Daily mycoprotein consumption for one week does not affect insulin sensitivity or glycaemic control but modulates the plasma lipidome in healthy adults: a randomised controlled trial

Mariana OC Coelho[1], Alistair J Monteyne[1], Marlou L Dirks[1], Tim JA Finnigan[2], Francis B Stephens[1] and Benjamin T Wall[1]

[1] *Department of Sport and Health Sciences, College of Life and Environmental Sciences, University of Exeter, Exeter EX1 2LU, United Kingdom*

[2] *Marlow Foods Ltd., Stokesley TS9 7AB, United Kingdom*

Running title: Mycoprotein and metabolic health

Keywords: Mycoprotein, protein, metabolic health, metabolomics, cholesterol, lipoproteins

Sources of support: Marlow Foods Ltd

Clinical trial registry: NCT02984358 (https://clinicaltrials.gov/ct2/show/NCT02984358)

Corresponding author:

Benjamin T. Wall, PhD

Department of Sport and Health Sciences

College of Life and Environmental Sciences

St Luke's Campus, Heavitree Road

University of Exeter

Exeter, EX1 2LU

UK

Tel: +44 (0)139 272 4774

Email: b.t.wall@exeter.ac.uk

Abbreviations

CGMS, continuous glucose monitoring system; CHO, carbohydrate; CON, control group; iAUC, incremental area under the curve; IL-6, interleukin 6; IS, insulin sensitivity; OGTT, oral glucose tolerance test; MYC, mycoprotein group; NRM, nuclear magnetic resonance; RM, repeated measures; RMR, resting metabolic rate; SCFA, short-chain fatty acid; SEM, standard error of the mean

Abstract

Mycoprotein consumption has been shown to improve acute postprandial glycaemic control and decrease circulating cholesterol concentrations. We investigated the impact of incorporating mycoprotein into the diet on insulin sensitivity (IS), glycaemic control and plasma lipoprotein composition. Twenty healthy adults participated in a randomised, parallel-group trial in which they consumed a 7 d fully-controlled diet where lunch and dinner contained either meat/fish (CON) or mycoprotein (MYC) as the primary source of dietary protein. Oral glucose tolerance tests were performed pre- and post- intervention, and 24h continuous blood glucose monitoring was applied throughout. Fasting plasma samples were obtained pre- and post- intervention and were analysed using quantitative, targeted NMR-based metabonomics. There were no changes within or between groups in blood glucose or serum insulin responses, nor in IS (Cederholm; 51±3 to 51±3 and 54±3 to 53±3 $mg.L^2/mmol.mU.min$ in CON and MYC, respectively; $P<0.05$) or 24 h glycaemic profiles. No differences between groups were found for 171 of the 224 metabonomic targets. Forty five lipid concentrations of different lipoprotein fractions (VLDL, LDL, IDL and HDL) remained unchanged in CON but showed a coordinated decrease (7-27 %; all $P<0.05$) in MYC. Total plasma cholesterol, free-C, LDL-C, HDL2-C, DHA and omega-3 fatty acids decreased to a larger degree in MYC (14-19 %) compared with CON (3-11 %; $P<0.05$). Substituting meat/fish for mycoprotein twice-daily for one week did not modulate whole-body IS or glycaemic control but resulted in changes to plasma lipid composition; the latter primarily consisting of a coordinated reduction in circulating cholesterol containing lipoproteins.

Introduction

Growing evidence suggests dietary protein consumption above the current reference daily allowances (i.e. 0.75-0.8 g/kg/day in the UK and the USA[1; 2]) may confer metabolic benefits relating to healthy ageing and weight management, such as improved glycaemic control[3; 4; 5; 6; 7; 8; 9]. In parallel, increasing data are accumulating concerning the environmental cost of intensive animal-derived dietary protein production[10], resulting in shifting social attitudes and government initiatives towards more sustainable sources. As a consequence, the efficacy of non-animal derived, sustainably produced dietary proteins to support glycaemic control and metabolic health is a pressing research focus.

Mycoprotein is a low-energy food source, rich in protein and fibre, derived from the continuous cultivation of the fungus *Fusarium venenatum*[11]. For the production of an equivalent amount of edible protein, mycoprotein requires less water and land usage, and has a reduced carbon footprint when compared with meat and dairy[12; 13; 14], positioning it as a sustainable alternative protein source.

Previous work has shown that the ingestion of a single mycoprotein-rich meal in combination with an oral glucose tolerance test results in reduced postprandial glycaemia and insulinaemia compared with isonitrogenous and isoenergetic control meals[16; 17]. The careful matching of nutritional conditions in these studies suggests that either mycoprotein was delaying intestinal glucose absorption or improving postprandial (peripheral) glucose uptake, with either effect plausibly linked to the amino acid composition or fibre content (and type) contained within mycoprotein. We have recently shown that protein digestion and amino acid absorption following mycoprotein ingestion is sustained during the acute postprandial period, highlighting the potential of this alternative protein source to modulate glycaemic control[15]. However, whether these findings translate to habitual mycoprotein consumption improving physiologically relevant, longer-term changes in insulin sensitivity and/or glycaemic control has not been investigated.

Studies that have investigated the incorporation of mycoprotein into the habitual diet (20-60 g dry weight per day for 3-8 weeks using either fully-controlled or supplemented free living nutritional interventions) have reliably shown a 0.4–0.8 mmol/L lowering of blood cholesterol concentrations and improvements in LDL:HDL ratios in healthy and hypercholesterolemic individuals[18; 19]. These studies designed the nutritional interventions in an energy- and macronutrient- matched manner, and therefore the higher fibre content of the mycoprotein conditions is likely the causative factor (27-39 g per day in the mycoprotein based diets vs 25-27 g per day in the control diets).

In the present study, we applied a one week fully controlled dietary intervention in healthy young adults where the major source of dietary protein at lunch and dinner was obtained from meat and fish (control group; CON) or from mycoprotein (intervention group; MYC) with energy and macronutrient (except fibre) content of the diets matched. We hypothesised that one week of mycoprotein consumption would improve whole-body insulin sensitivity and 24 h free living glycaemic control. We also applied a novel, targeted Nuclear Magnetic Resonance (NMR) based quantitative metabonomics approach of 224 relevant metabolites that has been epidemiologically validated as a biomarker of insulin sensitivity[20] and would allow further insight as to the impact upon metabolic profile of mycoprotein consumption.

Subjects and Methods

Participants and medical screening

Twenty healthy, recreationally active, young adults (age: 24±1 y; BMI: 23±1 kg/m^2; male = 8 and female = 12) participated in the present study. Subjects' characteristics are presented in **Table 1**. Prior to participating, each subject attended a screening visit to ensure eligibility. Blood pressure, body mass, height and body composition (determined by air displacement plethysmography; Bodpod; Life Measurement, Inc., Concord, CA, USA) were measured at screening. The participants also completed a general health questionnaire and the International Physical Activity Questionnaire (IPAQ)[21]. Vegetarians, vegans, smokers, and participants taking regular medication or suffering from chronic diseases were excluded. Participants regularly consuming >2.5 or <0.8 g/kg of protein per day were also excluded. Participants included were recreationally active (partook in regular exercise or sport at a non-competitive level, two to five days a week), were normotensive, and had a BMI between 18.5 and 30 kg/m^2. Half of the female participants (6/12) were taking hormonal contraceptives. When this was not the case, female participants were tested (and their habitual data collected) during the follicular phase of their menstrual cycle, to control for cycle variations in glucose and insulin responses[22]. All participants were informed of the study's purposes, procedures and risks, and provided written informed consent. The study was conducted at the Nutritional Physiology Research Unit, Department of Sport and Health Sciences, St. Lukes campus, University of Exeter, between January and December of 2017, and it was approved by the University of Exeter's Sport and Health Sciences Ethics Committee (Ref No: 161026/B/07) in accordance with the Declaration of Helsinki and registered at ClinicalTrials.gov (NCT02984358).

Experimental Protocol

The present study was a randomised, controlled, parallel design trial, with participants being randomly allocated into one of two dietary interventions which differed with respect to the primary source of dietary protein consumed: meat/fish derived dietary protein (CON; *n*=10) or mycoprotein (MYC; *n*=10). Participants were allocated sequential numbers at the time of screening which were then used as the only identifiable characteristic for all documents containing participant information, and were randomised into groups using an online randomiser (http://www.randomization.com/), with stratification by sex. **Figure 1** shows an overview of the study design. All subjects underwent a period of habitual data collection as well as data collection during their allotted intervention.

Habitual data collection

Habitual data collection took place either during the 2 weeks before (CON; *n*=7, MYC; *n*=7) or between 2 and 8 weeks following (CON; *n*=3, MYC; *n*=3) the experimental period. Subjects were asked to complete a 3-day food diary to assess their habitual dietary intake, following consultation with a qualified nutritionist concerning how to complete this in as much detail as possible. All food and drink consumed were recorded for three consecutive days, including two weekdays and one weekend day. The diaries were analysed for energy and macronutrient content using Nutritics (Nutritics Professional Nutritional Analysis Software, Swords, Dublin, Ireland). Participants wore a GENEActiv Original accelerometer (ActivInsights, Kimbolton, UK), a wrist-worn device to measure daily physical activity by intensity, on their non-dominant wrist, for 5 consecutive days (including both week and weekend days). Physical activity data from the GENEActiv monitors were processed using GENEActiv excel macros. The 5 days of habitual physical activity data were compiled into an individual average for each participant and the same was done for the 7 days of the intervention. Glucose sensors were placed subcutaneously at the side of the abdomen and connected to a continuous glucose monitoring system (CGMS; Dexcom G4 Platinum, San Diego, California, USA) to measure interstitial glucose concentrations (calibrated to blood glucose concentrations measured via finger prick 4 times per day) every 5 minutes for the same 5 days as those where accelerometry data were collected. During all habitual data collections, participants were instructed not to change their normal routines.

Experimental test days

Participants reported to the laboratory at ~08.00 on day 0 (prior to starting the dietary intervention) and on day 8 (the morning following the intervention) after an overnight fast and refraining from intense exercise and alcohol consumption for at least 24 hours, to undertake two identical experimental test days. A cannula was placed retrogradely in a dorsal hand vein and the hand was then placed in a heated box (55ºC) for arterialised venous blood sampling before a fasted arterialised-venous blood sample was collected[23]. Fasted measurements of oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) were collected using a facemask and the Metamax 3B (MM3B) portable indirect calorimetry system (Cortex, Leipzig, Germany) for 30 minutes. Carbohydrate and fat oxidation rates, as well as resting metabolic rate (RMR), were calculated using the Frayn equations[24]. Subsequently, an oral glucose tolerance test (OGTT) was performed. Briefly, participants ingested 75 g glucose (dextrose, BulkPowders, Colchester, United Kingdom) dissolved in 350 mL water in 5 minutes or less

(with the exact time being recorded for each participant in the first visit and replicated on the last test day). Arterialised venous blood samples were then collected for a 2 h period at 15 min intervals for the measurement of glucose and insulin concentrations and the subsequent calculation of glucose tolerance and insulin sensitivity. Indirect calorimetry was performed throughout the OGTT period with the exception of the first 15 minutes following glucose ingestion.

Dietary intervention

Basal metabolic rate (BMR) was estimated using the Henry equations based on age, gender, and weight[25]. The IPAQ was used to calculate a physical activity level (PAL) factor[26]. Individual energy requirements were then calculated by multiplying the participant's BMR and PAL. Thereafter, an individual 7-day meal plan was designed for each participant with all food prepared, weighed and packaged in-house in the department's research kitchen facility. Nutritional information for the two diets is provided in **Table 2**. Subjects consumed a diet containing 1.2 g of protein per kg of body weight per day (in order to reflect an average UK diet[27]), with 30% of their energy being provided by fat and the remainder from carbohydrates (~50–55%; variation due to different energy requirements and the clamping of protein intake). The meals were identical between the two groups, aside from meat or fish providing the primary protein source in lunches and dinners for the CON group (n=10) and this being replaced by Quorn Foods™ products (to provide the required amount of mycoprotein) in the MYC group (n=10). The CON group consumed meals based on chicken, ham, beef, tuna and salmon. In the MYC group, this was substituted for Quorn chicken pieces, Quorn mince, Quorn fillets and Quorn roast chicken slices. An additional line of interest was the impact of the mycoprotein diet on plasma short chain fatty acid concentrations. Acetate, for example, can be produced from gut microbial fermentation of dietary fibre (with the mycoprotein diet being high in dietary fibre) but also from hepatic metabolism of alcohol[28]. To isolate the impact of the diet, we therefore chose not to provide any alcohol during the intervention, and required participants to abstain from alcohol for 24 h prior the start of the intervention. All participants reported adhering to these guidelines. A document and diary detailing the plan were provided to the subjects in order to track compliance to the dietary intervention, log meal times and provide recipe information/instructions. While no formal data concerning tolerability and dietary preferences/liking were collected during the intervention, subjects informally reported no particular disliking of any foods, nor any adverse events (e.g. GI, nausea etc.), and compliance and feedback were similar across groups.

Participants were required to visit the laboratory at ~08.00 in the fasted state on days 2, 4 and 6 where body mass was measured wearing light clothing (seca 703 column scale, seca, Germany) and the next two days of food were provided. In these interim visits, the researchers discussed with the participants any questions or issues that may have arisen, and in the event of any substantial weight change (>0.5 kg, with the same upward or downward trend on two consecutive visits) the energy content of the next two days was adjusted. The GENEActiv accelerometer was worn for the duration of the one-week intervention and on day 2 a glucose sensor was placed and the CGMS connected to collect continuous glucose data for the last 5 days of the intervention. Following the one-week intervention (i.e. day 8), participants were required to repeat the experimental test day where a further OGTT was performed as described above.

Plasma and serum collection and analyses

One mL of each blood sample was collected into FX blood collection tubes (Becton Dickinson, Franklin Lakes, New Jersey, USA) containing powdered sodium fluoride and potassium oxalate, and glucose was immediately analysed using the YSI 2300 STAT PLUS Biochemistry Analyser (YSI, Yellow Springs, Ohio, USA). Four mL of blood were collected into LH (lithium heparin) plasma tubes (Becton Dickinson) and immediately centrifuged. The remaining 4 mL of each blood sample were collected into SST tubes (containing spray-coated silica and a polymer gel for serum separation; Becton Dickinson) and left at room temperature for at least 30 minutes. All tubes were centrifuged at 4º C and 4000 RPM, and aliquoted (one aliquot designated for each of the below analyses) plasma and serum were stored at -80º C.

One aliquot of each postabsorptive serum sample was transported to the Clinical Chemistry department of the Royal Devon & Exeter NHS Foundation Trust and analysed for uric acid concentrations using the Roche Cobas 702 module of the Cobas 8000 analyser (Roche, Basel, Switzerland) and Roche Uric Acid Kits (Cobas; UA2). Insulin concentrations were analysed in serum samples using DRG ELISA kits (DRG International, Springfield, New Jersey, USA). IL-6 concentrations were measured in plasma samples using Quantikine ELISA kits (R&D Systems, Minneapolis, Minnesota, USA).

Plasma samples were also sent to the MRC Integrative Epidemiology Unit at the University of Bristol for metabolomics analysis by nuclear magnetic resonance (NMR) spectroscopy. NMR spectroscopy and mass spectrometry (MS) are the key technologies in the metabolomics field, however, MS cannot analyse lipoproteins, making NMR currently the only high-throughput methodology capable of quantifying these metabolites in a cost-effective manner[29].

Biomarker concentrations quantified by this NMR approach have been shown to be highly consistent with concentrations obtained from standardised clinical chemistry analyses[30]. For a detailed description of the experimental protocol, including sample preparation and NMR spectroscopy please see references[29; 30; 31]. The data were then processed using the Nightingale Health's NMR-based blood biomarker analysis platform, which provides 224 quantified metabolomic measures per sample (142 primary concentrations plus 82 selected ratios and molecule diameters), including the lipid concentrations and composition of 14 lipoprotein subclasses, fatty acids, amino acids, glycolysis-related measures and ketone bodies. This approach has previously been used to establish large scale and cross-sectional plasma lipid metabolic profiles of more metabolically compromised populations compared with healthy controls[32; 33] but its use in human nutrition trials is a novel application as, to date, NMR spectroscopy has rarely been applied to investigate changes in response to nutritional interventions[34].

Insulin sensitivity

Five different insulin sensitivity indices[35; 36; 37; 38; 39], all validated against the hyperinsulinemic euglycemic clamp technique, were calculated pre and post intervention using the blood glucose and serum insulin concentrations measured in the fasting state and during the OGTTs. The homeostatic model assessment (HOMA-IR) is calculated from solely fasting concentrations of glucose and insulin and has been shown to provide a reasonable estimate of hepatic insulin sensitivity[35]. The Matsuda index uses OGTT glucose and insulin concentrations, as well as their corresponding fasting values, and represents a combined estimate of both hepatic and peripheral tissue sensitivity[37]. The Cederholm, OGIS and GUTT indices focus mainly on peripheral insulin sensitivity and muscular glucose uptake by measuring OGTT glucose clearance[36; 38; 39].

Continuous glucose monitoring system (CGMS)

The Dexcom G4 Platinum CGMS sensor was placed in the participants' abdominal subcutaneous fat, using a dedicated applicator. A transmitter was then attached to the sensor and glucose data, collected every 5 minutes, was automatically sent to a receiver. The participants were instructed to carry the receiver at all times and to calibrate the monitor 4 times a day at regular intervals by pricking their fingers with disposable lancets and using Contour Next blood glucose meters (Bayer, Leverkusen, Germany). Data from the days when the sensor was inserted and removed were excluded (i.e. days 2 and 8). Days with data for fewer than

70% of the total timepoints were also excluded. The remaining data were analysed for glycaemic control (24 h average glucose, glucose area under the curve (AUC) and two-hour postprandial glucose) and for glycaemic variability (SD, CONGA1 and CONGA2). To calculate the CONGA1 and CONGA2 indices, the SD of the differences between each glucose concentration reading and the reading obtained 1 (CONGA1) or 2 (CONGA2) hours prior was determined[40].

Statistical analyses

A power analysis based on the assumption of a 12% increase in the Matsuda Index with mycoprotein consumption (calculated based on previous research[16]) was performed and determined that 8 participants were needed in each group to provide a power of 80% and a 95% CI. Ten participants per group were recruited to account for a potential 20% dropout rate. Recruitment and testing was ended once the trial was fully recruited according to the a priori power calculation.

Participant baseline characteristics, dietary intake, and physical activity data were analysed using multiple unpaired t-tests. The two groups were compared, for most parameters, using a two-way ANOVA with repeated measures [RM] (with condition and time [RM] as factors). Bonferroni post hoc tests were performed in the event of significant main effects to detect individual differences. Blood glucose and serum insulin concentrations during the pre- and post- intervention OGTTs were analysed with three-way ANOVAs (condition, time and test day as factors). Additionally, for the aforementioned parameters, incremental Area Under the Curves (iAUC) were calculated and a one-way ANOVA was performed to detect any significant effect of treatment. Carbohydrate and fat oxidation data were averaged as fasting and fed responses and analysed with three-way ANOVAs (condition, fasted or fed state, and test day as factors). For the NMR metabolomics measures, a % change (Δ) from pre- to post-intervention was calculated for each of the 224 metabolites for each participant. The measures were divided into three groups (concentrations, ratios and dimensions) and analysed using multiple t-tests for the dimension measures (n = 3) and using Significant Analysis of Microarrays (SAM) for the concentration and ratio measures (n = 142 and n = 79, respectively). A heat-map was designed for the significant metabolites and these were organised into clusters. As an internal validation, a Bland-Altman plot and a Pearson correlation were used to analyse the agreement between the YSI and metabolomics fasting glucose data. Missing data were handled using imputation in a linear interpolation manner. Statistical significance was set at P<0.05. For the SAM analysis, the delta (tuning parameter which determined the False

Discovery Rate (FDR) threshold) was set at 1 for the analysis of metabolomics ratios, resulting in a FDR of 0.131 and at 0.8 for metabolite concentrations, resulting in a FDR of 0.095. A FDR of 0.1 was set for metabolite dimensions analysis. NMR metabolomics calculations were carried out in MetaboAnalyst 4.0 (Wishart Research Group, University of Alberta, Edmonton, Alberta, Canada). All other calculations were performed using GraphPad Prism version 7.0 (GraphPad Software, San Diego, California, USA).

Results

Nutritional intervention

Body mass was not different between habitual testing and at the outset of the intervention in either group (from 69±4 to 70±4 in CON and 69±6 to 70±6 kg in MYC; $P>0.05$), nor did body mass change during the intervention in either group (70±4 and 70±6 kg post intervention in CON and MYC, respectively; $P>0.05$) indicating participants remained in energy balance throughout the entirety of the study period in both groups.

The nutritional content of the prescribed diets, the actual food consumed during the intervention according to food logs, and participants' habitual diets are summarised in Table 2. Prescribed diets and actual food consumed did not differ in any parameter, and so all other comparisons were made using the habitual and actual intervention diets only. There were no significant differences in the energy and fat intakes between the groups' habitual diets (both $P>0.05$) nor did these parameters change between habitual intake and during the intervention in either group (all $P>0.05$). Additionally there were no significant differences in the carbohydrate and protein intakes between the groups' habitual diets nor between the groups' intervention diets (all $P>0.05$), but there was a reduction in protein intake and an increase in carbohydrate intake from their habitual diets to the intervention in both groups (time effect $P<0.05$). Although fibre intake was not different between groups (group effect; $P>0.05$), significant time and interaction effects were detected ($P<0.05$), such that fibre intake increased by 31±2 % in the MYC group only ($P<0.05$). The MYC group consumed 215±16 g of Quorn products daily, corresponding to 181±13 g wet weight (45±3 g dry weight) of mycoprotein per day. In the CON group, 38±1 and 6±1 % of the total protein consumed was provided by meat and fish, respectively, and in the MYC group, 38±2 % was provided by Quorn products. Dairy provided 13±1 % of protein in the CON group and 15±2 % in the MYC group ($P>0.05$), and 32±1 % and 36±2 % of protein in the CON and MYC groups, respectively, came from non-animal sources (not including mycoprotein; $P>0.05$). The remaining portion of dietary protein was provided by mixed (plant and animal) sources (e.g. chocolate bars, porridge oat pots, cakes, etc.).

Physical activity

Physical activity data are shown in **Table 3**. Habitual physical activity was not different between CON and MYC groups when expressed as average daily total activity time, light activity, moderate activity, vigorous activity, or sedentary time (all $P>0.05$). None of the

physical activity parameters changed during the intervention when compared with habitual levels in either group (all $P>0.05$).

Insulin sensitivity

Fasting blood glucose and serum insulin concentrations did not differ between groups at baseline (both $P>0.05$) and fasting serum insulin concentrations did not change throughout the intervention in either group (from 14.8±1.1 to 14.2±1.7 and from 12.3±2.4 to 12.7±1.7 $mU \cdot L^{-1}$ in CON and MYC, respectively; $P>0.05$). Pre- and post- intervention fasting blood glucose concentrations displayed a strong trend for an interaction effect (from 4.41±0.08 to 4.58±0.06 $mmol \cdot L^{-1}$, and from 4.55±0.11 to 4.47±0.07 $mmol \cdot L^{-1}$ in CON and MYC, respectively; $P=0.05$). Despite this, baseline insulin sensitivity reflected by the HOMA-IR was not different between groups (2.9±0.2 and 2.7±0.5 in CON and MYC, respectively; $P>0.05$) and did not change during the intervention in either group ($P>0.05$). Blood glucose and serum insulin concentrations during the two OGTTs performed pre- and post- intervention in the CON and MYC groups are shown in **Figure 2**. Both parameters increased with CHO ingestion ($P<0.0001$) and peaked between 30 and 45 minutes of the OGTT, at around 8 $mmol \cdot L^{-1}$ and 100 $mU \cdot L^{-1}$ for blood glucose and serum insulin concentrations, respectively, with no differences detected over time or between groups ($P>0.05$ for interaction and group effects). Blood glucose iAUC and serum insulin iAUC during the OGTT (displayed in Figure 2) also did not differ between groups or over time (both $P>0.05$). Consequently, there were also no differences between groups at baseline or over the intervention for any of the OGTT derived calculations of insulin sensitivity ($P>0.05$ for Cederholm, Matsuda, GUTT and OGIS). **Figure 3** displays these four indices and HOMA-IR for the two time points in the two groups.

Continuous glucose monitoring system (CGMS)

Average daily glucose values were aggregated for the habitual data (5.5±0.1 $mmol \cdot L^{-1}$ in CON and 5.4±0.1 $mmol \cdot L^{-1}$ in MYC) and for each of the intervention days, in the two groups (5.5±0.1, 5.5±0.2, 5.3±0.2, 5.4±0.1 and 5.4±0.1 $mmol \cdot L^{-1}$ in CON and 5.7±0.2, 5.5±0.1, 5.4±0.2, 5.3±0.2 and 5.6±0.1 $mmol \cdot L^{-1}$ in MYC, for days 3 to 7 of the intervention, respectively). Habitual data demonstrated no differences between groups ($P>0.05$) and this did not change throughout the intervention ($P>0.05$, for time and interaction effects). No differences were found between groups during the intervention in the average glucose concentrations in the two-hour postprandial period after the participants' evening meal (6.3±0.2, 6.1±0.4, 5.5±0.2, 5.3±0.2 and 5.5±0.2 $mmol \cdot L^{-1}$ in CON, and 6.0±0.3, 5.9±0.2,

342 5.6±0.2, 5.9±0.2 and 6.1±0.2 mmol·L^{-1} in MYC, for days 3 to 7 of the intervention, respectively;
343 P>0.05 for time and for interaction effects). There were also no differences in glycaemic
344 variability between groups, expressed as standard deviation (SD), CONGA1, or CONGA2 (all
345 P>0.05).
346
347 *Indirect calorimetry*
348 There were no differences in RMR between groups before the intervention (1539±114 kcal in
349 CON and 1692±119 kcal in MYC; P>0.05), and there were no main effects of time, condition
350 or an interaction effect (all P>0.05). An effect of CHO ingestion was detected for both
351 carbohydrate (increasing) and fat (decreasing) oxidation rates (P<0.0001). No interaction or
352 condition effects were found (all P>0.05). The relative contribution of fat and carbohydrate
353 oxidation to total energy expenditure in both the fasted and fed state are displayed in **Figure 4**.
354
355 *Plasma IL-6 and serum uric acid concentrations*
356 Fasting plasma IL-6 concentrations did not differ between groups at baseline (P>0.05) and did
357 not change throughout the intervention in either group (from 1.7±0.6 to 1.4±0.6 pg·mL^{-1}, and
358 from 2.1±0.6 to 1.3±0.4 pg·mL^{-1} in CON and MYC; P>0.05 for time and interaction effects).
359 Fasting serum uric acid concentrations were 297±20 µmol·L^{-1} in the CON group and 260±13
360 µmol·L^{-1} in the MYC group at baseline (P>0.05), and remained constant in both groups
361 throughout the study (main effects of time, condition and interaction; all P>0.05).
362
363 *Nuclear magnetic resonance (NMR) based metabolomics*
364 **The** 224 metabolites measured by NMR metabolomics are listed in Supplementary Table 1.
365 No differences between groups were found for 171 (93 concentrations, 76 ratios and 2
366 dimensions) of the quantified targets. **Figure 5** and **Table 4** summarise the significant changes
367 found in 53 of the targets (49 concentrations, 3 ratios and 1 dimension). Forty five lipid
368 concentrations of different lipoprotein fractions (including VLDL, LDL, IDL and HDL)
369 remained largely unchanged in the CON group (0 – 11 % change) but decreased significantly
370 in the MYC group (7-27 % decreases; all P<0.05). Plasma free cholesterol concentrations
371 decreased by 4.00±0.03 % in the CON group (from 0.89±0.06 to 0.86±0.07 mmol·L^{-1}) but by
372 significantly more (13.99±0.03 %) in the MYC group (from 0.75±0.07 to 0.64±0.06 mmol·L^{-1};
373 P<0.05) and, similarly, total (including VLDL, LDL, IDL and HDL) plasma cholesterol
374 concentrations decreased by 5.23±0.03 % in the CON group (from 3.00±0.19 to 2.86±0.25
375 mmol·L^{-1}) but to a significantly greater degree (by 14.28±0.03 %) in the MYC group (from

2.50±0.26 to 2.12±0.22 mmol·L^{-1}; P<0.05). Plasma LDL cholesterol concentrations decreased by 2.55±0.07 % in the CON group (from 0.88±0.09 to 0.85±0.11 mmol·L^{-1}) but to a greater degree, 19.33±0.07 %, in the MYC group (from 0.71±0.13 to 0.56±0.11 mmol.L-1; P<0.05) and plasma HDL2 decreased by 11.03±0.02 % in the CON group (from 0.91±0.08 to 0.82±0.08 mmol·L^{-1}) but by 18.58±0.03 % in the MYC group (from 0.72±0.07 to 0.58±0.05 mmol·L^{-1}; P<0.05). DHA and omega 3 fatty acids concentrations decreased by 3.04±0.05 % (from 0.110±0.014 to 0.107±0.015 mmol·L^{-1}) and 2.78±0.05 % (from 0.30±0.02 to 0.29±0.03 mmol·L^{-1}) in the CON group and by 17.26±0.03 % (from 0.085±0.009 to 0.070±0.008 mmol·L^{-1}) and 17.53±0.05 % in the MYC group (from 0.24±0.03 to 0.20±0.02 mmol·L^{-1}), respectively (both P<0.05). HDL dimensions decreased by 1.26±0.00 % in MYC but only by 0.17±0.00 % in CON (P<0.05). Interestingly, plasma glucose remained unchanged in the CON group (from 3.8±0.1 to 3.8±0.0 mmol·L^{-1}) but was reduced by 4.49±0.00 % (from 3.8±0.1 to 3.6±0.1 mmol·L^{-1}) in MYC, and plasma acetate concentrations increased by 8.5±0.1 % (from 0.055±0.005 to 0.059±0.006 mmol·L^{-1}) and 43.6±0.1 % (from 0.059±0.005 to 0.083±0.008 mmol·L^{-1}) in CON and MYC, respectively. These changes were not significant using the SAM multivariate analyses, but were significant when individually analysed (t-tests, P<0.05) which we deemed appropriate given their lack of involvement in the recognised pathways that the remainder of the metabolomics SAM analyses took into account. Changes in plasma total cholesterol, free cholesterol, LDL cholesterol, HDL2 cholesterol, DHA, omega-3 fatty acids, acetate and glucose concentrations are represented in **Figure 6**. A Bland-Altman analysis was performed in order to verify the trend for a decrease in blood glucose concentrations determined by YSI against the significant change in NMR derived analyses of plasma glucose in the MYC group. This also served as a verification of the robustness of the NMR based metabolomics approach. The Bland-Altman plot to analyse the levels of agreement between the YSI and metabolomics glucose data is represented in **Figure 7**. There was a strong positive correlation between the two measurements (r = 0.60; P<0.001; 95% limits of agreement: from 0.287 to 1.216).

Discussion

We investigated the impact of substituting meat and fish for mycoprotein as the major source of dietary protein at lunch and dinner during a fully controlled, energy and macronutrient balanced one week dietary intervention period on insulin sensitivity, glycaemic control and plasma lipid composition. We report that the mycoprotein intervention did not change indices of whole-body insulin sensitivity or 24 h free living glycaemic control. However, the mycoprotein intervention had a profound impact on the plasma lipidome, inducing changes generally assumed to be indicative of improvements in long-term cardio-metabolic health.

Earlier studies[16; 17] reported that bolus mycoprotein ingestion improved acute postprandial glucose handling, but no work had investigated whether this translated to longer term measures of metabolic health. We sought to test the hypothesis that chronic (one week), habitual (twice daily) mycoprotein consumption would improve whole body insulin sensitivity and/or daily habitual glycaemic control under carefully controlled conditions. We applied a nutritional intervention with no differences in energy or macronutrient consumption between groups (except for fibre; see Table 2) to young adults (who were well matched across groups; see Table 1). As a result, in our control group, despite a shift from habitual to controlled dietary conditions (which can often induce metabolic changes *per se*[19]), we observed no changes in any index of insulin sensitivity or glycaemic control. When substituting meat and fish for mycoprotein as the primary source of dietary protein in lunch and dinner, we also observed no changes in indices of liver or peripheral insulin sensitivity determined during an oral glucose tolerance test (Figures 2 and 3). Given the per meal mycoprotein consumption (~90 g wet weight) was equivalent/in excess of previous work demonstrating bolus mycoprotein consumption could improve acute glycaemic control[16; 17], this lack of support for our hypothesis was perhaps surprising. However, those previous studies also indicated the effect was likely mediated by acute postprandial interactions of mycoprotein with dietary carbohydrate, rather than an effect on insulin sensitivity *per se*. Of interest, epidemiological studies have shown total (postprandial) hyperglycaemia and/or the prevalence of (postprandial) hyperglycaemic excursions over the day to be better predictors of longer term cardio-metabolic health[41]. As such, to capture any effects of repeated mycoprotein ingestion on cumulative free-living postprandial glycaemic control (which could feasibly be independent of changes in insulin sensitivity), we applied continuous glucose monitoring throughout the study. However, whether we looked across the entire day or focussed on postprandial periods only, we did not observe any impact of the mycoprotein intervention (compared with either habitual conditions

436 or the control group). We therefore demonstrate that short-term mycoprotein consumption does
437 not impact insulin sensitivity or daily blood glucose control, at least in healthy young adults.
438 It is worth noting that our participants habitually consumed relatively high protein intakes (i.e.
439 ~1.5 g/kg/day) and the intervention therefore represented a ~20% decrease in habitual protein
440 intake. Since high protein diets have been shown to improve glycaemic control[42] we cannot
441 discount the possibility that the drop in protein intake obscured any potential changes in insulin
442 sensitivity or glycaemic control; though, if so, we would expect these effects across both groups
443 equivalently and our control group also remained unchanged. From a translational perspective,
444 the protein content of the diet plays a large role in determining free-living energy balance, both
445 directly via inducing dietary thermogenesis and indirectly based on the leverage of appetite[9].
446 Given mycoprotein is also a particularly satiating dietary protein source[43; 44; 45], attention
447 should also be paid when considering mycoprotein (and how much) as a dietary intervention
448 as to whether over- or under- eating is of primary concern for a particular population. It is
449 important that future work extends these findings to more (metabolically) compromised
450 individuals, where such dietary interventions are more likely to induce subtle, but clinically
451 relevant, alterations in indices of metabolic health.
452 Our findings are in line with previous work that has reported that nutritionally induced acute
453 beneficial effects on postprandial glucose handling do not necessarily translate to longer term
454 benefits on insulin sensitivity[46]. Noteworthy, however, is the lowering effect of the
455 mycoprotein diet on fasting blood glucose concentrations. As a recognised clinical marker of
456 insulin sensitivity, this suggests mycoprotein consumption may support metabolic health,
457 although it is difficult to explain why this was observed in the absence of effects on calculated
458 insulin sensitivity and/or 24 h glucose control. It is possible that mycoprotein consumption
459 specifically altered glucagon sensitivity (potentially due to mycoprotein's high fibre
460 content)[47], or induced early improvements in β-cell function[48], but clearly this warrants
461 further research. It is crucial such research examines the effects of mycoprotein consumption
462 during a longer time period and in various (more metabolically compromised) populations, as
463 these changes may simply have been too subtle to detect in healthy individuals during a
464 relatively short-term intervention. While our data did not largely support our hypothesis,
465 incorporating mycoprotein as a sustainably produced alternative to meat clearly does not
466 *negatively* impact on metabolic health over a one week period, an important perspective given
467 the impetus in various populations to reduce animal-derived protein consumption. Indeed no
468 gastrointestinal or other adverse effects were reported during the mycoprotein intervention, and
469 the food substitutes were generally well tolerated/liked. While data concerning food

preferences driving eating behaviour are necessary to evaluate the wider potential/application of such dietary interventions under free-living conditions, our data indicate mycoprotein containing products are a practical and feasible simple alternative to animal protein sources.

An existing body of work has demonstrated that 3-8 weeks of mycoprotein consumption reduces total plasma cholesterol and plasma LDL concentrations thereby resulting in improved HDL/LDL ratios (a robust marker of beneficial metabolic health outcomes[18; 19; 49]). To shed further light on this area we applied a novel NMR-based quantitative and targeted metabonomics approach[50]. Strikingly, we found that merely one week of mycoprotein consumption led to coordinated changes in 53 of our 224 targets (see Table 4 and Figure 6). Specifically, we report decreases in plasma lipoprotein lipid content and, importantly, in plasma total, free, LDL and HDL2 cholesterol. However, unlike in previous studies [18; 19], this reduction in cholesterol was ubiquitous across lipoprotein species, and therefore did not impact lipoprotein ratios (e.g. LDL/HDL)[51].

We[14] and others[18; 19] have previously argued that the cholesterol lowering effect of mycoprotein consumption is likely related to the fibre content (or type) it contains (the most obvious nutritional difference across the diets; 26 vs 34 g daily in CON and MYC, respectively). While our design (not fibre matched across groups) does not allow us to disentangle the interesting potential effects of fibre *quantity* vs *type*[14], the role of higher fibre intake in reducing circulating cholesterol concentrations is also in line with epidemiological[52; 53] and intervention[54] studies (2 to 10 g fibre supplementation per day), with these effects also translating to a reduced risk of coronary heart disease. We therefore provide evidence that an innocuous and feasible dietary intervention can provide a sufficient increase to dietary fibre intake to place individuals at the top end of this dose-response effect. Furthermore, we extend on previous observations[18; 19; 49] by demonstrating how rapidly this effect ensues consequent to mycoprotein consumption.

The mechanism(s) by which increased fibre intake lowers circulating plasma cholesterol may be related to large intestinal fermentation of insoluble fibre fractions via the gut microbiota[14; 55]. Short-chain fatty acids (SCFAs), such as acetate, propionate and butyrate, are primary products of fibre fermentation[56]. Though SCFAs have been shown to have a range of metabolic effects[57; 58; 59; 60; 61], of note is the reported effect of (propionate in particular) reducing hepatic cholesterol synthesis[62]. *In vitro* colonic models have shown mycoprotein's fibre (which is composed of approximately two thirds β-glucan and one-third chitin[14]) to be fermentable to propionate and butyrate, but at the expense of acetate[63], suggesting such end products could underpin the cholesterol lowering effect. It is, therefore, somewhat surprising

504 that we observed a considerable (40%) increase in plasma acetate (Figure 6) following one
505 week of mycoprotein consumption. However, this is in line with previous work showing the
506 acute consumption of other dietary fibres leading to increased postprandial serum acetate
507 concentrations[64], and that fibre (e.g. oat or bran rich diets) induced reductions in circulating
508 cholesterol are also associated with increases in blood acetate[65]. Whether acetate as an *in vivo*
509 end product of mycoprotein bacterial fermentation mediated the plasma cholesterol lowering
510 effect, either by inhibiting cholesterol synthesis, or by other unknown mechanisms (such as
511 reduced cholesterol absorption or increased peripheral clearance) is not clear, and warrants
512 future (human) research.
513 The metabolomics approach also revealed non-cholesterol related changes in the plasma
514 lipidome. Of note, mycoprotein consumption decreased plasma DHA and omega-3 fatty acid
515 concentrations (Figure 6), presumably due to a lower dietary load. However, given the reported
516 variance in plasma levels of these lipids tends to be related to an individual's last meal, this
517 may be an acute effect rather than reflective of a 'deficiency', particularly over only a one week
518 period[66]. Nevertheless, since omega-3 fatty acids in particular have been linked to various
519 desirable health outcomes[67] it would be prudent for future dietary interventions that involve
520 reducing dietary intake of omega-3 (and DHA) lipids to monitor such effects.
521 In conclusion, the present data show that substituting meat/fish for mycoprotein at lunch and
522 dinner for one week does not modulate whole-body insulin sensitivity or 24 h free living
523 (postprandial) glycaemic control, but considerably impacts upon the plasma lipidome.
524 Mycoprotein represents a sustainable dietary protein source that can be incorporated into the
525 daily diet without compromising short-term metabolic health and facilitating rapid and possibly
526 beneficial changes to the plasma lipidome.

Acknowledgements

None

Financial Support

The project was sponsored by Marlow Foods Ltd (BTW as grant holder). The University of Exeter were responsible for the study design, data collection and analysis, decision to publish and preparation of the manuscript. The private partners have contributed to the project through regular discussion.

Conflict of Interest

BTW has received research grants from Marlow Foods. MOCC and AJM receive PhD studentship funding from Marlow Foods and the College of Life and Environmental Sciences, University of Exeter. TJAF is an employee of Marlow Foods. Remaining authors declare no conflicts of interest.

Authors' contributions

MOCC and BTW designed research. MOCC recruited, randomised and assigned participants to interventions. MOCC, AJM and MLD conducted research. TJAF provided essential materials. MOCC analysed data. MOCC, FBS and BTW wrote the paper. BTW had primary responsibility for final content. All authors read and approved the final manuscript.

References

1. Committee on Medical Aspects of Food Policy (1991) *Dietary reference values for food energy and nutrients for the United Kingdom*: HM Stationery Office.
2. Trumbo P, Schlicker S, Yates AA *et al.* (2002) Dietary reference intakes for energy, carbohdrate, fiber, fat, fatty acids, cholesterol, protein and amino acids. *Journal of the Academy of Nutrition and Dietetics* **102**, 1621.
3. Phillips SM (2017) Current Concepts and Unresolved Questions in Dietary Protein Requirements and Supplements in Adults. *Frontiers in nutrition* **4**, 13.
4. Phillips SM, Chevalier S, Leidy HJ (2016) Protein "requirements" beyond the RDA: implications for optimizing health. *Applied Physiology, Nutrition, and Metabolism* **41**, 565-572.
5. Paddon-Jones D, Campbell WW, Jacques PF *et al.* (2015) Protein and healthy aging. *The American journal of clinical nutrition* **101**, 1339S-1345S.
6. Wall BT, Cermak NM, van Loon LJ (2014) Dietary protein considerations to support active aging. *Sports Medicine* **44**, 185-194.
7. Phillips SM (2017) Nutrition in the elderly: a recommendation for more (evenly distributed) protein?, pp. 12-13: Oxford University Press.
8. Wall BT, Morton JP, van Loon LJ (2014) Strategies to maintain skeletal muscle mass in the injured athlete: nutritional considerations and exercise mimetics. *European journal of sport science* **15**, 53-62.
9. Westerterp-Plantenga MS, Lemmens SG, Westerterp KR (2012) Dietary protein–its role in satiety, energetics, weight loss and health. *British journal of nutrition* **108**, S105-S112.
10. Ranganathan J, Vennard D, Waite R *et al.* (2016) Shifting diets for a sustainable food future. *World Resources Institute: Washington, DC, USA*.
11. Finnigan T (2011) Mycoprotein: origins, production and properties. In *Handbook of Food Proteins*, pp. 335-352: Elsevier.
12. Hoekstra AY (2013) *The water footprint of modern consumer society*: Routledge.
13. Carbon Trust (2014) *Quorn, beef and chicken footprints*. *Internal report*.
14. Coelho MOC, Monteyne AJ, Dunlop MV *et al.* (2019) Mycoprotein as a possible alternative source of dietary protein to support muscle and metabolic health. *Nutrition Reviews*.
15. Dunlop MV, Kilroe SP, Bowtell JL *et al.* (2017) Mycoprotein represents a bioavailable and insulinotropic non-animal-derived dietary protein source: a dose–response study. *British Journal of Nutrition* **118**, 673-685.

16. Turnbull WH, Ward T (1995) Mycoprotein reduces glycemia and insulinemia when taken with an oral-glucose-tolerance test. *The American journal of clinical nutrition* **61**, 135-140.
17. Bottin JH, Swann JR, Cropp E *et al.* (2016) Mycoprotein reduces energy intake and postprandial insulin release without altering glucagon-like peptide-1 and peptide tyrosine-tyrosine concentrations in healthy overweight and obese adults: a randomised-controlled trial. *British Journal of Nutrition* **116**, 360-374.
18. Turnbull WH, Leeds AR, Edwards DG (1992) Mycoprotein reduces blood lipids in free-living subjects. *The American journal of clinical nutrition* **55**, 415-419.
19. Turnbull WH, Leeds AR, Edwards GD (1990) Effect of mycoprotein on blood lipids. *The American journal of clinical nutrition* **52**, 646-650.
20. Del Coco L, Vergara D, De Matteis S *et al.* (2019) NMR-Based Metabolomic Approach Tracks Potential Serum Biomarkers of Disease Progression in Patients with Type 2 Diabetes Mellitus. *Journal of clinical medicine* **8**, 720.
21. Craig CL, Marshall AL, Sjorstrom M *et al.* (2003) International physical activity questionnaire: 12-country reliability and validity. *Medicine and science in sports and exercise* **35**, 1381-1395.
22. Brennan IM, Feltrin KL, Nair NS *et al.* (2009) Effects of the phases of the menstrual cycle on gastric emptying, glycemia, plasma GLP-1 and insulin, and energy intake in healthy lean women. *American Journal of Physiology-Gastrointestinal and Liver Physiology* **297**, G602-G610.
23. McGuire E, Helderman J, Tobin J *et al.* (1976) Effects of arterial versus venous sampling on analysis of glucose kinetics in man. *Journal of Applied Physiology* **41**, 565-573.
24. Frayn K (1983) Calculation of substrate oxidation rates in vivo from gaseous exchange. *Journal of applied physiology* **55**, 628-634.
25. Henry C (2005) Basal metabolic rate studies in humans: measurement and development of new equations. *Public health nutrition* **8**, 1133-1152.
26. Westerterp K (1999) Obesity and physical activity. *International Journal of Obesity* **23**, S59.
27. Bates B, Lennox A, Prentice A *et al.* (2014) *National diet and nutrition survey: Results from years 1, 2, 3 and 4 (combined) of the rolling programme (2008/2009-2011/2012): A survey carried out on behalf of public health England and the food standards agency*: Public Health England.

28. Korri UM, Nuutinen H, Salaspuro M (1985) Increased blood acetate: a new laboratory marker of alcoholism and heavy drinking. *Alcoholism: Clinical and Experimental Research* **9**, 468-471.

29. Soininen P, Kangas AJ, Würtz P *et al.* (2015) Quantitative serum nuclear magnetic resonance metabolomics in cardiovascular epidemiology and genetics. *Circulation: Cardiovascular Genetics* **8**, 192-206.

30. Würtz P, Kangas AJ, Soininen P *et al.* (2017) Quantitative serum nuclear magnetic resonance metabolomics in large-scale epidemiology: a primer on-omic technologies. *American journal of epidemiology* **186**, 1084-1096.

31. Soininen P, Kangas AJ, Würtz P *et al.* (2009) High-throughput serum NMR metabonomics for cost-effective holistic studies on systemic metabolism. *Analyst* **134**, 1781-1785.

32. Deelen J, Kettunen J, Fischer K *et al.* (2019) A metabolic profile of all-cause mortality risk identified in an observational study of 44,168 individuals. *Nature Communications* **10**, 1-8.

33. 't Hart LM, Vogelzangs N, Mook-Kanamori DO *et al.* (2018) Blood metabolomic measures associate with present and future glycemic control in type 2 diabetes. *The Journal of Clinical Endocrinology & Metabolism* **103**, 4569-4579.

34. Beynon RA, Richmond RC, Santos Ferreira DL *et al.* (2019) Investigating the effects of lycopene and green tea on the metabolome of men at risk of prostate cancer: The ProDiet randomised controlled trial. *International journal of cancer* **144**, 1918-1928.

35. Matthews D, Hosker J, Rudenski A *et al.* (1985) Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* **28**, 412-419.

36. Cederholm J, Wibell L (1990) Insulin release and peripheral sensitivity at the oral glucose tolerance test. *Diabetes research and clinical practice* **10**, 167-175.

37. Matsuda M, DeFronzo RA (1999) Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes care* **22**, 1462-1470.

38. Gutt M, Davis CL, Spitzer SB *et al.* (2000) Validation of the insulin sensitivity index (ISI0, 120): comparison with other measures. *Diabetes research and clinical practice* **47**, 177-184.

39. Mari A, Pacini G, Murphy E *et al.* (2001) A model-based method for assessing insulin sensitivity from the oral glucose tolerance test. *Diabetes care* **24**, 539-548.

40. McDonnell C, Donath S, Vidmar S *et al.* (2005) A novel approach to continuous glucose analysis utilizing glycemic variation. *Diabetes technology & therapeutics* **7**, 253-263.

41. Cavalot F, Petrelli A, Traversa M *et al.* (2006) Postprandial blood glucose is a stronger predictor of cardiovascular events than fasting blood glucose in type 2 diabetes mellitus,

particularly in women: lessons from the San Luigi Gonzaga Diabetes Study. *The Journal of Clinical Endocrinology & Metabolism* **91**, 813-819.

42. Farnsworth E, Luscombe ND, Noakes M *et al.* (2003) Effect of a high-protein, energy-restricted diet on body composition, glycemic control, and lipid concentrations in overweight and obese hyperinsulinemic men and women. *The American journal of clinical nutrition* **78**, 31-39.

43. Turnbull WH, Walton J, Leeds AR (1993) Acute effects of mycoprotein on subsequent energy intake and appetite variables. *The American journal of clinical nutrition* **58**, 507-512.

44. Burley V, Paul A, Blundell J (1993) Influence of a high-fibre food (myco-protein^*) on appetite: effects on satiation (within meals) and satiety (following meals). *European journal of clinical nutrition* **47**, 409-409.

45. Williamson D, Geiselman P, Lovejoy J *et al.* (2006) Effects of consuming mycoprotein, tofu or chicken upon subsequent eating behaviour, hunger and safety. *Appetite* **46**, 41-48.

46. Nestel P, Cehun M, Chronopoulos A (2004) Effects of long-term consumption and single meals of chickpeas on plasma glucose, insulin, and triacylglycerol concentrations. *The American journal of clinical nutrition* **79**, 390-395.

47. Bodnaruc AM, Prud'homme D, Blanchet R *et al.* (2016) Nutritional modulation of endogenous glucagon-like peptide-1 secretion: a review. *Nutrition & metabolism* **13**, 92.

48. Abdul-Ghani MA, DeFronzo RA (2009) Plasma glucose concentration and prediction of future risk of type 2 diabetes. *Diabetes Care* **32**, S194-S198.

49. Udall JN, Lo CW, Young VR *et al.* (1984) The tolerance and nutritional value of two microfungal foods in human subjects. *The American journal of clinical nutrition* **40**, 285-292.

50. German JB, Hammock BD, Watkins SM (2005) Metabolomics: building on a century of biochemistry to guide human health. *Metabolomics* **1**, 3-9.

51. Natarajan S, Glick H, Criqui M *et al.* (2003) Cholesterol measures to identify and treat individuals at risk for coronary heart disease. *American journal of preventive medicine* **25**, 50-57.

52. Rimm EB, Ascherio A, Giovannucci E *et al.* (1996) Vegetable, fruit, and cereal fiber intake and risk of coronary heart disease among men. *Jama* **275**, 447-451.

53. Wolk A, Manson JE, Stampfer MJ *et al.* (1999) Long-term intake of dietary fiber and decreased risk of coronary heart disease among women. *Jama* **281**, 1998-2004.

54. Brown L, Rosner B, Willett WW *et al.* (1999) Cholesterol-lowering effects of dietary fiber: a meta-analysis. *The American journal of clinical nutrition* **69**, 30-42.

55. Gunness P, Gidley MJ (2010) Mechanisms underlying the cholesterol-lowering properties of soluble dietary fibre polysaccharides. *Food & function* **1**, 149-155.
56. Cummings J, Pomare E, Branch W *et al.* (1987) Short chain fatty acids in human large intestine, portal, hepatic and venous blood. *Gut* **28**, 1221-1227.
57. den Besten G, van Eunen K, Groen AK *et al.* (2013) The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *Journal of lipid research* **54**, 2325-2340.
58. Zheng L, Kelly CJ, Battista KD *et al.* (2017) Microbial-derived butyrate promotes epithelial barrier function through IL-10 receptor–dependent repression of claudin-2. *The Journal of Immunology* **199**, 2976-2984.
59. Cheng D, Xu J-H, Li J-Y *et al.* (2018) Butyrate ameliorated-NLRC3 protects the intestinal barrier in a GPR43-dependent manner. *Experimental cell research* **368**, 101-110.
60. Kim Y, Keogh J, Clifton P (2018) Probiotics, prebiotics, synbiotics and insulin sensitivity. *Nutrition research reviews* **31**, 35-51.
61. Roshanravan N, Mahdavi R, Alizadeh E *et al.* (2017) Effect of butyrate and inulin supplementation on glycemic status, lipid profile and glucagon-like peptide 1 level in patients with type 2 diabetes: A randomized double-blind, placebo-controlled trial. *Hormone and Metabolic Research* **49**, 886-891.
62. Cheng H-H, Lai M-H (2000) Fermentation of resistant rice starch produces propionate reducing serum and hepatic cholesterol in rats. *The Journal of nutrition* **130**, 1991-1995.
63. Harris HC, Edwards CA, Morrison DJ (2019) Short Chain Fatty Acid Production from Mycoprotein and Mycoprotein Fibre in an In Vitro Fermentation Model. *Nutrients* **11**, 800.
64. Tarini J, Wolever TM (2010) The fermentable fibre inulin increases postprandial serum short-chain fatty acids and reduces free-fatty acids and ghrelin in healthy subjects. *Applied physiology, nutrition, and metabolism* **35**, 9-16.
65. Bridges SR, Anderson JW, Deakins DA *et al.* (1992) Oat bran increases serum acetate of hypercholesterolemic men. *The American journal of clinical nutrition* **56**, 455-459.
66. Sun Q, Ma J, Campos H *et al.* (2007) Comparison between plasma and erythrocyte fatty acid content as biomarkers of fatty acid intake in US women. *The American journal of clinical nutrition* **86**, 74-81.
67. Swanson D, Block R, Mousa SA (2012) Omega-3 fatty acids EPA and DHA: health benefits throughout life. *Advances in nutrition* **3**, 1-7.

Table 1 – Participants' characteristics

	CON	MYC	*P* value
Sex	6 F / 4 M	6 F / 4 M	-
Age (y)	24±1 [19 – 31]	24±1 [18 – 31]	0.63
Height (cm)	174±3 [162 – 188]	171±4 [152 – 189]	0.64
Body mass (kg)	69±4 [49 – 86]	69±6 [46 – 99]	0.93
BMI (kg/m^2)	23±1 [19 – 28]	23±1 [19 – 30]	0.70
Body fat (% of body mass)	21±4 [9 – 44]	21±3 [8 – 38]	0.95
Lean mass (kg)	53±4 [35 – 73]	55±5 [35 – 79]	0.82

Range of results for each measurement is displayed between [ ].
Multiple t-tests were used to compare each characteristic in CON and MYC.
Abbreviations: CON, control group; MYC, mycoprotein group.

Table 2 – Nutritional composition of participants' habitual diets, of the prescribed intervention diet and of their actual intake during the intervention according to the collected logs during the one week intervention

	Habitual dietary intake		Prescribed intervention diet		Actual intake during intervention	
	CON	**MYC**	**CON**	**MYC**	**CON**	**MYC**
Energy (MJ/d)	8.9±0.7	10.1±0.6	10.1±0.6	11.0±1.0	10.1±0.6	10.9±0.1
Energy (kcal/d)	2120±177	2414±150	2422±155	2624±237	2422±152	2598±247
Protein (g/d)	91±7	107±14	83±5*	84±7*	83±5*	82±7*
Protein (g/kg body weight)	1.4±0.1	1.6±0.2	1.2±0.0*	1.2±0.0*	1.2±0.0*	1.2±0.0*
Protein (% total energy)	18.8±1.5	17.6±1.7	13.7±0.4*	13.0±0.5*	13.7±0.4*	12.9±0.6*
Carbohydrate (g/d)	247±29	260±22	331±22*	355±35*	330±22*	350±37*
Carbohydrate (% total energy)	41.6±2.5	43.0±2.1	54.5±0.4*	53.9±0.7*	54.4±0.4*	53.4±1.0*
Fat (g/d)	94±8	99±7	82±5	87±8	82±5	87±8
Fat (% total energy)	36.8±2.0	37.0±1.7	30.3±0.2*	29.8±0.1*	30.5±0.2*	30.2±0.3*
Fibre (g/d)	23±2	26±2	26±2	34±2†	26±2	34±2†
Alcohol (g/d)	8.8±3.6	7.7±3.9	0±0	0±0	0±0	0±0

Separate two-way repeated measures ANOVAs were used to compare CON and MYC actual dietary intakes during the intervention with both the habitual diets and the prescribed intervention diets.
Abbreviations: CON, control group; MYC, mycoprotein group.
* Significantly different from habitual diet (time effect; $P<0.05$)

† Significantly different from habitual diet and from CON group (time and interaction effect; $P<0.05$)

Table 3 – Daily habitual physical activity and daily physical activity during the intervention

	Habitual		Intervention	
	CON	**MYC**	**CON**	**MYC**
Total activity (mins/day)	241 ± 19	251 ± 34	247 ± 45	295 ± 26
Light activity (mins/day)	83 ± 4	80 ± 7	85 ± 16	94 ± 9
Moderate activity (mins/day)	150 ± 15	158 ± 27	154 ± 27	186 ± 18
Vigorous activity (mins/day)	8 ± 3	12 ± 5	8 ± 3	15 ± 5
Sedentary (mins/day)	656 ± 24	661 ± 26	659 ± 42	654 ± 34

Multiple two-way ANOVAs were used to compare the different activity levels in CON and MYC habitually and during the intervention.
Abbreviations: CON, control group; MYC, mycoprotein group.

Table 4 – Significant NMR-based metabolomics features identified using either Significant Analysis of Microarrays (concentrations and ratios) or t-tests (dimensions)

Concentrations	% Δ change CON	SEM	% Δ change MYC	SEM	d.value	stdev	rawp	q.value
XL-HDL-FC	-0.48%	0.05	-25.85%	0.04	-2.4672	0.064471	0.00098592	0.050372
XL-HDL-C	-1.40%	0.04	-23.04%	0.03	-2.3885	0.052223	0.0011972	0.050372
XL-HDL-CE	-1.34%	0.04	-22.11%	0.03	-2.3173	0.051227	0.0016197	0.050372
XL-HDL-L	-1.91%	0.04	-22.18%	0.04	-2.1522	0.055798	0.0033099	0.070958
XL-HDL-P	-1.99%	0.04	-21.94%	0.04	-2.1173	0.055879	0.0038028	0.070958
XL-HDL-PL	-1.84%	0.05	-21.76%	0.04	-1.9285	0.064943	0.006831	0.091044
L-HDL-FC	-9.17%	0.03	-24.54%	0.04	-1.8228	0.045938	0.0088028	0.10266
L-HDL-C	-8.43%	0.02	-21.48%	0.04	-1.5961	0.043399	0.016901	0.12108
IDL-FC	-2.98%	0.04	-18.33%	0.04	-1.585	0.058474	0.017958	0.12108
L-HDL-L	-9.33%	0.02	-20.84%	0.03	-1.5517	0.035824	0.019648	0.12108
L-HDL-P	-9.33%	0.02	-20.62%	0.03	-1.5358	0.035138	0.02007	0.12108
L-HDL-CE	-8.21%	0.02	-20.67%	0.04	-1.5351	0.042784	0.02007	0.12108
DHA	-3.04%	0.05	-17.26%	0.03	-1.4892	0.057088	0.023239	0.12108
M-LDL-TG	-7.08%	0.03	-27.03%	0.09	-1.4817	0.096334	0.024014	0.12108
IDL-C	-2.64%	0.05	-17.21%	0.04	-1.4298	0.063572	0.028732	0.12108

M-LDL-P	-3.61%	0.06	-25.72%	0.10	-1.4276	0.11648	0.028803	0.12108
M-LDL-L	-3.29%	0.06	-25.32%	0.10	-1.4213	0.11663	0.029577	0.12108
XL-HDL-TG	0.33%	0.07	-18.45%	0.07	-1.4058	0.09517	0.031056	0.12108
M-LDL-C	-2.02%	0.08	-27.06%	0.12	-1.3926	0.14145	0.032676	0.12108
L-HDL-PL	-10.54%	0.02	-20.19%	0.03	-1.392	0.030936	0.032746	0.12108
L-LDL-FC	-2.12%	0.04	-14.96%	0.03	-1.3761	0.054964	0.034366	0.12108
L-HDL-TG	-3.43%	0.06	-19.38%	0.05	-1.3743	0.077665	0.034507	0.12108
IDL-CE	-2.46%	0.05	-16.74%	0.05	-1.3649	0.066219	0.035634	0.12108
L-LDL-C	-2.71%	0.06	-19.07%	0.06	-1.3617	0.081812	0.035915	0.12108
IDL-L	-2.71%	0.04	-15.08%	0.04	-1.347	0.053468	0.037535	0.12108
L-LDL-CE	-2.94%	0.07	-21.27%	0.07	-1.3285	0.099596	0.03993	0.12108
FAw3	-2.78%	0.05	-17.53%	0.05	-1.3162	0.073673	0.041338	0.12108
IDL-P	-2.70%	0.04	-14.47%	0.04	-1.309	0.051504	0.04162	0.12108
S-LDL-L	-4.29%	0.06	-24.90%	0.10	-1.3075	0.11925	0.04162	0.12108
IDL-PL	-2.38%	0.03	-13.66%	0.03	-1.306	0.047989	0.041761	0.12108
S-LDL-P	-4.59%	0.06	-25.05%	0.10	-1.3009	0.11887	0.042606	0.12108
L-LDL-L	-3.20%	0.05	-16.65%	0.05	-1.2903	0.065862	0.043592	0.12108
S-LDL-C	-2.61%	0.08	-26.81%	0.13	-1.2792	0.15082	0.045	0.12108

L-LDL-P	-3.44%	0.05	-16.59%	0.05	-1.2753	0.064748	0.045423	0.12108
LDL-C	-2.55%	0.07	-19.33%	0.07	-1.2438	0.096487	0.050775	0.12622
M-LDL-FC	-2.46%	0.04	-20.02%	0.09	-1.2437	0.10285	0.050775	0.12622
FreeC	-4.00%	0.03	-13.99%	0.03	-1.241	0.042177	0.051408	0.12622
S-LDL-FC	-3.64%	0.05	-20.72%	0.10	-1.1778	0.10661	0.060634	0.14192
M-LDL-PL	-3.03%	0.04	-19.60%	0.10	-1.1769	0.1024	0.060845	0.14192
XS-VLDL-CE	3.13%	0.04	-7.16%	0.03	-1.1534	0.050838	0.065352	0.14689
L-LDL-TG	-4.61%	0.03	-14.48%	0.03	-1.1497	0.047456	0.066127	0.14689
LDL-TG	-5.97%	0.03	-15.99%	0.04	-1.1321	0.050121	0.070986	0.1535
S-LDL-TG	-8.70%	0.03	-25.25%	0.10	-1.1253	0.10863	0.072394	0.1535
XS-VLDL-PL	-2.38%	0.03	-12.60%	0.04	-1.1087	0.053763	0.076197	0.15798
XS-VLDL-C	1.26%	0.04	-8.36%	0.03	-1.0857	0.050216	0.081761	0.16583
S-LDL-PL	-4.60%	0.04	-19.57%	0.10	-1.0608	0.10266	0.089507	0.17306
HDL2-C	-11.03%	0.02	-18.58%	0.03	-1.0577	0.03298	0.090563	0.17306
L-LDL-PL	-2.68%	0.04	-11.85%	0.03	-1.0553	0.048557	0.090915	0.17306
Serum-C	-5.23%	0.03	-14.28%	0.03	-1.0515	0.047617	0.092746	0.17306
Ratios	**% Δ change CON**	**SEM**	**% Δ change MYC**	**SEM**	**d.value**	**stdev**	**rawp**	**q.value**

XL-HDL-FC_%	1.11%	0.01	-4.74%	0.01	-3.1601	0.018531	0.0060759	0.18544
L-HDL-FC_%	0.08%	0.01	-5.07%	0.02	-2.9305	0.017575	0.008481	0.18544
IDL-FC_%	-0.38%	0.01	-3.93%	0.01	-2.7984	0.012681	0.010253	0.18544
Dimensions	**% Δ change CON**	**SEM**	**% Δ change MYC**	**SEM**	**t.stat**	**p.value**	**- log 10 (p)**	**FDR**
HDL_D	-0.17%	0.00	-1.26%	0.00	3.4955	0.0025822	2.588	0.0077467

Figure Legends

Figure 1 Overview of the experimental protocol.

Figure 2 Blood glucose (A, B and C) and serum insulin (D, E and F) concentrations during oral glucose tolerance tests (OGTT) on days 0 (A and D) and 8 (B and E) of a fully controlled dietary intervention with either a meat-based diet (CON) or a mycoprotein-based diet (MYC). OGTT data were analysed using three-way ANOVAs. Incremental area under the curve (iAUC) data were analysed using one-way ANOVAs. There was a significant effect of CHO ingestion for blood glucose and serum insulin ($P<0.0001$). No interaction effects or main effects of condition or time were found (all $P>0.05$). For both blood glucose iAUC and serum insulin iAUC, no statistically significant main effects of time or condition (both $P>0.05$), as well as no interaction effects ($P>0.05$) were found.

Figure 3 Insulin sensitivity indices (A: HOMA-IR, B: Cederholm, C: Matsuda, D: OGIS, E: GUTT) calculated with the blood glucose and serum insulin concentrations measured fasting and during oral glucose tolerance tests (OGTT) pre and post a one week fully controlled dietary intervention with either a meat-based diet (CON) or a mycoprotein-based diet (MYC). There were no differences between groups at baseline for any of the OGTT calculated insulin sensitivity indices (all $P>0.05$) and no changes resulted from the intervention (time and interaction effects; all $P>0.05$)

Figure 4 Relative contribution of fat and carbohydrate oxidation rates to energy expenditure calculated via indirect calorimetry using the Frayn equations, in the fasted and CHO fed states, pre- and post- a one week fully controlled dietary intervention with either a meat-based diet (CON) or a mycoprotein-based diet (MYC). An effect of CHO ingestion was found for both carbohydrate and fat oxidation rates ($P<0.0001$). No interaction or condition effects were found (all $P>0.05$).

Figure 5 Heat map and cluster representation of NMR based metabolomics analyses which exhibited significant changes between pre- and post- a one week fully controlled dietary intervention with either a meat-based diet (CON) or a mycoprotein-based diet (MYC),

calculated by the Δ change for each participant. Participants in CON are represented in red and participants in MYC are shown in green.

Figure 6 Selected metabolites from the metabolomics analysis considered of particular relevance. Total plasma cholesterol (A), plasma free cholesterol (B), plasma LDL cholesterol (C), plasma HDL2 cholesterol (D), plasma DHA (E), plasma omega-3 fatty acids (F), plasma acetate (G) and plasma glucose (H) as measured by NMR-based targeted metabolomics pre- and post- a one week fully controlled dietary intervention with either a meat-based diet (CON) or a mycoprotein-based diet (MYC). NMR metabolomics metabolite concentrations were analysed using Significant Analysis of Microarrays (SAM). Total plasma cholesterol, free cholesterol, LDL cholesterol, HDL2 cholesterol, DHA and omega-3 fatty acid were decreased to a larger degree in the MYC group (14-19% decrease) compared with the CON group (3-11 % decrease; $P<0.05$). Plasma glucose remained unchanged in the CON group but was reduced by 4.5±0.1 % in MYC and plasma acetate concentrations increased by 8.5±0.1 % and 43.6±0.1 % in CON and MYC, respectively. The changes in these two variables were significant when individually analysed (t-tests, $P<0.05$) but not when using the SAM multivariate analysis.

Figure 7 Bland-Altman analysis plot for blood glucose concentrations measured by the benchtop YSI biochemistry analyser and plasma glucose concentrations measured by NMR spectroscopy, representing the consistency of the variant results between the two techniques. Measurements for every participant's sample, pre- and post- a one week fully controlled dietary intervention with either a meat-based diet (CON) or a mycoprotein-based diet (MYC) in CON and MYC were aggregated. There was a strong positive correlation between the two techniques ($r = 0.60$; $P<0.001$).