More physiological absorption of a prolonged release Amino Acid supplement: preclinical evidence of an improved Amino Acid utilization

Giarratana N[1] | Draper K[2] | Giardino L[3] | Bighinati A[4] | Reiner G[1]

1 APR Applied Pharma Research, Balerna, Switzerland
2 Relief Therapeutics, Connecticut, United States
3 Department of Veterinary Medical Sciences, University of Bologna, Bologna, Italy
4 Department of Life Science, University of Modena and Reggio Emilia, 41125 Modena, Italy

Learning Objectives

- To assess the impact of a prolonged released amino acid mix formulation on nitrogen balance both in acute and long-term experimental studies
- To evaluate the impact of a prolonged release amino acid mix on muscle strength and glucose metabolism

Background

- Patients with PKU compensate their restricted dietary regimen with protein substitutes, mainly Phe-free L-amino acid (AA) mixtures, typically characterized by absorption kinetics different from those of natural dietary proteins.
- Slowly absorbed and digested proteins allow for better post-prandial utilization of dietary nitrogen1, in contrast with free AAs that are absorbed too rapidly to support anabolic requirements.[2]
  - ▶ Prolonged release of AAs from a protein substitute may be able to better support anabolic requirements and prevent or reduce catabolic episodes, potentially minimizing suboptimal outcomes in PKU patients.[3]
- Blood urea nitrogen (BUN) production reflects the pattern of AA utilization:
  - In the short-term it is a marker of oxidation of supplemented AAs into nascent proteins.[4]
  - In the long-term it is a marker of catabolic episodes (proteolysis) that occurs during the fasting state in order to maintain plasma AA homeostasis.[1]
- The muscle is the primary organ affected by catabolic episodes in PKU patients through proteolysis[5]
- Glucose metabolism is also influenced by blood levels of certain AAs, that in free form elicit earlier / stronger insulin responses and lower blood glucose levels more than physiologically digested proteins.[7]

A nitrogen source for PKU patients obtained with the Physiomimic technology™ (AA-PT), that can prolong AA release in the gut as well as mask odor and taste of AAs, has been shown to reduce peak AA concentrations (C_{max}) while maintaining similar areas under the AA concentration/time curve (AUC), suggestive of prolonged AA release.[8]

Materials and Methods

Healthy adult male Wistar rats (180-337 g) were treated by oral gavage to evaluate the metabolic impact of Amino Acids engineered with Physiomimic Technology (AA-PT) on muscle strength and glucose levels in acute and long-term studies.

Acute: 6-12 animals/group fasted for 12 hours were administered a single dose (700 mg AAs/kg body weight) of the nitrogen sources (AA-PT and natural protein casein CAS) and corresponding control (same mix done with free AAs) in a solution of 20% glucose (8 ml/kg body weight) with additional nutrients to mimic the marketed GOLIKE product.

- ▶ Blood glucose levels measured at baseline and 15, 30, 45, 60 and 90 minutes, post administration.
- BUN, ghrelin, gastric inhibitory polypeptide (GIP), glucagon-like peptide-1 (GLP-1), glucagon and insulin measured at 90 min, at sacrifice.

Long-term experiment: two rounds of 3-5 animals/group after 2 weeks of adaptation were treated 2 times/day for 2 weeks with AA-PT and its control (AA-PT-C). Each gavage, after 2 hrs fasting, was 1.25g/Kg body weight of protein plus other nutrients (i.e. glucose 5% and starch 5%) and Phe to guarantee a physiological diet. Rats in both treatment groups consumed the same quantity of feed and lost the same weight.

- ▶ Western blot of muscle catabolism markers: Atrogin-1 and BNIP3L/NIK and muscle anabolism markers: Myostatin and mTOR on femoral biceps and vastus lateralis using GADPH as the reference protein.
- Muscle strength was measured with a grip strength meter at Day 0 and after 15 days of treatment.
- Glucose tolerance (3g/kg body weight) was evaluated at Day 0, 7 and 14 at baseline, 30 min and 120 min after glucose ingestion.

Results

Acute Effect of the Physiomimic Technology™ on AA oxidation.
AA-PT produced (Figure 1):
- significantly less BUN than its corresponding control
- BUN was not significantly different from that produced by an equivalent amount of natural protein CAS

Acute Effect of the Physiomimic Technology™ on gylcemia.
The glycemia trend with AA-PT, did not differ significantly from that obtained with natural protein CAS

Long term Effect of the Physiomimic TechnologyTM on BUN.
AA-PT produced (Figure 2):
- less BUN than its corresponding control, confirming the same trend of acute result.

FIGURE 1: BUN after acute treatment

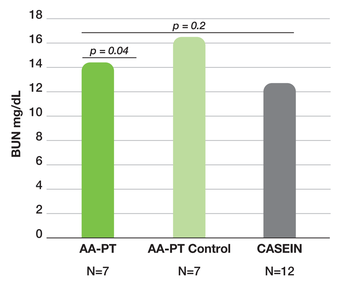
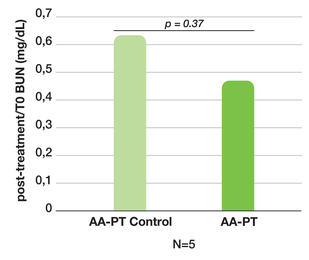


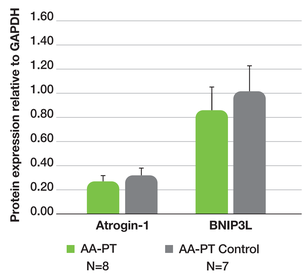
FIGURE 2: BUN after 2 weeks of treatment



Long term Effect of the Physiomimic Technology™ on muscle.
AA-PT produced:
- A significantly lower degradation marker BNIP3L (-46%) in femoral biceps (Figures 3).

A similar but non-significant trend observed in the vastus lateralis
- a significantly higher synthesis muscle protein Myostatin in vastus lateralis (+58%). A similar but non-significant trend observed in the femoral biceps (Figures 4).
- a significantly stronger grip strength relative to the baseline (+30%), whereas no significant increase in muscle strength was observed in animals treated with AA-PT-C (Figures 5).

FIGURE 3: VASTUS LATERALIS



FEMORAL BICEPS

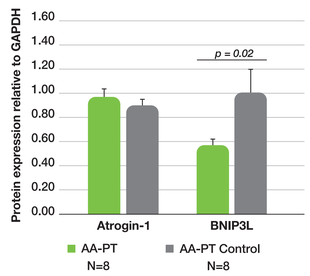
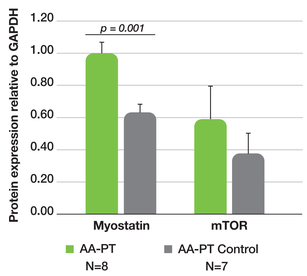


FIGURE 4: VASTUS LATERALIS



FEMORAL BICEPS

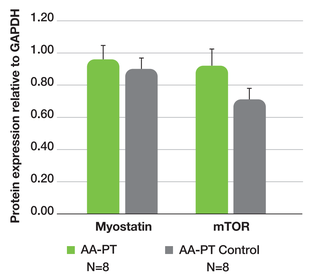
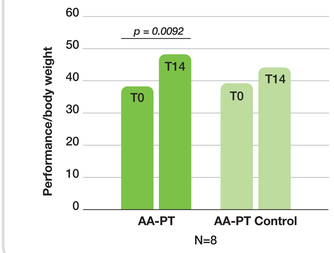


FIGURE 5: GRIP TEST



Long-term Effect of the Physiomimic Technology™ on glucose tolerance.
AA-PT produced (Figures 6):
- a significant better tolerance of glucose load at day 7 and 14

FIGURE 6: BEFORE TREATMENT

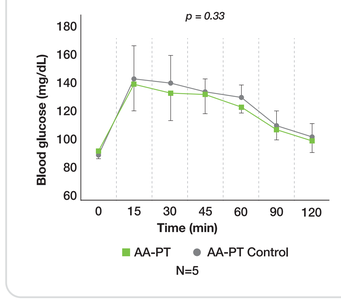


FIGURE 6: DAY 7

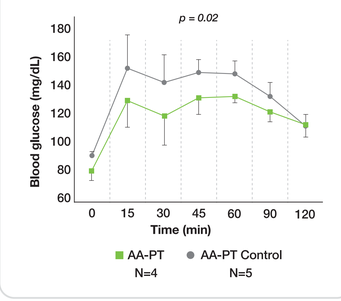
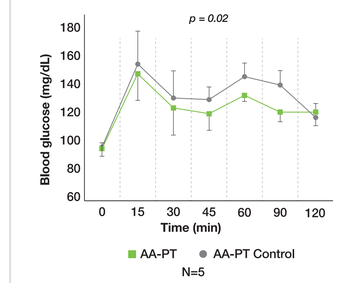


FIGURE 6: END OF TREATMENT



Discussion

Preliminary results suggest that the Physiomimic Technology™ amino acids have a more physiological absorption kinetic, with possible beneficial effects on AA oxidation and catabolism with a direct impact on muscle mass and strength and on glucose tolerance in normal healthy rats.

BUN results, after acute and long-term treatments, suggest better AA utilization, less AA oxidation and fewer catabolic episodes

Markers of muscle strength and anabolism / catabolism further support this hypothesis.

Improved glucose tolerance has been demonstrated with AA-PT treatment.

Based on these pre-clinical results, follow on clinical studies are being designed to evaluate the effects of prolonged absorption and better amino acid utilization on (i) body composition (ii) muscle performance (iii) markers of oxidative stress (iv) Phe / Tyr ratios and their fluctuation.

REFERENCES

1. Dangin M, Boirie Y, Garcia-Rodenas C, Gachon P, Fauquant J, Callier P, Ballèvre O, Beaufrère B. The digestion rate of protein is an independent regulating factor of postprandial protein retention. Am J Physiol Endocrinol Metab. 2001;280:E340-8.
2. Lacroix M, Bos C, Léonil J, Airinei G, Luengo C, Daré S, et al. Compared with casein or total milk protein, digestion of milk soluble proteins is too rapid to sustain the anabolic postprandial amino acid requirement. Am J Clin Nutr. 2006; 84: 1070-1079..
3. Ilgaz, F., et al., Long-Term Growth in Phenylketonuria: A Systematic Review and Meta-Analysis. Nutrients, 2019. 11(9).
4. Whang KY, Easter RA. Blood Urea Nitrogen as an Index of Feed Efficiency and Lean Growth Potential in Growing-Finishing Swine. Asian-Australas J Anim Sci. 2000;13:811-816
5. Gannon MC, Nuttall FQ. Amino acid ingestion and glucose metabolism--a review. IUBMB Life. 2010;62:660-8
6. Tessari P, Kiwanuka E, Cristini M, Zaramella M, Enslen M, Zurlo C, Garcia-Rodenas C. Slow versus fast proteins in the stimulation of beta-cell response and the activation of the entero-insular axis in type 2 diabetes. Diabetes Metab Res Rev. 2007 Jul;23(5):378-85
7. Giarratana N, Gallina G, Panzeri V, Frangi A, Canobbio A, Reiner G. A New Phe-Free Protein Substitute Engineered to Allow a Physiological Absorption of Free Amino Acids for Phenylketonuria. J Inborn Errors Metab Screen. 2018; 6: 1–9.
8. Scheinin, M., et al., Amino Acid Plasma Profiles from a Prolonged-Release Protein Substitute for Phenylketonuria: A Randomized, Single-Dose, Four-Way Crossover Trial in Healthy Volunteers. Nutrients, 2020. 12(6).

CONFLICT OF INTEREST

NG and GR are employees of APR Applied Pharma Research S.A. (Balerna/Switzerland); LC and AB work at DIMIVET, an Italian contract research organization associated with Bologna University, with no financial interest in APR Applied Pharma Research S.A.; J.C.R. is a member of the European Nutritionist Expert Panel (Biomarin), the Advisory Board for Applied Pharma Research and Nutricia, and has received honoraria as a speaker from APR, Merck Serono, Biomarin, Nutri-cia, Vitaflo, Cambrooke, PIAM, and Lifediet.