMP protein (13, 25). For the GMP diet, no attempt was made to duplicate the concentration of supplemental tyrosine found in the various formulas consumed by each subject because, in most cases, the tyrosine content of the formula was substantially greater than the estimated needs. Thus, for all subjects, tyrosine intake in the AA diet was greater than tyrosine consumed when GMP products were substituted.

Low-phenylalanine food products made with GMP as the protein source were developed for this study by the Wisconsin Center for Dairy Research, the University of Wisconsin-Madison. Before initiation of the study, each subject tasted a variety of food products made with GMP and selected 2 to 3 products that would be included in menus for the GMP diet. GMP beverages and foods included an orange-flavored sports beverage, a chocolate-flavored or caramel-flavored beverage, chocolate or strawberry pudding, and a cinnamon crunch bar (12). The range of phenylalanine content in the GMP food products varied with the purity of the GMP and the additional ingredients used to produce these foods and beverages, but, in general, a serving of GMP food products provided 5–10 g protein and 15–30 mg phenylalanine. Since this study was completed, improved recipes have been developed to further lower the phenylalanine content of all the GMP products used for the study (12).

Diet composition

The AA and GMP diets were calculated on the basis of a prestudy evaluation of each subject's phenylalanine allowance and were controlled for energy, protein, phenylalanine, and fat (**Table 3**). The AA diet (days 1–4) included a subject's usual AA formula, which was different for each subject. For the GMP diet

TABLE 3
Nutrient composition of amino acid (AA) and glycomacropeptide (GMP) diets[1]

	AA diet	GMP diet
Energy (kcal/kg)		
<18 y old	56 ± 6	57 ± 5
≥18 y old	35 ± 1	35 ± 2
Energy from protein (%)[2]	11 ± 1	10 ± 1
Energy from fat (%)[3]	24 ± 1	23 ± 1
Phenylalanine intake (mg · kg^{-1} · d^{-1})	13 ± 2	13 ± 2
Tyrosine intake (mg · kg^{-1} · d^{-1})	85 ± 9	51 ± 5[4]

[1] Values are means $\pm$ SEMs and are based on calculated dietary intake; $n = 11$.

[2] Protein from synthetic AAs represents 75% of the total protein in the AA diet and only 10% of the total protein in the GMP diet (from supplementing the GMP with limiting indispensable AAs). All other protein in the AA and GMP diets is from natural sources of intact protein.

[3] Total fat intake ranged from 18% to 31% of total energy. A low fat intake is typical in those with phenylketonuria, given their selection of carbohydrate-based foods and the low fat content of many AA formulas designed for older individuals with this disorder (28).

[4] Significantly different from the AA diet, $P < 0.0001$ (paired t test, pairing on subject).

(days 5–8), GMP products were substituted for a subject's entire daily intake of AA formula. The phenylalanine content of foods used to plan the menus was determined by AA analysis of selected foods and by calculation of phenylalanine content for the remaining foods (26, 27). Foods that were not analyzed were matched in quantity, brand, and packing lot in both diets, whereas foods analyzed for phenylalanine content were used in variable amounts to account for the phenylalanine content of the GMP products.

Because of the limitations in data to quantitate the phenylalanine content of foods, dietary composites were collected for phenylalanine analysis to verify calculations of phenylalanine content. Thus, a duplicate of all food, formula, and GMP food products consumed by each subject during a 24-h period was collected for 2 d during both the AA diet and the GMP diet. Each duplicate was ground and freeze-dried, and an aliquot of each composite was sent to the University of Missouri for AA analysis (24). When comparing the composite analyses for each subject, phenylalanine content in the AA diet and the GMP diet was not significantly different ($P = 0.061$).

Measurements

The blood spots collected by each subject to establish their phenylalanine allowance and on prestudy days 1 and 2 were analyzed for phenylalanine and tyrosine by tandem mass spectrometry (MS/MS; data not shown) (29). An AA profile was completed on all fasting and postprandial plasma samples collected on days 3–8 by using a Beckman 6300 amino acid analyzer (Beckman-Coulter Inc, Fullerton, CA) equipped with an ion chromatography system that uses postcolumn ninhydrin derivatization (30). The samples were deproteinized with sulfosalicylic acid, centrifuged ($14,000 \times g$; 5 min) and passed through a 0.2-μm syringe filter before adding an internal standard and injecting it into the column.

Serum chemistry profiles were analyzed by using standard techniques at the Clinical Laboratory, the University of Wisconsin-Madison Hospital and Clinics. Plasma insulin was measured in postprandial samples by using an radioimmunoassay specific for human insulin (Linco Research, St Charles, MO) on samples pooled within subjects for days 3 + 4 and days 7 + 8. Insulin-like growth factor I (IGF-I) was measured in postprandial plasma samples for days 4 and 8 after removal of IGF-binding proteins by HPLC; the recovery of IGF-1 was 85–90% (31).

Statistical analysis

All statistical analysis was conducted with the statistical program R for Mac OS X version 1.12 (R Project for Statistical Computing, Wirtschaftsuniversität, Vienna, Austria; http://www.r-project.org). After dietary composites were analyzed, AA values within each diet were averaged for each subject ($n = 2$), and then values between both diets were compared by using paired t tests. Also, paired t tests, pairing on subject, were conducted to compare plasma AA values from the last day of the AA diet (day 4) to the last day of the GMP diet (day 8) for both postprandial and fasting samples. Changes in the chemistry panel and liver function tests were compared by using the same method. In addition, paired t tests were conducted to compare fasting and postprandial AA concentrations within each diet in the subset of 6 subjects from whom fasting plasma was available. All comparisons were considered statistically significant if $P \leq 0.05$. On the basis of the primary endpoint comparing plasma phenylalanine concentration on the last day of the AA diet (day 4) with the last day of the GMP diet (day 8), the achieved sample size ($n = 11$) was sufficient to provide 80% power at $P = 0.05$ if the change in plasma phenylalanine concentration was 150 μmol/L.

RESULTS

Diet acceptability and AA composition

After consuming the GMP diet for 4 d, 10 of 11 subjects claimed that the GMP products were superior in sensory qualities to their usual AA formula. Moreover, at the conclusion of the study, 6 of the 7 adult subjects expressed a strong preference to consume GMP products rather than their usual AA formula, if GMP became available to them as a dietary option.

Compared with current recommendations, the analyzed intake (mg amino acid/g of dietary protein) of all indispensable AAs met requirements for both the AA and GMP diets (13, 14). However, AA analysis of the dietary composites indicated several significant differences in AA intake with ingestion of the AA diet compared with the GMP diet (**Table 4**). Because GMP contains a high concentration of the LNAAs threonine and isoleucine, mean intakes of both of these AAs were significantly higher with the GMP than the AA diet. Despite supplementation of GMP with tyrosine at 150% of DRI and leucine, histidine, tryptophan, and methionine at 130% of the DRI, the intake of these AAs, with the exception of methionine, was significantly lower with the GMP than the AA diet. The intakes of other AAs that were significantly lower with ingestion of the GMP diet compared with the AA diet included the indispensable AA lysine and the dispensable AAs arginine, alanine, glycine, and taurine.

TABLE 4
Analyzed profile of amino acids (AAs) from 24-h composites of AA and glycomacropeptide (GMP) diets[1]

AA	AA diet	GMP diet	P value[2]
	g amino acid/24-h diet		
Alanine	4.08 ± 0.32	3.15 ± 0.07	0.039
Arginine	4.07 ± 0.23	0.80 ± 0.09	<0.0001
Aspartic acid	6.09 ± 0.37	5.08 ± 0.25	0.059
Cysteine	1.43 ± 0.09	0.50 ± 0.05	<0.0001
Glutamic acid	10.3 ± 0.90	11.6 ± 0.64	0.219
Glycine	3.52 ± 0.26	0.99 ± 0.07	<0.0001
Histidine	1.89 ± 0.10	1.27 ± 0.09	0.001
Isoleucine	3.70 ± 0.16	4.75 ± 0.26	0.004
Leucine	6.51 ± 0.35	4.12 ± 0.28	<0.0001
Lysine	4.53 ± 0.21	3.09 ± 0.17	<0.0001
Methionine	1.24 ± 0.06	1.28 ± 0.08	0.605
Phenylalanine	0.79 ± 0.09	0.74 ± 0.08	0.061
Proline	5.27 ± 0.27	5.91 ± 0.32	0.198
Serine	3.27 ± 0.26	3.30 ± 0.18	0.883
Taurine	0.54 ± 0.08	0.25 ± 0.03	0.019
Threonine	3.00 ± 0.10	7.12 ± 0.41	<0.0001
Tryptophan	1.07 ± 0.07	0.57 ± 0.05	<0.0001
Tyrosine	4.40 ± 0.17	2.63 ± 0.21	<0.0001
Valine	4.50 ± 0.14	4.13 ± 0.21	0.105
BCAA	14.72 ± 0.62	13.00 ± 0.72	0.034

[1] Values are means ± SEMs; $n = 22$. BCAA, sum of leucine, isoleucine, and valine.
[2] Represents the difference between the AA and the GMP diets by paired t test.

Physical examination and blood chemistry

There were no physical concerns detected on exam or expressed by any subject to indicate any negative effect on health status when subjects consumed GMP as the primary protein source for a 4-d period. There were no significant differences among serum concentrations of albumin, prealbumin, or total protein as indicators of protein status or creatinine as an indicator of renal status measured on the last day of the AA diet (day 4) compared with the GMP diet (day 8; **Table 5**). However, BUN as an indicator of hepatic ureagenesis was significantly lower with ingestion of the GMP diet on both day 7 and day 8 than with the

TABLE 5
Effect of amino acid (AA) and glycomacropeptide (GMP) diets on postprandial indexes of protein and glucose metabolism[1]

Test	AA diet	GMP diet	P value[2]
Blood urea nitrogen (mmol/L)	4.2 ± 0.3	3.4 ± 0.2	0.02
Creatinine (μmol/L)	73 ± 5.5	73 ± 4.6	1.00
Total protein (g/L)	68 ± 1.4	67 ± 1.4	0.27
Albumin (g/L)	44 ± 0.9	44 ± 0.8	0.84
Prealbumin (g/L)	317 ± 7.5	310 ± 7.3	0.22
Insulin-like growth factor I (nmol/L)	13.5 ± 1.3	13.7 ± 1.5	0.14
Insulin (pmol/L)	84 ± 22	116 ± 34	0.05
Glucose (mmol/L)	4.5 ± 0.1	4.8 ± 0.1	0.14
CO_2 content (mmol/L)	26 ± 0.6	28 ± 0.6	0.01

[1] Values are means ± SEMs; $n = 11$, except total protein and insulin for which $n = 10$; all values are within normal range. Values are for serum except those for insulin-like growth factor I and insulin, which used plasma.
[2] Difference between the last day of the AA diet (day 4) and the GMP diet (day 8) by paired t test, pairing on subject.
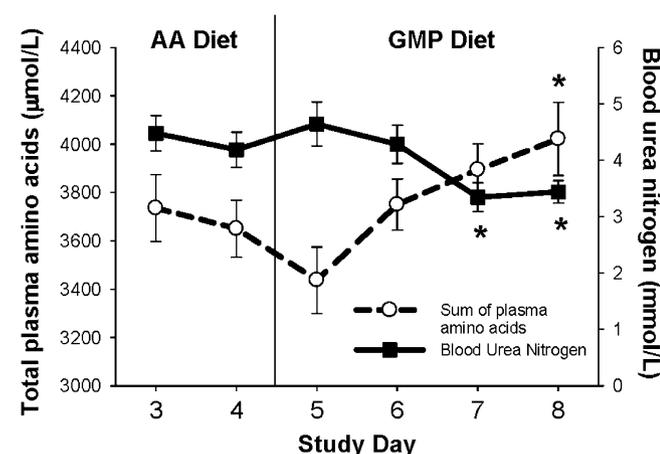
AA diet on day 4 (**Figure 1**). Plasma concentration of IGF-I was not significantly different with the AA and GMP diets, which suggests adequate protein nutrition in both diets (32). Plasma insulin concentration was higher and marginally significant with the GMP diet compared with the AA diet ($P = 0.053$), and serum glucose concentration was not significantly different. Serum carbon dioxide content, which is primarily bicarbonate, was significantly lower with the GMP diet compared with the AA diet, which is consistent with a lower systemic acid content. The mean concentrations of other standard chemistries, including electrolytes and liver function tests, remained within the normal range with both diets (data not shown). The exception was elevated concentrations of various liver function tests (alanine aminotransferase and γ-glutamyltranspeptidase) measured in subject 2, who was on anticonvulsant medications for his seizure disorder. However, further increases in these liver function tests were not detected with ingestion of the GMP diet compared with the elevations measured at admission to the study.
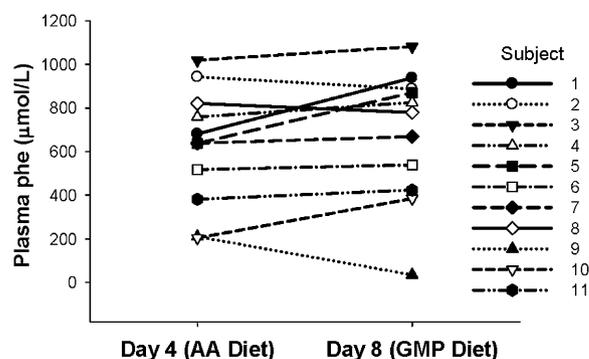
Plasma AA concentrations

The concentration of total AAs in plasma was significantly greater, and the concentration of BUN was significantly lower, with the GMP diet compared with the AA diet when measured 2.5 h after eating breakfast (Figure 1). This is consistent with slower absorption of AAs from an intact source of protein compared with synthetic AAs (33) and higher insulin concentrations with ingestion of GMP (34–36).

Phenylalanine and tyrosine

There was no significant difference ($P = 0.173$) in the mean postprandial concentration of phenylalanine in plasma with ingestion of the AA diet (day 4) compared with the GMP diet (day 8; **Figure 2**). The mean change in the concentration of phenyl-

FIGURE 2. Concentrations of phenylalanine in plasma of individual subjects with phenylketonuria ($n = 11$) after consuming the amino acid (AA) diet or the glycomacropeptide (GMP) diet for 4 d. Blood was obtained 2.5 h after eating breakfast, and plasma was isolated for analysis of the complete AA profile. Subjects showed a range of plasma phenylalanine concentrations after consuming the AA diet or the GMP diet for 4 d. There was no significant difference in the concentration of phenylalanine in plasma when the last day of the AA diet (day 4) was compared with the last day of the GMP diet (day 8); $P = 0.173$ by paired t test, pairing on subject. Group mean ± SEM was 619 ± 82 μmol/L (AA diet) and 676 ± 92 μmol/L (GMP diet). The mean change in the concentration of phenylalanine in plasma was 57 ± 52 μmol/L. phe, phenylalanine.
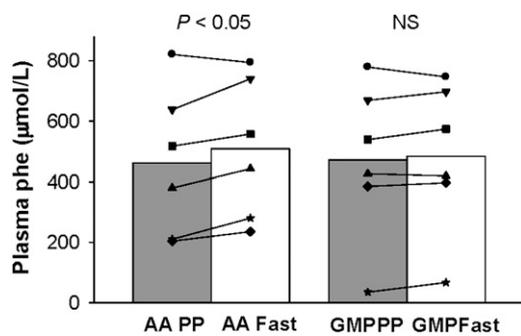
alanine in plasma was 57 ± 52 μmol phenylalanine/L. Among individual subjects, the response of plasma phenylalanine concentration to ingestion of the GMP diet was heterogeneous, ranging from a decrease of 175 μmol phenylalanine/L to an increase of 257 μmol phenylalanine/L. Overall, there was no consistent association between a change in the concentration of phe in plasma with ingestion of the AA diet compared with the GMP diet and sex, genotype, and age.

Concentrations of phenylalanine in both fasting and postprandial plasma were available on day 4 (AA diet) and on day 8 (GMP diet) for a subset of 6 adult subjects. The postprandial response to the GMP diet was not significantly different with this subset ($n = 6$) than with the first 5 subjects. Ingestion of the AA diet for 4 d resulted in a significant 10% increase in the concentration of phenylalanine in plasma obtained after an overnight fast compared with the concentration of phenylalanine in postprandial plasma obtained 2.5 h after eating breakfast ($P = 0.048$; **Figure 3**). In contrast, ingestion of the GMP diet for 4 d resulted in no significant change in the concentration of phenylalanine in plasma when comparing plasma obtained in a fasting state to plasma obtained in a postprandial state.

Tyrosine is an important AA in the PKU diet because it is indispensable and a precursor of adrenaline, norepinephrine, melanin, and thyroxine (1). Concentrations of tyrosine in plasma obtained in the postprandial or fasting samples were not significantly different with ingestion of the GMP or AA diets (**Table 6**). Concentrations of tyrosine in plasma after an overnight fast were decreased compared with postprandial concentrations with ingestion of both the GMP and the AA diet; however, the GMP diet resulted in a mean fasting tyrosine concentration that was below the normal range.

Additional AAs

The most dramatic change in the profile of AAs in plasma with ingestion of the GMP compared with the AA diet was the 2.25- to

FIGURE 1. The concentration of total amino acids (AAs) and blood urea nitrogen in postprandial plasma with ingestion of the glycomacropeptide (GMP) or the AA diet. Plasma was obtained 2.5 h after eating breakfast; $n = 11$ with the exception of blood urea nitrogen on study days 5 and 6 for which $n = 6$. Total plasma AAs indicate the sum of all AAs measured in plasma. Values are means ± SEMs. Total plasma AAs increased and blood urea nitrogen decreased with ingestion of the GMP diet when compared with day 4 of the AA diet. There was a significant effect of time in the repeated-measures ANOVA. *Significantly different from the AA diet on day 4, $P < 0.05$ (paired t test, pairing on subject).

FIGURE 3. The concentration of phenylalanine in postprandial (PP; 2.5 h after eating breakfast) compared with fasting (fast, overnight fast) plasma in subjects with phenylketonuria fed glycomacropeptide (GMP) compared with the amino acid (AA) diet for 4 d. Group means and the response of individual subjects are shown; $n = 6$ (day 4 compared with day 8). There was no significant change in plasma phenylalanine concentration comparing fasting with PP concentrations when consuming the GMP diet ($P = 0.349$); however, the AA diet showed a significant increase in plasma phenylalanine ($P = 0.048$) by paired t test, pairing on subject. phe, phenylalanine.

2.47-fold increase in postprandial concentrations of the nontoxic LNAA isoleucine and threonine (37, 38), which places these values above the normal clinical range (Table 6). A significant increase in plasma concentration of isoleucine and threonine with the GMP diet occurred within 24 h of ingesting the GMP diet and was consistent with the high concentrations of these AAs in GMP (**Figure 4**). However, there was no further significant increase in plasma concentration of isoleucine and threonine after days 5 and 7, respectively. The concentration of isoleucine was not different in plasma obtained after an overnight fast, whereas the concentration of threonine in fasting plasma remained ≈2-fold greater with ingestion of the GMP compared with the AA diet.

Consistent with the AA profile of the GMP and AA dietary composites, there were significantly lower postprandial concentrations of ornithine and tryptophan in plasma and significantly higher concentrations of isoleucine and threonine in plasma with consumption of the GMP compared with the AA diet. After an overnight fast, plasma concentration of arginine was significantly lower and concentration of threonine was significantly higher with the GMP compared with the AA diet (Table 6).

DISCUSSION

This is the first clinical trial to investigate the efficacy of substituting intact protein from GMP food products for synthetic AA formulas that are currently required for nutritional management of PKU. No adverse health problems were found, and blood chemistries remained normal when subjects with PKU consumed GMP as their primary protein source for 4 d in this controlled metabolic diet study (Table 5). Furthermore, the GMP products were preferred by the subjects, which confirms the results of blind taste tests comparing GMP to AA products in those with PKU (11). Thus, foods and beverages made with GMP are both safe and highly acceptable for use in the phenylalanine-restricted diet for PKU.

Over a period of 4 d, there was no significant change in plasma phenylalanine concentrations with ingestion of GMP products compared with the AA formula (Figure 2). Previous studies have shown that supplementation with LNAAs for 1–2 wk (17, 18) or with threonine for 8 wk (16) reduces plasma phenylalanine concentration, possibly via reduced intestinal phenylalanine absorption through the common LNAA transporter (20). Similar effects could be expected with administration of GMP because of its elevated threonine and isoleucine content (10, 11). Indeed, PKU mice fed GMP for 4–7 wk showed an 11–20% reduction in phenylalanine concentrations in plasma and brain compared with an AA diet (15). Furthermore, one individual with PKU who replaced his entire AA formula prescription with GMP food products for 10 wk at home showed significantly lower circulating concentrations of phenylalanine within 1–2 wk of initiation of the GMP diet (12). These findings suggest that longer-term consumption of GMP products will reduce concentrations of circulating phenylalanine and that the current 4-d study period was not sufficient to show this positive effect.

GMP, as an intact protein source, may delay absorption of AAs and improve utilization of phenylalanine and other AAs for protein synthesis when compared with a synthetic AA source. In this study, the AA diet showed a significantly higher mean fasting phenylalanine concentration compared with the postprandial phenylalanine concentration (Figure 3), whereas there was no significant difference in fasting and postprandial phenylalanine concentrations apparent with the GMP diet (Table 6). This suggests that the GMP diet induced less variation and potentially lower mean concentrations of phenylalanine in plasma over a 24-h period. Gropper and Acosta (33) suggested that protein retention improved with a lower maximal plasma concentration of total AAs and a slower decrease in these concentrations when the intact protein cottage cheese, rather than its component AAs, was ingested by healthy adults as their sole protein source. Moreover, Metges et al (39) showed significantly increased net protein synthesis and decreased leucine oxidation over 8 h by using an oral L-[1-^{13}C]leucine and an intravenous [^{2}H$_3$]leucine tracer in human subjects fed intrinsically labeled casein than those fed extrinsically labeled AAs simulating casein. Similar findings were reported using a single meal that contained either casein or free AAs mimicking casein (40). Thus, evidence is consistent with increased protein retention and decreased oxidation of AAs in association with a slower rate of absorption of AAs when the dietary protein source is an intact protein, such as GMP, compared with a free AA source, such as AA formula.

Evidence of improved protein retention with the GMP diet was also shown by a lower serum BUN and higher plasma insulin and total AA concentrations when measured 2.5 h after eating a breakfast containing GMP compared with one containing AAs (Table 5, Figure 1). Urea is produced linearly in response to plasma AA concentrations, and control of nitrogen balance is primarily regulated by urea production (41). BUN, as a measure of hepatic utilization of AAs for urea synthesis, would be expected to remain lower with slower splanchnic AA release (40, 42). Thus, a slower, more gradual and sustained elevation in plasma AA concentration with an intact protein source (33), in conjunction with a lower BUN concentration, suggests that fewer AAs are degraded for urea production and instead are retained for protein synthesis when GMP is substituted for synthetic AAs as the primary protein source. Postabsorptive AAs, including isoleucine and threonine (35, 36), are known to stimulate insulin release with subsequent stimulation of protein synthesis and inhibition of protein degradation (34). Because

TABLE 6
Effect of amino acid (AA) and glycomacropeptide (GMP) diets on fasting and postprandial (PP) concentrations of AAs in plasma[1]

AA	AA diet			GMP diet			Response to diet fasting compared with PP *P* value[4]
	PP[2]	Fasting[2]	*P* value[3]	PP[2]	Fasting[2]	*P* value[1]	
	μmol/L			*μmol/L*			
Alanine	455 ± 52	356 ± 25	0.029	514 ± 45	401 ± 50	0.001	0.743
Arginine	62 ± 14	57 ± 5	0.694	47 ± 5	47 ± 6	0.845	0.551
Citrulline	37 ± 4	26 ± 5	0.138	23 ± 3	26 ± 4	0.084	0.063
Cystine	43 ± 1	43 ± 2	0.899	37 ± 2	41 ± 3	0.019	0.062
Glutamic acid	40 ± 8	50 ± 10	0.207	43 ± 10	49 ± 9	0.556	0.692
Glutamine	635 ± 29	628 ± 15	0.747	659 ± 33	623 ± 28	0.025	0.095
Glycine	415 ± 62	399 ± 55	0.473	346 ± 40	371 ± 47	0.099	0.112
Histidine	85 ± 9	78 ± 5	0.206	82 ± 6	75 ± 4	0.066	0.681
Isoleucine	57 ± 6	49 ± 4	0.352	119 ± 15	54 ± 5	0.015	0.004
Leucine	120 ± 20	96 ± 3	0.313	86 ± 11	83 ± 4	0.823	0.264
Lysine	210 ± 19	181 ± 9	0.062	191 ± 24	171 ± 16	0.138	0.311
Methionine	24 ± 2	24 ± 1	0.832	31 ± 4	25 ± 3	0.150	0.144
Ornithine	74 ± 7	60 ± 8	0.004	50 ± 4	62 ± 18	0.460	0.129
Phenylalanine	462 ± 100	508 ± 95	0.048	472 ± 106	483 ± 101	0.349	0.037
Proline	200 ± 15	144 ± 12	0.001	234 ± 37	160 ± 20	0.012	0.322
Serine	131 ± 21	122 ± 16	0.123	127 ± 14	120 ± 11	0.517	0.821
Taurine	73 ± 19	82 ± 18	0.154	73 ± 13	63 ± 11	0.145	0.027
Threonine	158 ± 22	135 ± 17	0.013	354 ± 44	265 ± 29	0.030	0.064
Tryptophan	48 ± 5	44 ± 3	0.269	34 ± 3	42 ± 2	0.048	0.006
Tyrosine	81 ± 8	38 ± 3	0.001	56 ± 10	29 ± 3	0.027	0.155
Valine	240 ± 9	194 ± 13	0.015	241 ± 21	187 ± 5	0.048	0.521
BCAA	417 ± 28	347 ± 14	0.084	445 ± 45	324 ± 8	0.048	0.062

[1] Values are means ± SEMs; *n* = 6, except for cystine for which *n* = 5. BCAA, sum of leucine, isoleucine, and valine.

[2] PP plasma concentrations of ornithine (*P* = 0.019) and tryptophan (*P* = 0.003) were significantly lower, but within the normal range, and isoleucine (*P* = 0.003) and threonine (*P* = 0.004) were significantly higher with ingestion of the GMP diet than with the AA diet and above the normal range. The only significant differences in fasting AA concentrations were a decrease in arginine (*P* = 0.008) and an increase in threonine (*P* = 0.001) with ingestion of the GMP diet compared with the AA diet.

[3] There was a significant effect of time in the repeated-measures ANOVA. Statistical analysis by paired *t* test, pairing on subject, is from data collected on the last day of the AA diet (day 4) and the last day of the GMP diet (day 8).

[4] The response to a diet is calculated first by finding the difference between fasting and PP AA concentrations for each subject on the AA diet and on the GMP diet and then by comparing the differences by paired *t* test, pairing on subject.

GMP induces a slower and more prolonged release of amino acids, the insulin response and stimulus of net protein synthesis may be potentiated. In addition, whey protein has been shown to increase insulin concentration to a greater extent than other milk protein fractions or other intact protein sources (43). Thus, the ability of GMP to slow AA catabolism and ureagenesis may reflect increased postprandial concentrations of threonine and isoleucine acting as insulin secretagogues as well as delayed absorption of AAs.

Arginine has multiple functions, which include serving as a substrate for synthesis of protein, urea, and nitric oxide. The cofactor for *PAH*, tetrahydrobiopterin, is also the cofactor for nitric oxide synthetase (44, 45). Arginine is synthesized in the kidney from intestinal citrulline derived from glutamine, but it is considered to be a conditionally indispensable AA in severely stressed patients (46). Recent evidence in healthy adults fed a diet free of both arginine and precursors of arginine for 4 wk shows that arginine is a nutritionally dispensable AA; however, the functional requirement for arginine is unknown (42). Consistent with minimal arginine in GMP, plasma arginine concentrations were significantly lower with ingestion of the GMP compared with the AA diet. Taken together, we conclude that it is prudent to supplement GMP with arginine for utilization in the PKU diet.

For this study, GMP was supplemented with the following 5 limiting AAs on the basis of the 2002 DRI recommendations (13): histidine, leucine, methionine, tryptophan, and tyrosine. Plasma concentrations of histidine, leucine, and tryptophan remained within the normal range, which suggests adequate supplementation of these AAs in the GMP diet (Table 6). Recent findings of a lower minimum requirement for methionine plus cysteine for school-age children (47) and adults (48) suggest that methionine supplementation is not required in GMP. In contrast, plasma concentration of tyrosine was below the normal range when measured in the fasting state (Table 6), which suggests that additional tyrosine supplementation may be required in GMP. Indeed, additional tyrosine from a supplement providing 1000 mg/d allowed for plasma tyrosine concentrations to remain within the normal range for one subject who consumed GMP as his primary protein source for 10 wk (12). In summary, our data suggest that GMP must be supplemented with arginine, histidine, leucine, tryptophan, and tyrosine to provide a complete source of dietary protein in the PKU diet.

Lifelong adherence to the PKU diet is very difficult, often resulting in poor compliance and the neuropsychological consequences of hyperphenylalaninemia (1, 6–8). This research shows a new, improved paradigm for the PKU diet through the
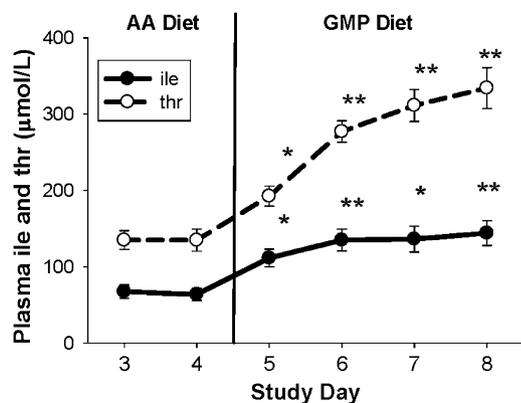
FIGURE 4. Concentrations of threonine and isoleucine in postprandial plasma after consuming the glycomacropeptide (GMP) diet for 4 d (days 5–8). Values are mean ± SEM; $n = 11$, of plasma obtained 2.5 h after breakfast. For study days 3 and 4, all subjects consumed an amino acid (AA) diet; on days 5–8, all AA formula was replaced with GMP food products. There was a significant effect of time in the repeated-measures ANOVA. *Significantly different from the last day of the AA diet (day 4), $P < 0.05$ (paired t test, pairing on subject). **Significantly different from the last day of the AA diet (day 4), $P < 0.0001$. There was no further significant increase in plasma concentration of isoleucine and threonine after days 5 and 7, respectively. ile, isoleucine; thr, threonine.

use of palatable foods and beverages made with the intact, low-phenylalanine protein GMP instead of synthetic AAs. When supplemented with limiting indispensable AAs, GMP appears to be a safe and acceptable alternative to synthetic AAs as the primary protein source for nutritional management of PKU. As an intact protein, GMP delays absorption of AAs and improves protein retention and phenylalanine utilization compared with a diet that provides the majority of nitrogen from AAs. Further research is required to investigate the long-term nutritional safety of GMP and its ability to reduce concentrations of phenylalanine in blood and brain and to improve compliance with the PKU diet.

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Erratum

Eaton SB, Konner MJ, Cordain L. Diet-dependent acid load, paleolithic nutrition, and evolutionary health promotion. Am J Clin Nutr 2010;91:295–7.

The article title in the print version of this article should have appeared as ''Diet-dependent acid load, Paleolithic nutrition, and evolutionary health promotion.'' The title has been corrected in the online version of the article.

doi: 10.3945/ajcn.2010.29198.

Erratum

Ashokkumar B, Mohammed ZM, Vaziri ND, Said HM. Effect of folate oversupplementation on folate uptake by human intestinal and renal epithelial cells. Am J Clin Nutr 2007;86:159–66.

In the first line of the abstract on page 159, a letter was inadvertently left out of the second word in the sentence. The word *aid* should be replaced with *acid* (ie, ''Folic acid''). The sentence should read as follows: ''Folic acid plays an essential role in cellular metabolism.''

doi: 10.3945/ajcn.2010.29240.

Erratum

van Calcar SC, MacLeod EL, Gleason ST, et al. Improved nutritional management of phenylketonuria by using a diet containing glycomacropeptide compared with amino acids. Am J Clin Nutr 2009;89:1068–77.

In the third full sentence on page 1073, the word *lower* should be replaced with *higher*. The sentence should read as follows: ''Serum carbon dioxide content, which is primarily bicarbonate, was significantly higher with the GMP diet compared with the AA diet, which is consistent with a lower systemic acid content.'' With this correction, the sentence will be in agreement with the data for carbon dioxide content presented in Table 5 on page 1072.

doi: 10.3945/ajcn.2010.29241.