**Methods**

*Plasma amino acid analyses*

To precipitate out proteins, 250 µl of acetonitrile, with 0.1% formic acid (LC-MS grade, Sigma-Aldrich), was added to 100 µl of plasma (i.e. a ratio of 2.5:1 acetonitrile:plasma). Vortexed samples were left to stand for 20 min at room temperature, then debris removed by centrifugation at 13K rpm, 4oC, and 20 min in a Beckman-Coulter benchtop centrifuge. The supernatant was filtered through a 0.2 µM syringe tip filter (Phenomenex, UK) into clean 1.5ml centrifuge tubes and frozen until ready for derivatization. An AccQ•Tag Ultra derivatization kit (Waters Corporation, Milford, MA, USA) was used for derivatizing 10µl of plasma sample, spiked with an internal standard containing stable isotope-labelled amino acids (L-amino acid mix (Sigma-Aldrich, Co., St. Louis, MO, USA) to give a concentration of 6 µM per sample. The sample was immediately vortexed followed by incubation for 15 min at 55oC. To maintain consistency between the time of extraction and time of analysis, due to the large scale of the project, derivatised samples were prepared and analysed in daily batches.

HPLC-ESI-MS/MS quantitative analysis of amino acids in plasma was performed using an Agilent 6420B triple quadrupole (QQQ) mass spectrometer (Technologies, Palo Alto, USA) hyphenated to a 1200 series Rapid Resolution HPLC system. 5 µl of sample extract was loaded onto an Eclipse Plus C18 (3.5 µm, 2.1 x 150 mm) reverse phase analytical column (Agilent Technologies, Palo Alto, USA) with an Eclipse Plus C18 (1.8 µm, 2.1x5mm) guard (Agilent Technologies, Palo Alto, USA). For detection using positive ion mode, mobile phase A comprised of 95% LC-MS grade H2O, with 0.1 % formic acid and 1 mM ammonium formate, and mobile phase B was 95% acetonitrile (LC-MS grade) with 0.1 % formic acid. The following gradient was used: 0 min – 0% B; 16 min – 20% B; 20 min – 100% B; 22 min – 100% B; 23 min – 0% B followed by 3 min re-equilibration time. The flow rate was 0.25 ml min-1 and the column temperature was held at 35 °C for the duration. The QQQ source conditions for electrospray ionisation were as follows: gas temperature 350 °C, drying gas flow rate of 11 l min-1, nebuliser pressure 35 psi, and capillary voltage 4 kV. All ions were scanned in positive ion mode and given a dwell time of 30 milliseconds. The fragmentor voltage and collision energies had previously been optimised for each compound. Data analysis was undertaken using Agilent Mass Hunter Quantitative analysis software for QQQ (Version B.07.01). Accurate quantification used the stable isotope labelled internal standards added during sample extraction.

**Results**

*Plasma biochemistry profile*

Plasma clinical biochemistry profiles are reported in Table 3. Fasting levels of all parameters were within normal clinical ranges, indicative of our healthy cohort of participants, and these ranges were not breached following protein consumption under any of the conditions despite any significant changes. Plasma Na+ concentrations and osmolality remained stable during all conditions. Plasma glucose concentrations declined following protein ingestion (time effect; *P*<0.001), becoming significantly lower than fasting values only in the late postprandial period in the MLK20, MYC20 and MYC40 conditions (all *P*<0.05), significantly lower in the late compared with early postprandial period in the MYC60 condition (*P*<0.01), and with no effect of treatment (*P*=0.86) or interaction (*P*=0.25). Plasma urea concentrations showed a significant interaction effect (*P*<0.001) such that there was a significant decrease from fasting levels by the late postprandial period in the MLK20 and MYC20 conditions (from 5.6±0.3 and 5.6±0.3 to 5.4±0.3 and 5.3±0.3 mmol.L-1, respectively; both *P*<0.01), which were also significantly lower than values obtained in the early postprandial phase (both *P*<0.01). While plasma urea concentrations did not change significantly over the experimental period in MYC40, there was a trend (*P*=0.09) for elevated levels in the late postprandial phase compared to both fasting and the early postprandial period in MYC60, and the MYC80 group displayed a significant increase from fasting levels (5.3±0.3 mmol.L-1) to 5.7 mmol.L-1 (*P*<0.001) in the early postprandial phase, which continued to rise by the late postprandial phase to 6.0 mmol.L-1 (*P*<0.001 compared with fasting and early postprandial phase). Plasma creatinine concentrations declined following protein ingestion (effect of time; *P*<0.001) with no effect of treatment (*P*=0.98) or any interaction (*P*=0.20) observed. Specifically, plasma creatinine concentrations decreased from fasting levels following protein ingestion in the MLK20 condition during the late phase (*P*<0.05), and during the MYC20 and MYC80 conditions at both the early and late phases (both *P*<0.05), but remained unaltered in the MYC40 and MYC60 conditions. The effects on plasma urea and creatinine concentrations translated to a time (*P*<0.01) and interaction effect (*P*<0.05) upon urea:creatinine ratios, such that the ratio rose in the MYC80 group only during both the early (*P*<0.05) and late (*P*<0.001) postprandial phases. Plasma K+ concentrations declined following protein ingestion (*P*<0.001) such that fasting levels were lower by the late postprandial phase in the MLK20 (*P*<0.05) and MYC20 (*P*<0.05) conditions only, with no effect of treatment (*P*=0.89) or any interaction (*P*=0.26) effects observed. Plasma pH changed with prandial state (time effect; *P*<0.01), with MYC60 showing a significant decline from fasting in the late postprandial phase (*P*<0.05), and MYC80 a decline during the early postprandial phase (*P*<0.05). An effect of protein ingestion was detected for plasma Mg++ concentrations (time effect; *P*<0.001) with MYC60 (*P*<0.05) and MYC80 (*P*<0.01) showing increases from fasting during the early postprandial phase, and MYC20 (*P*<0.05) and MYC60 (*P*<0.001) exhibiting elevations during the late postprandial phase. Fasting plasma lactate concentrations decreased with protein ingestion (time effect; *P*<0.001) during the early (MYC20; *P*<0.05) and the late (MYC20 and MYC40; *P*<0.05) postprandial phases, and declined from the early to the late postprandial phase in the MLK20 condition only (*P*<0.01). Fasting plasma Cl- concentration was not influenced by mycoprotein ingestion in any dose, though the ingestion of MLK20 resulted in a significant decrease during both early (*P*<0.001) and late (*P*<0.01) postprandial phases (time effect; *P*<0.001). Plasma Ca*++* concentrations were affected by protein ingestion (time effect; *P*<0.001) and a significant time *x* treatment interaction was also detected (*P*<0.05), indicating that concentrations rose from fasting levels throughout early (MLK20; *P*<0.01: MYC80; *P*<0.01) and late (MLK20; *P*<0.001) postprandial phases under certain conditions. A significant time *x* treatment interaction was observed for plasma TCO2 concentrations showing a rise with protein ingestion in the MYC80 condition only during both the early and late postprandial phases (both *P*<0.001).