Translating Discovery to Practice: Glycomacropeptide (GMP) Provides a “Whey” Forward for Phenylketonuria (PKU)

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The Beginning: Discovery, Concept & Outreach, 1999-2002

- **Discovery**: Mark Etzel, Prof of Food Science, method to isolate GMP from cheese whey
- **Concept**: article in PKU National News
  - Is there interest in GMP medical foods as an alternative to AA medical formula?
- **Outreach**: GMP for PKU Task Force formed
  - Faculty, Waisman Biochemical Genetics Clinic, PKU Families, Medical Foods Co, Whey Processing Co, WI Center for Dairy Research, and WARF

PKU community was essential for this translational research
GMP Medical Foods: Proof of Concept Research 2003-present

- Research Funding
  - WARF and USDA Hatch – PKU mice
  - PKU Community – Foundations & state chapters
  - NIH R03, Phase 1 study, 2005 - **KEY**
  - FDA Orphan Products Development, 2011
  - Skeletal Health in PKU – pending grants

**ICTR, GMP Task Force and a committed interdisciplinary research team enabled success**
Interdisciplinary PKU Research Team

Denise Ney    Mark Etzel    Greg Rice    Sally Gleason    Sandy van Calcar

Karen Hansen    Robert Blank    Sangita Murali    Erin MacLeod    Patrick Solverson
GMP Medical Foods: Patent and Commercialization

- Patent Disclosure and Application, 2009
- Commercialization
  - Cambrooke Foods executes option on patent
  - GMP formula “Bettermilk” comes to market, 2010
  - Cambrooke attracts venture capital, 2011
- US Patent “GMP Medical Foods” issued 2013

Patent protection was key to bring GMP medical foods to market
What is PKU?

12 oz soda with Aspartame = 100 mg phe

Classical PKU restricts phe to 300-500 mg/d
Phenylketonuria (PKU)

Dietary Protein

Tissue Protein

Phenylalanine

Phenylpyruvate

Phenyllactate

Phenylacetate

Tetrahydrobiopterin

Dihydrobiopterin

Phenylalanine hydroxylase

Tyrosine

Oxidation
Protein Synthesis
Catecholamines
Thyroxine
Melanin
PKU: A Public Health Success Story

- Profound cognitive impairment due to untreated PKU is rare in developed world
  - Mandated newborn screening programs, by 1970
  - Initiation of low-phe diet within first week of life

- 20,000 individuals with treated PKU in the US, and 50,000 worldwide

1934 Folling → 1953 Bickel → 1965 Guthrie
PKU discovered ↓ phe AA diet Newborn screening
Norway Germany & UK USA
PKU Management: Low-Phe Diet

- No high protein foods
  5-10 g pro/day from fruits, veg & low protein bread and pasta

- To meet protein & energy needs, 24-32 oz of phe-free AA formula is required daily
  AA formula provides >75% of protein needs.
Lifelong adherence to the low-phe diet is very difficult.

- 21 of 33 patients that attended Emory University’s Division of Medical Genetics Metabolic Camp from 2002 to 2008 were considered to be out of metabolic control.
- 40% of 33 patients < 12 years of age had >60% of blood phe levels within recommended range (Mol Genet Metab 108:255-58, 2013.)

Low-Phe Foods Can be Made with GMP as an Alternative to AA Formula

- Chocolate and Strawberry Pudding
- Flavored Beverages
- Sports Beverages
- Crackers
- Snack Bar
- Crisp Cereal
- Peanut Butter

*5-15 g protein & 15-25 mg phe/serving

High Protein, Low-Phe GMP Foods
WI Center for Dairy Research

GMP is a whey protein found in milk

<table>
<thead>
<tr>
<th>Protein</th>
<th>Percentage</th>
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<tbody>
<tr>
<td>β-lactoglobulin</td>
<td>35-40%</td>
</tr>
<tr>
<td>α-lactalbumin</td>
<td>20-25%</td>
</tr>
<tr>
<td>Glycomacropeptide</td>
<td>15-20%</td>
</tr>
<tr>
<td>Other Proteins</td>
<td>15-20%</td>
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</tbody>
</table>
GMP is produced when making cheese. Pure GMP contains no phe
GMP is Generally Recognized as Safe Food Ingredient (GRAS) found in:

- Whey-based infant formulas
- Toothpaste - prevents dental cavities
- Protein/Weight reduction supplements - suppresses appetite and reduces body fat
- PKU Medical Foods-Glytactin™, Cambrooke Foods – 2010
Medical Foods

Rethinking the formula

Health insurance covers drugs approved by regulatory agencies, but it often doesn’t pay for the products known as ‘medical foods’ needed to keep individuals alive and well. This lack of reimbursement means that many who cannot afford these life-saving diets suffer brain deterioration and disability—or worse. Roxanne Khamsi reports on the battle for medical foods and how it could affect the treatment of diseases as diverse as osteoporosis and Alzheimer’s.
GMP Medical Foods Provide Primarily Intact Protein

- Protein synthesis and N retention are improved with ingestion of intact protein compared with AA, in part, because intact protein is absorbed more slowly than AA.
Inpatient Metabolic Study

- 11 PKU subjects (12-31 yr age), admitted to CRU for 6 days
- Two 4-day treatments with an AA diet followed by a GMP diet

Summary Phase 1: GMP is a more physiologic source of low-phe protein than AAs

- GMP improves protein retention
- GMP promotes satiety – “feel fuller longer”
- GMP enhances taste and variety of PKU diet
- No effect on plasma phe level after meal

van Calcar SC & Ney DM. *J Acad Nutr Diet* 112:1201, 2012
Hypothesis: GMP medical foods will improve dietary compliance and metabolic control of PKU compared with the usual AA-based diet
PKU Clinical Trial – FDA OOPD

- 30 early-treated PKU subjects, ≥12 yr of age

- Diet treatments, 3 wk at home in randomized crossover design include
  - AA: Usual diet with AA formula
  - GMP: Replace protein provided from AA formula with GMP medical foods containing Glytactin™

www.clinicaltrials.gov  NCT 01428258
Camino pro low-phe GMP medical foods with Glytactin™
Teens and Adults with PKU: You are invited to take part in a research study

The purpose of this study is to compare the PKU diet with amino acid formula to the PKU diet with foods and beverages made with Glycomacropeptide (abbreviated GMP). This will help us to determine if the GMP diet is easier to follow and helps improve blood phe levels.

If you agree to participate, you will be on your usual diet for the first 3 weeks and then for another 3 weeks, you will stop taking your formula and replace it with products made from GMP. Or, you may be on the GMP diet for the first 3 weeks and then on your usual diet for the second 3 weeks.

The research study will involve 4 visits over 11 weeks. For each visit, you will come to the Waisman Center in Madison for about 3 hours. At each visit, blood will be drawn, neuropsychological tests will measure how well you think and questionnaires will ask you about your diet. You will also have a scan to measure your bone density at one of the visits. At home, you will collect blood on filter paper three times per week to measure your phe level and fill out records about your diet each day.

There is little risk to participating in the study, although blood draws can be painful and neuropsychological testing may be embarrassing or frustrating.

The study will help us learn if GMP foods and beverages can replace your usual PKU formula that you now take. This may help you and others with PKU improve their phe levels.

If you are interested in participating or have any questions about the research study please call Sandy van Calcar at the Biochemical Genetics Clinic at 608-263-5993 or Denise Ney in the Department of Nutritional Sciences at 608-262-4386.
Skeletal Health in PKU
Skeletal Fragility is a Complication of PKU

- Lower BMD compared to age, gender, and sibling matched controls
  - 30-50% have osteopenia

- Higher rates of fracture compared to siblings

- Trabecular bone preferentially affected

- No consistent relationship across ~20 cross sectional studies between BMD and
  - Blood levels of Phe and vitamin D
  - Energy, protein or calcium intake
  - Physical activity
Low Bone Strength Is a Manifestation of Phenylketonuria in Mice and Is Attenuated by a Glycomacropeptide Diet

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Abstract

Purpose: Phenylketonuria (PKU), caused by phenylalanine (phe) hydroxylase loss of function mutations, requires a low-phe diet plus amino acid (AA) formula to prevent cognitive impairment. Glycomacropeptide (GMP), a low-phe whey protein, provides a palatable alternative to AA formula. Skeletal fragility is a poorly understood chronic complication of PKU. We sought to characterize the impact of the PKU genotype and dietary protein source on bone biomechanics.

Procedures: Wild type (WT; Pah⁺/⁺) and PKU (Pah⁻/-/⁻) mice on a C57BL/6J background were fed high-phe casein, low-phe AA, and low-phe GMP diets between 3 to 23 weeks of age. Following euthanasia, femur biomechanics were assessed by 3-point bending and femoral diaphyseal structure was determined. Femoral ex vivo bone mineral density (BMD) was assessed by dual-energy x-ray absorptiometry. Whole bone parameters were used in principal component analysis. Data were analyzed by 3-way ANCOVA with genotype, sex, and diet as the main factors.

Findings: Regardless of diet and sex, PKU femora were more brittle, as manifested by lower post-yield displacement, weaker, as manifested by lower energy and yield and maximal loads, and showed reduced BMD compared with WT femora. Four principal components accounted for 87% of the variance and all differed significantly by genotype. Regardless of genotype and sex, the AA diet reduced femoral cross-sectional area and consequent maximal load compared with the GMP diet.

Conclusions: Skeletal fragility, as reflected in brittle and weak femora, is an inherent feature of PKU. This PKU bone phenotype is attenuated by a GMP diet compared with an AA diet.
High Dietary Acid Load Provided by AA diet = BAD FOR BONE

- Skeletal buffering by release of HCO₃⁻ along with calcium & phosphorus
- Increased renal calcium excretion
- Four RCTs document that neutralization of dietary acid load with potassium citrate or potassium bicarbonate leads to:
  - Lower bone resorption
  - Lower renal calcium excretion
  - Higher BMD (1 trial, published in 2013)
Conclusion: GMP is a more physiologic source of low-phe protein than AAs

- GMP may improve metabolic control and skeletal health for individuals with PKU

GMP Provides a “Whey” Forward for PKU
Disclosure

- Research funding: NIH/NIDDK, FDA OOPD, USDA, National PKU Alliance & PKU donors

- D Ney is a co-inventor on US Patent 8,604,168 B2, GMP Medical Foods for Nutritional Management of PKU, held by the Wisconsin Alumni Research Foundation and licensed to Cambrooke Therapeutics, LLC.
GMP Medical Foods for PKU: Concept to Commercialization

- Concept & Outreach to PKU Community, 1999
  - PKU National News and GMP for PKU Task Force

- Proof of Concept: Research, 2003-present
  - Studies in PKU mice and humans with PKU

  - Cambrooke Foods bring GMP Medical Foods to market (2010) and attracts venture capital
  - US Patent “GMP Medical Foods” issued 2013
GMP Increases Bone Size in PKU & WT Mice

Solverson et al; PLoS One 7(9):e45165; Sept 2012
GMP Improves Bone Strength in PKU and WT Mice Compared with AA Diet