Glycomacropeptide: An Alternative to Management of Phenylketonuria

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Abstract:

Phenylketonuria is a metabolic disorder categorized by high level of phenylalanine in blood due to mutations in a gene involved in phenylalanine metabolism. The primary management of disease is lifelong restriction of protein food along with utilizing food comprising of synthetic amino acids. Compliance with synthetic food is poor leading to neurological problems. Glycomacropeptide, 64 amino acid proteins, provide a new standard because it does not contain phenylalanine in its pure form. GMP provides more acceptable and physiological source of protein as compared to synthetic amino acids. GMP is probiotics, reduces harmful gut bacteria, decrease renal load, improves protein utilization and satiety and improves bone strength size and strength. Advances in biotechnology will ultimately lead transition from synthetic amino acids to intact protein for the dietary management of phenylketonuria.

Keywords: Phenylketonuria(PKU), Phenylalanine (Phe), Glycomacropeptide (GMP), amino acid supplements

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1. Introduction:

Inherited metabolic disorders (IMD) are those genetic disorders that are characterized by an increased amount of some intermediary metabolites due to lack of some specific enzymes. IMD are diagnosed in new born via screening programs using tandem mass spectrometry and genotyping. [1]. The individuals diagnosed with IMDs are treated by nutritional management with medical foods consisting of synthetic mixtures of amino acids.

Phenylketonuria occurs due to the deficiency of phenylalanine hydroxylase that converts phenylalanine to tyrosine [2]. In this case, the dietary and tissue protein is not converted to tyrosine but instead converts into phenylpyruvate as shown in figure 1. In the absence of dietary management of PKU, increase in amount of phenylalanine occurs that damages the developing central nervous system.

A highly restrictive and lifelong diet that limits the amount of phenylalanine to prevent cognitive impairment and brain damage is required for PKU[3, 4].

Phenylalanine dietary treatment comprises of PHL-free amino acid supplement that contains both essential and non-essential amino acid, vitamins, minerals and long chain fatty acids. Amino acids compete with phenylalanine at blood brain barrier and decrease the uptake of phenylalanine into brain. However, the long-term consumption of Amino acid supplements results in kidney impairment and it has less physiological utilization [5].Also, the diet is difficult to follow due to unpleasant odor and strong peer pressure to follow the diet.[3]

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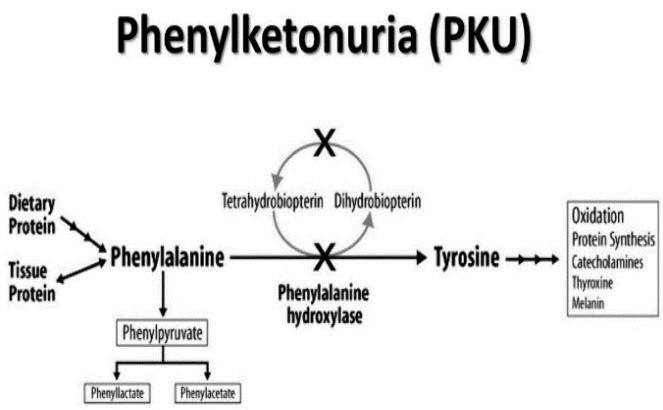

Fig 1 : Metabolism of phenylalanine in phenylketonuria [6]

This review illustrates the evidence of supporting the advantages of Glycomacropeptide as an alternative to nutritional management of PKU. Considerations with reference to biotechnology that are needed to obtain amino acid are also covered.

2. *Glycomacropeptide:*

Glycomacropeptide is a 64-amino acid protein containing no aromatic amino acid as shown in figure 2. It is naturally found in bovine milk in its whey fraction and is released in new born gastrointestinal tract by proteolysis caused by pepsin. Since 2010, it is considered as an alternative treatment for cognitive PKU impairment. At commercial level GMP is a byproduct of cheese production when chymotrypsin cleaved *k*-casein into para-*k*-casein which remains in the curd and k-casenio-GMP remains in whey fraction [3]. In vitro studies show that GMP inhibits toxicants of *Escherichia coli* and *Vibrio cholera* and balance immune response in humans. GMP improves intestinal health due to its prebiotic properties by reducing harmful Sulphur producing bacteria. GMP is also known to improve bone strength and size by increasing calcium absorption and decrease renal load.

GMP is the only naturally occurring protein that is free of phenylalanine (and all aromatic amino acids) in purified form. Pure GMP contains 47% indispensable amino acids but is free of amino acids such as histidine, tryptophan, arginine, cysteine or phenylalanine [7]. It also comprises of all branched chain and essential amino acid and reduces phenylalanine amount in brain. The decrease is due to increase amount of LNAA (large neutral amino acids) naturally present in GMP that competitively inhibit the update of phenylalanine in brain [3] .

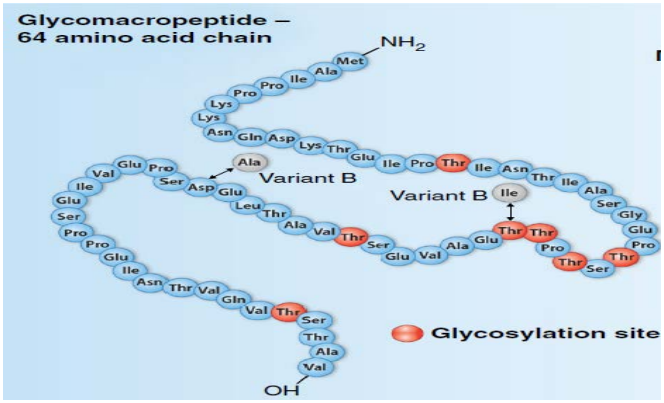

Fig 2: Structure of Glycomacropeptide [1]

During isolation of GMP, it is contaminated with other whey proteins such as beta-lactoglobin and alpha-lactalbumin that contains aromatic amino acids. Thus, commercially available GMP contains 2 to 5 mg of phenylalanine.

Many foods and beverages have been modified with the addition of GMP with reduced 2mg phenylalanine per gram of GMP protein. The modified medical foods include beverages, crackers, pudding, salad dressings and snack bar. These medical foods usually contain 12 to 15 g of protein equivalents and 2 to 3 grams of GMP.

GMP-modified low phenylalanine diet provides 70% of protein from intact protein in GMP and 30% from synthetic amino acids that are supplemented with GMP as compared to other amino acid based low phenylalanine diet that provides 80% of protein from synthetic amino acids and only 20% from intact proteins present in fruits and vegetables.

Comparison of the concentration of indispensable amino acids in pure GMP, in a commercial GMP food and in average protein food is shown in table:

Amino acid (grams per 100 g)	Pure GMP[a]	Lacprodan cGMP-20[b]	Average protein[c]
Histidine	0.0	0.2	2.6
Isoleucine	11.9	9.2	4.8
Leucine	1.7	2.0	7.8
Lysine	5.8	5.1	8.2
Methionine	2.0	1.6	2.0
Phenylalanine	0.0	0.16	4.7
Threonine	16.7	14.5	5.5
Tryptophan	0.0	0.03	1.9
Tyrosine	0.0	0.05	5.2
Valine	8.9	7.6	6.2
BCAA[d]	22.5	18.8	18.8

Fig 3: Comparison of phenylalanine in pure GMP, in a commercial GMP food and in average protein food [3]

3. *Physiological effects of Glycomacropeptide:*

In case of GMP supplementation of only 5 essential amino acid is required such as Leucine, methionine, histidine, tyrosine and tryptophan. [7]

- **GMP medical foods allows production of a variety of palatable foods:**

Intact protein is more suitable than synthetic amino acids due to better odor, taste and better functional properties for a range of food production.

- **Prebiotic properties of GMP to improve intestinal health:**

GMP possesses prebiotic properties due to a high degree of glycosylation by mucin-type carbohydrate chains. The prebiotic effects involve improving intestinal microbiota, reduces harmful Sulphur producing bacteria and production of short chain fatty acids (SCFA) that reduces colonic pH and prevent microbial colonization in gut.

- **Intact protein present in GMP reduces renal load, improves protein utilization:**

Ingestion of intact protein present in GMP in PKU patients results in increased post prandial plasma concentrations of amino acids, and a consistently low rate of absorption and digestion of amino acids that results in lower blood urea nitrogen concentrations

- **GMP improves bone strength and size:**

Skeletal delicateness categorized by low bone mineral density with greater risk of fractures emerged as a serious complication in PKU patients treated with amino acid food. It occurs due to increased uptake of dietary acid as in the case of amino acids that causes bone buffering of H^+ ions that results in decreased bone reabsorption and increased renal excretion of calcium with decreased bone density. The calcium absorption can be increased due to intake of GMP food. [1]

4. *Obtaining intact protein using biotechnology with defined amino acid profile:*

For obtaining peptide with no aromatic amino acid can take several routes. The protein synthesis technologies have developed since last decade but still there are some pitfalls for obtaining proteins as greater the size, lower will be the yield of protein. Due to lower yield, the extraction of protein becomes very expensive. Another strategy for obtaining protein with no aromatic amino acids is the expression of protein in the milk of life stock. Yet the cost is still 50 times more than the cost of GMP obtained naturally.

5. *Isolation strategies of Glycomacropeptide:*

Thus, Glycomacropeptide from cheese whey provides low cost protein, leaving only cost of purification, thus providing a significant advantage over recombinant proteins. Newer technologies such as charged ultracentrifugation membranes increases the purity at the same time lowering the cost. Purity can be increased using ion exchange chromatography in a single step.

Large-scale technologies currently available that are used to isolate GMP from cheese whey includes ion exchange chromatography and ultra-filtration. GMP has an isoelectric point below 3.8 as compared to whey protein that has an isoelectric point above 4.3 and this physiological difference plays a significant role in GMP separation. For ion exchange chromatography, a chromatography column packed with SP Sepharose big beads is used. A feed solution containing crude GMP with 10mM sodium lactate maintained at pH 4 is added in column. The proteins that bound to the column are desorbed at the end by adding elution buffer. Ultrafiltration is used to concentrate the

GMP and to remove proteins, salts and non-nitrogen proteins. At the end, the concentrated GMP was converted into powdered form by lyophilization. [7]

Two analytical methods such as tandem mass spectroscopy (MS/MS) and amino acid analyzer is used to measure the concentration of phenylalanine in blood. Tandem mass spectroscopy is used to analyze phenylalanine concentration obtained in blood spots on filter paper and Beckman 6300 amino acid analyzer is used to analyze amino acid profile of blood [3]

6. Analysis of phenylalanine level in models:

In an experiment, the amount of phenylalanine in the blood of two patients was determined after an overnight fast before breakfast for via tandem mass spectrometer and amino acid plasma analyzer.

A decrease of about 13-14% of blood phenylalanine concentrations was observed when GMP modified food was consumed as compared to amino acid supplements as in figure 4. The concentration was expressed relative to 100 mg of phenylalanine intake by known phenylalanine content, mean fasting plasma and blood phenylalanine concentrations. The absolute concentration plasma concentration of phenylalanine was reduced to 10% i.e. from 736µmol/L to 667µmol/L when GMP modified food was consumed. [3]

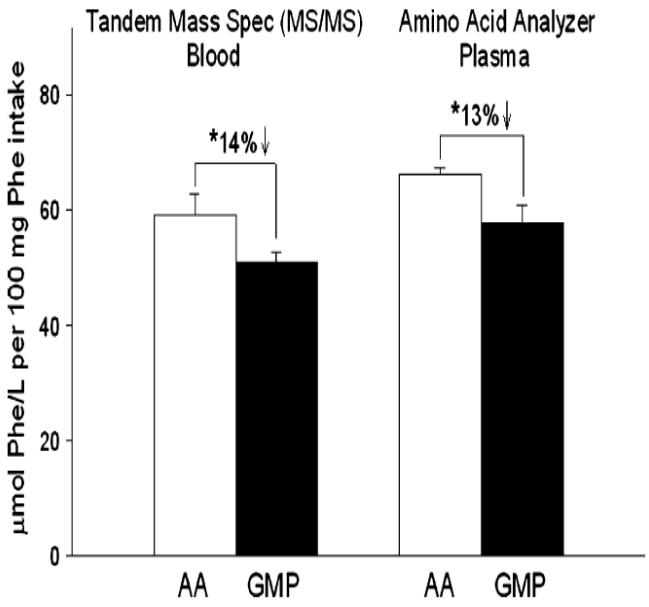

Fig 4: Mean Phenylalanine concentrations obtained after overnight fast and before breakfast [3]

The comparison of concentration of phenylalanine with amino acid supplements shows that the phenylalanine is much greater in amino acid supplements than in natural GMP diet as shown in figure 5.

Amino acid	Concentration (µmol/L)		
	Amino acid diet	GMP diet	Normal range
Alanine	296 ± 26	331 ± 5	177–583
Arginine	58 ± 2	62 ± 3	15–128
Citrulline	26 ± 2	48 ± 7*	12–55
Cystine	31 ± 1	42 ± 5	5–82
Glutamate	46 ± 10	53 ± 7	10–131
Glutamine	698 ± 22	858 ± 16**	205–756
Glycine	428 ± 7	428 ± 22	151–490
Histidine	69 ± 3	75 ± 2	41–125
Isoleucine	42 ± 3	69 ± 1**	30–108
Leucine	92 ± 3	91 ± 3	72–201
Lysine	189 ± 8	201 ± 5	48–284
Methionine	19 ± 1	19 ± 3	10–42
Ornithine	61 ± 7	51 ± 6	48–195
Phenylalanine	736 ± 13	667 ± 24[b]	35–85
Proline	153 ± 7	214 ± 13**	97–329
Serine	95 ± 1	88 ± 8	58–181
Taurine	65 ± 6	59 ± 13	54–210
Threonine	95 ± 4	246 ± 12**	60–225
Tryptophan	33 ± 2	35 ± 2	10–140
Tyrosine	53 ± 5	45 ± 6	34–112
Valine	241 ± 15	275 ± 14	119–336
BCAA[c]	374 ± 17	435 ± 15*	221–645

Fig 5: Phenylalanine concentration in amino acid diet and in GMP diet [3]

To evaluate the nutritional adequacy of GMP and control of phenylalanine concentrations in the brain and plasma, a mouse model with PKU was given GMP food as a sole source of protein for 47 days. The results show a decrease of 11% of phenylalanine in blood and 20% in various brain regions as compared to mouse who as given amino acid supplements as in figure 6. (Ney, Gleason et al. 2009)

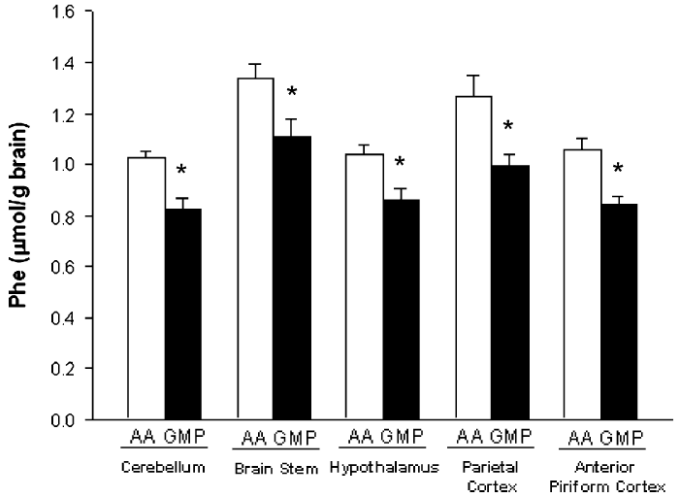

Fig 6: phenylalanine concentrations in different regions of a brain [3]

7. Conclusion:

Glycomacropeptide found naturally are preferred source of amino acids as compared to synthetic amino acids in phenylketonuria patients. Glycomacropeptide medical food increases human physiology and improve organ functions as compared to synthetic amino acid. The glycosylated regions of GMP act as prebiotics. Intact protein also stimulates new muscle synthesis, decreases renal load and improves protein utilization. GMP reduces skeletal fragility and improves bone size and strength. In future, advancement in food biotechnology will leads to the development of new separation technique for GMP that will speed up the shifting from synthetic amino acids to GMP medical food.

8. References:

1. Ney, D.M. and M.R. Etzel, *Designing medical foods for inherited metabolic disorders: why intact protein is superior to amino acids.* Current opinion in biotechnology, 2017. **44**: p. 39-45.
2. Blau, N., F.J. van Spronsen, and H.L. Levy, *Phenylketonuria.* The Lancet, 2010. **376**(9750): p. 1417-1427.
3. Ney, D., et al., *Nutritional management of PKU with glycomacropeptide from cheese whey.* Journal of inherited metabolic disease, 2009. **32**(1): p. 32-39.
4. van Spronsen, F.J. and G.M. Enns, *Future treatment strategies in phenylketonuria.* Molecular Genetics and Metabolism, 2010. **99**: p. S90-S95.
5. Pinto, A., et al., *Nutritional status in patients with phenylketonuria using glycomacropeptide as their major protein source.* European Journal of Clinical Nutrition, 2017.
6. Van Calcar, S.C. and D.M. Ney, *Food products made with glycomacropeptide, a low-phenylalanine whey protein, provide a new alternative to amino acid–based medical foods for nutrition management of phenylketonuria.* Journal of the Academy of Nutrition and Dietetics, 2012. **112**(8): p. 1201-1210.
7. LaClair, C.E., et al., *Purification and use of glycomacropeptide for nutritional management of phenylketonuria.* Journal of food science, 2009. **74**(4).