Category: 07. PKU; Number: P-133

Sustained release of amino acids in humans demonstrated with a uniquely engineered amino acid mixture for phenylketonuria

MacDonald Anita, *Birmingham Women's and Children's Hospital NHS Foundation Trust, Department of Dietetics, Birmingham, United Kingdom*
Scheinin Mika, *CRST Oy, Turku, Finland*
Barassi Anna, *Head of Clinical Affairs, APR Applied Pharma Research S.A., Balerna, Switzerland*

BACKGROUND

Ingestion of free amino acids (AAs) results in rapid non-physiological AA absorption that may exceed anabolic capacity and result in the loss of nitrogen.[1,2] In clinical practice, this inefficiency is overcome by feeding AAs in high doses.[3] However, AAs have important effects on the signals that control appetite, digestion and metabolism.[4,5] Therefore, more physiological absorption kinetics are desirable for AA products. A novel Physiomimic Technology™ (PT) was able to modify the release of free AAs in an animal model,[6] but its effects in humans have to be studied.

METHODS

We compared the absorption kinetic profiles of AA mixes in a randomized cross-over trial in 30 healthy adult volunteers (body weight 55-85 kg, body mass index (BMI) ≤ 30 kg/m²):
- TEST - AAs engineered with the Physiomimic Technology™ (PT);
- REFERENCE – a non-engineered AA mixture with the same composition as TEST.

These were also compared with a free AA mix from the market (MP) having an AA composition similar to TEST and with non-hydrolysed casein (Figure 1).

Interventions
AAs were administered in single doses in 300 mL of water. The nominal dose was 0.40 g AA/kg body weight. Subjects observed washout periods of 9 to 14 days during which they were on free diet, except on the two days prior to test day when they maintained a controlled (moderate protein) diet.

Measurements
Blood and urine samples were collected on test days and analyses performed as indicated in Figure 2. A light snack was provided 5 hours after product ingestion.

Primary Endpoint
The two co-primary endpoints were:
- Peak plasma essential AAs (EAAs) concentrations (C_{max})
- Area under the EAA concentration/time curve (AUC_{0-300}).

Primary hypothesis: ≥ 20% lower C_{max} of TEST vs REFERENCE, while maintaining bioequivalence (log-transformed AUC_{0-300} ratio with 90% CI within the range 0.80-1.25).
Tyrosine and arginine were treated as EAAs because tyrosine is essential in PKU. Arginine is required by infants and growing children on restricted diets.

Statistical methods
Sample size was calculated on the primary endpoints. Primary parameters were analysed on log-transformed data using a linear mixed effect model with sequence, period and treatment as fixed effects, and "subject within sequence" and a residual error term as random effects. A 90% CI was calculated from the model for AUC_{0-300} equivalence testing. These CIs were anti log converted to obtain ratios of geometric least square means. Descriptive statistics and period comparisons were used for the secondary parameters.

RESULTS

The study population consisted of 15 women and 15 men, mean ± SD age 27.4 ± 8.2 years (range 18.0-43.0), mean ± SD body weight 70.0 ± 8.48 kg.

Primary endpoints – both the two co-primary endpoints were met (Figure 3, Table 1):
1. EAA C_{max} was 27.4% lower with TEST compared to REFERENCE (ratio, 0.726, p<0.0001).
2. The two AA preparations were bioequivalent (AUC_{0-300} ratio, 0.890 [95% CI: 0.865, 0.915]).

Secondary endpoints – supported these findings:
Prolonged AA absorption up to 7 hours with lower C_{max} and bioequivalent AUC_{0-420} in TEST vs REFERENCE:
- Large Neutral AAs (32.3% C_{max} reduction; p<0.0001; Table 1)
- Branched-Chain AAs (42.1% C_{max} reduction; p<0.0001; Table 1)
- Total AAs (22.5% C_{max} reduction; p<0.0001; Figure 4, Table 1)

Similar results were observed when the PT-engineered AA mix was compared to MP and casein (Table 1).
Tyrosine was equally absorbed from TEST and REFERENCE (AUC_{0-420} ratio 1.052 [90% CI: 0.984, 1.125]), while MP and casein showed smaller AUC_{0-420} due to the different AA compositions.

Effect on Phe levels
Blood Phe fluctuations were less prominent with TEST, compared to REFERENCE (p=0.0046, ANCOVA; Figure 5).

Markers of metabolism
Markers of nitrogen balance, blood urea nitrogen (BUN) and urine urea, were significantly lower with TEST vs. REFERENCE:
- **BUN** p<0.0001, ANCOVA; Figure 6;
- **Urea** p=0.0023, ANCOVA; (data not shown).

AAs stimulate insulin secretion.[4] Insulin levels were lower and, subsequently, glucose levels decreased less with TEST, compared with REFERENCE (Table 1), with less fluctuations of both insulin and glucose over time (data not shown).

DISCUSSION

In healthy human volunteers, the AA mix engineered with Physiomimic Technology™ (TEST) significantly reduced the plasma C_{max} of EAAs, LNAAs, BCAAs and total AAs, while maintaining bioavailability compared to a control mix with an identical composition (REFERENCE).
Similar conclusions can be drawn for TEST vs MP. In this study, casein showed an unexpected AA absorption peak, not in line with published data. This anomalous peak made the kinetic profile of TEST prolonged also when compared to casein.
Indicators of nitrogen balance, BUN and urea, suggest that lower, more sustained blood AA levels may favour physiological utilization of ingested AAs.
Lower insulin peaks and more stable glucose levels are in line with lower AA C_{max} with TEST.
Interestingly, also the kinetics of phenylalanine, not present in the administered AA mixes, appeared to be more stable, with smaller fluctuations with the TEST product.

Conclusion
Physiomimic Technology™ applied to AAs provided prolonged absorption for up to 7 hours after ingestion of AA doses of 0.40 g/kg. Prolonged absorption was hypothesised as a statistically significantly lower C_{max} of AAs in blood while maintaining bioequivalence. The modified kinetic profile of AAs engineered with the Physiomimic Technology™ might influence other metabolic parameters.

Figure 1
Comparison made in this randomized crossover study

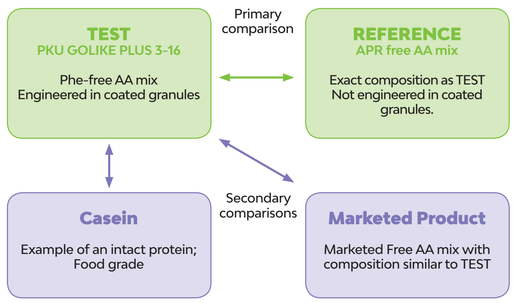


Figure 2
Sample collection and analysis scheme

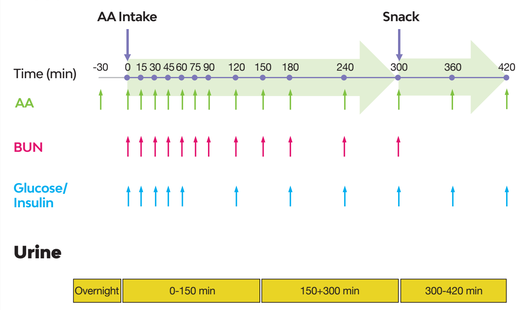
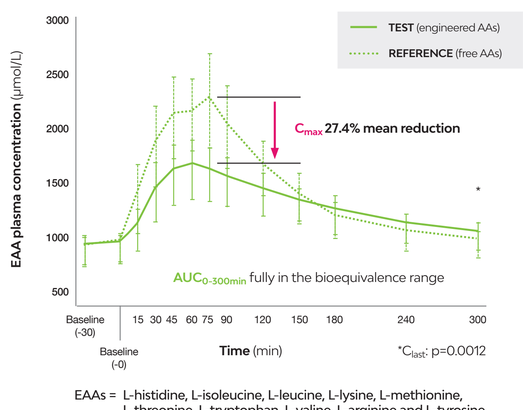


Figure 3
Plasma Essential AA concentrations with TEST vs REFERENCE at the indicated times after ingestion of AA mixes



EAAs = L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-threonine, L-tryptophan, L-valine, L-arginine and L-tyrosine.

Figure 4
Plasma Total AA concentrations with TEST vs REFERENCE at the indicated times after ingestion of AA mixes

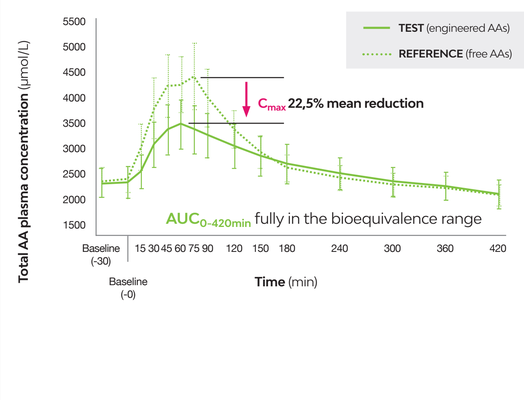
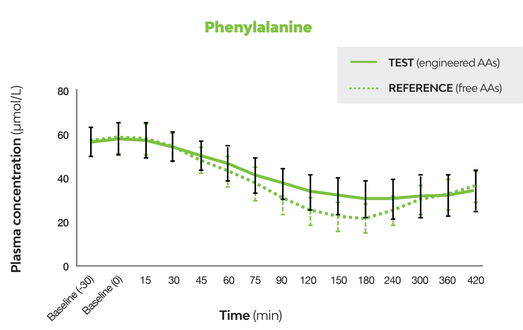


Figure 5
Plasma Phe concentrations with TEST vs REFERENCE at the indicated times after ingestion of AA mixes*



* Note that Phe is not present in the AA mixes.

Figure 6
Plasma BUN concentrations with TEST vs REFERENCE at the indicated times after ingestion of AA mixes

Table 1
Kinetic parameters with the 4 nitrogen sources in the 4 treatment periods for the 30 subjects in this this crossover study

Data for each nitrogen source						Comparisons			
Parameter		TEST	REFERENCE	Marketed product (MP)	Casein		TEST vs. REFERENCE*	TEST vs. MP	TEST vs. Casein
EAAs Primary endpoint	C_{max} µmol/L Mean (SD)	1768.2 (252.77)	2434.6 (367.52)	//	//	**C_{max} *p*-value**	**0.726** <0.0001	//	//
	$AUC_{0-300min}$ (µmol/L)*min Mean (SD)	396027.6 (44935.44)	443869.8 (46190.52)	//	//	**$AUC_{0-300min}$ (90% CI)**	**0.890** (0.865, 0.915)	//	//
EAAs	C_{max} µmol/L Mean (SD)	1768.2 (252.77)	2434.6 (367.52)	2124.2 (313.88)	2175.9 (379.34)	**C_{max} *p*-value**	**0.726** <0.0001	**0.835** <0.0001	**0.818** <0.0001
	$AUC_{0-420min}$ (µmol/L)*min Mean (SD)	508855.6 (57172.36)	549374.1 (55167.22)	555955.3 (54542.07)	542576.7 (55364.52)	**$AUC_{0-420min}$ (90% CI)**	**0.924** (0.900, 0.950)	**0.917** (0.892, 0.942)	**0.936** (0.910, 0.961)
LNAAs	C_{max} µmol/L Mean (SD)	1265.3 (175.15)	1872.4 (301.08)	1636.2 (225.55)	1679.4 (282.20)	**C_{max} *p*-value**	**0.677** <0.0001	**0.775** <0.0001	**0.759** <0.0001
	$AUC_{0-420min}$ (µmol/L)*min Mean (SD)	385042.3 (43215.11)	423787.3 (43934.14)	434652.2 (38995.24)	421698.21 (40276.07)	**$AUC_{0-420min}$ (90% CI)**	**0.908** (0.885, 0.932)	**0.887** (0.864, 0.910)	**0.911** (0.888, 0.935)
BCAAs	C_{max} µmol/L Mean (SD)	692.8 (106.48)	1201.1 (221.18)	1043.2 (148.85)	1031.9 (188.62)	**C_{max} *p*-value**	**0.579** <0.0001	**0.664** <0.0001	**0.676** <0.0001
	$AUC_{0-420min}$ (µmol/L)*min Mean (SD)	206603.5 (28352.92)	239573.9 (29549.71)	262872.4 (26146.87)	248022.8 (27320.31)	**$AUC_{0-420min}$ (90% CI)**	**0.860** (0.836, 0.885	**0.785** (0.763, 0.807)	**0.829** (0.806, 0.852)
Total AAs	C_{max} µmol/L Mean (SD)	3566.5 (468.10)	4586.6 (575.72)	4108.6 (584.69)	4161.0 (612.82)	**C_{max} *p*-value**	**0.775** <0.0001	**0.870** <0.0001	**0.860** <0.0001
	$AUC_{0-420min}$ (µmol/L)*min Mean (SD)	1106090.3 (129426.51)	1176026.8 (97284.78)	1170493.8 (122839.97)	1160090.0 (121539.14)	**$AUC_{0-420min}$ (90% CI)**	**0.937** (0.909, 0.966)	**0.945** (0.917, 0.974)	**0.952** (0.923, 0.981)
BUN	$AUC_{0-300min}$ (mmol/L)*min Mean (SD)	1357.4 (201.24)	1572.9 (265.88)	1423.3 (241.46)	1475.9 (291.83)	**Repeated measure of covariance**	P<0.0001 (ANCOVA)	p=0.0208 (ANCOVA)	//
Insulin	$AUC_{0-300min}$ (mU/L)*min Mean (SD)	2137.9 (1011.68)	2703.7 (1375.05)	2593.4 (1083.67)	2604.6 (1344.29)	**Repeated measure of covariance**	P<0.0001 (ANCOVA)	P=0.0160 (ANCOVA)	//
Glucose	$AUC_{0-300min}$ (mmol/L)*min Mean (SD)	1621.2 (91.49)	1609.4 (93.35)	1610.0 (86.38)	1600.0 (110.84)	**Repeated measure of covariance**	P=0.0068 (ANCOVA)	p=0.0139 (ANCOVA)	//

KEY REFERENCES
1 Dangin M, et al. Am J Physiol Endocrinol Metab. 2001; 280:E340-8.
2 Metges CC, et al. Am J Physiol Endocrinol Metab. 2000; 278E1000-9.
3 van Spronsen FJ, et al. Lancet Diabetes Endocrinol. 2017; 5:743-756.
4 Gannon MC, al. IUBMB Life. 2010; 62:660-8.
5 Calbet JA, et al. J Nutr. 2002; 132:2174-82.
6 Giarratana N, et al. J Inborn Errors Metab Screen. 2018; 6:1–9.

CONFLICT OF INTEREST
Anita MacDonald has received research funding and honoraria from Nutricia, Vitaflo International and Merck Serono. She is a member of the European Nutrition Expert Panel (Biomarin), member of Sapropterin Advisory Board (Biomarin), member of the Advisory Board entitled ELEMENT (Danone-Nutricia), and member of an Advisory Board for Arla and Applied Pharma Research.
Mika Scheinin is employee, board member and stock owner of CRST Ltd., a contract research organization that was commissioned by Applied Pharma Research to perform this clinical trial.
Anna Barassi is an employee of APR Applied Pharma Research s.a.