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Clinical science

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https://doi.org/10.1042/CS20231126

Clinical Science (2024) **138** 43–60
https://doi.org/10.1042/CS20231126

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Research Article

Nasogastric bolus administration of a protein-rich drink augments insulinaemia and aminoacidaemia but not whole-body protein turnover or muscle protein synthesis versus oral administration

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Nasogastric feeding of protein-rich liquids is a nutritional support therapy that attenuates muscle mass loss. However, whether administration via a nasogastric tube per se augments whole-body or muscle protein anabolism compared with oral administration is unknown. Healthy participants were administered a protein-rich drink (225 ml containing 21 g protein) orally (ORAL; n=13; age 21 ± 1 year; BMI 22.2 ± 0.6 kg · m^{-2}) or via a nasogastric tube (NG; n=13; age 21 ± 1 yr; BMI 23.9 ± 0.9 kg · m^{-2}) in a parallel group design, balanced for sex. L-[*ring*-$^{2}H_5$]-phenylalanine and L-[3,3-$^{2}H_2$]-tyrosine were infused to measure postabsorptive and postprandial whole-body protein turnover. Skeletal muscle biopsies were collected at −120, 0, 120 and 300 min relative to drink administration to quantify temporal myofibrillar fractional synthetic rates (myoFSR). Drink administration increased serum insulin and plasma amino acid concentrations, and to a greater extent and duration in NG versus ORAL (all interactions P<0.05). Drink administration increased whole-body protein synthesis (P<0.01), suppressed protein breakdown (P<0.001), and created positive net protein balance (P<0.001), but to a similar degree in ORAL and NG (interactions P>0.05). Drink administration increased myoFSR from the postabsorptive state (P<0.01), regardless of route of administration in ORAL and in NG (interaction P>0.05). Nasogastric bolus administration of a protein-rich drink induces insulinaemia and aminoacidaemia to a greater extent than oral administration, but the postprandial increase in whole-body protein turnover and muscle protein synthesis was equivalent between administration routes. Nasogastric administration is a potent intervention to increase postprandial amino acid availability. Future work should assess its utility in overcoming impaired sensitivity to protein feeding, such as that seen in ageing, disuse, and critical care.

Received: 15 September 2023
Revised: 15 December 2023
Accepted: 19 December 2023

Accepted Manuscript online: 19 December 2023
Version of Record published: 05 January 2024

Introduction

The loss of skeletal muscle mass during and after periods of bed rest and/or hospitalisation is associated with impaired functional capacity and reduced insulin sensitivity [1,2], and is the primary cause of persistent disability and reduced quality of life one year after hospitalisation [3]. Sustained negative net protein balance underpins muscle mass loss, which may occur at a rate of ∼2% per day in critically ill patients [4]. Thus, exploring interventions to improve net protein balance to subsequently prevent or reverse muscle loss is of clear clinical relevance.

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https://doi.org/10.1042/CS20231126

In both clinical and home care settings, nasogastric nutrient administration is commonly used to increase nutrient intake [5]. Bolus feeding, used by over one-third of adults receiving home care [6], is increasingly prevalent due to its ease of use and similarity to mealtimes. We have previously shown in a proof-of-concept study that nasogastric bolus versus continuous feeding does not influence lean mass loss and decline in metabolic health during one week of experimental bed-rest [7]. However, intriguingly, the loss of lean mass (∼0.5 kg loss) was less pronounced than in our previous (but similar) study, when participants consumed food orally (∼1.4 kg loss) [2]. Given that these studies were conducted in otherwise healthy people, free from the confounding influences of disease, inflammation, medication and/or malnutrition that influence protein turnover in clinical settings, it implies that the route of administration per se may permit a greater anabolic stimulus, manipulating either whole-body (i.e. reflecting total lean mass) and/or skeletal muscle (i.e. 'functional' lean mass) protein handling in the postprandial state at rest.

Despite relevance for patients in whom high rates of total body [8] or skeletal muscle [9] protein loss reduces survival, it remains unknown whether nasogastric administration of a protein-rich drink provides a more potent anabolic stimulus compared to when consumed orally. In the present study, healthy young adults were administered a protein-rich drink either orally or via a nasogastric tube, whilst being infused with the stable isotopes L-[*ring*-$^2H_5$]-phenylalanine and L-[3,3-$^2H_2$]-tyrosine to measure whole-body protein kinetics and rates of myofibrillar protein synthesis. Based on observed differences in plasma amino acid appearance and insulinaemia with different protein administration routes [10], we hypothesised that nasogastric drink administration would accelerate appearance of amino acids in the circulation when compared with oral administration. Moreover, we hypothesised that such accelerated plasma amino acid appearance would increase whole-body and skeletal muscle protein synthesis over a 5-h postprandial period.

Methods

Participants

Twenty-six healthy, recreationally active participants (14 females, 12 males; age: 21 ± 0 yr; body mass 69.2 ± 2.6 kg; BMI 23.1 ± 0.5 kg · m^{-2}) volunteered to take part in the present study. Prior to inclusion, participants attended the Nutritional Physiology Research Unit at the University of Exeter for routine medical screening to ensure that they did not contravene the following exclusion criteria: diagnosed metabolic impairment (e.g., Type 1 or Type 2 diabetes); cardiovascular disease; hypertension (≥140/90 mmHg); chronic use of prescribed or over-the-counter pharmaceuticals; personal or family history of epilepsy, schizophrenia or seizures; the presence of an ulcer in the stomach or gut; pre-existing condition with liver or kidneys; regular use of nutritional supplements; and/or an allergy to milk, lidocaine or amino acids. Medical history was discussed and all participants reported having had no previous gastrointestinal surgery. Written consent was obtained from all participants following the explanation of the experimental procedures, which were approved by the University of Exeter's Sport and Health Sciences Ethics Committee and conducted in accordance with the Declaration of Helsinki [11]. The present study was registered as a clinical trial on ClinicalTrials.gov (NCT03571425).

Experimental protocol

Following inclusion, participants were randomly assigned to undergo oral (ORAL) or nasogastric (NG) administration of a protein-rich, mixed macronutrient drink, in a parallel groups design counter-balanced for sex. Participants abstained from vigorous exercise, alcohol and caffeine on the day prior to each test visit. After consuming a standardised diet on the evening prior to the visit (976 kcal, 38 energy% [En%] carbohydrate, 17 En% protein, 45 En% fat), participants arrived in the laboratory at 0800 following a ≥10 h fast. A schematic of the experimental trial is shown in Figure 1. Participants rested in a semi-supine position whilst a Venflon cannula was inserted anterograde into an antecubital vein of one arm for stable isotope infusion. An initial blood sample was taken to measure background isotope enrichment ($t = -210$ min), after which the plasma phenylalanine and tyrosine pools were primed with 2.94 μmol · kg^{-1} L-[*ring*-$^2H_5$]-phenylalanine and 1.04 μmol · kg^{-1} L-[3,3-$^2H_2$]-tyrosine. Continuous infusion of 0.049 μmol · kg^{-1} · min^{-1} L-[*ring*-$^2H_5$]-phenylalanine and 0.017 μmol · kg^{-1} · min^{-1} L-[3,3-$^2H_2$]-tyrosine began thereafter and was maintained over the experimental trial (8.5 h).

A second Venflon cannula was inserted retrograde into a heated, dorsal hand vein and kept patent with a continuous 0.9% saline infusion. The hand was placed in a heated hand warmer at 55°C for arterialised venous blood collection [12] at $t = -120$, –60, −30, 0, 15, 30, 45, 60, 90, 120, 150, 180, 240 and 300 min. Muscle biopsies were collected at $t = -120$ and 0 from a randomly assigned leg (counter-balanced for leg dominance) for the calculation of postabsorptive myofibrillar protein synthesis rates. Further muscle biopsies were obtained at $t = 120$ and 300 min from the contralateral leg. This allowed muscle protein synthesis to be quantified over a full postprandial period (i.e.

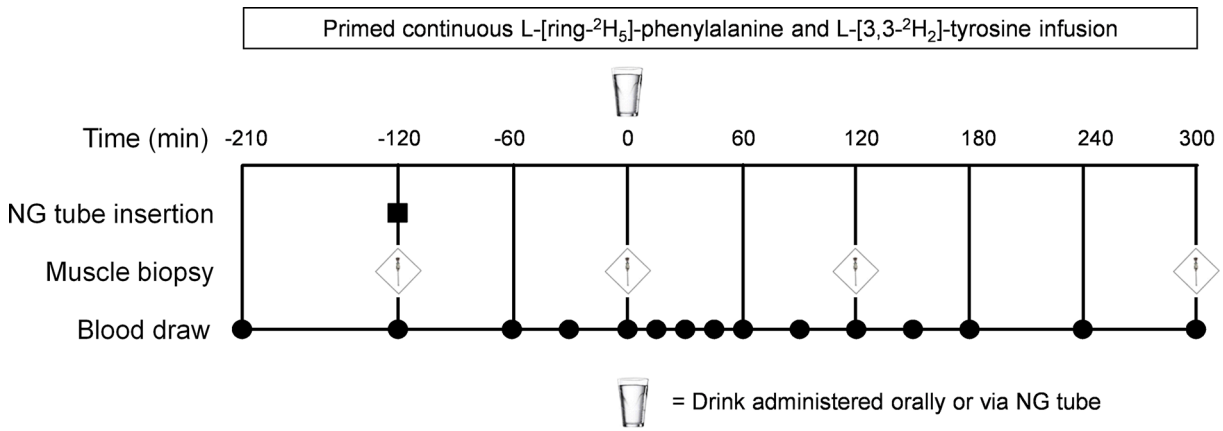

Figure 1. Graphical representation of the experimental protocol
NG, nasogastric.

0–300 min; equal to the time between meals used in our nasogastric bedrest study with nasogastric feeding [7], and in line with commonly used postprandial periods [13–17]), as well as calculation of an early (i.e. 0–120 min) and late (i.e. 120–300 min) period to increase resolution of this measure over time. The distinction between early versus late FSR was included because of hypothesized differences in digestion and absorption between ORAL and NG. Immediately after the second biopsy, a 3-min period began whereby participants were administered their allocated test drink (detailed below). An additional 50 ml water was used to rinse the drink containers and then administered to participants in the same manner.

For participants allocated to the NG group, a nasogastric tube (Flocare PUR tube Enlock, Ch8, 110 cm; Nutricia Advanced Medical Nutrition, Utrecht, The Netherlands) was inserted at the start of the postabsorptive measurement period. After anaesthetising the nasal cavity with xylocaine 10% spray, the nasogastric tube was inserted. To ensure placement in the stomach prior to administering drinks, the aspirate was tested to confirm pH <5.5. Given that continuous nasogastric feeding would likely influence aminoacidaemia, insulinaemia and the ensuing muscle protein synthetic response independently of the nasogastric tube [18], we administered the test drinks as a bolus over a standardised 3-min period for participants in both ORAL and NG groups.

Test drinks

A commercially available, protein-rich, mixed macronutrient drink (Recover, Beachbody LLC, Santa Monica, CA, U.S.A.), which contained a blend of whey, pea, and casein proteins with carbohydrate (full macronutrient and amino acid composition displayed in Table 1) was administered at $t=0$. This drink was selected for its high protein content, and we have previously shown it sufficient to stimulate muscle protein synthesis [19]. Sachets containing the powdered drink were made up to 225 ml in water as per manufacturer's instructions, and combined with 1500 mg powdered acetaminophen (paracetamol) in order to measure postprandial gastric emptying rates [20], as the appearance of exogenous amino acids is initially limited by gastric emptying and absorption in the gastrointestinal tract [13].

Blood sample collection and analyses

One aliquot of every blood sample (0.5 ml) was collected in a fluoride/oxalate tube (Vacutainer®, BD, Franklin Lakes, NJ, U.S.A.), rolled for 2 min to inhibit glycolysis, and then analysed for whole blood glucose concentrations (YSI 2300 PLUS, Yellow Springs, OH, U.S.A.). A second aliquot (3 ml) was collected in a serum separator tube (Vacutainer®, BD) and left to clot at room temperature for $\geq$30 min. A third aliquot (3 ml) was collected in a lithium heparin container (Vacutainer®, BD) and processed immediately. Lithium Heparin and serum separator tubes were centrifuged at 2850 × ***g*** for 10 min at 4°C to obtain plasma and serum samples, respectively, which were aliquoted and snap-frozen in liquid nitrogen prior to storage at −80°C for further analysis.

Arterialised serum samples were used to determine concentrations of insulin (Human insulin ELISA kit EIA-2935, DRG International Inc., Springfield, NJ, U.S.A.) and paracetamol (Paracetamol Assay Kit K8002, Cambridge Life Sciences Ltd., Cambridgeshire, U.K.).

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Table 1 Nutritional composition of the test drink

	Per serving
Energy (kcal)	128
Energy (kJ)	537
Macronutrients (g)	
Protein	21.1
Carbohydrate	8.9
Fat	0.6
Fibre	2.3
Amino acid content (mg)	
Alanine	1050
Arginine	990
Aspartic acid	2517
Glutamic acid	4331
Glycine	564
Histidine	483
Isoleucine	1345
Leucine	2364
Lysine	2016
Phenylalanine	976
Proline	1385
Serine	1179
Threonine	1289
Tyrosine	760
Valine	1377
EAA[1]	**9851**
NEAA[2]	**12776**
BCAA	**5087**

[1]Methionine and tryptophan not analysed
[2]Asparagine, cysteine and glutamine not analysed. BCAA, branched-chain amino acid; EAA, essential amino acid; NEAA, non-essential amino acid.

Enrichments of L-[*ring*-$^2H_5$]-phenylalanine, L-[3,3-$^2H_2$]-tyrosine and L-[*ring*-$^2H_4$]-tyrosine, and concentrations of phenylalanine, tyrosine, leucine, isoleucine, and valine were determined using gas chromatography mass spectrometry. Briefly, 500 μl of arterialised plasma was deproteinised on ice with an equal volume of 15% 5-sulfosalicylic acid and 10 μl of 2 mM norleucine as internal standard, and vortexed prior to centrifugation at 4000 × ***g*** for 10 min at 4°C. Supernatants were passed over cation exchange resin columns (100–200 mesh; H+ form; Dowex 50WX8; Sigma-Aldrich Company Ltd., Dorset, U.K.) with 6 ml of 0.5 M acetic acid. Amino acids were eluted with 6 M NH_4OH and dried under a vacuum for 8 h at 60°C (Savant™ SpeedVac™, ThermoFisher Scientific, Waltham, MA, U.S.A.).

Following derivatisation to tert-butyl-dimethylsilyl (TBDMS) esters, amino acid enrichments were determined using electron impact ionisation by monitoring labelled and unlabelled ions at mass/charge (m/z) of 341 and 336 for L-[*ring*-$^2H_5$]-phenylalanine, 468 and 466 for L-[3,3-$^2H_2$]-tyrosine, and 470 and 466 for L-[*ring*-$^2H_4$]-tyrosine. Amino acid concentrations were calculated from the mass charge corresponding to the unlabelled ion relative to that of the internal standard.

Muscle biopsy collection and analyses

Muscle biopsy samples were obtained under local anaesthesia (2% lidocaine) by the Bergström needle technique modified for suction [21] from the mid-section of the *m. vastus lateralis* approximately 15 cm proximal from the knee. All samples were rapidly dissected of visible fat and connective tissue, frozen in liquid-nitrogen-cooled isopentane, and stored at −80°C until subsequent analysis.

Myofibrillar protein fractions were isolated as previously described [19]. Briefly, ∼50 mg wet weight muscle tissue was homogenised using a glass pestle in 7.5 $\mu L \cdot mg^{-1}$ ice-cold homogenisation buffer (50 mM Tris · HCl pH 7.4, 1 mM EDTA, 1 mM EGTA, 10 mM β-glycerophosphate salt, 50 mM NaF and 0.5 mM activated Na_3VO_4; Sigma-Aldrich Company Ltd.) with a cOmplete™ protease inhibitor cocktail tablet (Roche, West Sussex, U.K.). Following centrifugation at 2200 × ***g*** for 10 min at 4°C, the pellet was washed with homogenisation buffer and the myofibrillar proteins

were solubilised in 0.3 M NaOH for 30 min at 50°C. The insoluble collagen fraction was separated by centrifugation at 10,000 × ***g*** for 5 min at 4°C and myofibrillar proteins were precipitated with 1 M perchloric acid. The myofibrillar pellet was washed twice in 70% ethanol and hydrolysed in 6 M HCl at 110°C for 24 h, before being dried under a vacuum (Savant™ SpeedVac™, ThermoFisher Scientific). The samples were reconstituted in 25% acetic acid and eluted from cation exchange resin columns (100–200 mesh; H^+ form; Dowex 50WX8; Sigma-Aldrich Company Ltd.) with 6 M NH_4OH. Samples were dried under vacuum prior to being suspended in 1:1 distilled water : 0.1% formic acid in acetonitrile and spun at 10,000 × ***g*** for 3 min at 4°C. The resultant supernatant was aliquoted, dried under a vacuum and stored at −20°C.

Samples were converted to their tert-butyl-dimethylsilyl (TBDMS) derivatives. The myofibrillar protein-bound amino acid phenylalanine enrichments were determined using electron impact ionisation by monitoring mass/charge (m/z) of 239 and 237. Standard curves were applied from a series of known standard enrichment values against the measured values in order to correct for any concentration dependant shift in the measured labelled to unlabelled m/z ratios.

Calculations

The time-course of phenylalanine appearance (R_a) and disappearance (R_d) rates at the whole-body level were calculated using modified Steele equations [22,23], taking in to account the change in amino acid enrichment and concentration, as follows (eqns 1 and 2):

$$Total\ R_a\ (t) = \frac{F - pV \times C\,(t) \times\ (dE\,(t)\,/dt)}{E\,(t)} \tag{1}$$

$$Total\ R_d\ (t) = Total\ R_a - pV \times \frac{dC\,(t)}{dt} \tag{2}$$

where $R_a(t)$ is the rate of appearance between two consecutive time points; F is intravenous tracer infusion rate ($\mu mol \cdot kg^{-1} \cdot min^{-1}$); pV is a constant representing the distribution volume ($0.125\ L \cdot kg^{-1}$); $C(t)$ is the mean plasma amino acid concentration between two consecutive timepoints; $dE(t)/dt$ is the time-dependent change in plasma amino acid enrichment; $E(t)$ is the mean plasma amino acid enrichment between two consecutive time points as mole percent excess (MPE), $R_d(t)$ is the rate of disappearance between two consecutive time points; and $dC(t)/dt$ is the time-dependent change in plasma amino acid concentration.

Postabsorptive and postprandial whole-body phenylalanine kinetics were calculated from plasma enrichment of L-[*ring*-$^2H_5$]-phenylalanine, L-[3,3-$^2H_2$]-tyrosine, and L-[*ring*-$^2H_4$]-tyrosine, using the following calculations [16,23] (eqns 3-8):

$$Total\ rate\ of\ appearance\ into\ plasma\ (R_a) = \frac{F}{E} \tag{3}$$

$$Fractional\ R_a\ \ of\ Tyr\ from\ Phe = \frac{E_{^2H_4\ Tyr}}{E_{^2H_5\ Phe}} \tag{4}$$

$$Phe\ hydroxylation\ rate = fractional\ R_a\ \ of\ Tyr\ from\ Phe\ \times Tyr\ R_a \tag{5}$$

$$Protein\ synthesis\ rate\ (PS) = \ Phe\ R_a - Phe\ hydroxylation\ rate \tag{6}$$

$$Protein\ breakdown\ rate\ (PB) = Phe\ R_a - F_{Phe} - Exogenous\ Phe\ R_a \tag{7}$$

$$Net\ protein\ balance = PS - PB \tag{8}$$

where for a given amino acid (phenylalanine, Phe; or tyrosine, Tyr), F is the venous tracer infusion rate ($\mu mol \cdot kg^{-1} \cdot min^{-1}$) and R_a is the rate of appearance. For calculations of PB, enrichment (E) is expressed as tracer-to-tracee ratio (TTR); for calculations for PS, mole per cent excess (MPE; calculated as TTR/(TTR+1)) is used. (eqns 1-8) do not distinguish between the tissues where phenylalanine was metabolised (which may include liver, kidney, skeletal muscle, and intestine [24,25]) and consider all tissues as one.

In order to quantify protein breakdown and net protein balance, free amino acids from the administered drink (i.e. exogenous phenylalanine) must be distinguished from that liberated from protein breakdown. Exogenous phenylalanine rate of appearance over the postprandial period was calculated by estimating bioavailability [26], as follows (eqn

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Table 2 Subjects' characteristics

	ORAL (*n*=13)	NG (*n*=13)
Sex (male:female)	6:7	6:7
Age (yr)	21 ± 1	21 ± 1
Body mass (kg)	66.5 ± 3.5	71.8 ± 3.9
Height (cm)	172 ± 3	173 ± 3
BMI ($kg \cdot m^{-2}$)	22.2 ± 0.6	23.9 ± 0.9
Energy ($MJ \cdot d^{-1}$)	7.9 ± 0.7	7.9 ± 0.5
Protein ($g \cdot d^{-1}$)	80 ± 8	85 ± 7
Protein ($g \cdot kg^{-1} \cdot d^{-1}$)	1.2 ± 0.1	1.2 ± 0.1
Carbohydrate ($g \cdot d^{-1}$)	203 ± 15	211 ± 14
Fat ($g \cdot d^{-1}$)	85 ± 10	81 ± 6

Values represent mean ± SEM. BMI, body mass index; NG, nasogastric administration of test drink; ORAL, oral consumption of test drink. Energy and macronutrient values are habitual dietary intake. All between group comparisons $P>0.05$.

9):

$$Exogenous\ Phe\ R_a = Total\ Phe\ consumed \times true\ illeal\ digestibility - absorbed\ Phe\ hydroxylation \quad (9)$$

where true ileal digestibility was assumed to be 0.96 based on the true ileal digestibility of phenylalanine from casein [27] and absorbed phenylalanine hydroxylation (representing phenylalanine hydroxylated in splanchnic bed) was calculated as the difference in postabsorptive to postprandial phenylalanine hydroxylation rate [26].

Skeletal muscle protein synthesis was determined by calculating the fractional synthetic rate of myofibrillar proteins (myoFSR) using the following precursor-product equation [23] (eqn 10):

$$myoFSR\ \left(\% \cdot h^{-1}\right) = \frac{E_{m2} - E_{m1}}{E_p \times (t_2 - t_1)} \times 100\% \quad (10)$$

where t represents time, ($E_{m2} - E_{m1}$) is the increase in myofibrillar protein-bound L-[*ring*-$^2H_5$]-phenylalanine enrichment from t_1 to t_2, and E_p is the mean plasma precursor enrichment between t_1 and t_2.

Statistical analyses

In the absence of any data concerning the effect of nasogastric tube feeding on skeletal muscle protein synthesis (i.e. primary outcome), an *a priori* power analysis was performed based on previous data from our laboratory [28], which determined that $n = 13$ per condition was sufficient to detect an additional 20% stimulation (i.e. due to the nasogastric tube) over and above the effects of feeding ($P<0.05$, power = 0.8, $f = 0.28$). We considered this difference clinically meaningful, as a ∼16% decline in postprandial muscle protein synthesis is thought to account entirely for muscle atrophy during aging [29]. A student's independent t-test was used to test for group differences in subject characteristics and the change in myoFSR rates from basal. Total incremental area under the curve (iAUC) was calculated for postprandial amino acid concentrations, with baseline set as the average of postabsorptive (−120 to 0 min) concentrations. Independent -tests were subsequently used to identify differences between groups, as well as total area under curve (AUC) for blood glucose, serum insulin and paracetamol concentrations. Peak concentration (C_{max}) and time at peak concentration (t_{max}) for glucose, insulin, individual amino acids, and exogenous phenylalanine R_a were analysed using independent t-tests. All other data were analysed using two-way mixed model ANOVA (time and group factors), with Sidak corrections for multiple comparisons applied when investigating post-hoc differences. Due to positive skew in myoFSR values, data were log-transformed prior to analysis. Statistical analysis was performed using GraphPad Prism 9 (GraphPad Software, Inc., San Diego, CA, U.S.A). Participant characteristics presented as mean ± SEM, with all other data presented as mean [lower, upper 95% confidence interval], with $P<0.05$ indicating statistical significance.

Results

Participant characteristics

No differences in age, height, weight, BMI, or body fat percentage were identified between groups (Table 2; all $P>0.05$). Furthermore, habitual diet intake was similar between groups (all parameters $P>0.05$). Due to unexpected

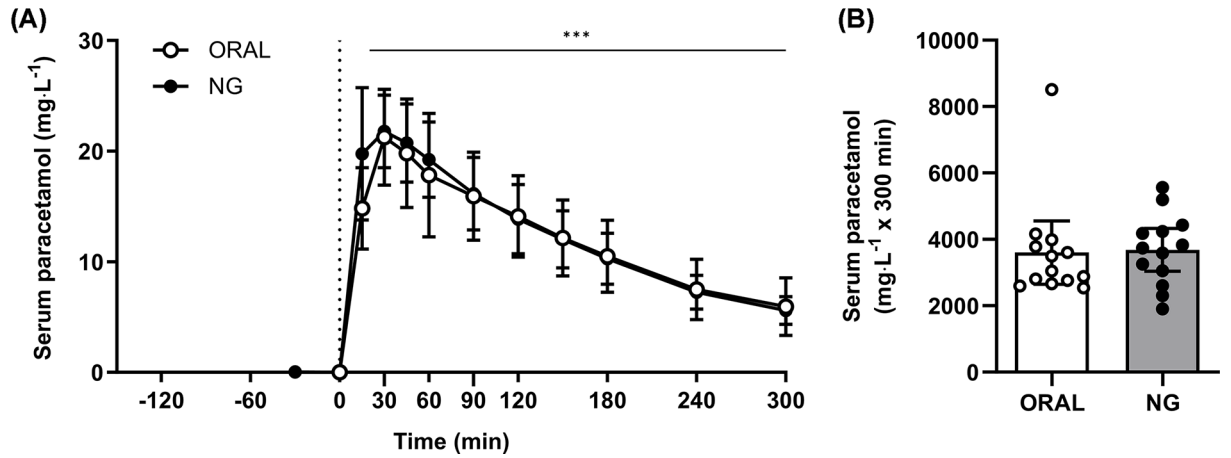

Figure 2. Serum paracetamol concentrations

Serum paracetamol concentrations after administering a test drink either orally (ORAL; n=13; open symbols) or via a nasogastric tube (NG; n=13; filled symbols), presented as (**A**) time-course prior to and over 300 min following drink administration; (**B**) Postprandial area under the curve (AUC) over 120 min. Data presented as mean with 95% confidence intervals. Time-course data were analysed by two-way analysis of variance (ANOVA) with time and group factors, and AUC was compared by independent-samples t-test. (A) significant main effect of time (P<0.001; post-hoc differences denoted by ***P<0.001 significantly different to 0 min).

technical issues, L-[3,3-$^2H_2$]-tyrosine was not infused into $n = 3$ participants in ORAL and $n = 3$ participants in NG, and so Figure 6 represents $n = 10$ in both groups.

Serum paracetamol, blood glucose, and serum insulin concentrations

Serum paracetamol concentrations (Figure 2) were elevated above fasting concentrations at all timepoints following drink administration (time effect, P<0.001), peaking after 30 min in both groups (21.2 [17.3, 25.1] and 21.8 [18.8, 24.7] mg · L^{-1} in ORAL and NG, respectively; interaction P>0.05). Mean AUC expressed over 120 and 300 min of the postprandial period did not differ between NG and ORAL (both P>0.05).

Drink administration increased blood glucose concentrations (time effect P<0.001, in Figure 3A) similarly between groups (interaction P>0.05), such that glucose was significantly elevated above postabsorptive concentrations between 15 and 45 min. Postprandial AUC was similar between conditions. Peak concentration (C_{max}) and time to peak concentration (t_{max}) was similar between conditions (both P>0.05), at 5.2 [5.1, 5.4] and 5.3 [5.0, 5.5] mmol · L^{-1} in ORAL and NG, occuring at 29 [24, 34] and 28 [22, 33] min in ORAL and NG, respectively.

Drink administration increased serum insulin in both groups (time P<0.001) but to a greater extent in NG (interaction P<0.001; Figure 3B). Specifically, serum insulin increased from postabsorptive concentrations earlier and were sustained for longer, from 15 to 60 min following drink administration (all P<0.01), versus increases at 30 and 45 min in ORAL (P<0.05). This did not translate to a difference in AUC over the full 300 min postprandial period, as mean AUC was 5538 [4129, 6947] mU · L^{-1} · 300 min^{-1} in ORAL and 6649 [5646, 7653] mU · L^{-1} · 300 min^{-1} in NG (P>0.05). Although t_{max} did not differ between conditions, occuring at 30 [24, 36] and 25 [20,31] min in ORAL and NG, respectively, C_{max} was greater with NG versus ORAL (76 [64, 88] versus 49 [38, 60] mU · L^{-1}, P<0.01).

Plasma amino acid concentrations

Individual branched chain amino acid (BCAA; i.e. leucine, isoleucine, and valine), phenylalanine, and tyrosine concentrations are shown in Figure 4. Drink administration increased plasma concentrations of all amino acids (all time effects P<0.001), with a prolonged postprandial increase observed in NG versus ORAL (all interaction effects P<0.05). Specifically, drink administration in ORAL increased plasma concentrations of leucine, isoleucine (both 15–120 min; P<0.05), and valine (15–150 min; P<0.05) compared with 0 min. Plasma phenylalanine and tyrosine concentrations also increased from 15 to 60 min and 15 to 90 min, respectively, compared with 0 min (both P<0.05). Following drink administration in NG, plasma leucine increased to 251 [220, 281] μmol · L^{-1} at 15 min (versus 245 [218, 272] μmol · L^{-1} in ORAL; P>0.05) and remained elevated compared with 0 min throughout the entire postprandial period (P<0.05). Both valine and isoleucine concentrations increased from 0 min between 15 and 240 min (P<0.05). Phenylalanine and tyrosine concentrations increased from 0 min between 15–90 min and 15–150 min,

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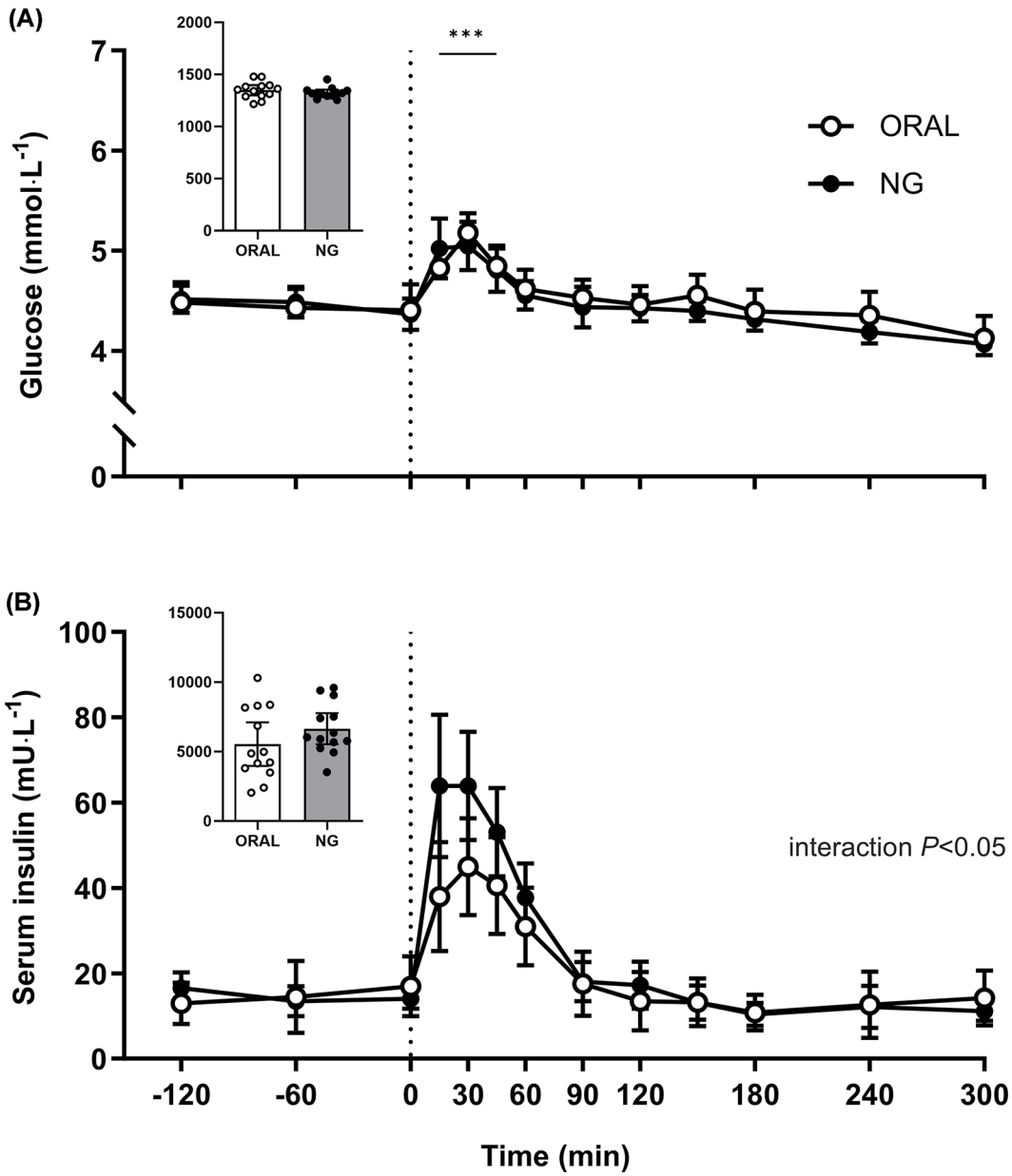

Figure 3. Blood glucose and serum insulin concentrations

Blood glucose (**A**) and serum insulin (**B**) concentrations after administering a test drink either orally (ORAL; n=13; open symbols) or via a nasogastric tube (NG; n=13; filled symbols). Insets are total postprandial area under the curve (AUC). Data presented as mean with 95% confidence intervals. Time-course data were analysed by two-way analysis of variance (ANOVA) with time and group factors, and AUC was compared by independent-samples t-test. (A) Significant main effect of time (P<0.001; post-hoc differences denoted by ***P<0.001 significantly different to 0 min). (B) Significant main effect of time (P<0.001). Significant time × group interaction effect (P<0.001). Post hoc differences from 0 min between 30 and 45 min in ORAL (P<0.05) and 15 and 60 min in NG (P<0.01).

respectively (P<0.01), with tyrosine concentration greater in NG than in ORAL at 45 min (111 [100, 121] versus 79 [67, 92] $\mu mol \cdot L^{-1}$, P<0.05). In NG, C_{max} of phenylalanine (83 [77, 89] versus 71 [64, 78] $\mu mol \cdot L^{-1}$), tyrosine (115 [103, 128] versus 85 [73, 98] $\mu mol \cdot L^{-1}$), and isoleucine (286 [256, 316] versus 245 [220, 269] $\mu mol \cdot L^{-1}$, all P<0.05) was greater versus ORAL, with borderline difference observed for leucine (389 [359, 419] versus 344 [313, 375] $\mu mol \cdot L^{-1}$, P=0.05). There were no differences in t_{max} or iAUC between groups for the concentration of any amino acid.

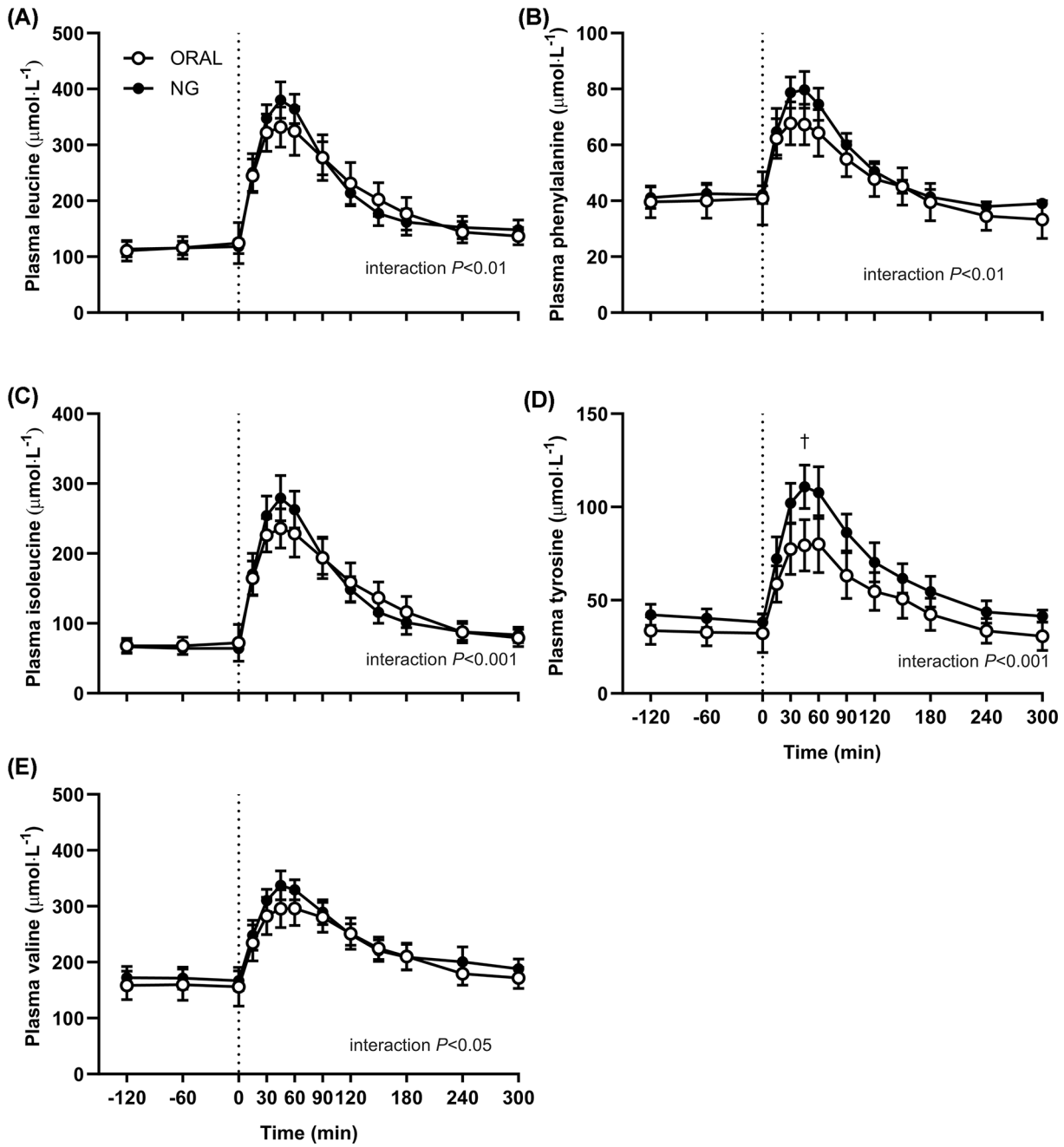

Figure 4. Plasma amino acid concentrations

Time course of plasma (**A**) leucine, (**B**) phenylalanine, (**C**) isoleucine, (**D**) tyrosine and (**E**) valine concentrations during a 120-min postabsorptive period and 300-min postprandial period after administering a test drink either orally (ORAL; n=13; open symbols) or via a nasogastric tube (NG; n=13; filled symbols). Insets are total postprandial incremental area under the curve (iAUC). Data presented as mean with 95% confidence intervals. Time-course data were analysed by two-way analysis of variance (ANOVA) with time and group factors, and iAUC was compared by independent-samples t-test. Significant main effect of time (P<0.001) for all amino acids. Significant main effect of group for tyrosine (P<0.01). Significant time × group interaction effect (P<0.001 for isoleucine and tyrosine; P<0.01 for leucine and phenylalanine; P<0.05 for valine). Post-hoc differences from 0 min in (A) leucine between 15 and 120 min in ORAL (P<0.05) and 15 and 300 min in NG (P<0.05); (B) phenylalanine between 15 and 60 in ORAL (P<0.05) and 15 and 90 min in NG (P<0.01); (C) isoleucine between 15 and 120 min in ORAL (P<0.05) and 15 and 240 min in NG (P<0.05); (D) tyrosine between 15 and 90 min in ORAL (P<0.05) and 15 and 150 min in NG (P<0.01); and (**E**) valine between 15 and 150 min in ORAL (P<0.05) and 15 and 240 min in NG (P<0.05). Post-hoc differences between group at a given timepoint denoted by $^{\dagger}P$<0.05.

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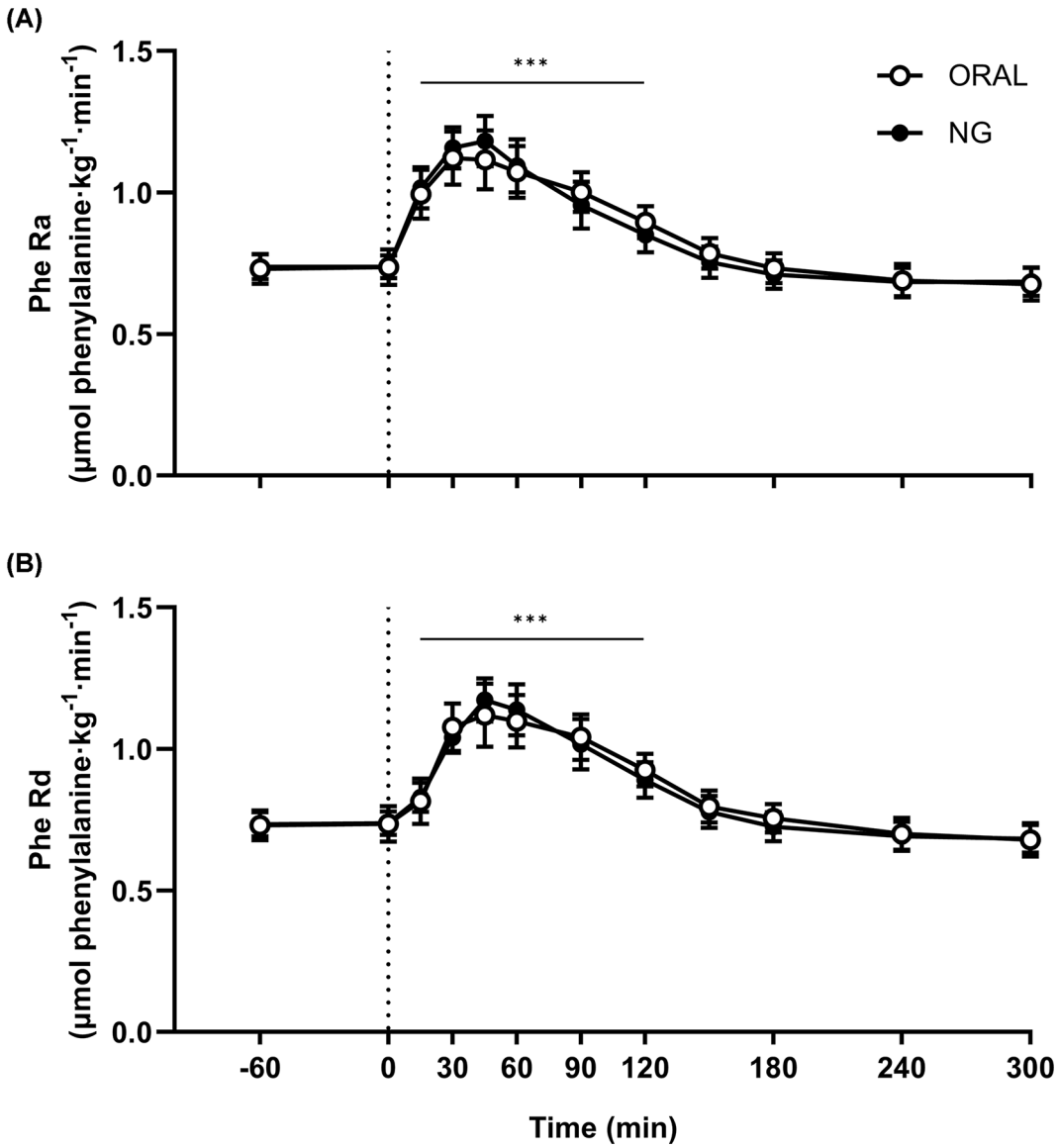

Figure 5. Phenylalanine rates of appearance and disappearance

Total phenylalanine (**A**) rate of appearance (R_a) and (**B**) rate of disappearance (R_d) in the plasma pool during a 60-min postabsorptive period and 300-min postprandial period after administering a test drink either orally (ORAL; n=13; open symbols) or via a nasogastric tube (NG; n=13; filled symbols). Data presented as mean with 95% confidence intervals. Data were analysed by two-way analysis of variance (ANOVA) with time and group factors. (A,B) Significant main effect of time (P<0.001; post-hoc differences denoted by ***P<0.001 significantly different to 0 min).

Whole-body protein kinetics

Total phenylalanine rate of appearance (R_a) and disappearance (R_d) are displayed in Figure 5. Following drink administration, both R_a and R_d increased between 15 and 120 min (both P<0.001 versus 0 min) to a similar extent in ORAL and NG (interaction P>0.05), before returning to postabsorptive values between 150 and 300 min (both P>0.05 versus 0 min).

Whole-body protein kinetics (Figure 6) were calculated from the plasma L-[*ring*-$^2H_5$]-phenylalanine, L-[3,3-$^2H_2$]-tyrosine, and L-[*ring*-$^2H_4$]-tyrosine enrichments in n = 10 per group. Whole-body protein synthesis increased 5 [1, 8]% in ORAL following drink administration, with a similar increase observed in NG (4 [1, 7]%; time P<0.01; interaction P>0.05). Whole-body protein breakdown decreased 20 [11, 28]% following drink administration in ORAL, with a similar decrease observed in NG (17 [14, 20]%; time P<0.001; interaction P>0.05). Phenylalanine

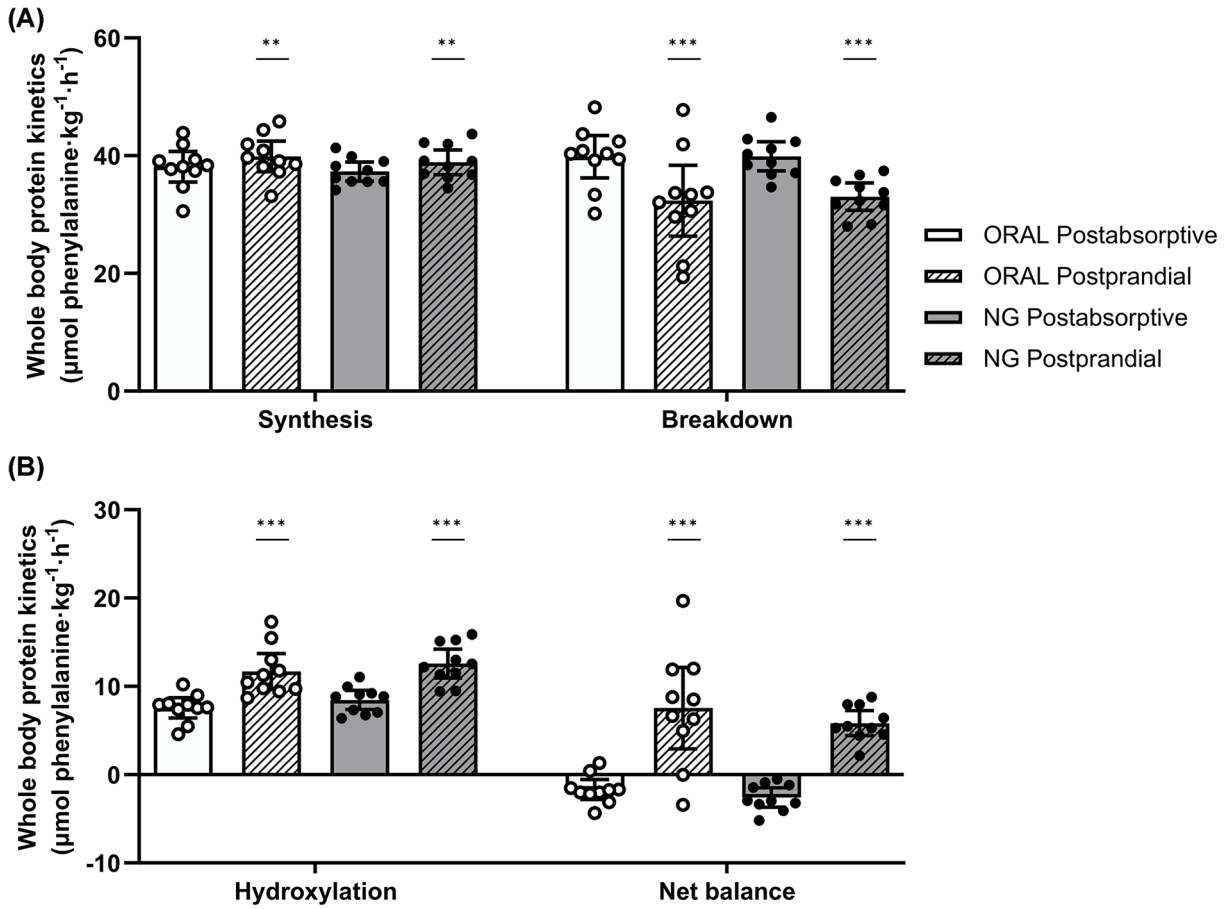

Figure 6. Whole-body protein kinetics

Whole-body phenylalanine kinetics representing (**A**) protein synthesis and breakdown; and (**B**) hydroxylation and net protein balance; measured in the plasma pool during the postabsorptive (60 min; solid bars) and postprandial (300 min; hashed bars) periods after administering a test drink either orally (ORAL; n=10; open symbols) or via a nasogastric tube (NG; n=10; filled symbols). Data presented as mean with 95% confidence intervals. Each process (i.e. synthesis, breakdown, hydroxylation and net protein balance) was analysed separately by two-way analysis of variance (ANOVA) with time and group factors, but presented on the same figures for clarity. Significant main effect of time denoted by ***P<0.001, **P<0.01, significantly different to postabsorptive period.

hydroxylation to tyrosine increased following drink administration by 56 [41, 71]% and 49 [40, 59]% in ORAL and NG, respectively (P<0.001), with no difference between groups (group P>0.05; interaction P>0.05). As a result, drink administration increased whole-body net protein balance from negative to positive (P<0.001) to a similar extent between ORAL and NG (interaction P>0.05). Estimated exogenous phenylalanine R_a over the postprandial period was unaffected by route of administration, at 85 [74, 96] $\mu mol \cdot kg^{-1} \cdot 300\ min^{-1}$ in ORAL and 83 [75, 91] $\mu mol \cdot kg^{-1} \cdot 300\ min^{-1}$ in NG (P>0.05).

Skeletal muscle protein synthesis

Skeletal muscle protein L-[*ring*-$^2H_5$]-phenylalanine enrichment increased over time in both groups (P<0.001), but to a greater extent in NG (interaction P<0.01). Between −120 and 0 min, enrichment increased from 0.0033 [0.0024, 0.0041] to 0.0054 [0.0035, 0.0073] MPE in ORAL and to a greater extent in NG (0.0024 [0.0004, 0.0044] to 0.0051 [0.0030, 0.0073] MPE; interaction P<0.001). At 120 min, enrichment had increased to 0.0078 [0.0060, 0.0096] MPE in ORAL and 0.0094 [0.0055, 0.0132] MPE in NG (post-hoc P>0.05 between groups). At 300 min, enrichment increased again to 0.0146 [0.0116, 0.0176] and 0.0215 [0.0181, 0.0249] MPE in ORAL and NG, respectively (both P<0.05 versus 120 min; P>0.05 between groups).

Mean plasma L-[*ring*-$^2H_5$]-phenylalanine enrichments between biopsy timepoints, which were used as the precursor to calculate myoFSR, did not differ between ORAL and NG (group P>0.05). Plasma enrichment decreased

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from 6.78 [6.29, 7.27] and 6.69 [6.39,7.00] MPE during the postabsorptive period to 4.98 [4.67, 5.29] and 5.01 [4.72, 5.29] MPE during the early postprandial period (120 min after drink administration; $P<0.01$ vs. postabsorptive) in ORAL and NG, respectively. This increased to 6.98 [6.51, 7.46] and 7.05 [6.72, 7.37] MPE during the later postprandial period (300 min; $P<0.001$ vs. 0–120 min), such that mean postprandial plasma L-[*ring*-$^2H_5$]-phenylalanine enrichments were 6.18 [5.79, 6.57] and 6.23 [5.93, 6.53] MPE in ORAL and NG, respectively ($P<0.001$ vs. postabsorptive).

Skeletal muscle myoFSR (Figure 7) was overall higher in NG compared with ORAL (group $P<0.05$). Drink administration increased myoFSR in both groups to a similar extent (time $P<0.01$). Specifically, myoFSR increased from 0.016 [0.005, 0.027] (postabsorptive) to 0.030 [0.020, 0.040] $\% \cdot h^{-1}$ (300-min postprandial) and from 0.020 [0.013, 0.026] (postabsorptive) to 0.053 [0.043, 0.062] $\% \cdot h^{-1}$ (300-min postprandial) in ORAL and NG, respectively (interaction $P>0.05$). With the postprandial period separated into early (0–120 min) and late (120–300 min) phases, myoFSR increased over time ($P<0.05$), such that myoFSR was significantly greater during the late compared with postabsorptive period in ORAL and NG (0.032 [0.016, 0.048] and 0.053 [0.044, 0.069] $\% \cdot h^{-1}$, respectively; $P<0.05$ versus postabsorptive). The change from the postabsorptive to postprandial, early, and late periods did not differ between ORAL and NG (all $P>0.05$; Figure 7C). For all myoFSR comparisons, the statistical outcomes did not differ when data were analysed with or without log-transformation.

Discussion

The present study shows for the first time that nasogastric protein-rich drink administration increases insulinaemia and aminoacidaemia to a greater extent than when the same drink was administered orally, in healthy young adults. Whilst whole-body net protein balance and skeletal muscle protein synthesis robustly increased following drink administration in both groups, contrary to our hypothesis, the greater insulinaemia and aminoacidaemia with nasogastric administration did not further increase whole-body net protein balance and skeletal muscle protein synthesis compared with oral administration.

Nasogastric drink administration augments aminoacidaemia and insulinaemia

In the present study, administering a protein-rich, mixed macronutrient drink containing 21 g protein increased plasma amino acid concentrations ∼2- to 4-fold (Figure 4). Notably, nasogastric administration led to overall higher and more sustained plasma amino acid concentrations than oral administration, despite identical drink composition (Figure 4 and Table 1). The appearance of amino acids in the circulation following protein ingestion is typically limited by digestion speed and gastric emptying [13,30]. For example, nasojejunal protein feeding demonstrably increases peak plasma amino acid concentrations by ∼19% over and above those observed with nasogastric feeding [10], likely as the point of delivery is beyond the stomach. To establish gastric emptying rates in the present study we administered 1.5 g paracetamol with each drink, as plasma paracetamol appearance is limited by gastric emptying rate rather than intestinal absorption [31]. However, here we report a comparable rise in serum paracetamol concentrations regardless of route of drink administration, as well as similar mean AUC over both 120 and 300 min, suggesting that gastric emptying does not explain the present differences in circulating amino acid concentrations.

In concert with greater and more sustained plasma amino acid concentrations, serum insulin concentrations were greater after nasogastric compared with oral drink administration (Figure 3B). Interestingly, serum insulin concentrations increased earlier with nasogastric versus oral administration (i.e. increased from fasting at 15 min in NG, vs. 30 min in ORAL), whilst circulating amino acid and glucose concentrations increased from fasting at similar time points between groups. Moreover, serum insulin concentrations peaked by 30 min in both groups, preceding the leucine peak at 45 and 60 min with nasogastric and oral drink administration, respectively. Although amino acids, particularly leucine [32], are insulinotropic, these relative timings implicate another factor in driving this insulin response following nasogastric tube placement, which may then serve to manipulate circulating amino acid concentrations. Indeed, insulin influences circulating amino acid availability through increasing net amino acid balance across tissues [33–35] and by suppressing amino acid release from tissues in a dose-dependent manner [34,36]. Given that total phenylalanine appearance (i.e. derived both from drink and that released from tissues; Figure 5A) was equivalent between administration routes, this opens up the possibility of increased intestinal amino acid absorption or reduced first-pass splanchnic extraction with nasogastric protein administration, countering any possible suppression of amino acid release from tissues. Together with greater peak amino acid concentrations and an extended duration of aminoacidaemia, it appears nasogastric protein administration increases overall amino acid availability, making this a potent strategy to induce an anabolic stimulus to support muscle mass maintenance.

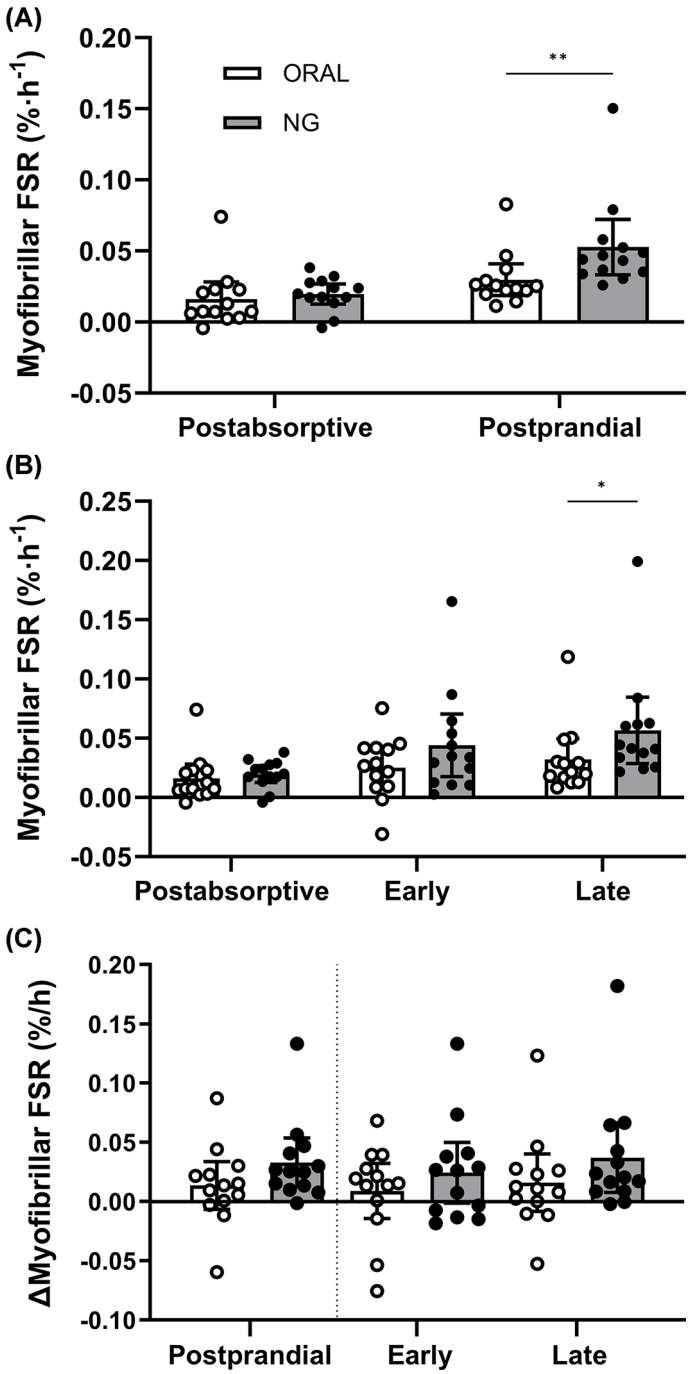

Figure 7. Myofibrillar protein synthesis rates

Myofibrillar protein fractional synthetic rate (FSR) calculated over (**A**) the postabsorptive and total postprandial period; (**B**) the postabsorptive, early (0–120 min) and late (120–300 min) postprandial periods; and (**C**) change (Δ) in myofibrillar FSR from postabsorptive to postprandial, early, and late periods; after administering a test drink either orally (ORAL; n=13; open symbols) or via a nasogastric tube (NG; n=13; filled symbols). Data presented as mean with 95% confidence intervals. Data were analysed by two-way analysis of variance (ANOVA) with time and group factors (A,B), or by separate independent t-tests (C). (A) significant main effect of time denoted by $^{**}P<0.01$; significant main effect of group ($P<0.05$). (B) Significant main effect of time ($P<0.05$; post-hoc differences denoted by $^{*}P<0.05$ significantly different to postabsorptive period); significant main effect of group ($P<0.05$).

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Nasogastric drink administration does not augment whole-body protein turnover or muscle protein synthesis

In light of our prior observations that nasogastric feeding may attenuate whole-body lean mass loss during bed rest [7] compared with oral feeding [2], we measured whole-body protein kinetics prior to and following protein administration. As anticipated, administration of a protein-rich drink increased whole-body protein synthesis by ∼4% and suppressed protein breakdown by ∼18% (Figure 6A). This resulted in a shift from negative to positive whole-body net protein balance from the postabsorptive to the postprandial period. However, the greater aminoacidaemia and insulinaemia observed with nasogastric administration was not associated with higher whole-body net protein balance over the 300-min postprandial period. Whilst somewhat unexpected, not least considering that protein synthesis could feasibly be lower given the augmented aminoacidaemia with nasogastric administration, this discrepancy is consistent with previous work. That is, hydrolysed casein has been shown to induce greater aminoacidaemia versus a protein-matched bolus of intact casein, yet total whole-body protein turnover measured over 360 min remained similar between groups [30]. Given that larger protein doses robustly and routinely increase total postprandial whole-body net protein balance [16,37,38], strategies to increase protein provision, rather than influencing digestion kinetics, are likely more effective at mitigating muscle mass loss.

Despite the relevance of increasing whole-body net protein balance in hospitalized patients and its role in establishing dietary guidelines for protein intake, alterations in whole-body protein kinetics do not necessarily reflect changes in skeletal muscle protein turnover [39]. Indeed, muscle mass is important for function and glucose homeostasis, and is also a clinical predictor for morbidity and mortality [9,40]. Thus, we quantified the postabsorptive and postprandial muscle protein synthetic response to nasogastric and oral administration. In accordance with previous work [14,15,41,42], administration of the protein-rich drink in the present study increased myoFSR by ∼2-fold from postabsorptive conditions (Figure 7). Remarkably, however, there was no further effect of nasogastric feeding on myoFSR, supporting our whole-body level measurements of protein synthesis and rate of disappearance. Although it is widely accepted that the degree of aminoacidaemia [17,43] and extracellular amino acid availability [44] (namely leucine) predict the muscle protein synthetic response, recent investigations have shown that manipulating postprandial amino acid concentrations do not necessarily translate to greater rates of muscle protein synthesis when amino acid provision is matched [45,46]. This suggests that the impact of rate and/or magnitude of plasma aminoacidaemia on muscle protein synthesis may depend more on the total dose of protein and/or specific amino acids ingested.

Relevance of augmenting aminoacidaemia

Discordance between postprandial amino acid concentrations and rates of muscle protein synthesis may arise from the protein dose (including that used in the present study) already inducing a maximum synthetic response, as it is well established that ingesting 20 g protein is sufficient to maximise postprandial muscle protein synthesis in young, healthy individuals [47,48]. Nonetheless, in clinical situations where impairments in protein digestion and absorption are present [49], suboptimal amounts or protein are consumed or administered [50], and/or a larger stimulus is required to overcome anabolic resistance to protein feeding (e.g. as in intensive care or in chronic disease [51–53]), the more rapid and higher rise in postprandial insulin and amino acid concentrations with nasogastric protein administration may be advantageous to stimulate whole-body and/or muscle protein synthesis. In support, a recent systematic review from our laboratory identified a relationship between ingested leucine dose and postexercise muscle protein synthesis rates only in older but not younger individuals [54]. This could imply that the degree of amino acid availability is important only in anabolic-resistant populations. Moreover, the greater insulin response observed may contribute to the the suppression of elevated muscle protein breakdown generally observed in critical illness [4,55], thereby potentially improving whole-body net protein balance. Therefore, the present data raise the possibility that nasogastric feeding could be used to (partially) overcome anabolic resistance during hospitalisation, given that fast-digesting proteins and larger protein boluses are currently recommended to overcome this [56,57]. This needs further investigation, as current ESPEN guidelines for feeding in critical care are to prioritise oral over enteral (e.g. nasogastric) feeding where possible [58].

Strengths and limitations

The use of a parallel group design and inclusion of merely tracer naïve participants improves the reliability of our dual tracer approach and therefore strengthens our conclusions, as it reduces risk of violating assumptions that tracer is not recycled and that tracer appears only from the infusion. As our groups were well matched (Table 2), we are confident that nasogastric administration does not influence muscle protein synthesis or whole-body turnover versus oral administration.

We acknowledge possible limitations with using paracetamol to determine gastric emptying rather than gold-standard scintigraphy. However, paracetamol appearance is validated and shows good agreement with scintigraphy [20]; our finding that gastric emptying does not differ therefore appears to be robust. In support, previous work has shown that gastric emptying rates measured by scintigraphy are similar between nasogastric and oral ingestion of a mixed macronutrient bolus [59], and in the present study, blood glucose concentrations increased similarly between groups following drink administration (Figure 3A), reflecting the modest carbohydrate content of the drinks.

Although our present study design prevents us from identifying the precise factors underpinning the augmented insulinaemia and aminoacidaemia, gastric emptying (Figure 2), as well as appearance from endogenous protein (i.e. whole-body protein breakdown; Figure 6A) and disappearance into whole-body and/or muscle protein (Figures 6A and 7) can likely be excluded as contributing factors. Thus, we identify an exciting area for further work, where the present findings can be built upon by a comprehensive endocrine investigation into the effects of administration route on gut- and pancreas-derived hormones, and how this would be influenced by bolus versus continuous (or sip) feeding.

Conclusion

Nasogastric administration of a protein-rich, mixed macronutrient drink markedly increases insulinaemia and aminoacidaemia over and above that observed following oral administration. Whilst nasogastric administration did not lead to greater whole-body net protein balance or skeletal muscle protein synthesis in healthy individuals, the increased availability of postprandial amino acids shows that nasogastric administration is a potent intervention to induce an anabolic stimulus, potentially alongside suppressing muscle protein breakdown. This may be of particular utility in conditions characterised by a reduced sensitivity to protein feeding.

Clinical perspectives

- Nasogastric tube feeding supports muscle mass maintenance, even under controlled experimental conditions, but little is known about its acute effect on nutrient absorption and subsequent handling.
- Compared with consuming a test drink orally, nasogastric feeding administration augmented insulinaemia and aminoacidaemia but did not influence whole-body protein turnover or rates of skeletal muscle protein synthesis.
- Whilst not influencing whole-body amino acid metabolism or skeletal muscle protein synthesis in a healthy population, the greater aminoacidaemia and insulinaemia induced by nasogastric feeding is notable. Future work is needed to assess the impact of nasogastric feeding in clinical populations displaying impaired anabolic sensitivity to protein ingestion. Enteral feeding in these populations could feasibly support muscle mass maintenance not only by ensuring sufficient dietary intake but also by improving the protein synthetic response and/or suppressing muscle protein breakdown following each meal.

Data Availability

Upon submission, authors agree to make any materials, data, and associated protocols available upon request.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Funding

A.J.M. and D.R.A. are supported in part by National Institute of Aging Grant [grant number P30-AG024832]. This work was performed as part of a PhD studentship grant supported by the University of Exeter and Beachbody LLC.

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https://doi.org/10.1042/CS20231126

CRediT Author Contribution

George F. Pavis: Conceptualization, Resources, Data curation, Software, Formal analysis, Supervision, Validation, Investigation, Visualization, Methodology, Writing—original draft, Writing—review & editing. **Doaa R. Abdelrahman:** Resources, Formal analysis, Investigation, Methodology, Writing—review & editing. **Andrew J. Murton:** Resources, Data curation, Formal analysis, Investigation, Methodology, Writing—review & editing. **Benjamin T. Wall:** Conceptualization, Resources, Data curation, Formal analysis, Supervision, Investigation, Methodology, Writing—original draft, Project administration, Writing—review & editing. **Francis B. Stephens:** Conceptualization, Resources, Data curation, Formal analysis, Supervision, Funding acquisition, Investigation, Methodology, Writing—original draft, Project administration, Writing—review & editing. **Marlou L. Dirks:** Conceptualization, Data curation, Formal analysis, Supervision, Investigation, Visualization, Methodology, Writing—original draft, Project administration, Writing—review & editing.

Ethics Approval

Ethical approval was granted by the University of Exeter's Sport and Health Sciences Ethics Committee (proposal reference number: 180509/B/03) and carried out in accordance with the World Medical Association Declaration of Helsinki. This study was registered as a clinical trial with ClinicalTrials.gov (NCT03571425). All participants provided oral and written informed consent prior to enrolment in the study.

Abbreviations

ANOVA, analysis of variance; AUC, area under the curve; BCAA, branched chain amino acid; EAA, essential amino acid; FSR, fractional synthetic rate; iAUC, incremental area under the curve; MPE, mole per cent excess; NEAA, non-essential amino acid; NG, nasogastric tube; TBDMS, tert-butyl-dimethylsilyl.

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