

Randomized Control Trials

Postprandial metabolic responses to a glycomacropeptide-based protein substitute in different meal contexts – Implications for phenylketonuria (PKU)

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SUMMARY

Background: Casein glycomacropeptide supplemented with amino acids (CGMP-AA) is used in the treatment of phenylketonuria (PKU). However, its acute metabolic effects when consumed with other foods remain poorly understood. This study investigated the impact CGMP-AA consumed alone or with two low-protein meals providing carbohydrates with either fibre or fat.

Methods: In this randomised crossover trial, fifteen healthy adults (27.0 ± 7.9 y; 7 females) consumed: A) CGMP-AA alone; B) CGMP-AA with low-protein bread and unpeeled apple; or C) CGMP-AA with low-protein bread and olive oil. Blood samples were collected over 120 min to assess glucose, insulin, C-peptide, amino acids, and appetite-related hormones (glucose-dependent insulintropic polypeptide (GIP), glucagon-like peptide-1 (GLP-1), and peptide YY (PYY)).

Results: Co-ingestion with low-protein foods had limited effects on total essential and large neutral amino acid concentrations over 120 min, but increased Tyr incremental area under the curve (iAUC_(0-120min)) and peak concentration (C_{max}) compared with CGMP-AA alone. Meal B elicited the highest glucose, insulin, and C-peptide iAUC_(0-120min) and C_{max}, followed by meal C, whereas meal A induced minimal glycaemic and insulinaemic excursions. Meal C produced the greatest GIP response, whereas GLP-1 and PYY did not differ between test meals.

Conclusions: In healthy adults, CGMP-AA elicited distinct acute postprandial responses depending on whether it was consumed alone or with low-protein foods. CGMP-AA consumed with food is not metabolically equivalent to CGMP-AA consumed alone, and carbohydrate-with-fibre and carbohydrate-

Abbreviations: ALT, alanine aminotransferase; ANOVA, analysis of variance; AST, aspartate aminotransferase; BMI, body mass index; CGMP-AA, low-phenylalanine casein glycomacropeptide supplemented with rate-limiting amino acids; C_{max}, peak concentration; DPP4, dipeptidyl peptidase-IV; EAAs, Essential amino acids; GC-FID, gas chromatography with flame ionization detection; GIP, glucose-dependent insulintropic polypeptide; GLP-1, glucagon-like peptide-1; HDL, high-density lipoprotein; iAUC, incremental area under the curve; IU, international units; L-AAs, Phe-free L-amino acids; LNAAs, large neutral amino acids; LDL, low-density lipoprotein; mTOR, mammalian target of rapamycin; Phe, phenylalanine; PKU, phenylketonuria; PYY, peptide YY; SD, standard deviation; T_{max}, time to reach peak concentration; Tyr, tyrosine; γGT, gamma-glutamyl transferase.

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with-fat meal contexts affect distinct aspects of the postprandial response. These findings should be confirmed in individuals with PKU before being translated into dietary recommendations.

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1. Background

Phenylketonuria (PKU; OMIM #261600), is an inherited metabolic disorder of phenylalanine (Phe) metabolism, mostly caused by a deficiency of the hepatic enzyme phenylalanine hydroxylase. Impaired conversion of Phe to tyrosine (Tyr) results in elevated blood Phe concentrations, which, if untreated, are associated with severe neurological impairment. Management of PKU primarily relies on a Phe-restricted diet that limits natural protein intake to limit dietary Phe exposure. To ensure adequate protein intake, patients with PKU require supplementation with a Phe-free/low Phe protein substitute, taken in small and evenly distributed doses throughout the day [1].

Protein substitutes are essential to support normal growth, prevent protein deficiency, help maintain metabolic control, and provide Tyr, which becomes an essential amino acid in individuals with PKU due to impaired endogenous synthesis from Phe [2,3]. Tyr has important biological roles as a precursor for catecholamine neurotransmitters, including dopamine, adrenaline and noradrenaline, as well as for melanin and thyroid hormone synthesis, further supporting the importance of adequate Tyr provision in PKU [4,5].

Historically, protein substitutes have been formulated using Phe-free L-amino acids (L-AAs). More recently, low-Phe casein glycomacropeptide (CGMP) supplemented with rate-limiting amino acids (CGMP-AA) has emerged as an alternative source of protein equivalent. CGMP-AA has gained considerable interest because of its improved palatability and acceptability compared with conventional L-AA formulations, which may support adherence to prescribed protein substitute intake [1,6,7].

In addition to its nutritional role, CGMP demonstrates several biological properties, including immunomodulatory, antimicrobial, and prebiotic effects, and it has also been shown to inhibit oral bacterial adhesion, thereby contributing to anticariogenic activity [8]. In children with PKU, CGMP-based protein substitutes have been associated with significant improvements in gastrointestinal symptoms such as stomach pain, discomfort during eating, heartburn and reflux, wind, bloating, and constipation, when compared with L-AA-based formulations [9]. In addition, some studies have reported higher median blood Tyr concentrations throughout the day following CGMP-AA compared with L-AAs intake, suggesting that CGMP-AA may influence Tyr availability in PKU [10,11]. However, the metabolic significance of these changes, and the extent to which they are influenced by meal context, remains poorly characterised.

Meal-induced metabolic responses are critical determinants of nutrient utilisation, protein synthesis, appetite regulation, and overall metabolic homeostasis [12–16]. These acute postprandial processes are elicited repeatedly throughout the day and collectively contribute to long-term metabolic regulation and overall health. Their magnitude and pattern vary substantially depending on the macronutrient composition of a meal [13–16]. Although carbohydrates are the main drivers of postprandial glycaemic and insulinaemic excursions [15], dietary fat can also influence these

parameters by delaying gastric emptying and altering intestinal glucose absorption kinetics [17]. Amino acids also contribute to postprandial metabolic regulation, with certain amino acids, particularly leucine, stimulating insulin secretion through direct pancreatic β -cell activation, thereby influencing postprandial glycaemic control and amino acid utilisation [18]. In parallel, macronutrient composition of a meal affects the secretion of appetite-regulating hormones such as glucose-dependent insulinotropic polypeptide (GIP), glucagon-like peptide-1 (GLP-1), and peptide YY (PYY) [13,19,20].

Although protein substitutes are routinely consumed with or around meals in PKU, the acute metabolic effects of CGMP-AA when consumed alone or alongside foods differing in macronutrient composition remain incompletely understood. This is clinically relevant because the food matrix accompanying a protein substitute may influence postprandial insulin secretion, glycaemic responses, amino acid absorption kinetics and Tyr availability. Therefore, the metabolic response to CGMP-AA is likely to depend not only on its amino acid composition, but also on the macronutrient composition of the meal with which it is consumed.

Thus, the aim of this randomised crossover study was to investigate, in healthy individuals, the acute postprandial effects of CGMP-AA consumed alone or in combination with two low-protein meals differing in macronutrient composition: one providing carbohydrate with fibre, and the other providing carbohydrate with fat. Amino acid absorption kinetics, including Tyr availability, as well as glycaemic and insulinaemic excursions and gut-derived hormones related to appetite regulation, were assessed as key postprandial outcomes.

Healthy individuals were selected to allow a controlled investigation of acute postprandial metabolic responses under standardised conditions. This approach is supported by previous stable isotope work suggesting that some aspects of whole-body protein metabolism in adults with PKU may be comparable to those of healthy controls [21]. However, this model does not reproduce the altered Phe and Tyr metabolism characteristic of PKU. Therefore, the present study provides a mechanistic framework to examine whether meal composition modulates the acute metabolic response to CGMP-AA, and to generate testable hypotheses for future studies in individuals with PKU.

2. Methods

2.1. Participants

Fifteen healthy volunteers were recruited between February and April 2025, through advertisements posted on websites, social media platforms, and printed posters displayed in public locations. Recruitment was open to the general public and included both university students and individuals from outside the university. Eligibility criteria included age ≥ 18 years old and body mass index (BMI) between 18.5 and 25.0 kg/m². Individuals were excluded if they had any clinically significant metabolic or endocrine disorder, or other conditions known to affect postprandial metabolism,

reported recent changes (within the past month) in the frequency, duration, or intensity of physical activity, used protein supplements, were pregnant or breastfeeding, or reported smoking or recreational drug use.

2.2. Study design

This was a randomised crossover trial. Participants attended the research facility (at NOVA Medical School, NOVA University of Lisbon) on three separate occasions, with a washout period of at least one week in between. On the day preceding each study visit, participants were instructed to avoid structured physical activity and to consume a standardised evening meal 10–12 h before the visit, containing a moderate amount of protein; they were asked to replicate the same meal before all three visits. During each session, participants consumed one of the three test meals: (A) CGMP-AA (PKU Sphere 20® - red berry, Vitaflo International Ltd.®, Liverpool, UK), consisting of a 35 g sachet reconstituted according to manufacturer instructions, and 100 mL of water, (B) CGMP-AA (35 g sachet), with two slices of white low-protein bread (Bez-gluten®, Malopolskie, Poland) (50 g), unpeeled apple (160 g; selected to provide a realistic acute fibre load), and 100 mL of water, and (C) CGMP-AA (35 g sachet), with two slices of white low-protein bread (50 g), olive oil (10 g; fat source), and 100 mL of water.

These combinations were designed to reflect realistic low-protein breakfast contexts in which a protein substitute may be consumed either with bread and fruit or with bread and a fat source. Olive oil was selected as the fat source to reflect Mediterranean dietary habits, given that the study was conducted in Portugal. All components of each test meal were consumed together, with no predefined order of consumption. Participants consumed the assigned test meal within 15 min under supervision. The nutritional composition of the test meals is summarised in Table 1.

The sequence of meal consumption was determined by simple randomisation. The postprandial amino acid response was defined *a priori* as the primary outcome of interest. Accordingly, the duration of the postprandial assessment was established based on preliminary pilot observations, which indicated that a 120-min period was sufficient to capture the amino acid response following CGMP-AA ingestion. This time-course was therefore selected to adequately assess the primary outcome while minimising participant burden compared with a longer 180-min protocol. Venous blood samples were collected at baseline (0 min, immediately before meal ingestion) and at 30, 45, 60, 90, and 120 min after the start of the meal.

Table 1
Nutritional composition of the test meals.

	Meal A	Meal B	Meal C
Calories, kcal	120.0	353.4	340.9
Protein, g	20.0	20.6	20.3
Of which Phenylalanine, mg	36.0	62.0	46.0
Carbohydrates, g	6.6	51.5	30.1
Of which sugars, g	2.8	26.8	5.4
Fibre, g	0.0	6.8	3.5
Fat, g	1.6	5.7	14.8
Of which saturated fat, g	0.4	0.9	2.2
Salt, g	0.71	1.5	1.5

Meal A: CGMP-AA and 100 mL of water. Meal B: CGMP-AA, with two slices of white low-protein bread (50 g), unpeeled apple (160 g), and 100 mL of water. Meal C: CGMP-AA, with two slices of white low-protein bread (50 g), olive oil (10 g), and 100 mL of water. **Abbreviations:** CGMP-AA, casein glycomacropeptide with amino acids.

2.3. Biochemical measurements

At each time point, venous blood was collected via a peripheral venous catheter (Introcan Safety® 3 Closed IV Catheter - 22G, B. Braun, Melsungen, Germany), inserted and handled by experienced nursing staff. Samples were drawn into serum tubes (BD Vacutainer® SST II Advance, Berkshire, United Kingdom), allowed to clot for 30 min at room temperature, centrifuged at 1.300×g for 15 min, and the resulting serum stored at −80 °C for subsequent analysis of glucose, insulin, C-peptide, and amino acids.

At baseline, 60 (expected peak), and 120 (end of the study time-course) mins, additional blood samples were collected into EDTA tubes (BD Vacutainer® EDTA Tubes) containing a dipeptidyl peptidase-IV (DPP4) inhibitor (Merck, Darmstadt, Germany), kept on ice, centrifuged immediately, and plasma stored at −80 °C for determination of GIP, GLP-1, and PYY concentrations.

A fasting metabolic profile was also obtained during one of the study visits. This included lipid profile (total cholesterol, high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), and triglycerides), liver function enzymes (alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (γGT)), renal function markers (urea, creatinine and estimated glomerular filtration rate), and C-reactive protein.

All analyses were performed using gold-standard methods. Glucose, insulin, C-peptide, and standard clinical chemistry parameters were determined by an outsourced certified medical laboratory. Free amino acids were extracted using a solid-phase extraction step with cation-exchange cartridges (Strata X-C, Phenomenex), following a protocol based on the EZ:faast™ kit from Phenomenex (Phenomenex Inc., CA, USA), with minor in-house modifications to suit laboratory procedures. Amino acids were subsequently analysed by gas chromatography with flame ionization detection (GC-FID), as propyl chloroformate derivatives. Appetite-regulating hormones were measured using ELISA kits (Millipore, Massachusetts, USA) following the manufacturer's instructions.

2.4. Anthropometry and body composition

Standing height was measured using a wall-mounted stadiometer, with participants barefoot and positioned according to standard guidelines. All measurements were performed by a registered dietitian to ensure consistency and reliability. Body composition was assessed in the fasting state using bioelectrical impedance analysis (InBody® model 770), following the standardised protocol described previously [22]. The following parameters were obtained: body weight, lean body mass, skeletal muscle mass, body fat mass, percentage of body fat, visceral fat area, total body water, extracellular water to total body water ratio, basal metabolic rate, whole-body phase angle, and waist-to-hip ratio.

2.5. Statistical analysis

As the postprandial amino acid response was the primary outcome of interest, the sample size was determined based on the study by Gropper et al. [23]. To detect a mean difference in total plasma amino acid concentrations between groups of 639 μmol/L, with a standard deviation (SD) of 572.8 μmol/L, at 80% power and a 1% significance level, 12 participants were required. To account for potential dropouts or loss to follow-up (~20%), a total of 15 participants were recruited. Analyses of appetite-regulating hormones were considered exploratory.

Incremental areas under the curves (iAUC_{0–120 min}), excluding areas below baseline, were calculated for each participant using

the trapezoidal rule. Postprandial hormonal concentrations (GLP-1, GIP and PYY), were expressed as percentage change from baseline. Data distribution was assessed using the Shapiro–Wilk test. Variables with a normal distribution are presented as mean $\pm$ SD, unless otherwise stated, and non-normally distributed variables as median (interquartile range). Comparisons between test meals were performed using repeated-measures (paired) analysis of variance (ANOVA) or equivalent non-parametric tests when assumptions were not met. Statistical significance was defined as $p < 0.05$. Statistical analysis was performed using GraphPad Prism V8.0 (GraphPad Software, Inc., California, USA).

2.6. Ethics

The study protocol was approved by the Ethics Research Committee of NMS/FCM–UNL (approval number 140/2024) and registered on [ClinicalTrials.gov](https://www.clinicaltrials.gov) (NCT06794515). All participants provided written informed consent prior to enrolment. The study was conducted in accordance with the principles of the Declaration of Helsinki.

3. Results

3.1. Participant characteristics

Fifteen healthy adults completed the study, and no adverse events occurred during the study visits. Participants had a mean age of 27.0 ± 7.9 years, a mean BMI of 21.4 ± 2.1 kg/m² and seven (46.7%) were female. Baseline anthropometric and biochemical characteristics are summarised in Table 2. No clinically relevant abnormalities were observed in lipid profile, liver or renal function markers, or C-reactive protein levels. Thirteen participants (86.7%) reported engaging in regular physical activity, including a combination of aerobic, resistance training, and recreational exercises, with frequencies ranging from one to four times per week.

3.2. Amino acid kinetics

Global postprandial amino acid patterns across the three meals are summarised in Tables 3 and 4, which provide $iAUC_{(0-120min)}$ and peak concentration (C_{max}) values, respectively. Most amino acids exhibited similar postprandial profiles across meals, with only a subset showing significant meal-dependent differences. Specifically, meal B produced significantly higher $iAUC_{(0-120min)}$ and C_{max} for alanine, asparagine, and proline, as well as a higher C_{max} for glycine. Both meals B

and C generated significantly greater Tyr $iAUC_{(0-120min)}$ and C_{max} compared with meal A.

Essential amino acids (EAAs) (Fig. 1A) increased postprandially, reaching their peak at 45 min. The increase was significantly more pronounced following meal B (2175.2 ± 205.4 μ mol/L) compared with meal A (1877.9 ± 484.0 μ mol/L). Concentrations subsequently declined, with meal B showing slightly lower values than meal A at 90 min, although this did not reach statistical significance ($p = 0.053$). No significant differences were observed in the $iAUC_{(0-120min)}$ (Fig. 1B) nor the C_{max} between the three meals (Tables 3 and 4), indicating that the early differences in EAAs concentrations did not translate into differences in total EAAs exposure over 120 min.

Large Neutral Amino Acids (LNAAs) (Fig. 1C) exhibited a similar temporal pattern, peaking at 45 min for all meals. Meal B induced significantly higher LNAAs concentrations than meal A at both 30 and 45 min. Levels progressively declined thereafter until 120 min. While no significant differences were observed in $iAUC_{(0-120min)}$ between meals (Fig. 1D), Meal B showed a significantly higher C_{max} than meal A ($p = 0.030$) for LNAAs (Tables 3 and 4). Thus, meal composition influenced the early LNAAs response, reflected by a higher C_{max} after meal B, but did not significantly alter overall LNAAs exposure over 120 min.

Postprandial concentrations of Phe and Tyr were also assessed (Fig. 2). In healthy adults, Phe concentration (Fig. 2A) gradually decreased over the 120-min postprandial period, with no differences between meals. Consistently, no between-meal differences were detected in Phe $iAUC_{(0-120min)}$ (Fig. 2B). In contrast, postprandial Tyr concentration (Fig. 2C) diverged from 60 min onward, with significantly higher values after meal C compared with meal A. Consistent with this, Tyr $iAUC$ (Fig. 2D) was significantly higher after both meals B and C compared with meal A. This indicates greater Tyr availability when CGMP-AA was co-ingested with low-protein foods, irrespective of whether the meal provided carbohydrate with fibre or carbohydrate with fat, compared with CGMP-AA consumed alone.

Collectively, co-ingestion of CGMP-AA with low-protein meals had limited effects on total EAAs and LNAAs exposure over 120 min, but significantly increased Tyr availability compared with CGMP-AA consumed alone.

3.3. Glycaemic and insulinaemic excursions

Postprandial glucose, insulin, and C-peptide concentrations differed across the test meals (Fig. 3). Glucose (Fig. 3A) increased

Table 2

Anthropometric and biochemical characteristics of study participants.

Characteristics	N = 15	Characteristics	N = 15
Age, years	27.0 $\pm$ 7.9	Total Cholesterol, mg/dL	168.5 $\pm$ 31.4
Female, n (%)	7 (46.7)	HDL Cholesterol, mg/dL	62.9 $\pm$ 15.1
Weight, Kg	63.9 $\pm$ 9.4	LDL Cholesterol, mg/dL	91.1 $\pm$ 19.5
Height, m	1.7 $\pm$ 0.1	Triglycerides, mg/dL	65.0 [59.0; 85.0]
BMI, Kg/m ²	21.4 $\pm$ 2.1	Fasting Glucose, mg/dL	82.2 $\pm$ 4.8
Lean Body Mass, Kg	49.4 $\pm$ 10.8	Insulin, μ U/mL	6.6 $\pm$ 1.9
Skeletal Muscle Mass, Kg	29.3 $\pm$ 6.9	C Peptide, ng/mL	1.43 $\pm$ 0.36
Body Fat Mass, Kg	11.4 $\pm$ 5.7	Urea, mg/dL	32.7 $\pm$ 6.6
Percent Body Fat, %	18.4 $\pm$ 9.3	Creatinine, mg/dL	0.89 $\pm$ 0.17
Visceral Fat Area, cm ²	49.6 [29.3; 57.7]	Estimated Glomerular Filtration Rate, mL/min/1.73m ²	103.5 $\pm$ 11.6
Total Body Water, L	38.4 $\pm$ 8.3	C-Reactive Protein, mg/L	0.18 $\pm$ 0.07
Extracellular Water/Total Body Water	0.38 $\pm$ 0.00	Aspartate Aminotransferase, U/L	23.0 [18.0; 25.0]
Basal Metabolic Rate, Kcal/day	1502.4 $\pm$ 244.6	Alanine Aminotransferase, U/L	8.0 [7.0; 12.0]
Whole Body Phase Angle, °	5.8 $\pm$ 0.9	Gamma-Glutamyl Transferase, U/L	14.5 $\pm$ 3.9
Waist-Hip Ratio	0.84 $\pm$ 0.05		

Abbreviations: BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Table 3iAUC_(0–120 min) for plasma amino acids following ingestion of the test meals.

	iAUC _(0–120min)			
	Meal A	Meal B	Meal C	p
Alanine, $\mu\text{mol/L}\cdot\text{min}$	12,257.3 [11,243.5; 18,573.8]	27,987.4 [22,408.5; 30,031.4] **	13,066.8 [10,755.2; 20,224.4]	<0.001
Asparagine, $\mu\text{mol/L}\cdot\text{min}$	3789.0 $\pm$ 902.4	4740.2 $\pm$ 1166.4 **	3905.8 $\pm$ 1278.0	0.016
Aspartate, $\mu\text{mol/L}\cdot\text{min}$	527.6 $\pm$ 349.6	505.8 $\pm$ 234.0	534.5 $\pm$ 273.5	0.957
Glutamate, $\mu\text{mol/L}\cdot\text{min}$	773.8 [65.2; 1333.6]	409.4 [24.9; 1852.2]	324.3 [9.6; 1234.1]	0.282
Glutamine, $\mu\text{mol/L}\cdot\text{min}$	8827.4 $\pm$ 2637.5	9412.9 $\pm$ 3583.2	7946.7 $\pm$ 4443.4	0.531
Glycine, $\mu\text{mol/L}\cdot\text{min}$	5639.3 $\pm$ 1432.4	6906.6 $\pm$ 2054.6	5353.6 $\pm$ 2378.6	0.086
Histidine, $\mu\text{mol/L}\cdot\text{min}$	2695.7 [2030.4; 3420.3]	3177.5 [2612.6; 4047.1]	2907.9 [1520.8; 3713.2]	0.449
Isoleucine, $\mu\text{mol/L}\cdot\text{min}$	11,825.1 $\pm$ 2832.1	12,117.7 $\pm$ 2102.6	11,985.8 $\pm$ 2879.1	0.862
Leucine, $\mu\text{mol/L}\cdot\text{min}$	26,804.9 $\pm$ 5766.3	26,090.7 $\pm$ 3401.6	25,273.0 $\pm$ 6004.6	0.572
Lysine, $\mu\text{mol/L}\cdot\text{min}$	8288.9 $\pm$ 3471.1	9242.8 $\pm$ 3533.1	8353.1 $\pm$ 4295.8	0.648
Methionine, $\mu\text{mol/L}\cdot\text{min}$	1047.9 $\pm$ 304.0	1294.3 $\pm$ 304.0	1067.0 $\pm$ 394.6	0.077
Ornithine, $\mu\text{mol/L}\cdot\text{min}$	2502.7 $\pm$ 1358.9	2422.4 $\pm$ 920.3	2262.7 $\pm$ 1313.3	0.815
Phenylalanine, $\mu\text{mol/L}\cdot\text{min}$	27.1 [0; 37.1]	51.8 [0; 111.5]	0.0 [0; 72.5]	0.199
Pipecolic acid, $\mu\text{mol/L}\cdot\text{min}$	16.2 [3.3; 47.5]	12.0 [2.8; 31.2]	6.8 [0.0; 32.6]	0.799
Proline, $\mu\text{mol/L}\cdot\text{min}$	17,030.3 $\pm$ 4211.3	20,407.4 $\pm$ 3918.4 **	17,454.2 $\pm$ 5024.8	0.008
Serine, $\mu\text{mol/L}\cdot\text{min}$	23.6 [0.0; 137.7]	0.0 [0.0; 380.1]	0.0 [0.0; 44.8]	0.770
Threonine, $\mu\text{mol/L}\cdot\text{min}$	14,240.7 $\pm$ 6958.3	17,839.1 $\pm$ 6320.3	17,353.9 $\pm$ 6834.5	0.065
Tryptophan, $\mu\text{mol/L}\cdot\text{min}$	4910.5 [4227.0; 5534.3]	4973.5 [4499.5; 5869.3]	4960.9 [4282.8; 5306.6]	0.282
Tyrosine, $\mu\text{mol/L}\cdot\text{min}$	2947.9 $\pm$ 1520.9	4883.3 $\pm$ 989.7 *	4618.8 $\pm$ 1690.8 *	<0.001
Valine, $\mu\text{mol/L}\cdot\text{min}$	18,092.1 $\pm$ 4994.3	17,979.6 $\pm$ 3497.0	16,821.2 $\pm$ 5106.8	0.460
EAA, $\mu\text{mol/L}\cdot\text{min}$	89,172.4 [63,524.2; 103,143.8]	92,204.4 [78,687.7; 100,116.6]	80,131.7 [62,999.2; 109,543.3]	0.936
LNA, $\mu\text{mol/L}\cdot\text{min}$	81,554.5 $\pm$ 18,921.8	87,030.3 $\pm$ 11,467.7	82,638.3 $\pm$ 19,736.9	0.494

Data are presented as mean $\pm$ SD or a as median [interquartile range]. Statistical differences between meals were assessed by repeated-measures ANOVA or Friedman test with post-hoc testing. * $p < 0.05$ vs meal A and # $p < 0.05$ vs meal C. **Meal composition:** meal A - CGMP-AA and 100 mL of water; meal B - CGMP-AA, with two slices of white low-protein bread (50 g), unpeeled apple (160 g), and 100 mL of water; meal C - CGMP-AA, with two slices of white low-protein bread (50 g), olive oil (10 g), and 100 mL of water. **Abbreviations:** CGMP-AA, casein glycomacropeptide with amino acids; EAA, essential amino acids; iAUC, incremental area under the curve; LNA, large neutral amino acids; SD, standard deviation.

Table 4C_{max} for plasma amino acids following ingestion of the test meals.

	C _{max}			
	Meal A	Meal B	Meal C	p
Alanine, $\mu\text{mol/L}$	551.8 $\pm$ 108.7	696.3 $\pm$ 107.0 *	602.6 $\pm$ 121.1	0.008
Asparagine, $\mu\text{mol/L}$	102.7 $\pm$ 19.5	126.5 $\pm$ 17.0 *	115.2 $\pm$ 20.6	<0.001
Aspartate, $\mu\text{mol/L}$	18.1 [10.4; 29.1]	18.5 [11.3; 24.7]	20.5 [14.2; 28.3]	0.169
Glutamate, $\mu\text{mol/L}$	89.5 $\pm$ 33.0	98.5 $\pm$ 33.3	100.5 $\pm$ 31.3	0.357
Glutamine, $\mu\text{mol/L}$	538.0 $\pm$ 84.9	580.3 $\pm$ 63.5	570.8 $\pm$ 78.7	0.168
Glycine, $\mu\text{mol/L}$	277.1 $\pm$ 70.5	320.9 $\pm$ 70.8 *	292.6 $\pm$ 17.1	0.007
Histidine, $\mu\text{mol/L}$	129.5 $\pm$ 31.0	140.0 $\pm$ 12.5	136.0 $\pm$ 19.9	0.232
Isoleucine, $\mu\text{mol/L}$	218.8 $\pm$ 57.9	245.4 $\pm$ 38.8	233.6 $\pm$ 45.1	0.129
Leucine, $\mu\text{mol/L}$	466.8 $\pm$ 111.3	511.9 $\pm$ 69.1	470.5 $\pm$ 82.3	0.124
Lysine, $\mu\text{mol/L}$	312.9 $\pm$ 73.6	341.5 $\pm$ 71.0	340.0 $\pm$ 70.9	0.310
Methionine, $\mu\text{mol/L}$	40.5 $\pm$ 12.4	46.6 $\pm$ 9.8	42.8 $\pm$ 12.5	0.094
Ornithine, $\mu\text{mol/L}$	103.1 $\pm$ 36.1	109.8 $\pm$ 39.4	116.6 $\pm$ 31.7	0.324
Phenylalanine, $\mu\text{mol/L}$	54.8 [48.2; 65.1]	51.7 [50.7; 69.2]	55.3 [50.1; 68.9]	0.381
Pipecolic acid, $\mu\text{mol/L}$	2.8 [1.9; 3.9]	2.8 [2.1; 3.8]	3.3 [2.0; 5.2]	0.854
Proline, $\mu\text{mol/L}$	380.0 $\pm$ 95.0	435.6 $\pm$ 81.8 *	405.2 $\pm$ 82.5	0.035
Serine, $\mu\text{mol/L}$	88.8 [77.4; 110.1]	105.3 [86.7; 165.9]	103.5 [85.5; 115.4]	0.057
Threonine, $\mu\text{mol/L}$	264.7 $\pm$ 121.6	318.5 $\pm$ 115.5	323.4 $\pm$ 136.0	0.053
Tryptophan, $\mu\text{mol/L}$	115.1 $\pm$ 20.6	125.7 $\pm$ 14.3	117.2 $\pm$ 20.0	0.125
Tyrosine, $\mu\text{mol/L}$	93.6 $\pm$ 21.3	114.9 $\pm$ 15.0 *	119.3 $\pm$ 30.1 *	0.007
Valine, $\mu\text{mol/L}$	474.6 $\pm$ 102.6	510.2 $\pm$ 72.3	472.9 $\pm$ 61.4	0.123
EAA, $\mu\text{mol/L}$	2026.0 $\pm$ 422.1	2241.3 $\pm$ 253.1	2124.8 $\pm$ 267.5	0.078
LNA, $\mu\text{mol/L}$	1805.4 $\pm$ 374.7	2008.3 $\pm$ 228.4	1895.9 $\pm$ 253.8	0.054

Data are presented as mean $\pm$ SD or a as median [interquartile range]. Statistical differences between meals were assessed by repeated-measures ANOVA or Friedman test with post-hoc testing. * $p < 0.05$ vs meal A and # $p < 0.05$ vs meal C. **Meal composition:** meal A - CGMP-AA and 100 mL of water; meal B - CGMP-AA, with two slices of white low-protein bread (50 g), unpeeled apple (160 g), and 100 mL of water; meal C - CGMP-AA, with two slices of white low-protein bread (50 g), olive oil (10 g), and 100 mL of water. **Abbreviations:** CGMP-AA, casein glycomacropeptide with amino acids; C_{max}, peak concentration; EAA, essential amino acids; iAUC, incremental area under the curve; LNA, large neutral amino acids; SD, standard deviation.

rapidly after meal ingestion, reaching peak concentrations (T_{max}) at 30 min for all meals. Meal B induced the highest C_{max} (96.2 $\pm$ 11.0 mg/dL), significantly higher than meal A (85.6 $\pm$ 5.1 mg/dL, $p = 0.031$). Thereafter, glucose concentrations fell below baseline from 45 min onward for all meals, although a slight increase was observed from 60 min for meals A and B and

from 45 min for meal C. Consistent with this, the glucose iAUC_(0–120min) (Fig. 3B) was significantly higher following meal B compared with meal A (489.5 $\pm$ 431.6 vs 70.6 $\pm$ 60.7 mg min/dL; $p = 0.006$), whereas the increase following meal C (408.6 $\pm$ 567.9 mg min/dL) did not reach statistical significance ($p = 0.095$) compared to meal A.

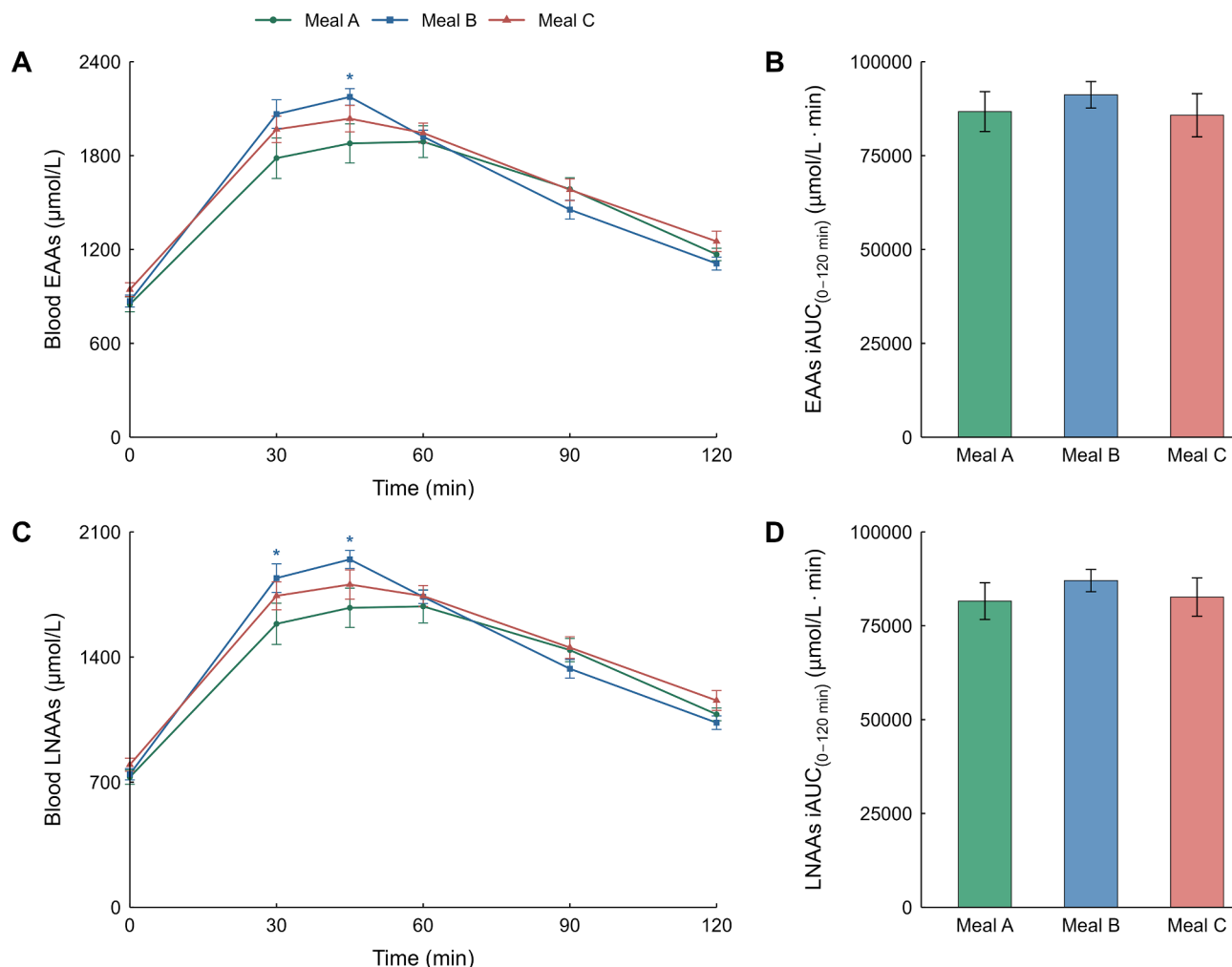


Fig. 1. Postprandial essential and large amino acids responses following ingestion of Meals A, B, and C. Essential (A) and large (C) amino acids concentrations over 120 min after the three test meals. iAUC_(0-120 min) for postprandial essential (B) and large (D) amino acids after the three meals. Data are presented as mean $\pm$ SEM ($n = 15$). Statistical differences between meals were assessed by repeated-measures ANOVA or Friedman test with post-hoc testing. For time-course analyses, * $p < 0.05$ vs meal A at corresponding time points. **Meal composition:** meal A - CGMP-AA and 100 mL of water; meal B - CGMP-AA, with two slices of white low-protein bread (50 g), unpeeled apple (160 g), and 100 mL of water; meal C - CGMP-AA, with two slices of white low-protein bread (50 g), olive oil (10 g), and 100 mL of water. **Abbreviations:** CGMP-AA, casein glycomacropeptide with amino acids; iAUC, incremental area under the curve; SEM, standard error of the mean.

Postprandial insulin (Fig. 3C) peaked at 30 min, with meal B eliciting a significantly higher concentration (78.9 ± 27.2 μ IU/mL) compared to meals A (43.6 ± 20.8 μ IU/mL) and C (57.5 ± 21.0 μ IU/mL). After the peak, insulin declined gradually for all meals; however, levels remained significantly higher for meal B compared with meal A throughout the postprandial period. Meal C also showed higher insulin levels than meal A from 60 min onward. Accordingly, insulin iAUC_(0-120 min) (Fig. 3D) was significantly higher following the consumption of meals B and C compared with meal A ($p < 0.001$), with meal B also exceeding meal C ($p < 0.05$).

As expected, C-peptide concentrations (Fig. 3E) followed a similar pattern to insulin, peaking at 30 min. Meal B induced a significantly higher peak (5.6 ± 1.4 ng/mL), compared to meals C (4.5 ± 1.0 ng/mL) and A (4.0 ± 1.1 ng/mL). From 45 min onward, C-peptide concentrations after meals B and C remained significantly higher than after meal A, and the iAUC_(0-120 min) (Fig. 3F) was also significantly greater for both meals B and C compared with meal A.

Generally, co-ingestion of CGMP-AA with low-protein meals enhanced postprandial insulin and C-peptide responses compared with CGMP-AA consumed alone, with the carbohydrate-with-fibre

meal producing the most pronounced glycaemic and insulinaemic excursions.

3.4. Appetite-regulating hormones concentrations

Postprandial GIP, GLP-1, and PYY were expressed as percentage of change from baseline (Fig. 4). Postprandial GIP concentrations (Fig. 4A) differed between the three meals. At 60 min, meals B (343.9% [246.4; 648.4]) and C (615.6% [289.4; 954.2]) produced larger increases in GIP than meal A (255.1% [125.9; 328.7]), with the response to meal C reaching statistical significance. By 120 min, GIP levels had fallen after meal A but continued to rise relative to 60 min after meals B and C. Consequently, GIP concentrations at 120 min were significantly higher after both meals B and C compared with meal A.

Postprandial GLP-1 concentrations (Fig. 4B) were broadly similar across the three meals. At 60 min, meals B (182.1% [32.0; 362.3]) and C (73.4% [-2.4; 340.3]) showed a greater increase compared with meal A (43.7% [-33.7; 67.1]), although these differences did not reach statistical significance. By 120 min, GLP-1

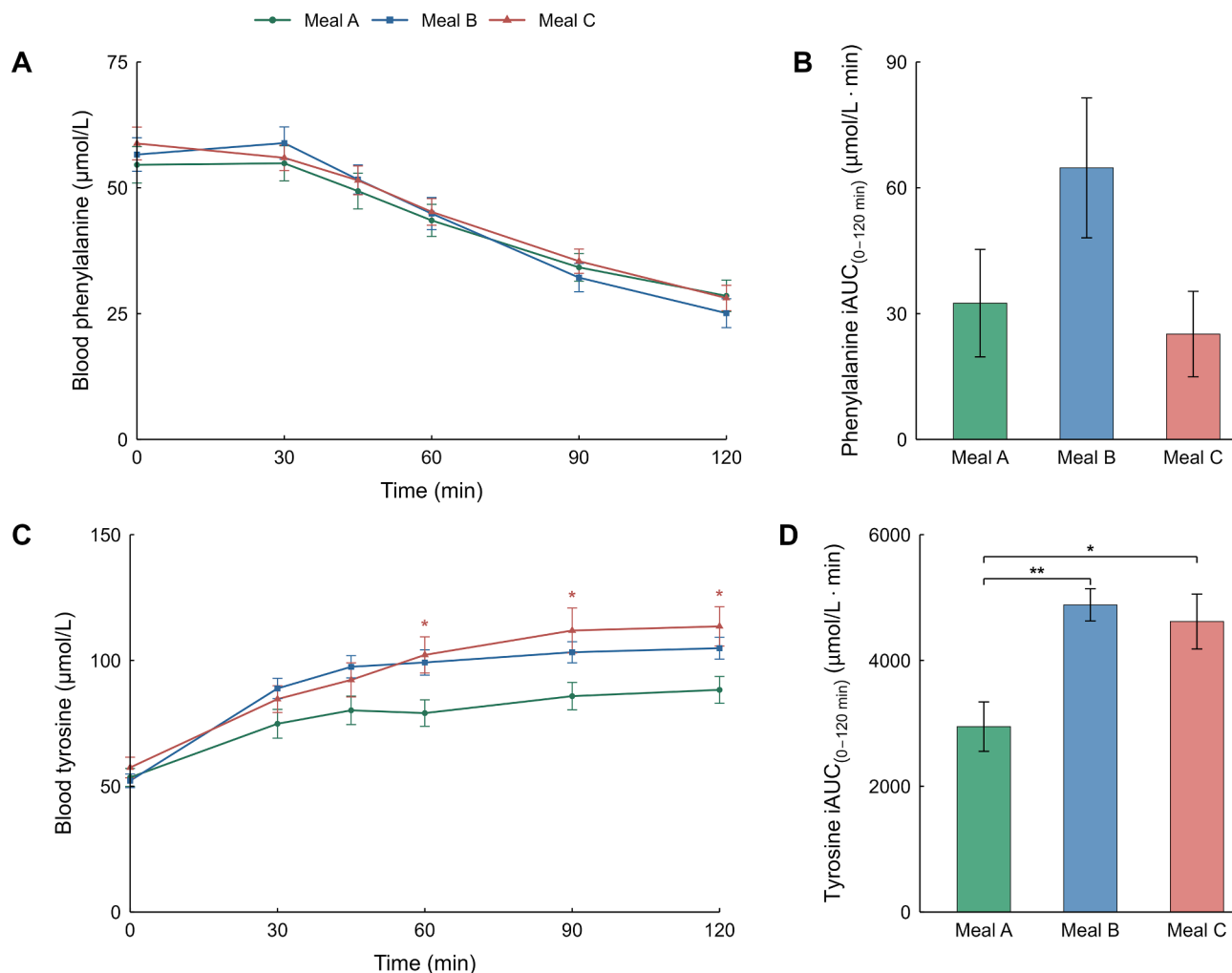


Fig. 2. Postprandial phenylalanine and tyrosine responses following ingestion of Meals A, B, and C. Phenylalanine (A) and tyrosine (C) concentrations over 120 min after the three test meals. iAUC_(0-120 min) for postprandial phenylalanine (B) and tyrosine (D) after the three meals. Data are presented as mean $\pm$ SEM (n = 15). Statistical differences between meals were assessed by repeated-measures ANOVA or Friedman test with post-hoc testing. For time-course analyses, *p < 0.05 vs meal A at corresponding time points. For iAUC_(0-120 min) analyses, *p < 0.05 and **p < 0.001 between meals. **Meal composition:** meal A - CGMP-AA and 100 mL of water; meal B - CGMP-AA, with two slices of white low-protein bread (50 g), unpeeled apple (160 g), and 100 mL of water; meal C - CGMP-AA, with two slices of white low-protein bread (50 g), olive oil (10 g), and 100 mL of water. **Abbreviations:** CGMP-AA, casein glycomacropeptide with amino acids; iAUC, incremental area under the curve; SEM, standard error of the mean.

levels after meal A were close to baseline, meal C showed a slight decrease, and meal B remained relatively unchanged.

Finally, postprandial PYY concentrations (Fig. 4C) did not differ significantly between the three meals. At 60 min, meal A did not produce a meaningful change in PYY concentrations (0.0% [−20.7; 25.9]), whereas meals B and C showed greater interindividual variability, with meal B producing the largest median increase (15.8% [−2.8; 53.5]) and meal C a more modest response (3.8% [−10.1; 87.8]). PYY concentration declined across all three meals between 60 and 120 min.

Taken together, co-ingestion of CGMP-AA with low-protein meals mainly affected GIP responses, particularly following the carbohydrate-with-fat meal, whereas GLP-1 and PYY did not differ significantly between test meals.

4. Discussion

This randomised crossover study investigated whether the acute postprandial metabolic response to CGMP-AA is influenced by the macronutrient composition of the foods with which it is consumed. Despite similar protein equivalent content (~20 g), the three meals produced distinct amino acid absorption kinetics,

glycaemic, insulinaemic, and appetite-regulating hormone responses. These findings highlight the significant role of non-nitrogen energy sources and the broader food matrix in shaping postprandial physiology following CGMP-AA ingestion.

4.1. Amino acid kinetics

Despite similar protein and amino acid intake across the three meals, a small subset of amino acids exhibited significantly higher iAUC_(0-120min) and C_{max} following meal B. Moreover, EAAs and LNAA reached higher concentrations, at 45 min, after meal B compared with meal A. It is possible that carbohydrate-derived energy provided by meal B reduced the need for amino acid oxidation, allowing greater transient accumulation of circulating amino acids. In contrast, when CGMP-AA was consumed alone (meal A), the limited availability of non-nitrogen energy sources may have favoured greater amino acid utilisation for energy metabolism, resulting in lower early postprandial amino acid concentrations [24,25]. These findings suggest that consuming CGMP-AA in isolation, without additional energy sources, could create a suboptimal postprandial metabolic environment for amino acid retention and utilisation. However, this interpretation

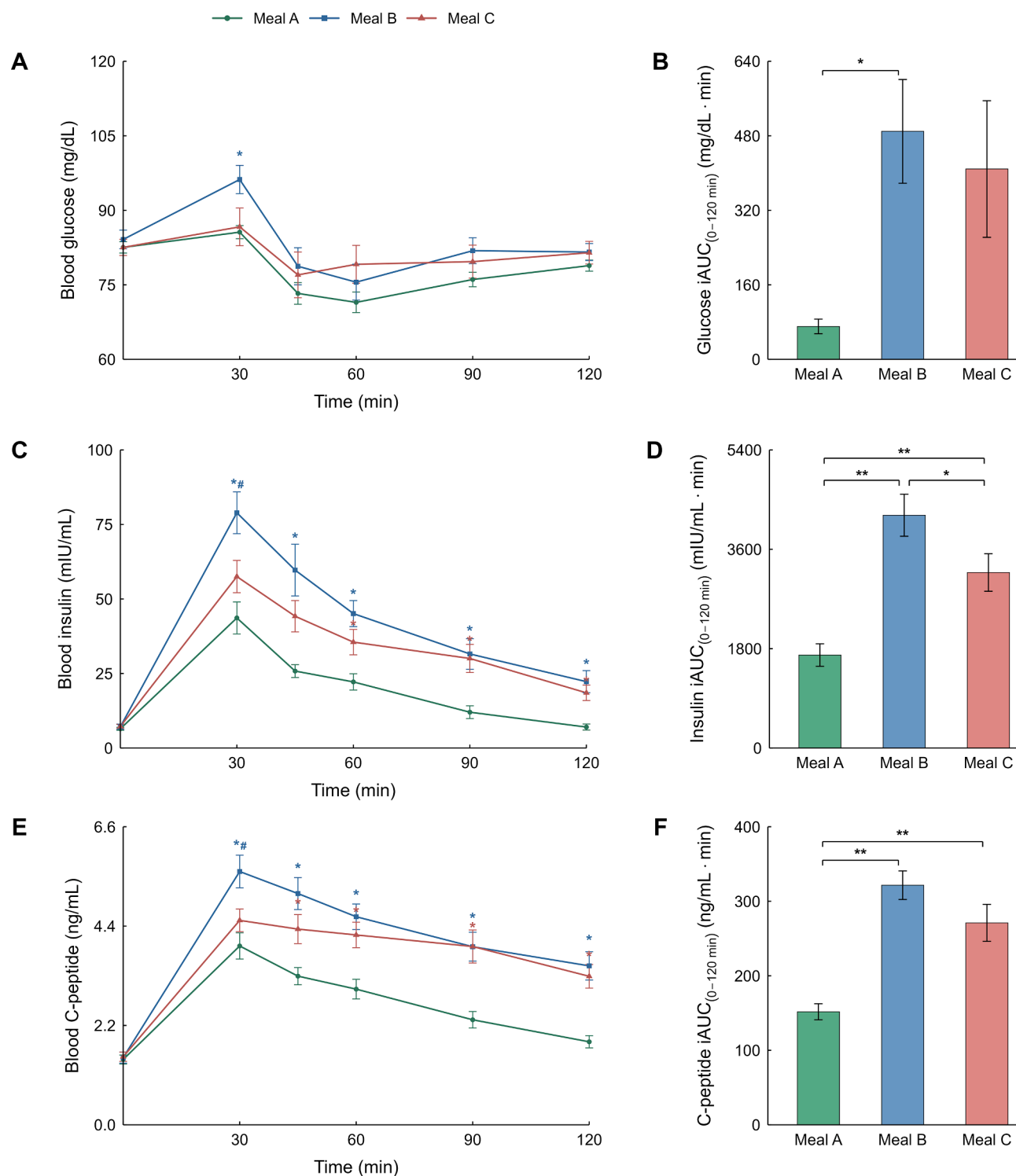


Fig. 3. Postprandial glycaemic, insulin, and C-peptide responses following ingestion of meals A, B, and C. Blood glucose (A), insulin (C), and C-peptide (E) concentrations over 120 min after the three test meals. iAUC_(0-120 min) for postprandial blood glucose (B), insulin (D), and C-peptide (F) after the three meals. Data are presented as mean $\pm$ SEM (n = 15). Statistical differences between meals were assessed by repeated-measures ANOVA with post-hoc testing. For time-course analyses, * $p < 0.05$ vs meal A and # $p < 0.05$ vs meal C at corresponding time points. For iAUC_(0-120 min) analyses, * $p < 0.05$ and ** $p < 0.001$ between meals. **Meal composition:** meal A - CGMP-AA and 100 mL of water; meal B - CGMP-AA, with two slices of white low-protein bread (50 g), unpeeled apple (160 g), and 100 mL of water; meal C - CGMP-AA, with two slices of white low-protein bread (50 g), olive oil (10 g), and 100 mL of water. **Abbreviations:** CGMP-AA, casein glycomacropeptide with amino acids; iAUC, incremental area under the curve; SEM, standard error of the mean.

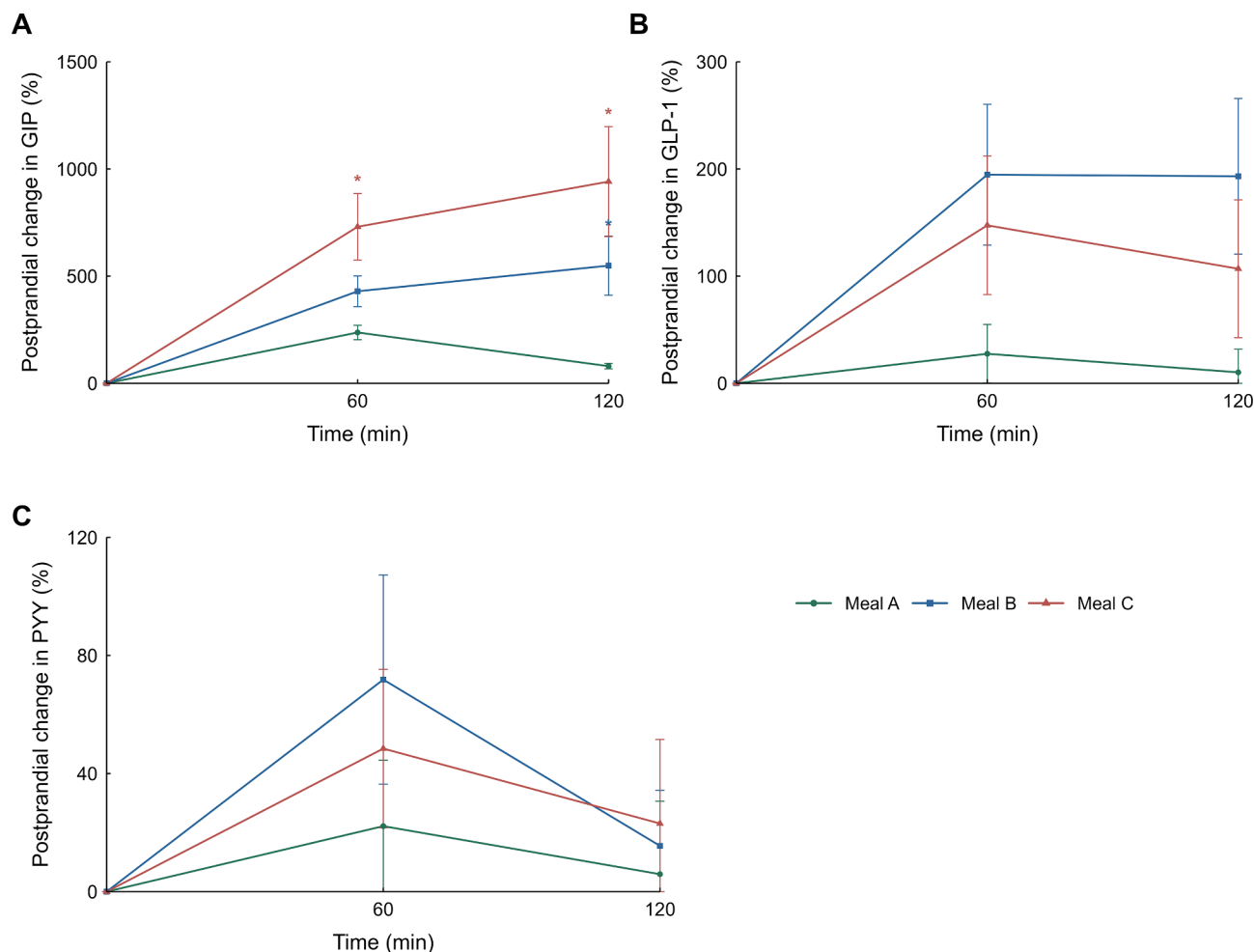


Fig. 4. Postprandial appetite-related hormones expressed as percentage change from baseline following ingestion of Meals A, B, and C. GIP (A), GLP-1 (B) and PYY (C) concentrations over 120 min after the three test meals. Data are presented as mean $\pm$ SEM ($n = 15$). Statistical differences between meals were assessed by repeated-measures ANOVA or Friedman test with post-hoc testing. * $p < 0.05$ vs meal A at corresponding time points. **Meal composition:** meal A - CGMP-AA and 100 mL of water; meal B - CGMP-AA, with two slices of white low-protein bread (50 g), unpeeled apple (160 g), and 100 mL of water; meal C - CGMP-AA, with two slices of white low-protein bread (50 g), olive oil (10 g), and 100 mL of water. **Abbreviations:** CGMP-AA, casein glycomacropeptide with amino acids; GLP-1, glucagon-like peptide-1; GIP, glucose-dependent insulinotropic polypeptide. PYY, peptide YY; SEM, standard error of the mean.

remains speculative, as amino acid oxidation, nitrogen balance and whole-body protein synthesis were not directly measured.

Additionally, the greater insulin rise observed after meals B and C, particularly meal B, may also have contributed to a more anabolic postprandial response. Insulin promotes amino acid uptake from the peripheral circulation, facilitating intracellular protein synthesis. This process is mediated in part through activation of the mammalian target of rapamycin (mTOR) pathway [26–28]. As insulin and amino acids act synergistically in the postprandial state, the higher insulin concentrations observed after co-ingestion with low-protein foods could theoretically support a greater peripheral amino acid uptake. In line with this, EAAs and LNAA showed a more pronounced decline beyond 60 min after meal B, although this did not reach statistical significance.

4.2. Phe and Tyr kinetics

Given their clinical relevance in PKU, the postprandial kinetics of Phe and Tyr warranted particular attention. In the present cohort of healthy individuals, as expected, no significant differences were observed in blood Phe concentrations, despite the

meals provided different amounts of Phe. This contrasts with findings from Daly et al. [10], who reported that even small differences in residual Phe intake from CGMP-AA formulations resulted in measurable changes in blood Phe concentrations in children with PKU over a 24-h period. The discrepancy with the present findings likely reflects differences in population, metabolic context, and study duration: unlike children with PKU, healthy adults efficiently metabolise Phe, and the acute 2-h postprandial assessment may have limited the ability to detect small meal-related differences in Phe. Thus, the absence of Phe differences in this healthy adult cohort should not be extrapolated directly to individuals with PKU.

In contrast to Phe, postprandial Tyr availability was significantly higher when CGMP-AA was co-ingested with low-protein foods compared with CGMP-AA consumed alone. Since all three meals provided the same amount of CGMP-AA, this difference suggests that the accompanying food matrix may influence Tyr absorption kinetics and postprandial availability. This may reflect a more gradual and sustained release of nutrients into circulation [29], although these mechanisms were not directly assessed.

Previous clinical studies in both patients with PKU and healthy volunteers have shown that, despite similar Tyr intake, blood Tyr concentrations are higher following CGMP-AA consumption compared with L-AAs [10,11,30]. This may be explained, at least in part, by formulation-related differences in Tyr bioavailability, including the limited solubility of Tyr in L-AA-based formulations [30], and potentially by gut microbiota-mediated effects [31]. However, the influence of the surrounding meal matrix on Tyr availability has not been adequately explored. The present findings extend previous work by suggesting that, even when the same CGMP-AA formulation and dose are consumed, the foods ingested alongside it may further influence postprandial Tyr availability.

This observation is particularly relevant in the context of PKU, where Tyr deficiency is directly implicated in disease pathophysiology [32]. Despite major advances in early diagnosis and lifelong dietary treatment, individuals with PKU may still present neurocognitive and neuropsychological difficulties, particularly in domains such as attention and executive function, and the underlying mechanisms are likely multifactorial [33–35]. Tyr availability may contribute to this complex metabolic–neurocognitive relationship, because this amino acid is the precursor of catecholamine neurotransmitters, including dopamine and noradrenaline, which are involved in neurocognitive processes such as attention, executive function, and mood regulation [4,5]. In addition, blood Tyr concentrations may fluctuate throughout the day in individuals with PKU [10,36], suggesting that strategies able to support a more stable Tyr profile may be clinically relevant.

Therefore, a meal-related increase in postprandial Tyr availability could be clinically relevant if it contributed to more stable Tyr exposure throughout the day, improved Phe/Tyr ratio, and improved substrate availability for catecholamine neurotransmitter synthesis, with potential implications for neurocognitive function. The relevance of Tyr and other LNAAs is further supported by LNAAs therapy in PKU, which aims to reduce brain Phe influx through competition at the blood–brain barrier and may improve brain availability of other LNAAs, including Tyr [37,38]. In the present study, however, acute blood Tyr concentrations were assessed in healthy adults, and brain Tyr uptake, competition with Phe and other LNAAs at the blood–brain barrier, neurotransmitter synthesis, and neurocognitive outcomes were not measured. Thus, whether the observed increase in Tyr availability translates into meaningful clinical benefits in individuals with PKU requires direct investigation.

4.3. Glycaemic and insulinaemic excursions

The most pronounced differences between meals were observed in glucose, insulin, and C-peptide levels. C-peptide is co-secreted with insulin from pancreatic β -cells, serving as a reliable marker of endogenous insulin secretion [39]. As expected, given the large variation in carbohydrate content (6.6 g in meal A, 51.5 g in meal B and 30.1 g in meal C), meal B produced both the highest glucose $C_{\max}$ and the largest glucose $iAUC_{(0-120\text{min})}$ closely followed by meal C, whereas meal A produced minimal glycaemic excursions. This is consistent with the well-established glycaemic physiology – meals with higher carbohydrate content produce larger and earlier increases in blood glucose [40]. The higher insulin and C-peptide concentrations after meal B are also aligned with the greater carbohydrate load, as insulin secretion is strongly dependent on glucose-stimulated β -cell activation [27].

Meal B also contained the highest amount of dietary fibre (6.8 g), derived from an unpeeled apple, providing a combination of soluble and insoluble fibre. Soluble fibre, such as pectin, can form a viscous gel in the intestine, slowing carbohydrate digestion

and absorption and thereby reducing postprandial glucose peaks [41,42]. Insoluble fibre, present in the apple peel, contributes mainly to intestinal bulk and transit, with limited direct impact on acute glycaemic responses [43]. In the present study, the higher fibre content of meal B was not associated with a lower glycaemic peak, possibly because the amount of fibre in the meal was insufficient to counterbalance the higher carbohydrate load. Indeed, postprandial benefits of soluble fibre are typically observed with approximately 10 g per meal in adults [44]. While no conclusions can be drawn regarding the efficacy of fibre in modulating glycaemic responses in this context, these findings suggest that simply adding a modest amount of fibre may be insufficient to attenuate postprandial glycaemic excursions when carbohydrate load is high. Future formulation strategies for low-protein foods should therefore consider carbohydrate quality alongside fibre dose, type, solubility and viscosity.

Glucose, insulin and C-peptide concentrations produced by meal C were intermediate between those of meals A and B. This reflects its moderate carbohydrate content and higher fat load. The presence of fat stimulates the release of gut hormones and slows gastric emptying, resulting in a more gradual appearance of glucose in the bloodstream and, consequently, a delayed and blunted insulin secretion [29,45].

Notably, glucose concentration fell below baseline approximately 45 min postprandially for all three meals. This may be attributable to the insulinotropic properties of specific amino acids, such as leucine, thus rapidly lowering glucose levels [18]. This earlier decline, however, may be disproportionate and not fully physiological [40]. Such a response may limit the establishment of a sustained anabolic postprandial environment [46], especially when protein substitutes are rapidly absorbed [11]. The subsequent increase in glucose levels may indicate activation of counter-regulatory processes, including *de novo* glucose production (gluconeogenesis) and glycogen breakdown (glycogenolysis), which typically occurs when circulating glucose falls below fasting levels [47].

Collectively, these findings reinforce that postprandial glycaemic and insulinaemic excursions are predominantly determined by carbohydrate load and nutrient-mediated modulation of gastric emptying. From a practical perspective, this suggests that CGMP-AA should not be evaluated solely in terms of protein equivalent, but also in relation to the concomitant low-protein foods consumed and their respective carbohydrate load, fat content, and the dose and functional properties of added fibre.

4.4. Appetite-regulating hormone responses

Finally, gut-derived hormone responses further illustrated the key role of the non-protein energy sources in shaping acute postprandial physiology. Postprandial GIP secretion was clearly meal dependent. Meal C elicited the most pronounced increase, consistent with the potent stimulatory effect of dietary fat on GIP release [48–50]. Meal B also induced higher GIP concentration than meal A at 120 min, likely reflecting the modest stimulatory effect of carbohydrates on GIP secretion [48,50].

No significant differences were observed in postprandial GLP-1 secretion across the three meals. Although meals B and C tended to elicit larger increases at 60 min compared with meal A, these differences did not reach statistical significance, potentially due to the limited temporal resolution of sampling (0, 60, 120 min) and the small sample size. In fact, given the higher carbohydrate and fibre content of meal B, one might have expected greater GLP-1 concentrations [51].

Similarly, postprandial PYY concentrations did not differ significantly between meals, although variability across

participants was considerable. Given that PYY secretion is closely linked to caloric load [52], the slightly greater increases after meals B and C are consistent with their higher energy content. However, these differences were not statistically significant and should therefore be interpreted cautiously.

Together, these findings suggest that including carbohydrates and, particularly, fat alongside CGMP-AA may enhance postprandial satiety signals through increased GIP concentrations. Importantly, these findings do not demonstrate improved satiety, as subjective appetite ratings and subsequent energy intake were not assessed. Nevertheless, they suggest that the meal context in which CGMP-AA is consumed may influence incretin responses. This may be particularly relevant for patients on a Phe-restricted diet, who often report low satiety and persistent hunger, potentially resulting in frequent snacking or overconsumption of other foods [53,54]. Whether these hormonal changes translate into meaningful effects on satiety or eating behaviour remains to be determined.

4.5. Integrated interpretation

All together, these findings show that the acute metabolic response to CGMP-AA depends not only on the protein substitute itself, but also on the broader meal context in which it is consumed. Although each test condition provided a similar protein equivalent, co-ingestion with low-protein foods altered the postprandial profile, supporting the concept that non-nitrogen energy sources can determine the metabolic responses to protein substitute intake.

This integrated response is particularly relevant in light of the recent trend toward developing protein substitutes with reduced carbohydrate and overall energy content [55]. While this approach may be appropriate when clinically indicated, meta-analytic evidence suggests that individuals with PKU do not have a higher prevalence of obesity compared with the general population [56]. Therefore, reducing non-protein energy sources in protein substitutes should not be assumed to be metabolically neutral, particularly if these products are consumed in isolation rather than alongside meals or snacks.

From a practical perspective, these findings support a more comprehensive approach to nutritional research in PKU and product formulation. Protein equivalent and Phe content remain central considerations, but carbohydrate quantity, fat content, and fibre characteristics may also influence the postprandial metabolic profile. However, because this study was conducted in healthy adults, these implications should be viewed as hypothesis-generating rather than directly clinically actionable.

4.6. Limitations

Certain limitations should be considered when interpreting these findings. First, this study was conducted in healthy adults rather than individuals with PKU. Although this allowed a controlled investigation of acute postprandial metabolic responses, healthy adults do not reproduce the impaired Phe hydroxylation and altered Phe/Tyr homeostasis characteristic of PKU. Therefore, extrapolation to individuals with PKU should be made with caution, and the clinical implications of these findings should be considered hypothesis-generating rather than directly actionable. Previous work by Van Rijn and colleagues [21] reported no significant differences in aspects of protein metabolism between adults with PKU and matched healthy controls using an L-[1-¹³C] valine tracer method,

supporting the use of healthy adults as a mechanistic model. However, this does not eliminate the need for PKU-specific studies, particularly when interpreting Phe and Tyr responses. Indeed, because Tyr is conditionally essential in PKU, individuals with PKU may have different Tyr requirements or utilisation patterns, and it cannot be excluded that postprandial increases in circulating Tyr could be attenuated by greater tissue uptake or metabolic use.

Second, the postprandial follow-up period of 120 min captured only the early metabolic phase. This time-course was selected based on preliminary pilot observations indicating that it was sufficient to capture the amino acid response to CGMP-AA while minimising participant burden. Nevertheless, it may not fully reflect late-phase glycaemic, amino acid, and gut hormone responses, particularly after the fat-containing meal. In addition, the hormone sampling intervals may have limited the detection of transient GLP-1, GIP, or PYY peaks.

Third, the sample size was determined based on amino acid outcomes, which were the primary outcome of interest. Therefore, analyses of appetite-regulating hormones should be interpreted as exploratory, and the study may have been underpowered to detect differences in GLP-1 and PYY responses.

Fourth, although the test meals were designed to reflect realistic low-protein breakfast contexts, they do not capture the full variability of habitual dietary patterns in individuals with PKU. The meals also differed in carbohydrate, fat, fibre, glycaemic load, and energy content, which reflects real-world meal differences but limits the ability to isolate the independent effect of each macronutrient.

Finally, the absence of direct measurements of amino acid oxidation, nitrogen balance, gastric emptying, protein synthesis, brain Tyr uptake, subjective appetite, and subsequent energy intake prevents definitive conclusions regarding amino acid metabolic fate, anabolic responses, satiety, or clinical outcomes.

These limitations reinforce the need for further studies in patients with PKU. Future trials should include longer postprandial monitoring to capture delayed metabolic responses, and indirect calorimetry combined with urinary nitrogen excretion measurements to estimate substrate oxidation rates. Stable isotope tracer approaches would also be particularly useful to clarify amino acid absorption, oxidation, tissue utilisation, and incorporation into protein. In addition, subjective appetite measures and subsequent food intake should be assessed to determine whether hormonal responses translate into meaningful effects on satiety. Such studies are needed to establish whether meal composition can inform practical dietary strategies regarding the co-ingestion of CGMP-AA with low-protein foods.

5. Conclusion

In healthy individuals, CGMP-AA elicited different acute postprandial responses depending on whether it was consumed alone or with low-protein foods. Compared with CGMP-AA alone, co-ingestion with either carbohydrate-with-fibre or carbohydrate-with-fat low-protein meals increased postprandial Tyr availability, while the carbohydrate-with-fibre meal produced the greatest glycaemic and insulinaemic response and the carbohydrate-with-fat meal elicited the most pronounced GIP response. These findings indicate that meal context is an important determinant of the acute metabolic effects of CGMP-AA and should be confirmed in individuals with PKU before being translated into dietary recommendations.

Authors contributions

Conceptualization: CR, AM, AF, JCR. Data curation: CR. Methodology: CR, AM, AF, JCR. Investigation: CR, DJP, IBM, SI, GMS, TN, MJA, IC, JS, CF, AFG, JC, CC, DP, JRA, AM, AF, JCR. Funding acquisition: AM, AF, JCR. Supervision: AM, AF, JCR. Writing - original draft: CR. Writing - reviewing & editing: CR, DJP, IBM, SI, GMS, TN, MJA, IC, JS, CF, AFG, JC, CC, DP, JRA, AM, AF, JCR.

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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 Nutritionist Expert Panel (Biomarin), a member of Sapropterin Advisory Board (Biomarin), a member of the advisory board entitled ELEMENT (Danone-Nutricia), and a member of an advisory board for Arla Foods Ingredients Group P/S and Applied Pharma Research. Catarina Rodrigues received honoraria as a speaker from PIAM, and her travel to conferences has been supported by both PIAM and PTC. Júlio César Rocha is member of the European Nutrition Expert Panel (Biomarin) and of the advisory boards of Applied Pharma Research, BioMarin, PTC and Nutricia. He has received speaker's fees from Applied Pharma Research, Merck Serono, BioMarin, Nutricia, VitaFlo, Cambrooke, PIAM, Lifediet, and PTC.

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