Preliminary indications of metabolic modulation by provision of amino acids engineered to allow physiological absorption

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Aims

To assess the metabolic impact of 2 nitrogen sources with different delivery formulations and to evaluate their impact on selected metabolic parameters as compared to a natural slow release protein.

Background

- Patients with PKU compensate their restricted dietary regimen with protein substitutes, mainly Phe-free L-amino acid (AA) mixtures, characterized by absorption kinetics different from those of natural dietary proteins.
- Slowly digested proteins provide better post-prandial utilization of dietary nitrogen[1], while free AAs are absorbed too rapidly to support anabolic requirements.[2]
  - ► Prolonged release of AAs by a protein substitute may be able to better support anabolic requirements and prevent or reduce catabolic episodes.
- Urea production reflects the pattern of AA utilization, with higher levels in fasting rats fed free AAs, compared to those fed a slow-release protein.[3]
- Blood urea nitrogen (BUN), an indicator of AA oxidation, is a useful indirect measure of the ability to retain dietary nitrogen.[4]
- Glucose metabolism is also influenced by blood levels of certain AAs[5], that in free form elicit earlier / stronger insulin responses and lower blood glucose levels than slowly digested proteins.[6]

A nitrogen source for patient with PKU obtained with the Physiomimic technology™ - a technology engineered to prolong the AA release in the gut and 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.[7]

Materials and Methods

We evaluated in rats the metabolic impact of the slow release protein casein and two protein substitutes with different delivery formulations for dietary treatment of PKU (Table 1).

Animals and Interventions

Groups of 7-12 adult male Wistar rats (200-250 g) fasted for 12 hours were administered a single dose (700 mg AAs/kg b.w.) of the nitrogen sources and corresponding controls (Table 1) in a solution of 20% glucose (8 ml/kg b.w.) via oral gavage under light anesthesia.

Outcome Measures

- Blood glucose was measured at baseline and then at 15, 30, 45, 60 and 90 minutes.
- Other metabolic markers including BUN, ghrelin, gastric inhibitory polypeptide (GIP), glucagon-like peptide-1 (GLP-1), glucagon and insulin were all measured at 90 min, at sacrifice.

TABLE 1

Test	Control
P1 AAs engineered with the Physiomimic technology™ *	Control of P1 Free AA + additives with the same composition as P1
P2 AAs in the form of microtabs**	Control of P2 Free AA + additives with the same composition as P2
CAS Casein***	Control of CAS Free AA in the same amount and composition as CAS

* engineered to prolong the AA release in the gut and to mask odor and taste of AAs
** claiming delayed release kinetics and neutral taste
*** known natural protein with a slow absorption profile
Controls: Each control consisted of a free AA mixture containing the same amount and ratio of AAs, without the application of any technology. For P1 and P2 the functional additives were also present in the control formulation

Results

Effect of the Physiomimic technology™ on AA oxidation

P1 produced (Figure 1)

- Significantly less BUN than its corresponding control (P1 vs Control of P1; p= 0.044*)
- BUN that was not significantly different from that produced by an equivalent amount of intact CAS (P1 vs CAS; p=0.171)

P2 produced (Figure 2)

- BUN that was not significantly different from its corresponding control (P2 vs Control of P2; p=0.149)
- BUN that was significantly higher than that produced by an equivalent amount of CAS (P2 vs CAS; p=0.006*).

Effect of the Physiomimic technology™ on glycemia

- The glycemia trend with P1, engineered with the Physiomimic technology™, did not differ significantly from that obtained with intact CAS (Figure 3 & 5).
- Differently, the trend with P2, AAs in form of microtabs, was significantly different from intact CAS (Figure 4 & 5).
- While the glycemic trend with P2 did not differ significantly from that of its Control, the glycemia trend in rats fed P1 vs its Control seemed to go in the same direction as the glycemia trend of rats fed CAS vs its Control (Figure 5).

The other metabolic markers did not differ significantly at 90 min in any of the groups [data not shown].

FIGURE 1 — P1

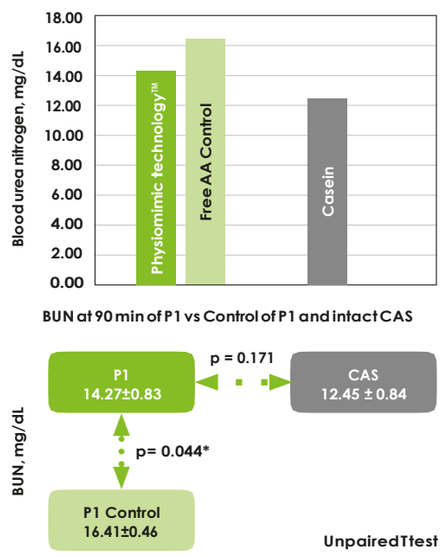


FIGURE 2 — P2

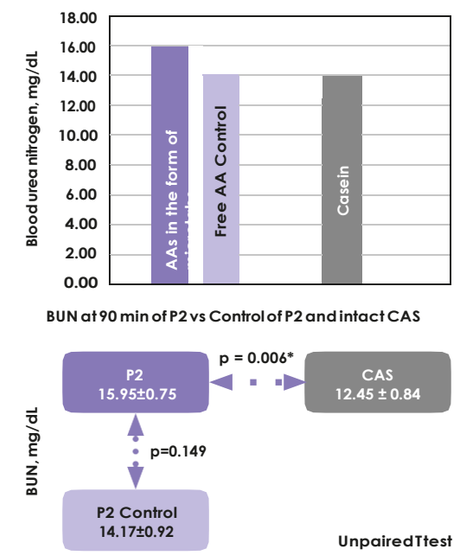


FIGURE 3 — P1

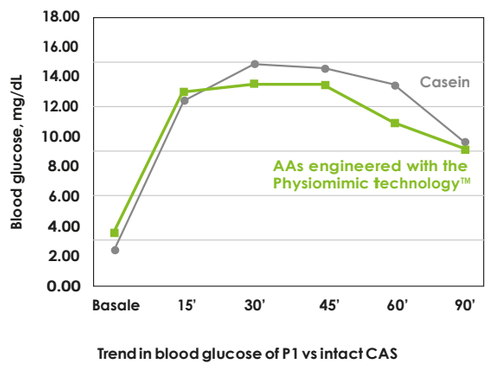


FIGURE 4 — P2

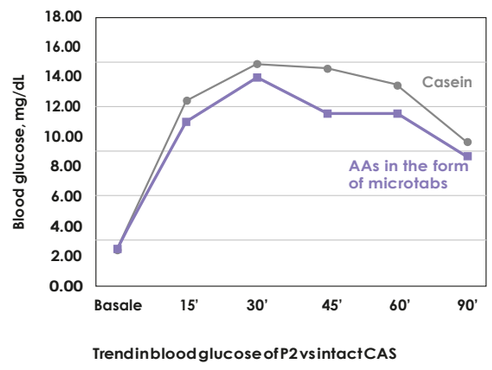
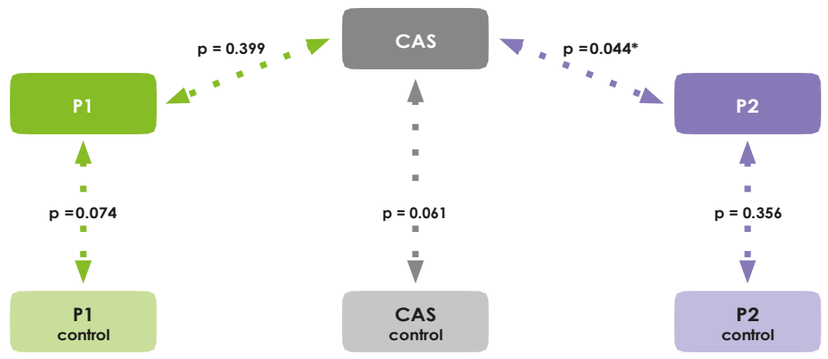


FIGURE 5

P-values for the differences between glycaemic trends — two-way ANOVA



Discussion

Preliminary results from the present study suggest that the protein substitute engineered with the Physiomimic technology™ (P1) may modulate BUN and glycemia in the same direction as intact casein protein.

Particularly, the BUN results suggest a potential benefit towards better AA utilization, with less AA oxidation with P1, although further research is warranted. As a comparison, the effect of P2 on these parameters was essentially similar to that of free AAs, without showing an impact on nitrogen metabolism.

Based on previous results showing that AAs engineered with the Physiomimic technology™ can promote a physiological pattern of AA absorption mimicking that of an intact protein[7], further research in this direction will be aimed at confirming whether P1 supports anabolic requirement in animal models and ultimately in patients with PKU.

KEY REFERENCES

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CONFLICT OF INTEREST

Julio C Rocha is member of APR's Advisory Board and received consultancy/speaker fees from APR, Vitaflo, Nutricia and Cambrooke. Julio C Rocha is also member of the European Nutrition Expert Panel (Biomarin).
Nadia Giarratana is an employee of Applied Pharma Research sa.
L Giardino, A Giuliani and M Fernandez do not report a conflict of interest for this work.