**METHODS**

*Dietary intervention*

****Meals were prepared by a staff member that was not involved in the study and were served on a white plate provided with the randomization code, making them blinded to both participants and researchers. The appearance of the 2 products was similar and presented in an identical form (small, diced pieces), see pictures below. In addition, we instructed the participants not to comment on the smell and taste and not to make any speculations of what they were consuming.

The amino acid content of the raw products were determined by the Dumas combustion method was used to determine nitrogen content using the Vario MAX cube CN (Elementar Analysensysteme, Germany). Protein content was calculated by multiplying the determined nitrogen content by 6.25 as the standard nitrogen-to-protein conversion factor. Amino acid profile of the products was determined by acid hydrolysis in triplicate of the raw products (~500 mg wet weight). Specifically, the amino acids were liberated by adding 2 mL of 6M HCl and heating to 110°C for 12 h. The hydrolysed free amino acids were subsequently dried under a nitrogen stream while heated to 120°C. Before analysis by ultraperformance liquid chromatography-mass spectrometry (UPLC-MS; ACQUITY UPLC H-Class with QDa; Waters, Saint-Quentin, France), the hydrolysate was dissolved in 5 mL of 0.1 M HCl and 20 µL of AccQ/Tag derivatizing reagent solution (Waters, Saint-Quentin, France) was a for the plasma amino acid concentration analysis.

*Plasma analysis*

Plasma glucose and insulin concentrations were analysed using commercially available kits (ref. no. A11A01667; glucose HK CP, ABX Diagnostics, Montpellier, France, and Human Insulin specific RIA, ref. no. HI-14K, Millipore, Billerica, USA, respectively).

Plasma amino acid concentrations were measured by using ultra-performance liquid chromatograph mass spectrometry (UPLC-MS, ACQUITY UPLC H-Class with QDa; Waters, Saint-Quentin, France). 50 µL of blood plasma was deproteinized using 100 µL of 10 % SSA with 50 µM of MSK-A2 internal standard (Cambridge Isotope Laboratories, Massachusetts, USA). Subsequently, 50 µL of ultra-pure demineralized water was added and samples were centrifuged (15 min at 14000 rpm). After centrifugation, 10 µL of supernatant was added to 70 µL of Borate reaction buffer (Waters, Saint-Quentin, France). In addition, 20 µL of AccQ-Tag derivatizing reagent solution (Waters, Saint-Quentin, France) was added after which the solution was heated to 55 °C for 10 min. An aliquot of 1 µL was injected and measured using UPLC-MS. The amino acids analysed were alanine, arginine, asparagine, aspartic acid, beta-alanine, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine.

For plasma L-[ring-13C6]-phenylalanine enrichment measurements, plasma phenylalanine was derivatized to the tert-butyldimethylsilyl (TBDMS) derivative with N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA), and the 13C enrichments were determined by electron ionization gas chromatography-mass spectrometry (GC-MS; Agilent 7890A GC/5975C MSD; Agilent Technologies) using selected ion monitoring of masses 336 and 342 for unlabelled and labelled (ring-13C6) phenylalanine, respectively. Standard regression curves were applied from a series of known standard enrichment values against the measured values to assess the linearity of the mass spectrometer and to account for any isotope fraction which may have occurred during the analysis. Phenylalanine enrichments were corrected for the natural level of 13C isotopes.

Mixed plasma proteins from blood samples at *t=*-180 min were used to determine baseline L-[ring-13C6]-phenylalanine enrichments to allow calculation of basal muscle protein synthesis rates using the single biopsy approach (1). Mixed plasma proteins were isolated from blood by using perchloric acid (PCA) to a final concentration of 2 %. Samples were centrifuged at 1000 *g* at 4 °C for 10 min, and the supernatants were removed. The mixed plasma protein pellet was washed 3 times with 2 % PCA and dried. Amino acids were liberated by adding 6 M HCl and were heated at 120 °C for 15–18 h. Thereafter, the enrichments in hydrolysed mixed plasma protein samples were assessed using the same procedures as the muscle protein-bound samples.

*Muscle analysis*

Mixed muscle protein L-[ring-13C6]-phenylalanine enrichments were extracted from ~50 mg of wet muscle tissue.  After the muscle was freeze-dried, collagen, blood, and other non-muscle fibres were removed from the muscle fibres under a light microscope. The isolated muscle fibre mass was weighed and 35 volumes (7x wet weight of isolated muscle fibres x wet-to-dry ratio 5:1) of ice-cold 2% PCA was added and the sample was homogenized and centrifuged. The tissue protein pellet was washed three times with 1.5 mL of ice-cold 2% PCA and hydrolysed in 3 mL of 6 M HCl overnight at 120°C. The free amino acids were then dissolved in 50% acetic acid solution and passed over cation exchange AG 50W-X8 resin [mesh size: 100–200, ionic form: hydrogen (Bio-Rad Laboratories)] columns. The amino acids were eluted with 2 M NH4OH for measurement of L-[ring-13C6]-phenylalanine enrichment in tissue protein. To determine the L-[ring-13C6]-phenylalanine enrichment of tissue protein, the purified amino acids were derivatized into their *N*(O,S)-ethoxycarbonyl ethyl ester derivatives with ethyl chloroformate (ECF). The derivatives were then measured by GC-C-IRMS (Thermo Fisher Scientific Delta V, Bremen, Germany) using a DB-17MS-column (30 m x 0.25 mm x 0.5 µm; Agilent J+W scientific GC column, Santa Clara, CA, USA) and monitoring of ion masses 44, 45, and 46. Standard regression curves were applied to assess the linearity of the mass spectrum and to account for isotopic fractionation.

Western blot analysis was performed on muscle tissue samples at t = 0, 120, and 300 min. A portion (∼20 mg) of each muscle sample frozen for biochemical analyses was homogenized in seven volumes Tris buffer (20 mM Tris‐HCL, 5 mM EDTA, 10 mM Na pyrosphospate, 100 mM NaF, 2 mM Na3VO4, 1% Nonident P‐40; pH 7.4) supplemented with protease and phosphatase inhibitors: aprotinin 10 µg∙mL-1, leupeptin 10 µg∙mL-1, benzamidin 3 mM and phenylmethylsulphonyl fluoride 1 mM. After homogenization, each muscle extract was centrifuged for 10 min at 10,000 g (4 °C) and sample buffer (final concentration: 60 mM Tris, 5% glycerol, 20 mg/mL SDS, 0.1mM DTT, 20 µg∙mL-1 bromophenol blue) was added to the supernatant. The supernatant was extracted and boiled for 5 min at 100 °C and put on ice after sample buffer was added to the sample. Immediately before analyses, the muscle extraction sample was warmed to 50°C and centrifuged for 1 min at 1,000g at room temperature (RT). Based upon a Bradford protein assay 50 μg protein was loaded for each sample and the amount of protein transferred was checked with a Coomassie staining. With the exception of mTOR, protein samples were run on a Criterion Precast TGX 4–20% gel (Bio‐Rad, Order No. 567–1094) for 90 min at 150V (constant voltage) and transferred onto a Trans‐blot Turbo 0.2μm nitrocellulose membrane (Biorad Order No. 170-4159) in 7 min at 2.5 A and 25 V. mTOR samples were run and blotted on a Criterion Precast XT 3–8% Tris‐acetate gel (Biorad Order No. 345-0130) and transferred onto a Trans-blot Turbo 0.2 µm nitrocellulose membrane (Biorad Order No. 170-4159) in 10 min at 2.5 A and 25 V. Specific proteins were detected by overnight incubation at 4°C on a shaker with specific antibodies in 50% PBS Odyssey blocking buffer (Part No. 927‐40 000; Li‐Cor Biosciences, Lincoln, NE, USA) after blocking for 60 min at RT in 50% Odyssey blocking buffer in PBS. Polyclonal primary phospho‐specific antibodies were purchased from Cell Signaling Technology (Danvers, USA) and included anti-total mammalian target of rapamycin (mTOR; no. 2972S), anti-phosphorylated mTOR Ser2448 (no. 2971L), anti-p70 ribosomal protein S6 kinase (p70S6K; no. 9202L) and anti-phosphorylated p70S6K Thr389 (no. 9205L), anti-total S6 ribosomal protein (no. 2217L), anti-phosphorylated S6 ribosomal protein Ser235/236 (no. 4856S), anti-total eukaryotic initiation factor 4E binding protein-1 (4E-BP1; no. 9452L) and anti-phosphorylated 4E-BP1 Thr37/46 (no. 9459L). Following incubation, membranes were washed 3 times 10 min in 0.1% PBS Tween 20 and once for 10 min in PBS. Next, samples were incubated on a shaker (1 h at RT) with infrared secondary antibodies, donkey anti-rabbit IRDYE 680 (Li-Cor, Cat. No. 926-32223, dilution 1:50000) and donkey anti-mouse IRDYE 800CW (Li-Cor, Cat. No. 926-32212, dilution 1:10000) dissolved in 50% PBS Odyssey blocking buffer. After a final wash step (3 x 10 min) in 0.1% Tween 20-PBS and once 10 min in PBS, protein quantification was performed by scanning on an Odyssey Infrared Imaging System (LI-COR Biotechnology, Lincoln, NE). Ponceau S staining was performed after blotting to assess blot efficiency. Phosphorylation as a proxy of activation of the signalling proteins of mTOR, p70S6K, ribosomal S6, and 4E-BP1 was expressed as a ratio between phosphorylated/total protein content (arbitrary units; AU).

**RESULTS**

Qualitative measurements on palatability were taken directly after consumption of the meals by providing participants with visual analogue scales (VAS), VAS scores are presented in **Supplemental Table 1**. Questions on ‘general taste experience’, ‘taste sensation in the mouth’, ‘taste addiction’, and ‘texture experience’ did not differ between treatment groups (*P*>0.05), while questions as ‘I have eaten food that has a similar taste’, ‘I have eaten foods like this before’, ‘I am satisfied with the taste’, and ‘Would you use the product more regular?’ were lower in Plant when compared with Chicken (*P*<0.05).

**Supplemental Table 1.** VAS scores on palatability questions

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Plant (*n*=12) | | Chicken (*n*=12) | |
|  | *Mean* | *SD* | *Mean* | *SD* |
| General taste experience | 43.3 | 21.0 | 58.8 | 19.0 |
| General texture experience | 48.7 | 16.7 | 60.6 | 18.3 |
| Is the taste addictive? | 21.3 | 15.9 | 30.3 | 18.8 |
| Would you have another bite of this meal? | 41.0 | 22.7 | 50.1 | 23.0 |
| Are you satisfied with the taste? | 46.4 | 20.1 | 67.2 | 23.3\* |
| How would you rate the taste in your mouth? | 47.3 | 15.1 | 49.7 | 15.4 |
| Have you ever eaten foods with a similar taste? | 38.0 | 20.7 | 63.2 | 20.1\* |
| Have you ever eaten foods like this? | 37.9 | 28.5 | 64.8 | 23.5\* |
| How likely would your family or friends like this meal? | 37.9 | 19.5 | 60.3 | 25.2\* |
| Would you use this product more often? | 39.1 | 23.5 | 58.1 | 19.9\* |
| How likely would you buy this product? | 37.1 | 24.7 | 55.0 | 19.9 |

VAS: visual analogue scale. Values are mean ± SD. Data were analysed by unpaired Student’s t-test. \*Indicates a significant difference between treatments, *P*<0.05.

**REFERENCES**

1. Burd NA, Groen BB, Beelen M, Senden JM, Gijsen AP, van Loon LJ. The reliability of using the single-biopsy approach to assess basal muscle protein synthesis rates in vivo in humans. Metabolism: clinical and experimental 2012;61(7):931-6. doi: 10.1016/j.metabol.2011.11.004.