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Purification and Use of Glycomacropeptide for Nutritional Management of Phenylketonuria

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Abstract

Individuals with phenylketonuria (PKU) cannot metabolize phenylalanine (Phe) and must adhere to a low-Phe diet in which most dietary protein is provided by a Phe-free amino acid formula. Glycomacropeptide (GMP) is the only naturally occurring protein that does not contain Phe, and is of interest as a source of protein for dietary management of PKU. However, commercially available GMP contains too much Phe from residual whey proteins and does not contain adequate levels of all the indispensable amino acids to provide a nutritionally complete protein. The aim of this study was to increase purity of GMP and develop a mass balance calculation for indispensable amino acid supplementation of GMP foods. Cation exchange chromatography, ultrafiltration/diafiltration, and lyophilization were used at the pilot plant scale to decrease Phe. Enough purified GMP (5 kg) was manufactured to provide 15 PKU subjects with a 4-d diet in which the majority of protein was from GMP foods. A mass balance was used to supplement GMP foods so that all indispensable amino acids met or exceeded the daily recommended intake. GMP foods were tested in a human clinical trial as a replacement for the traditional amino acid formula. Nutritionally complete GMP foods created with high purity GMP provide individuals with PKU with more options to manage PKU, which may lead to improved compliance and quality of life.

Keywords

foods; glycomacropeptide; phenylalanine; phenylketonuria; purification

Introduction

Dietary restrictions of phenylketonuria

Phenylalanine (Phe) is an indispensable amino acid that is converted to tyrosine (Tyr) by the enzyme phenylalanine hydroxylase (PAH; EC 1.14.16.1) in an individual with normal metabolism. About 1 in every 15000 infants born annually have absent or impaired function of this enzyme and are diagnosed with the metabolic disorder phenylketonuria (PKU) (Scriver 2001). If the diet of an individual with PKU is not modified within the first 20 d of

life, Phe and its breakdown products accumulate in the blood and brain, causing neurological damage and mental retardation (Scriver 2001). Foods such as meat, dairy, legumes, and bread must be avoided by individuals with PKU because of the high Phe content (Rutherford and Poustie 2005). Dietary management of PKU requires a low-Phe diet, suggested for life, wherein the majority of dietary protein is supplied by an amino acid formula, free of Phe. Natural foods are allowed in the low-Phe diet and are based mainly on fruits and vegetables (Weetch and MacDonald 2006). A daily tally of total Phe consumption for adults older than 19 y of age must not exceed a target value from 220 to 770 mg/d for female or 290 to 1200 mg/d for male (Acosta and Yanicelli 2001). The diet for PKU is difficult to follow, restrictive, and unpalatable; noncompliance can create neuropsychological impairment (Smith and others 1993; Shaw and Lawson 1994; Poustie and Rutherford 2000). Palatable foods that provide protein but are low in Phe are needed to increase compliance and quality of life for individuals with PKU.

Glycomacropeptide

Glycomacropeptide (GMP) is the only naturally occurring protein that contains no Phe. GMP is formed during cheese making when chymosin specifically cleaves κ -casein between the 105 to 106 amino acid residues. Para- κ -casein (residues 1 to 105) coagulates, forming cheese curd, while GMP (residues 106 to 169) remains in the whey (Walstra and others 2006). GMP is the 3rd most abundant protein in whey, after β -lactoglobulin (BLG) and α -lactalbumin (ALA) and makes up 15% to 25% of the total whey protein. GMP is present at a concentration of 1.2 to 1.5 g/L in whey (Thoma-Worringer and others 2006). GMP is highly polar and is glycosylated by galactosamine, galactose, and α -sialic acid at one or more threonine amino acid sites (Walstra and others 2006). Pure GMP contains 47% (w/w) indispensable amino acids, but contains no histidine (His), tryptophan (Trp), Tyr, arginine (Arg), Cysteine (Cys) or Phe.

Isolation of GMP from whey

Current large-scale technologies to isolate GMP from whey use ion exchange chromatography or ultrafiltration (Kawasaki and others 1994; Etzel 2004). GMP has an isoelectric point (pI) below 3.8 (Etzel 2004), whereas other major whey proteins have pI values above 4.3 (Walstra and others 2006). This physicochemical difference between GMP and other whey proteins is commonly used in isolation processes to separate GMP from whey (Thoma-Worringer and others 2006). Commercially available GMP isolated by ion exchange chromatography is not pure enough for PKU foods because it contains too much Phe from residual whey proteins (5 mg Phe/g product, manufacturer literature, Davisco Foods Intl., Eden Prairie, Minn., U.S.A.). Traditional amino acid formula is free of Phe, which allows an individual with PKU to consume natural foods that contain Phe to meet their daily allowance. In order for GMP to be a feasible protein replacement for amino acid formula in the PKU diet, improved processes are needed to increase GMP purity and reduce Phe content.

Protein and amino acid requirements in the PKU diet

Daily recommended intakes (DRI) of indispensable amino acids for individuals older than 1 y have been established based on the nitrogen content of foods (Table 1). DRI values for amino acids are often reported on a protein equivalent (PE) basis defined as total nitrogen (N) times a conversion factor of 6.25 (IOM 2005). Most proteins contain about 16% nitrogen and a conversion factor of 6.25 (100 g protein/16 g N = 6.25 g protein/g N) is appropriate (Nielsen 2003). However, the nitrogen to protein conversion factor varies among foods. For example, the nitrogen to protein conversion factor used for most dairy proteins is 6.38 (Nielsen 2003). GMP is a heterogeneous peptide, some GMP molecules are glycosylated and others are not. Because of this, the conversion factor for a GMP molecule

can range from 6.70 to 9.55 depending on the extent of glycosylation present (Dziuba and Minkiewicz 1996). Ambiguity in the definition of protein mass is illustrated by one manufacturer of GMP that uses a conversion factor of 6.47 to report protein and 7.07 to report GMP (Davisco Foods Intl.).

The actual protein content of foods is difficult to measure when the precise nitrogen to protein conversion factors are unknown. Proteins may contain nonprotein nitrogen, or basic amino acids, which are higher in nitrogen than other amino acids. For GMP to provide the recommended levels of indispensable amino acids in the diet, the protein composition of foods consumed must be known using an unambiguous definition of what constitutes 1 g of protein. In this study, a conversion factor of 6.25 was used to comply with the Inst of Medicine (IOM 2005) definition of protein used to establish the DRI for indispensable amino acids for individuals older than 1 y (Table 1).

GMP as a suitable source of protein in the PKU diet

GMP contains no His, Tyr, Trp, Cys, Arg, or Phe, and is low in methionine (Met) and leucine (Leu). His, Trp, Phe, Met, and Leu are all indispensable amino acids. Cys, Tyr, and Arg are conditionally indispensable amino acids because Met is a precursor to Cys, Phe is a precursor to Tyr, and glutamate, proline and aspartate are precursors to Arg. As a result, GMP must be supplemented to provide a nutritionally complete protein. Supplemented GMP has been found to be a safe alternative to an amino acid diet in a murine model, and the use of GMP foods holds promise to improve the quality of and compliance to the PKU diet (Ney and others 2008a, 2008b).

To conduct a human clinical trial to test the safety and feasibility of GMP foods for individuals with PKU, a pilot scale process was developed to prepare highly purified GMP sufficient to feed 15 individuals for 4 d. Food-grade materials and food-approved facilities were utilized to manufacture 5 kg of purified GMP using the following unit operations in sequence (1) cation exchange chromatography, (2) ultrafiltration and diafiltration (UF/DF), and (3) lyophilization. Furthermore, a mass balance calculation was developed to provide a clearly defined basis for amino acid supplementation. Purified, supplemented GMP was used to prepare GMP foods consumed in the human clinical trial.

Materials and Methods

This section has been divided into 3 subsections: (1) unit operations used to manufacture purified GMP using food-grade materials, (2) mass balance calculations used to determine GMP recovery and the mass of amino acids for supplementation of purified GMP, and (3) preparation and analysis of a GMP food consumed in the clinical trial and 1 patient response.

GMP purification process

Contaminating whey proteins in crude GMP (BioPure GMP, Davisco Foods Intl.) were trapped by adsorption onto a cation exchange resin and GMP was collected in the flow-through fraction. Ultrafiltration/diafiltration (UF/DF) was used to concentrate the GMP and wash out peptides, salts, and nonprotein nitrogen. Lyophilization was used to dry the purified, concentrated GMP.

Cation exchange chromatography—A 20-cm-dia chromatography column (INdEX, GE Healthcare, Piscataway, N.J., U.S.A.) was packed with SP Sepharose Big Beads (GE Healthcare). The column volume (CV) was 5.34 L, and the bed height was 18 cm.

Feed solution (75 g/L) was prepared by mixing crude GMP (BioPure GMP, Davisco Foods Intl.) with 10 mM sodium lactate, pH 4, and filtering through a 0.45- μ m pore size filter (Sartobran P, Sartorius, Edgewood, N.Y., U.S.A.). Equilibration and elution buffers were food-grade 50 mM sodium lactate, pH 4, and 10 mM NaOH, pH 12, respectively. Equilibration buffer and GMP feed solution were held at 4 °C to inhibit microbial growth. Elution buffer was held at 22 °C. In previous studies, temperature did not affect the outcome of the chromatography procedure (Tek and others 2005). Flow rate was 950 mL/min. Each cation exchange cycle consisted of 4 steps: (1) the column was brought to pH 4 using 2 CV of equilibration buffer, (2) 0.5 CV of feed solution was applied to the column, (3) the column was rinsed with equilibration buffer wherein the first 0.3 CV of effluent was discarded (column dead volume) and the next 2 CV of purified GMP was collected, and (4) bound proteins were desorbed from the column using 2.5 CV of elution buffer. Each of the 5 campaigns consisted of 9 to 11 consecutive cycles. About 100 L of dilute GMP protein solution were generated from each campaign. The cation exchange column was cleaned by pumping in 0.2 M NaOH, holding for 1 h, and then storing the column in 10 mM NaOH.

Ultrafiltration and diafiltration—GMP effluent from the cation exchanger was adjusted to pH 7 by addition of 1 M NaOH, and concentrated at 60 °C using a hollow fiber ultrafiltration membrane (3 kDa, 3.3 m², UFP-3-C-55, GE Healthcare). The applied pressure was 1.4 bar. GMP solution was concentrated from 100 to 10 L, and then 20 L of distilled water added and the solution was concentrated again to 10 L. The concentrate was filtered using a 0.45- μ m pore size filter (Sartorius) into a sanitized container and stored at 4 °C. Before and after each use, the UF membrane was cleaned for 30 min using 0.2 M NaOH containing 100 ppm NaOCl (bleach) at 50 °C. The UF membrane was stored in 10 ppm NaOCl.

Lyophilization—Concentrated, sterile-filtered GMP solution was frozen into a thin layer onto 1.2 or 2 L glass lyophilization flasks and dried for 48 h (Lyphlock6, Labconco, Kansas City, Mo., U.S.A.). GMP powder was recovered, weighed, and a portion used for analysis.

Composition analysis—Crude protein analysis (CP) and complete amino acid profiling (AAP) were conducted by the Experiment Station Chemical Laboratories (Univ. of Missouri-Columbia, Columbia, Mo., U.S.A.). AOAC official method 982.30 was used for AAP, and AOAC official method 990.03 was used for CP (AOAC 2005). Results were reported as gram per 100 g dry weight of purified GMP. A conversion factor of 6.25 times total nitrogen was used to express the results on a PE basis. Duplicate analyses were performed for all samples.

Mass balance calculations

Mass balance calculations are presented to describe (1) the calculations used to determine GMP recovery from the manufacturing process, (2) the lysine basis used for amino acid supplementation, and (3) the method used to determine the required amount of supplemental amino acids.

GMP recovery—Recovery was calculated on a PE basis. Grams of PE in the feed solution ($M_{PE,feed}$) were determined by multiplying the GMP feed concentration (75 g/L) by the grams of PE per gram of powder (from CP analysis) and then multiplying by the total volume of feed solution processed. The total grams PE recovered ($M_{PE,recovered}$) was obtained by multiplying the mass of purified GMP powder times the grams of PE per gram of purified GMP (from CP analysis). GMP recovery (%) was equal to $M_{PE,recovered}/M_{PE,feed} \times 100$.

Lysine basis for amino acid supplementation—Amino acid supplementation is required for purified GMP to meet the nutritional targets set by the DRI and the clinical trial (Figure 1). Lysine (Lys) was chosen as the basis for the mass balance supplementation calculation, because it was closest to the target value (Table 2, column C compared with D). Only 5 indispensable amino acids for PKU required supplementation: His, Leu, Met, Trp, and Tyr. Dispensable and conditionally indispensable amino acids were not supplemented.

Free amino acids are absorbed and degraded faster than those provided by intact proteins (Ney and others 2008b). Therefore, the target amino acid composition for the clinical trial was set above the DRI levels. Targets for His, Leu, Met, and Trp were set at 130% of the DRI level. Tyr was supplemented at 150% of the DRI level, because amino acid formulas are often enriched with high levels of Tyr (Spronsen and others 2001). In the tables and figures pertaining to the supplementation of GMP to meet the DRI, Phe and Tyr are listed together, as are Met and Cys, because Tyr and Cys are conditionally dispensable amino acids that can be synthesized from Phe and Met, respectively (IOM 2005). However, in a PKU patient, Tyr cannot be synthesized from Phe.

Amino acid supplementation calculations—A challenge with supplementation is that adding amino acids to the purified GMP changes the amino acid target (mg amino acid/g PE) by changing both the numerator (mg amino acid) and the denominator (g PE). Two methods will be illustrated for the supplementation calculation: one includes the change in the denominator and the other does not. Both methods account for the change in the numerator. The steps used to calculate amino acid supplementation are presented in Table 2. The experimental results for AAP were used to obtain the milligram of each amino acid per gram of purified GMP (column A). The experimental results for CP (column B) were divided into column A to obtain the GMP amino acid composition on a PE basis (column C). Conversion to a PE basis (column C) was needed to compare the purified GMP amino acid composition to the clinical trial target values (column D). The amino acid values of purified GMP were subtracted from each of the clinical trial target values to yield the required mass of each supplemented amino acid (column E). By adding the required amino acid values (column E) to the purified GMP amino acid values (column C), the composition of the supplemented GMP was calculated (column F). This method for the supplementation calculation ignored the impact of adding amino acids on the denominator of the amino acid composition (mg/g PE).

To account for the change in the denominator, the increase in the grams of PE per gram of purified GMP due to supplementation must be taken into account (Table 2, columns G and H). To do this, the total nitrogen contribution of the supplemental amino acids needed for 1 g of purified GMP was determined from the molecular formula and multiplied times 6.25 to yield the total grams of PE contributed by the added amino acids. The grams of PE from the supplemented amino acids were added to the grams of PE per 1 g of purified GMP (column B) to yield the corrected grams of PE per gram of purified GMP (column G). The supplemented composition (column F) was multiplied by column B and divided by column G to account for the change in the denominator (column H).

The corrected supplemented GMP composition was not statistically significantly different from the uncorrected composition (column H compared with F) ($P > 0.05$). Therefore, the calculation method used to supplement purified GMP in this study assumed that the contribution of added amino acids to the denominator was negligible.

Preparation, analysis, and safety of GMP foods

Preparation and analysis of GMP foods—The recipe for GMP strawberry pudding is presented in Table 3. Purified GMP, supplemental amino acids, and nondairy creamer

(Flavorite Non-Dairy Creamer, SuperValu, Eden Prairie, Minn., U.S.A.) contributed amino acids to the GMP strawberry pudding. Amino acid contribution from purified GMP, nondairy creamer, and added amino acids were used to calculate the amino acid composition of the pudding on a PE basis using the method presented in Table 2.

Safety of GMP foods for PKU subjects—Individuals with PKU were involved in an 8-d study to test if purified supplemented GMP was a safe and palatable protein source for individuals with PKU. The first 4 d of the study, subjects consumed their usual amino acid formula diet. The last 4 d of the study, the amino acid formula was replaced with GMP foods. The 2 diets were isocaloric and contained the same amount of protein and Phe. Blood samples were collected as described by Ney and others (2008a). Plasma measurements were completed 2.5 h after eating breakfast. Plasma amino acid profiles were determined for 4 female subjects with PKU, 19 to 29 y old.

Statistical analysis

Statistical analysis was performed using a one-way analysis of variance (ANOVA) (Minitab Statistical Software, Release 13.32, State College, Pa., U.S.A.) to compare the composition of supplemented GMP with and without taking the denominator into account (Table 2). Confidence intervals were constructed to compare the calculated composition of GMP strawberry pudding to the observed composition and the DRI values. A one-way ANOVA was used to compare plasma amino acid concentrations of each amino acid during the GMP foods diet compared to the amino acid diet. A 95% confidence level was used to construct confidence intervals, and statistical significance was declared at $\alpha < 0.05$.

Results

The goal of this study was to produce purified GMP with reduced Phe in quantities great enough to supply 15 PKU subjects with GMP foods during their participation in an 8 d clinical trial. Purified GMP required supplementation with indispensable limiting amino acids to provide a nutritionally complete protein source for use in GMP foods. The safety and efficacy of GMP foods as a palatable source of protein for the PKU diet was tested. The following sections discuss how these objectives were met and have been separated into the effect of the pilot plant process on the recovery and Phe content of purified GMP, the mass balance on amino acids in GMP strawberry pudding and comparison of the amino acid composition of the GMP strawberry pudding to the DRI and an amino acid formula, and the effect of the purified, supplemented GMP foods on the plasma amino acid levels of PKU subjects.

Effect of purification process on Phe content and GMP recovery

Table 4 contains the Phe content, recovery, and number of cation exchange cycles for each campaign. Phe in crude GMP was reduced 47% by the purification process, from 4.7 ± 0.5 mg/g PE to 2.7 ± 0.4 mg/g PE. Average GMP recovery was $52 \pm 4\%$. The low recovery of GMP was attributed primarily to a portion of GMP that bound to the cation exchange column and was not recovered in the flow through fraction. The purification process used to increase the purity of commercially available GMP gave consistent, repeatable Phe concentrations, and there were no statistical differences between Phe concentrations in purified GMP produced by the 5 campaigns ($P > 0.05$).

GMP transmission through the UF membrane for each campaign is presented in Table 5. Overall, GMP retention by the UF membrane was $96 \pm 2\%$. Although GMP has a molecular weight of about 7 kDa (Mikkelsen and others 2005), it has an apparent molecular weight of 45 kDa at pH 4 and above (Ayers and others 2003). The pH of GMP solution was increased

to 7 for the UF/DF step to minimize GMP transmission through the 3 kDa membrane. This likely explains the high recovery found for the UF step.

Comparison of amino acid supplementation calculations and GMP food composition

The GMP strawberry pudding was analyzed and the composition compared to the calculated amino acid composition (Table 6). Purified GMP (Table 6, column A) was supplemented with amino acids (column B), determined using the mass balance method that ignored the change in denominator due to added amino acids; similarly for the added amino acids from the nondairy creamer (column C). The sum of columns A, B, and C was the calculated composition of the GMP pudding ignoring changes in the denominator (column D).

For comparison, the amino acid composition of the GMP strawberry pudding was calculated to include the changes in the denominator (column E). Ignoring the contribution to the denominator resulted in a 30% overestimation, on average, of the calculated amino acid composition compared to the observed values (column D compared with F). By including the contribution to the denominator, the corrected calculated amino acid composition was not statistically different from the observed composition ($P > 0.05$), except for Tyr ($P < 0.05$) (column E compared with F). The observed amino acid composition of the GMP strawberry pudding (column F) met or exceeded all of the DRI target values (Table 1).

The amino acid composition of GMP strawberry pudding was compared to an amino acid formula (Figure 2). The amino acid formula contained significantly more His, Leu, Met + Cys, Tyr, and Trp than the GMP strawberry pudding ($P < 0.05$). However, both amino acid formula and GMP strawberry pudding met or exceeded DRI targets for all indispensable amino acids ($P > 0.05$, Table 1).

Effect of purified, supplemented GMP foods diet on PKU subjects

Plasma amino acid titers are an indication of proper nutrition and metabolism. Despite differences between plasma amino acids on the 2 diets, all amino acids (except Phe) were within the normal range ($P > 0.05$) and the GMP diet was safe (Table 7). Ile and Thr were statistically significantly higher for the GMP diet than the amino acid formula diet ($P < 0.05$), which was attributed to the high concentration of Thr and Ile in GMP. Cys, Tyr, and Trp were statistically significantly lower for the GMP diet than the amino acid diet ($P < 0.05$).

Discussion

Manufacture of purified GMP

The low overall recovery of GMP ($52 \pm 4\%$) was attributed to interactions between GMP and the cation exchange column and resulted in a portion of GMP binding to the column. The binding of GMP to the column was attributed to heterogeneity of GMP. Some GMP molecules were less negatively charged than others at the operating pH of 4 and therefore bound to the cation exchange column. Operating at a higher pH may have minimized GMP binding to the column by increasing the negative charge on GMP. This would also cause a decrease in electrostatic attraction between residual whey proteins and the cation exchange column as the positive charge on these proteins would be decreased. Increasing the operating pH to greater than 4 would have compromised purity. Purity was a priority over recovery in the production of GMP used in the clinical trial.

UF/DF removed low molecular weight solutes and concentrated the GMP prior to the final drying step. UF/DF cannot remove contaminating whey proteins, such as ALA and BLG, because these proteins are too large to permeate a 3 kDa membrane. On the other hand, low

molecular weight solutes, such as whey peptides, are small enough to be removed by UF/DF and may contain Phe.

Lyophilization produced a fine white powder with no flavor or odor and dissolved clearly in water (data not shown). However, the disadvantage of this drying method was the long processing time. Each other step in the process could be completed in 1 d, but lyophilization took several days to complete. Despite the intensive time requirement, lyophilization was the most practical choice to dry purified GMP for use in this study. Spray drying was not used because of the potential losses of GMP and limitations of the method when drying small quantities of product. In large-scale manufacture, spray drying would be the method of choice.

Amino acid supplementation of manufactured GMP

The calculation method used for supplementation of GMP ignored the grams of PE from added amino acids in the denominator, but was easily implemented and resulted in a GMP strawberry pudding that met or exceeded the DRI targets for all indispensable amino acids (Table 6). For GMP alone, ignoring the change to the denominator from added amino acids did not result in a statistically significantly different GMP composition (Table 2, column F compared with H).

Ignoring the change in the denominator from addition of both nondairy creamer and supplemented amino acids resulted in a 30% overestimation of amino acids compared to the observed value and 6 of the 9 amino acids were statistically significantly below the observed value (Table 6, column D compared with F). On the other hand, when the PE contributed by added amino acids were accounted for in the denominator, the corrected calculated values matched the observed values ($P > 0.05$) except for Tyr ($P < 0.05$) (Table 6, column E compared with F). However, Tyr was not statistically significantly different than the observed value at $\alpha < 0.01$ ($P > 0.01$). The lower than expected Tyr value was attributed to low purity of the Tyr supplement. Photodegradation of Tyr can occur (Kerwin and Remmele 2007) and could have taken place at some point during the manufacture or storage, which would lead to a lower than expected purity. Although the DRI for Tyr was met in the GMP food, increased Tyr supplementation would provide a higher level of Tyr.

Simplifications were reasonable for the supplementation calculations for GMP alone, due to the negligible impact on amino acid composition and ease of implementation, but were not reasonable to make when performing the mass balance calculations on the GMP food. GMP plus supplemented amino acids made up 15% (w/w) of the GMP pudding, whereas the nondairy creamer made up nearly 40% (w/w) of the pudding, and had a significant impact on the final composition.

For both diets, plasma amino acids of subjects were statistically significantly outside of the normal range for Phe only, which is a characteristic of PKU. However, there was no difference in the plasma Phe between the GMP and amino acid diets. This was anticipated, since the diets were controlled to provide equivalent amounts of Phe. All other indispensable amino acids were statistically within the normal range. Plasma Ile and Thr were approximately 2-fold greater in subjects during the GMP diet compared to the amino acid diet (Table 7). This was expected due to the high concentration of these amino acids in GMP compared to amino acid formula (Figure 2). A 2- to 3-fold increase in plasma Ile and Thr occurred in a murine model that was fed a supplemented GMP diet compared with amino acid or casein diets and was associated with a decrease in Phe levels in both plasma and brain (Ney and others 2008b). Dietary supplementation with large neutral amino acids, such as Ile and Thr has been shown to block Phe transport into brain tissue in mice (Ney and

others 2008b) and humans (Pietz and others 1999) with PKU and it is of interest to explore whether dietary GMP has a similar effect.

The nutritional requirement for amino acids is not a static subject. For example, recent evidence indicates it may be prudent to supplement GMP with the conditionally indispensable amino acid Arg for utilization in the PKU diet, and that lower requirements for Met and Cys may obviate the need for Met supplementation of GMP (van Calcar and others 2009). The mass balance calculation method of the present study is generically useful to supplement GMP foods to meet or exceed the nutritional needs of the human diet based on the most recent science.

Conclusions

GMP was purified and used, with amino acid supplementation, in a clinical trial to test the safety of purified, supplemented GMP as a source of protein in the PKU diet. Cation exchange chromatography, UF/DF, and lyophilization were used to produce 5 kg of high purity GMP from commercially available GMP to provide 15 PKU subjects with GMP foods for 4 d. Phe content of the GMP was reduced 47% by purification, allowing individuals to participate in the study who otherwise might not have been able to meet their protein needs without exceeding their Phe tolerance. Supplementation of GMP was required for limiting amino acids and was determined using a simplified calculation method. GMP foods met or exceeded the DRI for all indispensable amino acids. Production of increased purity GMP at the commercial scale holds promise to improve quality of life of PKU patients and their families for the dietary management of PKU.

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Figure 1.

Amino acid pattern of DRI, adjusted target for human clinical trials, and protein composition of purified (not supplemented) GMP produced in the present study. Values are mean $\pm$ SD. Sample size was $n = 10$.

Figure 2.

Amino acid profile of GMP strawberry pudding compared to amino acid formula (Phlexy-10 Drink Mix, SHS North America, Rockville, Md., U.S.A.) Values are mean $\pm$ SD. Sample size was $n = 2$. Same letter above GMP strawberry pudding and amino acid bars indicates that values are not statistically different ($P > 0.05$).

Table 1

Daily recommended intake (DRI) of indispensable amino acids for children 1 y of age and all other age groups. Adapted from IOM (2005).

Amino acid	DRI mg/g N	DRI mg/g PE ^a
His	114	18
Ile	156	25
Leu	341	55
Lys	320	51
Met + Cys	156	25
Phe + Tyr ^b	291	47
Thr	170	27
Trp	43	7
Val	199	32

^aPE = N × 6.25.

^bOnly Tyr was used to supplement GMP.

Table 2

Supplemental amino acid (AA) calculation method.

A	B	C = A/B	D	E = D - C	F = C+E	G	H = F × B/G
Amino acid	GMP AA composition ^A mg/g purified GMP	GMP PE ^A g PE/g purified GMP	GMP composition on PE basis mg/g PE	Clinical target trial mg/g PE	Ignores PE of added AA (not included in denominator)		Includes PE of added AA (included in denominator)
					AA supplementation required mg/g PE	Supplemented composition mg/g PE	
His	1.15 ± 0.07	---	1.5 ± 0.1	23	22	23.5 ± 0.1 ^a	21 ± 2 ^a
Ile	75.5 ± 4.2	---	102 ± 2	25	0	102 ± 2 ^a	90 ± 10 ^a
Leu	17 ± 1	---	23 ± 0.2	72	49	72 ± 0.2 ^a	66 ± 7 ^a
Lys	44 ± 3	---	60.1 ± 0.8	51	0	60.1 ± 0.8 ^a	55 ± 6 ^a
Met + Cys	15 ± 2	---	20.5 ± 0.5	33	12	32.5 ± 0.8 ^a	30 ± 3 ^a
Phe + Tyr	3 ± 1	---	2.6 ± 0.6	71	68	70.6 ± 0.6 ^a	64 ± 6 ^a
Thr	120 ± 10	---	161 ± 2	27	0	161 ± 2 ^a	150 ± 10 ^a
Trp	0 ± 0	---	0 ± 0	9	9	9 ± 0 ^a	8 ± 0.8 ^a
Val	62 ± 3	---	85 ± 2	32	0	85 ± 2 ^a	78 ± 8 ^a
PE	---	0.74 ± 0.05	---	---	---	---	0.81 ± 0.06

^AComposition obtained by analysis. Values are mean ± SD. Sample size was $n = 2$.Same letters in column F and H indicate no significant statistical difference ($P > 0.05$).

Table 3

GMP strawberry pudding recipe.

Ingredients	Dry mix percent % (w/w)
Purified GMP ^a	12.94
Supplemented amino acids ^a	
His	0.22
Leu	0.70
Met	0.11
Tyr	0.68
Trp	0.09
Food ingredients	
Nondairy creamer ^a	39.73
Sucrose	32.22
Starch	8.60
Strawberries, dried	2.13
Citric acid	1.59
Sodium chloride	0.57
Strawberry flavor	0.41
Red color	0.01
Dry mix total	100

^aPurified GMP, supplemental amino acids, and nondairy creamer contribute amino acids to final product.

Table 4

Phe content of purified and commercially available GMP.

Campaign	Cycles	Phe ^a mg/g PE	GMP recovery %
1	10	3.3 ± 0.2	51
2	11	2.6 ± 0.2	55
3	10	3 ± 0	55
4	9	2.2 ± 0.5	53
5	9	2.7 ± 0.1	44
Average purified GMP	---	2.7 ± 0.4	52 ± 4
Commercially available GMP	---	4.7 ± 0.5	---

^aComposition obtained by analysis. Values are mean ± SD. Sample size was $n = 2$.

Table 5

GMP transmission through UF membrane.

GMP campaign	GMP transmission (%)
1	3.4
2	6.8
3	2
4	3.6
5	2
Mean $\pm$ SD ($n = 5$)	4 ± 2

Table 6

Calculated and analyzed amino acid (AA) composition of GMP strawberry pudding.

A	B	C	D = A + B + C	E	F	
Amino acid	Purified GMP ^A mg/g PE GMP	Added AA from supplemented amino acids ^B mg/g PE GMP	Added AA from nondairy creamer ^A mg/g PE GMP	(Added AA not included in denominator)	(Added AA included in denominator)	
				Calculated AA composition ^B mg/g PE GMP	Corrected, calculated AA composition ^C mg/g PE Total	Analyzed AA composition ^A mg/g PE Total
His	1.5 ± 0.1	22	2.9 ± 0.6	26.4 ± 0.6 ^a	21 ± 3 ^a	18 ± 3 ^a
Ile	102 ± 2	0	5.6 ± 0.5	108 ± 2 ^a	83 ± 7 ^{a,b}	70 ± 10 ^b
Leu	23 ± 0.2	69	10 ± 1	102 ± 1 ^a	81 ± 6 ^b	77 ± 2 ^b
Lys	60.1 ± 0.8	0	6 ± 1	66 ± 1 ^a	51 ± 5 ^{a,b}	47 ± 8 ^b
Met + Cys	20.5 ± 0.5	11	4 ± 1	36 ± 1 ^a	28 ± 2 ^b	22 ± 3 ^b
Phe + Tyr	2.6 ± 0.6	66	8.7 ± 0.7	70.6 ± 0.9 ^a	63 ± 4 ^b	45 ± 3 ^c
Thr	161 ± 2	0	4.1 ± 0.7	165 ± 2 ^a	130 ± 10 ^a	120 ± 30 ^a
Trp	0 ± 0	9	1.7 ± 0.1	10.7 ± 0.1 ^a	8.7 ± 0.6 ^a	10 ± 2 ^a
Val	85 ± 2	0	7 ± 1	91 ± 3 ^a	70 ± 6 ^{a,b}	70 ± 10 ^b

^AComposition obtained by analysis. Values are mean ± SD. Sample size was $n = 2$.

^BCalculated without including added amino acids in the denominator (Method of Table 2, column F).

^CCalculated including added amino acids in the denominator (Method of Table 2, column H).

Same letter between the columns D, E, and F indicates that no difference was detected between means ($P > 0.05$).

A statistically significant difference was detected between columns E and F for Tyr + Phe at $\alpha < 0.05$ but not at $\alpha < 0.01$.

Table 7Postprandial plasma amino acids after consumption of amino acid diet or GMP foods diet.^A

Amino acid diet	Amino acid μmol/L	GMP foods diet μmol/L	Normal range μmol/L
His	90 ± 20 ^a	80 ± 10 ^a	41 to 125
Ile	60 ± 10 ^a	120 ± 50 ^b	30 to 108
Leu	120 ± 40 ^a	90 ± 30 ^a	72 to 201
Lys	220 ± 40 ^a	190 ± 60 ^a	48 to 284
Cystine	44 ± 6 ^a	38 ± 5 ^b	5 to 82
Met	23 ± 4 ^a	30 ± 10 ^a	10 to 42
Phe ^B	400 ± 200 ^a	400 ± 300 ^a	35 to 85
Tyr	90 ± 20 ^a	60 ± 30 ^b	34 to 112
Thr	170 ± 40 ^a	400 ± 100 ^b	60 to 225
Trp	50 ± 8 ^a	37 ± 7 ^b	10 to 140
Val	260 ± 30 ^a	240 ± 70 ^a	119 to 336

^A Determined from the last 2 d of the amino acid diet and the GMP diet, respectively. Plasma measurements completed 2.5 h after eating breakfast. Values are mean ± SD. Sample size was $n = 4$. Same letter between the amino acid diet and the GMP foods diet indicates that values are not statistically significantly different ($P > 0.05$).

^B Phe statistically significant outside of the normal range ($P < 0.05$).