Safety, efficacy and benefits of GMP-based medical foods

In order to facilitate and clarify the use of glycomacropeptide (GMP) in medical foods available to phenylketonuria (PKU) individuals, and specifically the protein substitutes developed by Ajinomoto Cambrooke, the following document provides literature reports on the impact of GMP in individuals with PKU and demonstrates the safety profile, efficacy, and benefits of GMP-based medical foods.

Background

Phenylketonuria (PKU) is an inherited metabolic disorder characterized by an absence of the enzyme phenylalanine hydroxylase (PAH) that breaks down the amino acid phenylalanine (Phe) (Scriver CR 2001). If left untreated, Phe levels accumulate in the blood and cause neurological and behavior disabilities (Scriver CR 2001). Current treatment is predominantly through dietary restriction of Phe to the minimum required for normal growth. A Phe restricted diet excludes all foods high in protein (i.e. meat, fish, poultry, dairy products, legumes and nuts). Due to the severe restriction of protein intake, PKU individual's diet include medical foods free from or low in Phe with the right mix of essential amino acids, vitamins, minerals and trace elements (Acosta PB 2010).

The ultimate goal of diet management is to achieve plasma Phe levels within recommended concentrations while maintaining adequate nutrition for normal growth and development (MRC 1993, NIH 2001). For individuals with PKU, the amount of Phe in the diet is restricted depending upon the level of PAH activity. Those with classical PKU must reduce dietary Phe to 200-500mg/day, whereas those with hyperphenylalanemia can tolerate more than 500mg/day (Scriver CR 2001). With the exception of many fruits and vegetables, one gram of protein in food contains approximately 50mg of Phe (Weetch E 2006).

Amino acid (AA)-based medical foods

Medical foods low or devoid of Phe have been manufactured since the 1960s. As mentioned above, these medical foods typically provide all the essential amino acids, with the exception of Phe, as well as the micronutrients missing in a diet which eliminates high protein foods. Most of these AA-based products have a bitter taste and smell which can be offensive, making the diet unpalatable and poor compliance a common issue. Many individuals with PKU frequently drink the formula in one sitting, despite research-based evidence where evenly spreading consumption throughout the day improves protein utilization and metabolic control (Schoeffer A 1994). Consumption of the AA-based medical food in one sitting at breakfast may

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also result in hunger throughout the day, which is compensated with intake of phe containing food resulting in poor control of Phe levels which may lead to neurological or psychosocial issues and poor quality of life (Simon E 2008).

Compliance in adolescent and adult patients is a common issue because of the detrimental neuropsychological consequences of high blood Phe levels. Finding a product which will support growth, health and compliance is an important part of treatment. Glycomacropeptide (GMP) is an alternative to traditional amino acid based medical foods and meets many of the medical and compliance needs of individuals with PKU.

GMP and GMP-based medical foods

GMP is a 64-amino acid peptide derived from cheese whey that is naturally low in Phe and is rich in valine, isoleucine and threonine (Etzel MR 2004) and a viable alternative to synthetic AAs in the PKU diet (reviewed in Ney DM 2014 and Hafid NA 2015).

GMP contains two to three times the amount of LNAA (Large Neutral Amino Acids) isoleucine, threonine and valine compared with other dietary proteins. Studies reported reductions in Phe concentrations in plasma or brain in individuals with PKU given supplementation with LNAAs (Pietz J 1999). Because LNAAs compete with Phe at the blood-brain barrier and for intestinal absorption, as they use the same transporter, this may explain the observed reductions on Phe (Pietz J 1999).

Purified GMP containing less than 2mg Phe per gram of protein is used for formulation of GMP-based medical food for PKU (Lim K 2007). Ajinomoto Cambrooke developed, in collaboration with the University of Wisconsin-Madison USA, a PKU protein substitute that is a proprietary blend of GMP and essential amino acids, under the brand name *Glytactin®*. One serving of such Glytactin® products contains **less than 20mg pf Phe**. As noted above, the daily allowed Phe concentration in Classical PKU is between 200-500mg. Therefore, each serving of the Glytactin® products is well below this range, thus allowing additional dietary intake. This amount of Phe can be accounted for in each individual's daily Phe prescription. Glytactin has been safely consumed by PKU individuals over the last 10 years in North America, South America, Australia and many countries in Europe (Italy, Portugal, Germany, UK, and Denmark). The use of GMP, specifically Glytactin, is also included in practice guidelines from GMDI (Genetic Metabolic Dietitians International) (see Figure 1) (Singh RH 2014).

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Classification	Amino acids, fat, carbohydrate, vitamins, and minerals	Amino acids, carbohydrate, vitamins, and minerals	Amino acids	Glycomacropeptide	Large neutral amino acids
Nutrient profile	Most complete	Most vitamins and minerals, no fat	Few or no vitamins and minerals and no fat	Variable depending on product; contains PHE	Variable depending on product
Energy/protein ratio (kcal/g protein)[b]	High to medium	Medium to low	Low	Variable	Low
Forms	Powder	Powder, ready-to-drink	Powder, capsules, tablets	Powder, ready-to-drink, bars, pudding	Powder, tablets
Products designed for infants	Periflex Infant[e], Phenex-1[f], Phenyl-Free 1[g]	None	None	None	None
Products designed for children[c]	Periflex Junior[e], Phenex-2[f], PhenylAde Essential[e], Phenyl-Free 2[g]	Lophlex[e], PhenylAde 40[e], PKU Coolers[h], PKU Gel[h], Maxamaid XP[e]	None	BetterMilk <12[i], Complete bars <12[i], Restore[i]	None
Products designed for adolescents and adults[d]	Periflex Advance, Phenex-2[f], PhenylAde Essential[e], Phenyl-Free 2[g], Phenyl-Free 2HP[g]	Lophlex LQ[e] PhenylAde RTD[e], PhenylAde 40[e], PhenylAde 60[e], PKU Coolers[h], Maxamum XP[e]	PhenylAde Amino Acid Blend[e] and Amino Acid Blend MTE[e], Phlexy-10[e]	BetterMilk 12+[i], Complete bars[i], Restore[i], Restore Lite[i], Swirl[i]	Lanaflex, PheBloc[e]

Figure 1: ACMG (American College of Medical Genetics), NIH, and GMDI recognize Glytactin GMP-MF as an Option for the Nutritional Management of PKU. Recommendations for the nutrition management of phenylalanine hydroxylase deficiency (Singh RH 2014).

The potential benefits of having GMP in the PKU diet have been explored in a number of studies.

The first human study with GMP was published in 2009 and is summarized in Figure 2. (Van Calcar SC 2009).

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Authors	Date	Title of Article and Findings
Van Calcar et al *American Journal of Clinical Nutrition*	Jan 2009	**Improved nutritional management of phenylketonuria by using a diet containing glycomacropeptide compared with amino acids.** • Eleven subjects participated in an inpatient observational metabolic study with two 4-day treatments: a current AA diet followed by a diet that replaced the AA formula with GMP supplemented with limiting AAs. • The GMP diet was **preferred** to the AA diet in 10 of 11subjects with PKU, and there were **no adverse reactions to GMP.** • When comparing fasting with postprandial, plasma Phe concentration increased significantly with the AA but not with the GMP diet. • There was no significant difference in Phe concentration in postprandial plasma with the GMP diet compared with the AA diet. • **Blood urea nitrogen was significantly lower with the GMP diet**, which suggests decreased ureagenesis • Plasma insulin was higher with the GMP diet than with the AA diet.

Figure 2: Improved nutritional management of phenylketonuria by using a diet containing glycomacropeptide compared with amino acids (Van Calcar SC 2009).

Evidence from studies in PKU indicates that GMP is a more physiologic source of low-Phe dietary protein compared with synthetic AA (Ney DM 2009, Van Calcar SC 2012). The most notable feature is that GMP-based medical foods provide predominantly intact protein compared with AA-based medical foods. It was previously shown that an intact protein is utilized more slowly than free AA and may increase utilization of AA for protein synthesis when compared with synthetic AA (Figure 3) (Gropper SS 1991).

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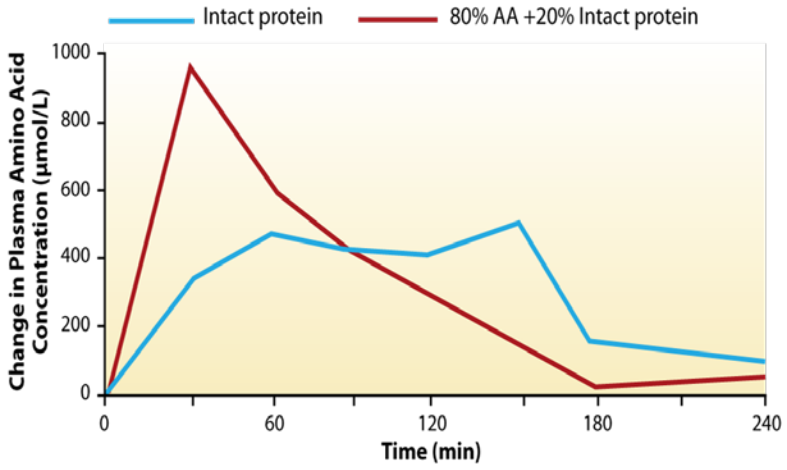

Figure 3: Intact Protein is Absorbed More Slowly than Free AA [Adapted from Gropper S et al. J Parenter Enteral Nutr. 1991 15(1):48-53.]

GMP consumption therefore mimics ingestion of intact proteins, because of slower absorption and decreased hepatic degradation of dietary intact protein compared with free AA (Dangin M 2001). Similar benefits were observed in a study with PKU individuals. The GMP diet showed significantly higher postprandial plasma AA absorption after a meal and significantly lower blood urea nitrogen concentration, suggesting decreased ureagenesis compared with the AA diet (Dangin M 2001, Van Calcar SC 2009). GMP may therefore delay absorption of AA and improve their utilization for protein synthesis.

When comparing fasting with postprandial Phe levels, fasting Phe concentrations were significantly greater with the AA but not with the GMP diet, while postprandial plasma Phe levels were not different with the AA and GMP diets, suggesting less daily variation in the Phe concentration over 24h with the GMP diet (Van Calcar SC 2009). Reduced fluctuation in Phe levels is important since a study suggests that long-term variation in plasma Phe concentrations may have significant impact on cognitive outcome (Anastasoaie V 2008). Sensory studies in individuals with PKU find medical foods containing GMP more palatable and of improved variety than their usual AA formulas and are acceptable alternatives to AA medical foods (Lim K 2007, Ney DM 2009, Van Calcar SC 2009).

Further evidence supporting the use of GMP in the PKU diet suggests that consuming GMP diet at breakfast promotes satiety in PKU individuals as reflected by decreased levels of the postprandial ghrelin concentrations (associated with greater feeling of fullness) when compared with the AA diet (MacLeod EL 2010).

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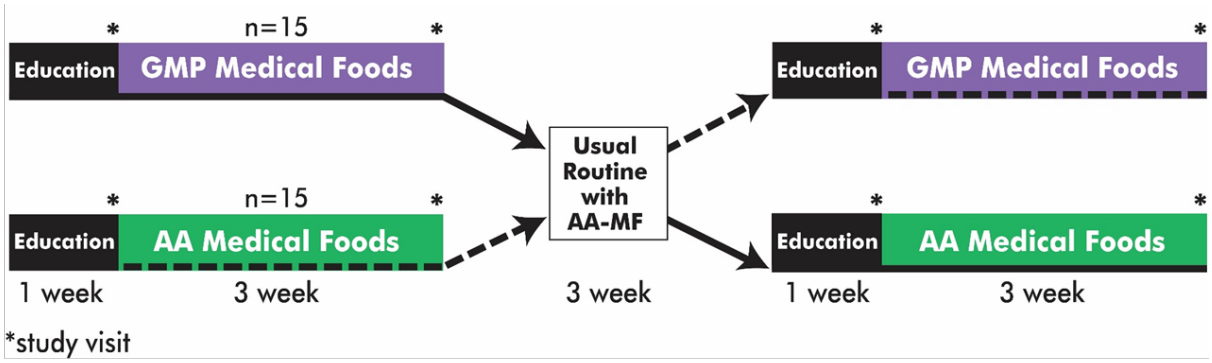
A recently published randomized, controlled, crossover trial investigated the efficacy and safety of a low-Phe diet combined with **Cambrooke's Glytactin** GMP-based medical foods (GMP-based MF) or AA-based MF providing the same quantity of protein equivalents in PKU individuals [(Ney DM 2016)]. The experimental design is demonstrated in Figures 4 and 5.

Demographics:

- 19 subjects at the University of Wisconsin-Madison Waisman Center
- 11 at Boston Children's Hospital
- 20 subjects with Classic PKU and 10 with Hyper-Phe
- 5 teens (15-17 years) and 25 adults (18-49 years)



Funded by FDA Office of Orphan Product Development, R01-FD003711; registered www.clinicaltrials.gov as NCT 01428258
* Glytactin products donated by Cambrooke Therapeutics

Figure 4: Experimental design of the randomized, controlled, crossover trial [(Ney DM 2016)].

Subjects were randomly assigned to a diet order of GMP diet, then wash-out for 3 weeks with AA diet and then AA diet, wash out for 3 weeks with AA diet and then GMP diet.

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Nutrient Intake	AA Diet	GMP Diet	P-value
Energy			
kcal/d	2113 ± 87	2265 ± 121	0.253
% energy from medical food/d	23% ± 2%	32% ± 2%	0.002
Total Protein, g/d	80 ± 3	79 ± 4	0.892
Protein from Natural Foods, g/d	26 ± 3	27 ± 3	0.651
Medical Food or Protein Substitute			
g protein from medical foods/d	54 ± 3	52 ± 3	0.527
% protein intake from medical food/d	68% ± 4%	66% ± 3%	0.603
Number of servings/d*	2.43 ± 0.2	3.74 ± 0.2	0.001

Values are mean ± SE, n=30 based on 3-d food records. *Daily medical food logs, visits 3-4, n=15

Figure 5: The diets were similar except for the type of MF (Ney DM 2016).

Calories and Protein Equivalents (PE)/day were consistent demonstrating a controlled study. Higher frequency of MF intake with GMP vs AA: 3.7/day vs 2.4/day.

As shown in Figure 6, there was no significant increase in plasma Phe, despite an increase in Phe intake from GMP, while blood Phe concentrations across time were not significantly different, suggesting similar Phe control (Ney DM 2016).

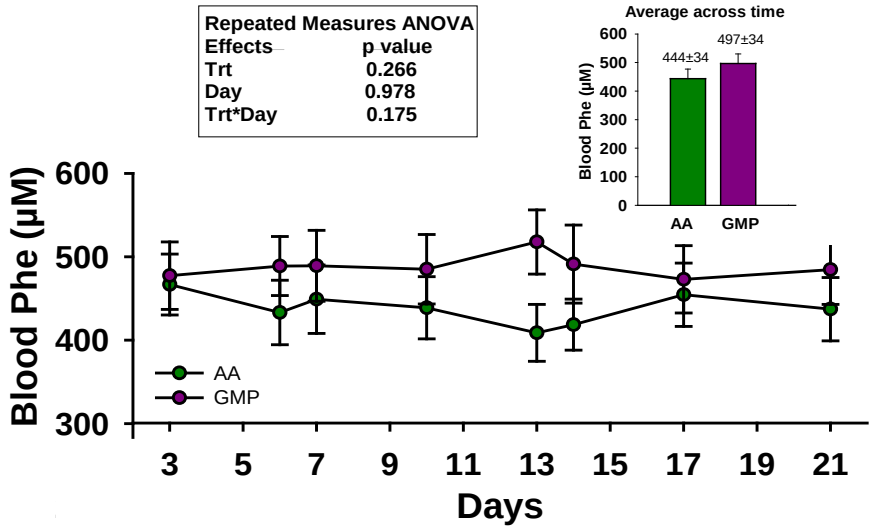

Figure 6: Similar Control of Blood Phe Over 3 Weeks with AA-based MF and Glytactin GMP-based MF (Ney DM 2016).

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A Retrospective chart review of 11 subjects utilizing Cambrooke's Glytactin GMP-based MF as part of their standard dietary therapy for PKU [Pinto A 2017 (i)].

- Ages 13 to 42 years of age (1 hyper Phe, 4 mild and 6 classical)
- Prescribed Glytactin GMP-based MF for 13 ± 7 months
- Dietary Phe intake was not decreased for Phe in Glytactin GMP-based MF (mean of 34 mg/day)
- Protein intake, nutritional intake, anthropometrics, and body composition remained similar

It was shown that serum Phe levels did not change significantly despite increased Phe intake from Glytactin GMP-based MF (see Figure 7), while serum Tyr levels were better with Glytactin GMP-based MF despite significantly lower intake vs AA-based MF (see Figure 8) [Pinto A 2017 (i)].

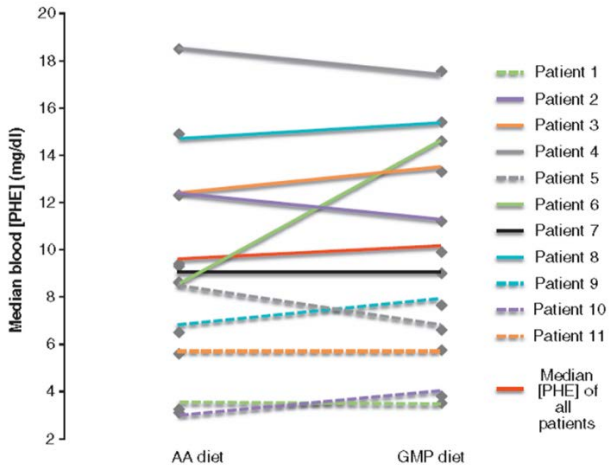

Figure 7: Comparing Serum Phe for AA-based MF vs Glytactin GMP-based MF [Pinto A 2017 (i)].

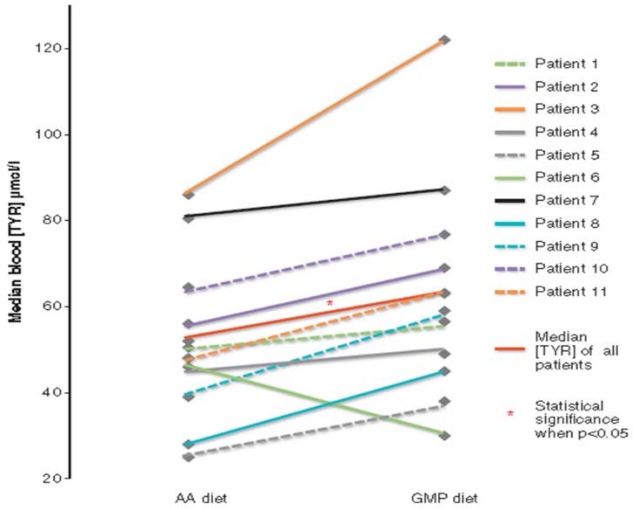

Figure 8: Comparing Serum Tyr levels for AA-based MF vs Glytactin GMP-based MF [Pinto A 2017 (i)].

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Serum Phe/Tyr ratio decreased which may indicate improved executive function, inhibitory control, and may reflect serotonin levels in the brain [(Pinto A 2017 (i))].

A retrospective study published in 2021 [(Pena MJ 2021)] evaluated the long-term (29 months) impact of GMP-based MF in 11 patients (ages 15-43). The products used were **Cambrooke's Glytactin** product range. Metabolic control, anthropometry, body composition and biochemical parameters were evaluated in patients taking L-AA versus GMP-based MF with different percentages of contribution (mean 66%) to the total protein substitute. 6 out of 11 patients took GMP-based MF for over 2 years.

There were no differences in biochemical markers between the L-AA and GMP-based groups and the overall percentage of overweight and obesity in taking L-AA versus GMP-based MF remained unchanged [(Pena MJ 2021)]. Blood Phe concentrations with GMP were not significantly changed from baseline and metabolic control remained unchanged between baseline and last assessment (Figure 9).

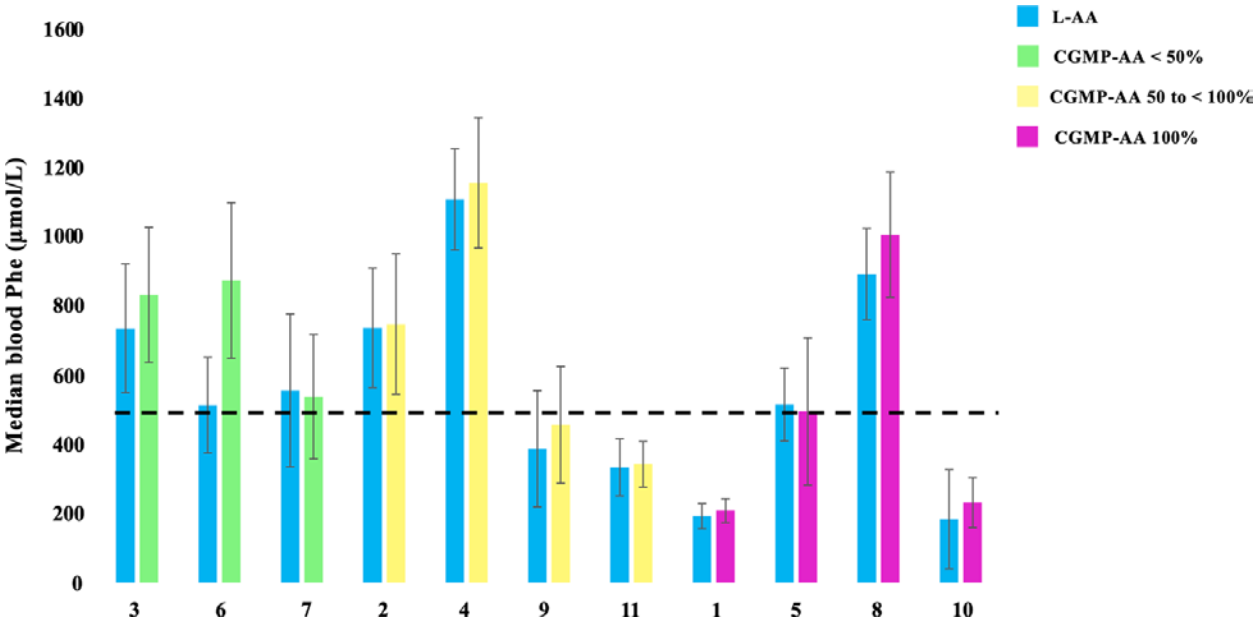

Figure 9: Median blood Phe levels of 11 patients with PKU taking L-AA versus GMP-based MF with different percentages of contribution to the total protein substitute. [(Pena MJ 2021).]

Blood Tyr however significantly increased with GMP, despite no difference in intake, but possibly because of better adherence with the GMP protein substitute and maybe due to lower solubility properties of the L-AA MF.

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In conclusion, the metabolic control and biochemical nutritional status of patients with PKU did not change with long-term use of GMP-based MF, suggesting that they are safe to use and that they improve long-term adherence and compliance with protein substitutes.

Pinto et al [(Pinto A 2017 (ii))] published the first report using **Cambrooke's Glytactin** GMP-based MF in maternal PKU.

- 31-year old G1P0 female with classical PKU and long history of poor metabolic control
- Glytactin GMP-based MF was started 18 months prior to pregnancy and provided 30 g/day protein equivalent (46 mg/ day Phe)
- ➢ Dietary PHE intake was not reduced to compensate for the PHE content of Glytactin GMP-based MF (46 mg Phe/day)
- Pre-conception, median blood PHE was 462 µmol/L
- Total protein equivalent (PE) from MF increased from 58 to 86 g/day during pregnancy but AA-MF provided all additional PE intake

It was concluded that GMP-based MF and AA-based MF were well tolerated with no morning sickness. Median blood Phe was within target range throughout the pregnancy while maternal weight gain was within normal expected range. And more importantly, the child was born without symptoms of MPKU syndrome [(Pinto A 2017 (ii))]

In a recent prospective, interventional free-living crossover study, absorption of **Cambrooke's Glytactin** GMP-based MF or AA-based MF with food was compared with the aim of evaluating short-term effects on plasma AA and other biomarkers [(Ahring KK 2018)]. The design of the study is described below:

- 8 patients age 16-48 years with classic PKU (7 females, 1 male)
- 4 different drink mixtures and breakfast of low pro bread, butter, & jam to provide 25% of daily requirement consumed at 4 different visits
    - DM1 = pure GMP DM 2 = FAA to mirror DM1 including Phe
    - DM3 = GMP + FAA DM4 = FAA to mirror DM3 without Phe

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- Between intervention consumed standard low protein diet with AA products: LNAA tablets x 4 pts, AA tablets x 2 pts, and standard AA medical food with full calories x2 pts
- Additional evaluation at baseline and 2 hours post prandial of serum glucose, insulin, glucagon like peptide (GLP-1), BUN (blood urea nitrogen), peptide tyrosine-tyrosine (PYY), and cholecystokinin (CCK)

The short-term findings showed that the residual Phe in the GMP-based MF did not change Phe levels compared to the Phe-free AA-based MF, while delayed peaks of serum AA demonstrated slower absorption with GMP (Ahring KK 2018). BUN was not significantly lower with GMP which may suggest more efficient utilization (Ahring KK 2018) and supports previous research studies (van Calcar SC 2009). As shown in Figure 10, levels of GLP-1, which promotes insulin secretion and reduces appetite and levels of PYY, which also reduces appetite, were highest with the complete GMP-based MF, which may support the finding of greater satiety (Ahring KK 2018).

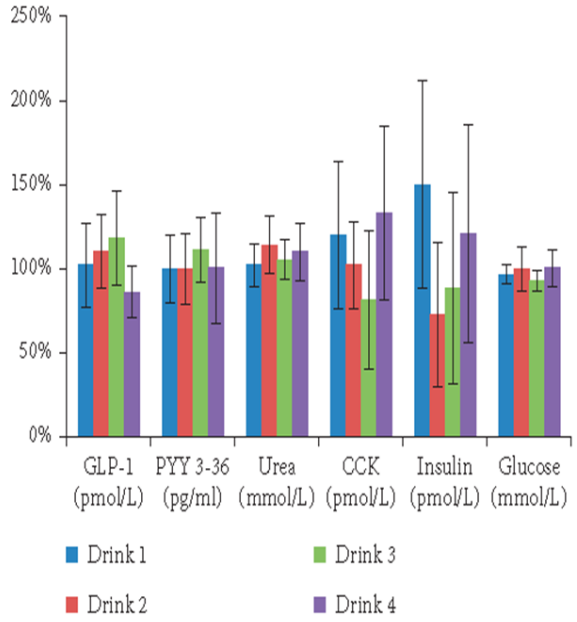

Figure 10: Comparing biomarkers between Glytactin GMP-based MF and AA-based MF (Ahring KK 2018).

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In another recently published study, GMP-based MF was evaluated in a longitudinal, parallel, controlled study over 12 months, where it was compared with a Phe-free AA-based MF in children with PKU ages 5-16 (Daly A 2019). No significant difference was found for serum Phe or Tyr levels when 75% of daily PE intake was provided by GMP-based MF and it was concluded that no dietary adjustments need to be made to compensate for the Phe provided by GMP-based MF (Daly A 2019). The effect of a GMP medical food on blood Phe control was investigated in a different study in 10 children (ages 4-16) with PKU (Zaki 2016). There were 2 phases during the study, with a 9-week duration in each phase: in phase I the children consumed 50% AA formula and 50% GMP and in Phase II they only consumed 100% AA formula. Phe levels and Phe/Tyr ratio during both phases of the study were not significantly different (Zaki 2016). The authors also reported that throughout the study, all patients preferred the GMP-supplemented diet over the classical AA formula due to better taste and satiety (Zaki 2016).

Several studies (Pinheiro de Oliveira 2016, Verduci 2018) indicated that the intestinal microbiota is altered in PKU patients influencing gastrointestinal homeostasis predisposing the patients to chronic inflammation. A unifying feature of GMP is its role as a prebiotic based on specific modulation of the GI microbiota that is beneficial due to increased concentrations of SCFA (small chain fatty acids) and lower indexes of inflammation (Sawin 2015).

In addition to the benefits of GMP-based MF mentioned above, recent evidence indicates that GMP-based MF can also support bone health in individuals with PKU (Stroup B 2017). Skeletal fragility characterized by low bone mineral density (BMD) and increased fracture is a poorly understood complication of PKU (Demirdas S 2015). There is no consensus on the incidence, etiology, implications and risk factors for low BMD. Low BMD was reported in 40-50% of adults with PKU (Choukair D 2015), while 33% of children have BMD at least two standard deviations below the expected range for age (De Croot MJ 2012). In a prospective, randomized, crossover study, PKU individuals consumed a low Phe diet combined with AA-based MF or **Cambrooke's Glytactin** GMP-based MF, each for 3 weeks. 25% of participants had low BMD for age, while dietary protein, calcium and magnesium intake were similar in both groups (Stroup B 2017). Unexpectedly the participants with the lowest BMD were all men who consumed the highest levels of protein from AA-based MF per day (Stroup B 2018). PRAL (Potential Renal Acid Load) is an equation that predicts the amount of diet derived acid that the body will have to manage to maintain acid-base homeostasis. A chronic high (positive) PRAL diet leads to high dietary acid load which may cause increased urinary excretion of bone

minerals (calcium, magnesium, phosphorous). The AA-based MF in the study [Stroup B 2017] provided 1.5-2.5-fold higher PRAL that the GMP-based MF resulting in a 3-fold higher renal net acid excretion. Cambrooke's Glytactin GMP-based MF were shown to significantly reduce urinary excretion of calcium by 40% and magnesium by 30%. Compared to the GMP-based MF, AA-based MF increase dietary acid load and cause increased urinary calcium and magnesium excretion and likely contribute to skeletal fragility in PKU [Stroup B 2017].

Conclusions

Findings over the recent years reinforce the safety and efficacy of GMP-based MF.

Four separate research groups representing four different countries* with a total of 116 subjects (40 adults and 76 children/ adolescents) reported the following:

- ✓ GMP-based Medical Foods are safe and tolerated
- ✓ Weight was stable for adults and growth was normal for children
- ✓ The Phe intake from foods does not need to be reduced when using a GMP-MF in adults
- ✓ In children, with 50-75% daily protein equivalent intake from GMP-based medical foods and remainder from AA-based medical foods, no dietary adjustment in Phe intake is necessary to maintain blood Phe control
- ✓ The Phe inherent to GMP-MF does not cause serum Phe levels to significantly increase despite increased daily Phe intake
- ✓ Tyrosine (Tyr) levels increased and remained within treatment range (desirable in PKU) despite a lower intake of Tyr with GMP-MF vs AA-MF

***Ney/Stroup (US)** [Ney DM 2016], **Pinto/Rocha (Portugal)** [Pinto A 2017 (i)], **Daly/Mac Donald (UK)** [Daly A 2019], **& Ahring (Denmark)** [Ahring KK 2018].

A low-Phe diet remains the cornerstone of PKU management. GMP-based medical foods, such as Ajinomoto Cambrooke's Glytactin® products, represent a new paradigm to the current PKU diet from synthetic AA as the primary source of protein to a more physiologically normalized diet based on intact protein. There is ample evidence that Glytactin® products containing minimal Phe are safe, efficacious, and help improve compliance with dietary therapy, while also supporting both digestive and bone health.

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