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Original article

A four-week dietary intervention with mycoprotein-containing food products reduces serum cholesterol concentrations in community-dwelling, overweight adults: A randomised controlled trial

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SUMMARY

Background: Substituting dietary meat and fish for mycoprotein, a fungal-derived food source rich in protein and fibre, decreases circulating cholesterol concentrations in laboratory-controlled studies. However, whether these findings can be translated to a home-based setting, and to decrease cholesterol concentrations in overweight and hypercholesterolemic individuals, remains to be established.

Objective: We investigated whether a remotely-delivered, home-based dietary intervention of mycoprotein-containing food products would affect various circulating cholesterol moieties and other markers of cardio-metabolic health in overweight (BMI >27.5 $kg \cdot m^{-2}$) and hypercholesterolaemic (>5.0 $mmol \cdot L^{-1}$) adults.

Methods: Seventy-two participants were randomized into a controlled, parallel-group trial conducted in a free-living setting, in which they received home deliveries of either meat/fish control products (CON; $n = 39$; BMI 33 ± 1 $kg \cdot m^{-2}$; 13 males, 26 females) or mycoprotein-containing food products (MYC; $n = 33$; BMI 32 ± 1 $kg \cdot m^{-2}$; 13 males, 20 females) for 4 weeks. Fingertip blood samples were collected and sent via postal service before and after the dietary intervention period and analysed for concentrations of serum lipids, blood glucose and c-peptide.

Results: Serum total cholesterol concentrations were unchanged throughout the intervention in CON, but decreased by 5 ± 2 % in MYC (from 5.4 ± 0.2 to 5.1 ± 0.2 $mmol \cdot L^{-1}$; $P < 0.05$). Serum low-density lipoprotein cholesterol and non-high-density lipoprotein cholesterol concentrations were also unchanged in CON, but decreased in MYC by 10 ± 3 % and 6 ± 2 % (both by 0.3 ± 0.1 $mmol \cdot L^{-1}$; $P < 0.05$). Serum high-density lipoprotein cholesterol and free triglyceride concentrations were unaffected in CON or MYC. Post-intervention, MYC displayed lower mean blood glucose (3.7 ± 0.2 versus 4.3 ± 0.2 $mmol \cdot L^{-1}$) and c-peptide (779 ± 76 vs. 1064 ± 86 $pmol \cdot L^{-1}$) concentrations ($P < 0.05$) vs. CON.

Conclusions: We show that a home-based dietary intervention of mycoprotein-containing food products effectively lowers circulating cholesterol concentrations in overweight, hypercholesterolemic adults. This demonstrates that mycoprotein consumption is a feasible and ecologically valid dietary strategy to improve markers of cardio-metabolic health in an at-risk population under free living conditions.

Clinical trial registration: NCT04773483 (https://classic.clinicaltrials.gov/ct2/show/NCT04773483).

Abbreviations: ANOVA, analysis of variance; BMI, body mass index; CON, control group; CVD, cardiovascular disease; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; MYC, mycoprotein group; RDV, Recommended daily value; TC, total cholesterol; TG, triglyceride.

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1. Introduction

The prevalence of cardiovascular disease (CVD) has doubled between 1990 and 2019, and at 18.6 million deaths worldwide, is the leading cause of mortality from chronic, non-communicable disease [1]. Most established risk factors of CVD, such as hyperglycaemia, hyperinsulinaemia, hypercholesterolaemia and high body mass index (BMI), are modifiable by lifestyle [1,2]. As such, effective lifestyle interventions, including increased physical activity and/or healthier eating habits, are the bedrock of evidence-based approaches to reduce the prevalence and burden of CVD [3–5].

Dietary interventions are attractive approaches to reduce CVD risk factors due to consistently favourable clinical outcomes [3,6], patient/practitioner preference over other lifestyle interventions and pharmaceuticals [7,8], and broader commercial and societal feasibility. Dietary interventions targeting modest (<10 %) weight loss consistently report reductions in CV biomarker risk factors, including circulating total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-c) concentrations [4,9,10]. However, long-term compliance to weight loss is poor; more than half of lost weight is reportedly regained within two years, rising to 80 % regained within five years [11]. Alternative dietary strategies to manage CVD risk target reducing consumption of dietary (trans and saturated) fats and/or increasing dietary fibre intake to $\geq$30g•day^{-1} [3,12,13] to reduce circulating cholesterol concentrations and improve insulin sensitivity. Practically, this can be achieved by following Mediterranean [14] and plant-based diets [15,16], meta-analyses of which have been shown to reduce total cholesterol concentrations by ~0.2 mmol•L^{-1} over >12 weeks in healthy [14] and at-risk [15,16] individuals, and reduce incidence of stroke by ~40 % [14]. Despite this utility, adopting such wholesale dietary changes in non-Mediterranean countries is difficult due to myriad geographic and socioeconomic factors, including higher costs of ingredients, unfamiliarity of foods, and reduced availability of specialist products [17–19]. Thus, easy-to-implement dietary substitutions targeted at improving cardiometabolic risk factors may be a more attractive strategy.

Mycoprotein is a high-protein, high-fibre food source produced by the continuous fermentation of the fungus *Fusarium venenatum*. A series of studies have shown that daily consumption of mycoprotein and/or mycoprotein-containing food products reduces total circulating cholesterol concentrations by ~0.6 mmol•L^{-1} in healthy [20] and hypercholesterolaemic [21,22] individuals, predominantly during laboratory-based interventions lasting between 1 and 8 weeks [20–23]. These beneficial effects have been attributed to reducing saturated fat intake from meat and/or increasing dietary fibre intake (by ~10 g from mycoprotein alone, to 30–40 g•d^{-1}, bringing intakes in line with or above recognized RDVs; [24]). We recently demonstrated that commercially available mycoprotein meat replacement products can feasibly be incorporated within omnivorous or plant-based diets [20,25] and lower concentrations of 45 circulating cholesterol moieties (and other lipid fractions) by 7–27 % [20].

With the established evidence-base for mycoprotein as a dietary intervention to reduce hypercholesterolaemia arising from laboratory-based studies, it is prudent to translate this to a home-based setting to evaluate real-world feasibility and efficacy. In the present study, we hypothesized that the provision of four weeks of daily mycoprotein-containing meat-replacement products to be consumed at home by members of the community with a high BMI and hypercholesterolaemia (and therefore at greater risk of CVD) would reduce circulating cholesterol, glucose and c-peptide (reflecting insulin production, and thus pancreatic beta cell function) concentrations compared with the provision of daily meat and fish products. This intervention was applied entirely remotely during the period of the SARS-CoV-2 pandemic UK lockdowns, with the use of food delivery services and novel postal blood-spot micro-analyses techniques to minimize participant contact thereby making it feasible within the legal restrictions whilst maximizing ecological validity.

2. Methods

2.1. Participants

Eighty-two overweight participants (age: 43 ± 2 y; BMI: 33 ± 1 kg•m^{-2}; 29 males, 53 females) were recruited to take part in this study, which was conducted entirely remotely (no face-to-face contact between participants and experimenters or visits to the University throughout the entire study period) between November 2020 and December 2021 during the UK's SARS-CoV-2 pandemic and associated social restrictions. Recruitment was conducted via social media advertising and invitations from Exeter Clinical Research Facility's Peninsula Research Bank.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human participants were approved by the University of Exeter's Sport and Health Sciences Ethics Committee (proposal reference number: 201021-B-02) and Peninsula Research Bank Steering Committee (proposal reference number: CRF473). This study was registered as a clinical trial at ClinicalTrials.gov (NCT04773483). Written informed consent was obtained from all participants.

Prior to inclusion, prospective participants met with a member of the research team using online videoconferencing platforms to discuss the study and experimental procedures in detail. Participants then completed an electronic health screening and dietary preference questionnaire via email. Inclusion criteria were: aged 18–70 y; BMI >27.5 kg•m^{-2} (with the assumption that this would also result in a hypercholesterolemic population [26]); and currently following an omnivorous diet. Participants were sent body mass scales if they did not have access to any, whereas height was determined by self-report. Exclusion criteria were: allergies/intolerances to penicillin or mycoprotein; using cholesterol lowering medication at time of recruitment or at any point during data collection; and/or prior intention to lose or gain weight during the study.

2.2. Study design and protocol

A schematic of the study design is presented in Fig. 1. Following enrolment, participants were randomly allocated on an intention to treat basis into one of two groups, the mycoprotein intervention (MYC) or control condition (CON), in a parallel groups study design. Group characteristics are shown in Table 1. Prior to the dietary intervention, participants were sent a bespoke, self-implemented, fingertip blood sampling kit prepared by Blood Sciences Academic Department at the Royal Devon & Exeter NHS Foundation Trust. Following an >8 h overnight fast and with a member of the research team assisting via a videoconferencing platform, participants obtained a postabsorptive fingertip blood sample. A multiple-pass 24 h dietary recall [27] was then obtained to ensure high response rate and performed under guidance of a dietician from the research team. Participants then obtained a second, postprandial fingertip blood sample 3 h after consuming their habitual breakfast. For fingertip blood collection, the initial bleed was wiped away and 4 drops were allowed to fall from the fingertip on to a blood spot collection card to determine blood glucose and c-peptide concentrations. To determine serum lipid concentrations, >500 μL whole blood was collected in a serum collection tube (BD Microtainer; BD, New Jersey, USA). Body mass was measured prior to breakfast

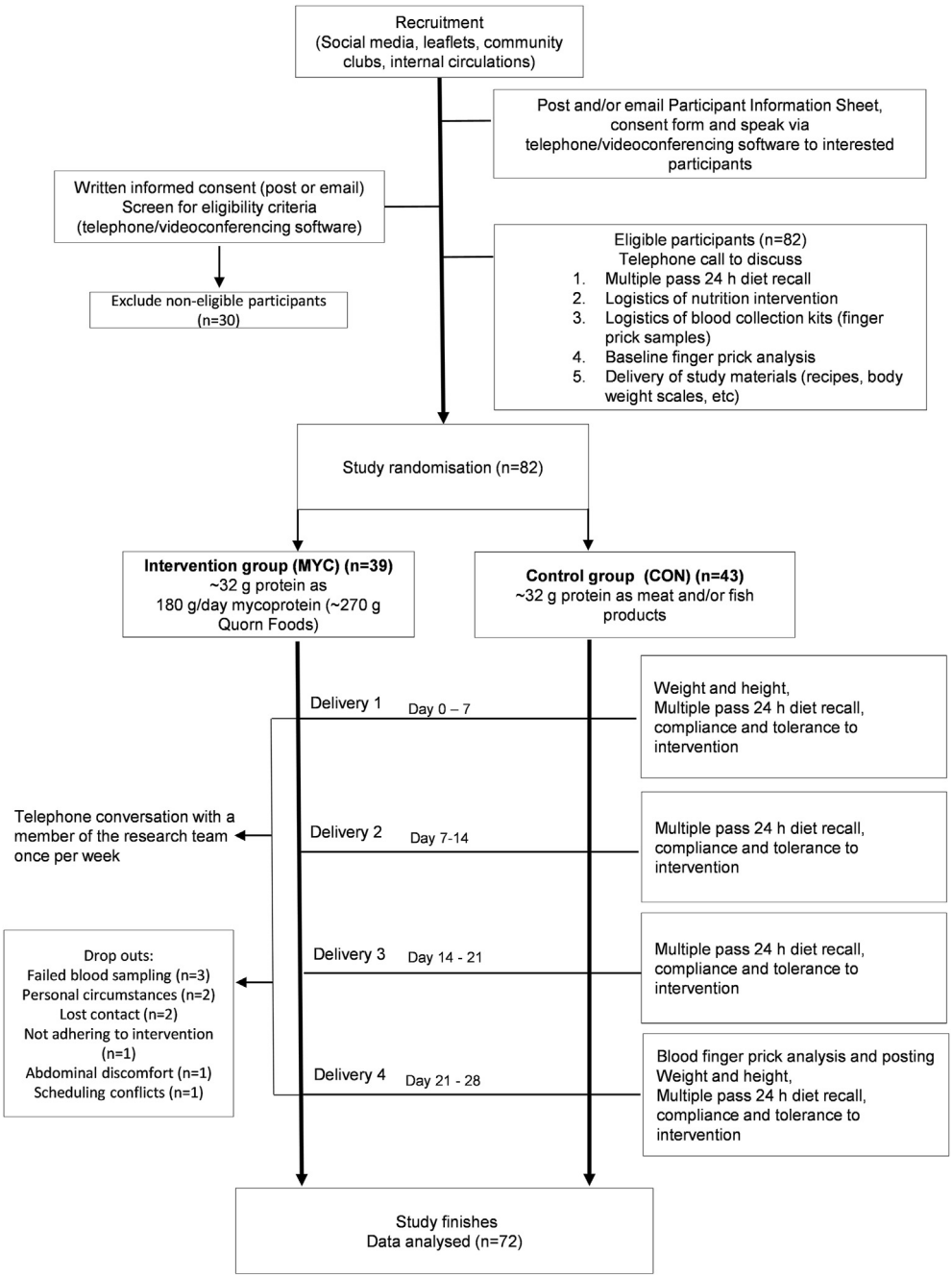

Fig. 1. Overview of the experimental protocol.

consumption and recorded on kit inserts, which were returned alongside blood samples on the same day using prepaid postage bags.

Once a successful fingertip blood collection was confirmed by analysis, participants then began receiving weekly supplies of either mycoprotein-rich food products (MYC) or comparable (both with respect to macronutrient content as well as practicality regarding meal incorporation) meat/fish alternatives (CON) over a continuous 4-week period. Food deliveries were organized by the research team to support adherence and delivered to the participants' home making use of local couriers and supermarket delivery services. This permitted the research team to amend diets in close to real-time in case of any supply issues or substitutions. Participants were able to contact the research team at any time via telephone or email in case of any queries or problems. Each week, participants met virtually with a member of the research team to conduct further multiple-pass 24 h dietary recalls, and to provide feedback on the foods provided, as well as establishing and providing encouragement for adherence. Upon completing the 4-week dietary intervention period, participants collected additional postabsorptive and (3 h) postprandial blood samples, and recorded their body mass, in the same manner as before the intervention.

2.3. Dietary intervention

In the MYC group, target daily mycoprotein consumption was in line with our previous work [20]; for participants between 60 and

Table 1
Participant characteristics and baseline serum lipid, blood glucose and blood c-peptide concentrations measured in a fingertip blood sample in the postabsorptive state.

	CON		MYC	
	(n = 39)		(n=33)	
Sex (male:female)	13:26		13:20	
	Mean	*SEM*	*Mean*	*SEM*
Age (y)	46	2	41	3
Body mass pre (kg)	95.2	3.2	92.7	2.8
Body mass post (kg)	94.2	3.2	92.0	2.7
Height (cm)	170	2	171	2
BMI ($kg \cdot m^{-2}$)	33	1	32	1
TC ($mmol \cdot L^{-1}$)	5.8	0.2	5.4	0.2
LDL-c ($mmol \cdot L^{-1}$)	3.6	0.1	3.3	0.1
HDL-c ($mmol \cdot L^{-1}$)	1.5	0.1	1.3	0.1
TG ($mmol \cdot L^{-1}$)	1.6	0.1	1.8	0.2
Glucose ($mmol \cdot L^{-1}$)	3.58	0.17	3.44	0.18
C-peptide ($pmol \cdot L^{-1}$)	732	67	642	73

Values represent mean ± SEM. CON, control dietary intervention; MYC, mycoprotein-based dietary intervention; BMI, body mass index; TC, total cholesterol; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol; TG, triglycerides. All between group comparisons $P > 0.05$.

80 kg this was set at 180 g (wet weight) mycoprotein consumption per day, which was provided as Quorn Foods products (~270 g total weight, depending on the product[s]). This target amount was adjusted to 150 or 210 g per day for participants <60 or >80 kg, respectively. Participants in CON were provided with a protein-matched quantity of equivalent meat and fish products (27, 32 and 37 g protein for participants <60, 60–80 and >80 kg, respectively). A list of all possible foods provided and their nutritional composition is detailed in Table 2. Participants were instructed to consume all foods provided, and to maintain body mass as closely as possible to minimize any influence of weight change *per se* on primary and secondary variables. No other dietary restrictions or advice were applied, and participants were encouraged to continue their habitual diets and physical activity levels as normal. Recipe guidebooks, which contained step-by-step instructions on preparing meals using the Quorn Foods products (MYC), or the equivalent meals using the meat/fish products (CON), were provided at request for inspiration and adherence purposes.

2.4. Dietary assessment

Multiple-pass 24 h dietary recalls [27] were performed prior to and during each week of the dietary intervention. Interview days were chosen at random for each participant and conducted using online videoconferencing platforms. Participants provided a list of all foods eaten on the previous day (first-pass). This list was then probed for additional details, such as condiments, side dishes, cooking oils, etc. (second-pass). In a third pass, the list of foods was read back to the participant for review, prompting recall of any missed items. To standardize the recall, dietary intakes were recorded using a form separated into meal moments (i.e. breakfast, morning snacks, lunch, afternoon snacks, dinner and evening snacks), and included a list of follow-up prompts. Energy and macronutrient intakes were calculated using online licensed software (Nutritics, Swords, Dublin, Ireland). All recalls were performed and analysed by the same investigator (GFP). Compliance was determined as a percentage, calculated as grams of protein consumed from products consumed versus the daily target (i.e. 27, 32 and 37 g protein targets for participants <60, 60–80 and >80 kg, respectively).

2.5. Blood collection and analysis

Fingertip blood sample analysis was carried out by the Blood Sciences Academic Department at the Royal Devon & Exeter NHS Foundation Trust on the Cobas 8000 automated platform (Roche Diagnostics, Rotkreuz, Switzerland). Following centrifugation at $4000 \times g$ for 10 min at 22 °C, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c) and triglycerides (TG) were determined in serum using enzymatic colourimetric assays on the Cobas c 702 module (using CHOL2, HDLC3 and TRIGL packs, respectively; Roche Diagnostics). Low-density lipoprotein cholesterol (LDL-c)

Table 2
Nutritional composition of meat/fish control products and mycoprotein-containing alternatives provided to participants.

	Energy ($kj \cdot 100g^{-1}$)	Fat ($g \cdot 100g^{-1}$)	Carbohydrate ($g \cdot 100g^{-1}$)	Fibre ($g \cdot 100g^{-1}$)	Protein ($g \cdot 100g^{-1}$)
CON					
Chicken breast	630	1.9	<0.5	<0.5	32.9
Roast chicken slices	513	3.0	1.3	<0.5	22.3
Beef mince	869	12.0	<0.5	<0.5	25.0
Beef meatballs	258	18.9	1.5	<0.5	20.5
Cured ham slices	559	4.4	<0.5	<0.5	23.3
Pork sausages	1207	23.6	3.1	1.0	16.1
Fish fingers	895	9.3	20.0	0.8	12.0
Cod fillet	410	1.0	<0.5	<0.5	21.9
Salmon fillet	1003	15.2	0.8	<0.5	25.0
Beef chilli and rice	469	2.6	14.8	2.4	5.9
Beef lasagne	582	6.6	11.5	1.2	7.8
Cottage pie	504	5.6	9.2	1.0	7.7
MYC					
Quorn Pieces	417	2.6	1.7	7.1	14.0
Quorn Vegan Chicken Free Slices	393	2.3	4.1	6.2	11.0
Quorn Southern Fried Bites	807	7.1	20.0	6.5	8.6
Quorn Vegetarian Fillets	353	1.6	1.9	5.5	13.0
Quorn Mince	384	1.7	2.3	7.5	13.0
Quorn Swedish Style Balls	545	4.6	7.4	3.0	13.0
Quorn Vegan Ham Free Slices	420	2.5	1.7	8.5	14.0
Quorn Vegetarian Sausages	827	11.0	1.5	5.5	11.0
Quorn Vegan Fishless Fingers	898	7.8	0.6	4.2	4.5
Quorn Chilli Bean Bowl	424	0.4	17.0	4.4	5.1
Quorn Lasagne	386	2.7	11.0	3.2	4.4
Quorn Cottage Pie	416	3.6	11.0	4.0	4.2

CON, control dietary intervention; MYC, mycoprotein-based dietary intervention.

was calculated using the Friedewald formula as previously described [28]. Non-high-density lipoprotein cholesterol (non-HDL-c) was determined from TC minus HDL-c.

Dried blood spot eluents were analysed using the hexokinase reference method (GLUC3 pack; Roche Diagnostics) to determine glucose concentrations. C-peptide concentrations were analysed on the Cobas e 801 module from dried blood spot eluents using a sandwich electrochemiluminescence immunoassay (C-Peptide pack; Roche Diagnostics).

2.6. Statistical analyses

An *a priori* power analysis was performed to determine the number of participants required to detect a 5 % reduction in total cholesterol concentrations in MYC ($P < 0.05$, power = 0.8, $f = 0.13$; G*Power version 3.1). Given that this initial effect size was based on a conservative estimation from previous research [20–22], we re-performed the power analysis after data had been collected from 25 participants to provide an accurate effect size estimate based on the current study conditions ($P < 0.05$, power = 0.8, $f = 0.18$). The resultant sample size ($n = 64$) was adjusted to account for a dropout rate of 25 %, resulting in 85 participants to be recruited.

Missing data analyses (regression imputation) were used for minimal missing data points, and in cases where there were significant missing data within a participant for a given variable, they were excluded from that analysis. A Student's independent *t*-test was used to test group differences in baseline characteristics. A two-way mixed model analysis of variance (ANOVA) with time and group factors was used to detect differences in serum TC concentrations (primary outcome). For secondary outcomes, a mixed-effects analysis with time (pre vs. post intervention), group (MYC vs. CON) and prandial status (postabsorptive vs. postprandial) as fixed effects were used to detect differences in blood glucose and C-peptide concentrations. Concentrations of LDL-c, HDL-c, non-HDL-c, TG, TC:HDL-c and LDL-c:HDL-c ratios (secondary outcomes), and dietary intakes and compliance to the intervention (tertiary outcomes) were analysed using two-way mixed model ANOVAs with time and group factors. Sidak corrections for multiple comparisons applied to follow up post hoc differences. Effect sizes (r) for primary and secondary outcomes were calculated from the *F*-ratio and residual degrees of freedom, with 0.10, 0.30, and 0.50 indicating small, medium and large effects, respectively. Statistical analysis was performed using GraphPad Prism 9 (GraphPad Software, Inc., San Diego, CA, USA).

All data are presented as mean ± SEM with $P < 0.05$ indicating statistical significance.

3. Results

3.1. Participants' characteristics

Of 82 participants enrolled in the study, 3 participants could not obtain a pre intervention blood sample (2 from MYC, 1 from CON), 2 participants terminated their involvement with the study due to personal circumstances (both MYC), 2 participants became uncontactable (CON and MYC), 1 participant reported abdominal discomfort during the intervention (MYC), 1 participant did not want to consume the foods allocated (CON) and 1 participant had difficulty scheduling the 4-week intervention period. Therefore, 72 participants were included in the final analyses.

No differences in age, height, body mass or BMI were identified between groups at baseline (Table 1; all $P > 0.05$). Baseline post-absorptive serum TC, LDL-c, HDL-c, TG, glucose and c-peptide concentrations did not differ between groups (Table 1; all $P > 0.05$). Both groups characteristics' indicate the presence of overweight (i.e. BMI >25 $kg \cdot m^{-2}$; [29]) and mild hypercholesterolaemia (i.e. >5.0 $mmol \cdot L^{-1}$; [30]). Of these, 23 participants in CON and 18 participants in MYC had BMI >30 $kg \cdot m^{-2}$, indicating obesity. Body mass modestly decreased pre to post intervention (time effect; $P < 0.001$) by 1.0 ± 0.3 kg in CON and 0.8 ± 0.3 kg in MYC but not differently between groups (group and group-by-time interaction; $P > 0.05$).

3.2. Dietary intervention

Energy and macronutrient consumption over the 4-week dietary intervention period is presented in Table 3. There were no differences between groups at baseline or at any timepoint during the study in energy, carbohydrate, protein, fat or alcohol intakes expressed as absolute quantities or as percentage of total energy intake (all comparisons; $P > 0.05$). Similarly, there were no changes in any of these nutritional parameters over the time course of the intervention period (all time effects; $P > 0.05$). Fibre consumption was comparable between groups pre intervention (19 ± 1 and 22 ± 2 $g \cdot d^{-1}$, in CON and MYC, respectively), and whilst this remained unchanged throughout the intervention period in CON (average across weeks of 19 ± 1 $g \cdot d^{-1}$), fibre intake increased in MYC by week 1 to33 ± 2 $g \cdot d^{-1}$, and remained equivalently higher throughout the study (35 ± 2 $g \cdot d^{-1}$, 36 ± 3 $g \cdot d^{-1}$ and 36 ± 3 $g \cdot d^{-1}$ during weeks 2, 3 and 4, respectively; group-by-time interaction; $P < 0.001$).

In CON, participants were provided with 38 ± 1 $g \cdot d^{-1}$ of protein from meat and fish items, and reported consuming 101 ± 11, 93 ± 10, 98 ± 11, and 104 ± 11 % of protein provided on weeks 1, 2, 3 and 4, respectively. Participants in MYC were provided with 36 ± 1 $g \cdot d^{-1}$ protein from mycoprotein-containing food products and reported consuming 75 ± 6, 76 ± 6, 82 ± 7 and 96 ± 6 % of protein provided on weeks 1, 2, 3 and 4, respectively (all comparisons within and between groups; $P > 0.05$). This equated to 160 ± 28 $g \cdot d^{-1}$ (wet weight) mycoprotein consumed for participants in MYC, from an allocation of 202 ± 3 $g \cdot d^{-1}$. Fibre from mycoprotein consumed contributed 10 ± 2 $g \cdot d^{-1}$ to the total fibre intake, with a further 5 ± 1 $g \cdot d^{-1}$ originating from other ingredients in the mycoprotein products (i.e. pea fibre, wheat flour, pinto and kidney beans).

Dietary lipid intakes, and lipid sub-classes, are shown in Table 4. Despite participants consuming more saturated fat from the products provided in CON versus MYC (4.6 ± 0.5 vs. 2.4 ± 0.2 $g \cdot d^{-1}$, respectively; $P < 0.001$), total saturated fat intake, as well as that of mono-unsaturated fats, poly-unsaturated fats and Ω-6 fatty acids were similar between groups and unchanged over time (all comparisons; $P > 0.05$). Intake of Ω-3 fatty acids were lower in MYC vs. CON both pre and throughout the intervention period (0.6 ± 0.1 vs. 0.9 ± 0.1 $g \cdot d^{-1}$; group effect, $P < 0.05$), and therefore was unaffected by the mycoprotein intervention (time and group-by-time interaction; $P > 0.05$). Intake of trans-fatty acids generally decreased over time ($P < 0.05$), but post hoc testing failed to identify further differences between pairs of timepoints. Dietary cholesterol intake was similar between groups at baseline (CON: 236 ± 36 $mg \cdot d^{-1}$; MYC: 248 ± 42 $mg \cdot d^{-1}$). This remained stable in CON (mean across intervention period 229 ± 20 $mg \cdot d^{-1}$), but reduced in MYC (group-by-time interaction; $P < 0.05$) from baseline at weeks 1 and 4 (135 ± 37 and 126 ± 23 $mg \cdot d^{-1}$, respectively; $P < 0.05$ vs. baseline).

3.3. Serum lipids

Concentrations of serum TC, LDL-c, non-HDL-c and TG could not be determined in 1 participant in MYC (multiple failures on post intervention blood spots; resulting in $n = 32$). Serum TC

Table 3
Nutritional composition of diets pre and during the 4 week dietary intervention period.

	Energy		Protein		Fat		Carbohydrate		Fibre	Alcohol	
	MJ·d^{-1}	kcal·d^{-1}	g·d^{-1}	EN%	g·d^{-1}	EN%	g·d^{-1}	EN%	g·d^{-1}	g·d^{-1}	EN%
CON											
Pre	7.9 ± 0.4	1884 ± 90	86 ± 4	19 ± 1	79 ± 6	36 ± 1	195 ± 11	42 ± 1	19 ± 1	7 ± 2	3 ± 1
Week 1	7.5 ± 0.3	1784 ± 81	90 ± 4	21 ± 1	70 ± 4	35 ± 2	189 ± 12	42 ± 2	19 ± 1	6 ± 2	2 ± 1
Week 2	7.7 ± 0.4	1826 ± 93	89 ± 5	20 ± 1	72 ± 5	34 ± 1	194 ± 11	43 ± 1	20 ± 1	7 ± 3	2 ± 1
Week 3	7.7 ± 0.4	1829 ± 91	92 ± 4	21 ± 1	72 ± 5	35 ± 1	193 ± 11	43 ± 1	19 ± 1	6 ± 2	2 ± 1
Week 4	8.3 ± 0.4	1982 ± 97	99 ± 8	21 ± 1	80 ± 6	35 ± 2	197 ± 11	41 ± 1	20 ± 1	10 ± 3	4 ± 1
MYC											
Pre	8.1 ± 0.4	1924 ± 95	90 ± 9	18 ± 1	77 ± 5	35 ± 1	207 ± 10	44 ± 1	22 ± 2	6 ± 3	2 ± 1
Week 1	7.4 ± 0.4	1773 ± 96	80 ± 5	19 ± 1	67 ± 6	32 ± 2	204 ± 11	47 ± 2	33 ± 2*†	5 ± 2	2 ± 1
Week 2	7.5 ± 0.4	1789 ± 103	81 ± 6	19 ± 1	68 ± 6	33 ± 2	204 ± 12	47 ± 2	35 ± 2*†	6 ± 2	2 ± 1
Week 3	8.0 ± 0.4	1902 ± 106	88 ± 6	19 ± 1	68 ± 6	31 ± 2	221 ± 14	47 ± 2	36 ± 3*†	8 ± 2	3 ± 1
Week 4	8.0 ± 0.6	1897 ± 133	89 ± 7	20 ± 1	74 ± 7	33 ± 2	212 ± 15	46 ± 2	36 ± 3*†	4 ± 2	1 ± 1

CON, control dietary intervention; MYC, mycoprotein-based dietary intervention; EN%, percentage contribution toward total energy intake. Group-by-time interaction denoted by *$P < 0.001$ different to Pre value within group, †$P < 0.001$ different to CON at that timepoint.

Table 4
Dietary intakes of lipid sub-classes composition pre and during the 4 week dietary intervention period.

	Saturated fats		Mono-unsaturated fats		Poly-unsaturated fats		Ω−3 fatty acids		Ω−6 fatty acids		Trans-fatty acids		Cholesterol	
	g·d^{-1}		g·d^{-1}		g·d^{-1}		g·d^{-1}		g·d^{-1}		g·d^{-1}		mg·d^{-1}	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
CON														
Pre	30	3	19	2	8	1	1.2	0.3	3.4	0.6	0.9	0.1	236	36
Week 1	25	2	13	1	6	1	0.6	0.1	3	0.3	0.5	0.1	185	34
Week 2	27	2	18	2	7	1	0.8	0.2	3.3	0.5	0.6	0.1	144	25
Week 3	27	2	15	2	7	1	1	0.2	3.6	0.5	0.8	0.2	167	24
Week 4	29	2	17	1	7	1	0.8	0.2	3.7	0.5	0.5	0.1	211	29
MYC														
Pre	26	2	19	2	8	1	0.5	0.1	3.6	0.6	0.7	0.1	248	42
Week 1	23	2	15	2	7	1	0.7	0.1	4.2	0.7	0.5	0.1	135	37*
Week 2	21	2	15	2	6	1	0.5	0.1	3.6	0.6	0.5	0.2	153	41
Week 3	26	3	14	2	7	1	0.5	0.1	3.4	0.8	0.8	0.2	141	35
Week 4	26	3	16	2	8	2	0.8	0.2	4.4	1.3	0.6	0.1	136	23*†

Values represent mean ± SEM. CON, control dietary intervention; MYC, mycoprotein-based dietary intervention. Main effect of group ($P < 0.05$) for Ω-3 and trans fatty acids. Group-by-time interaction denoted by *$P < 0.05$ different to Pre value within group, †$P < 0.05$ different to CON at that timepoint.

concentrations are displayed in Fig. 2. From similar baseline values across groups, serum cholesterol concentrations decreased over time (time effect; $P < 0.05$, $r = 0.28$) and were lower throughout in MYC compared with CON (group effect: $P < 0.05$, $r = 0.30$). The change over time was also greater in MYC (−0.3 ± 0.1 vs. 0.0 ± 0.1 mmol·L^{-1} in MYC and CON, respectively; group-by-time interaction; $P < 0.05$, $r = 0.24$). Additionally, serum cholesterol was lower in MYC than CON post intervention ($P < 0.01$).

Serum LDL-c concentrations (Fig. 3A) decreased pre-to post-intervention (time effect: $P < 0.01$, $r = 0.32$) and were lower in MYC compared with CON (group effect; $P < 0.05$, $r = 0.29$). However, the decrease was greater in MYC at −0.3 ± 0.1 mmol·L^{-1} vs. 0.0 ± 0.1 mmol·L^{-1} in CON (group-by-time interaction; $P < 0.05$, $r = 0.24$). Additionally, serum LDL-c was lower in MYC than CON post intervention ($P < 0.01$).

Serum HDL-c concentrations (Fig. 3B) decreased pre-to post-intervention (time effect: $P < 0.01$, $r = 0.34$) and were lower in MYC compared with CON (group effect; $P < 0.05$, $r = 0.24$). However, the change in MYC (−0.1 ± 0.0 mmol·L^{-1}) did not differ compared with CON (0.0 ± 0.0 mmol·L^{-1}; group-by-time interaction; $P > 0.05$, $r = 0.19$). The total cholesterol:HDL-c ratio was unaffected by group and unchanged over time (all comparisons $P > 0.05$, $r < 0.10$), at 4.2 ± 0.2 and 4.2 ± 0.2 in CON and MYC respectively pre-intervention, and 4.3 ± 0.2 and 4.2 ± 0.2 in CON and MYC respectively, post-intervention. The LDL-c:HDL-c ratio was similarly

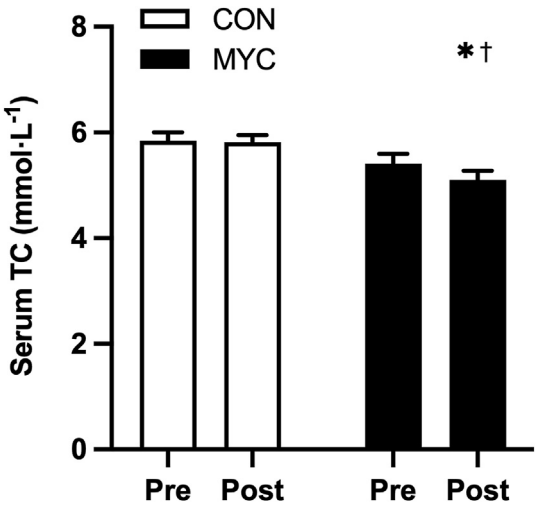

Fig. 2. Serum total cholesterol (TC) concentrations pre and post a 4-week dietary intervention period with meat and fish control products (CON; $n = 39$) or a protein matched quantity of mycoprotein products (MYC; $n = 32$). Data analysed by two-way analysis of variance (ANOVA) and expressed as mean ± SEM. Main effects of time ($P < 0.05$) and group ($P < 0.05$). Interaction effect ($P < 0.05$) denoted by *$P < 0.01$ significantly reduced from pre in MYC group, †$P < 0.01$ significantly lower than CON at given timepoint.

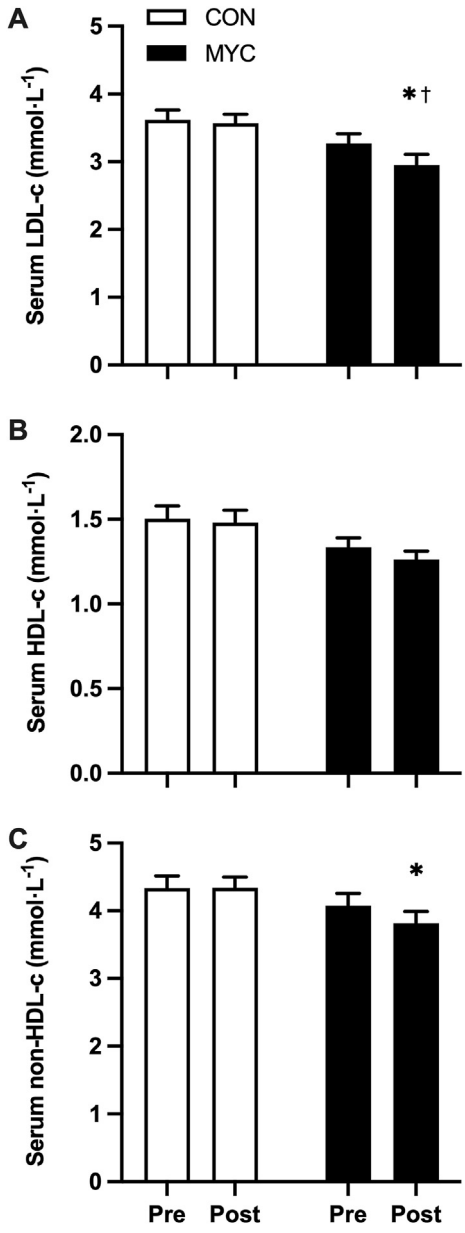

Fig. 3. Serum concentrations of *A*: low-density lipoprotein cholesterol (LDL-c), *B*: high-density lipoprotein cholesterol (HDL-c), and *C*: non-HDL-c, pre and post a 4 week dietary intervention period with meat and fish control products (CON; $n = 39$) or a protein matched quantity of mycoprotein products (MYC; $n = 32$ for LDL-c and non-HDL-c, $n = 33$ for HDL-c). Data analysed by two-way analysis of variance (ANOVA) and expressed as mean ± SEM. *A*: Main effects of time ($P < 0.01$) and group ($P < 0.05$). Interaction effect ($P < 0.05$) denoted by $^{*}P < 0.01$ significantly reduced from pre in MYC group, $^{\dagger}P < 0.01$ significantly lower than CON at given timepoint. *B*: Main effects of time ($P < 0.01$) and group ($P < 0.05$) only. *C*: Interaction effect only ($P < 0.05$), denoted by $^{*}P < 0.05$ significantly reduced from pre in MYC group.

unchanged (CON: 2.7 ± 0.2 to 2.7 ± 0.2; MYC: 2.5 ± 0.2 to 2.4 ± 0.2; pre to post, respectively; all comparisons $P > 0.05$), despite there being a borderline small effect size for time ($r = 0.13$) and group-by-time interaction ($r = 0.11$).

Serum concentrations of non-HDL-c (Fig. 3C) did not differ pre-to post-intervention (time effect; $P > 0.05$, $r = 0.23$) and were not different between CON and MYC groups (group effect; $P > 0.05$, $r = 0.19$). However, non-HDL-c decreased in MYC only (-0.3 ± 0.1 mmol·L^{-1}), vs. 0.0 ± 0.1 mmol·L^{-1} in CON (interaction $P < 0.05$, $r = 0.23$).

Serum TG concentrations did not differ pre-to post-intervention ($P > 0.05$, $r = 0.13$) and did not differ between CON and MYC groups (CON: 1.6 ± 0.1 to 1.7 ± 0.1 mmol·L^{-1}; MYC: 1.8 ± 0.2 to 1.9 ± 0.2 mmol·L^{-1}; group effect; $P > 0.05$, $r = 0.12$; group-by-time interaction; $P > 0.05$, $r < 0.10$).

3.4. Blood glucose and c-peptide

In CON, blood glucose could not be determined in 4 post-absorptive samples and 1 postprandial sample due to difficulties obtaining viable samples, so the final data reflect $n = 35$ and $n = 38$, respectively. In MYC, blood glucose could not be determined in 1 postabsorptive sample and 2 postprandial samples, and so data reflect $n = 32$ and $n = 31$, respectively.

Blood glucose concentrations (Fig. 4A) were overall not different between CON and MYC (group effect; $P > 0.05$, $r = 0.12$). Blood glucose concentrations increased over the 4-week dietary intervention period (time effect; $P < 0.05$, $r = 0.30$) in CON (by 23 ± 5 %), but did not change in MYC (group-by-time interaction; $P < 0.01$, $r = 0.34$), such that concentrations were lower post-intervention in MYC vs. CON (3.7 ± 0.2 vs. 4.3 ± 0.2 mmol·L^{-1}, respectively, with postabsorptive and postprandial pooled; $P < 0.05$). Blood glucose concentrations were greater in the postprandial (i.e. 3 h following breakfast) vs. postabsorptive state (prandial effect; $P < 0.05$, $r = 0.27$), but this rise was not altered by the dietary intervention period or group (all other interactions; $P > 0.05$, $r < 0.10$).

In CON, blood c-peptide concentrations could not be determined in 8 postabsorptive samples and 4 postprandial samples, so data reflect $n = 31$ and $n = 35$, respectively. In MYC, blood c-peptide concentrations could not be determined in 4 postabsorptive

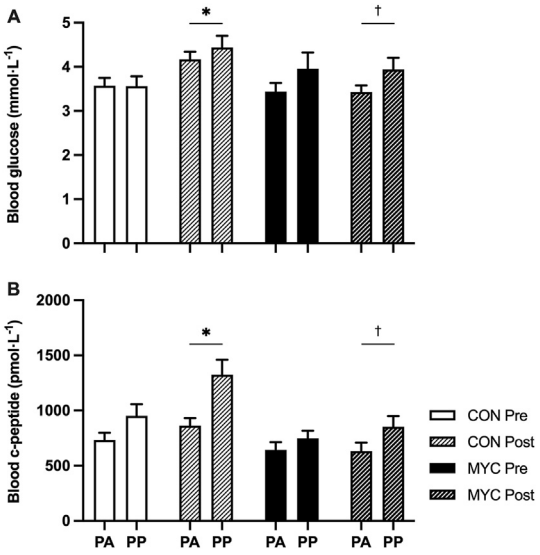

Fig. 4. Concentrations of blood *A*:glucose, and *B*: c-peptide in the postabsorptive (PA; after an >8 h overnight fast) and postprandial state (PP; 3 h after consuming breakfast), pre and post a 4 week dietary intervention period with meat and fish control products (CON) or a protein matched quantity of mycoprotein products (MYC). Data analysed by a mixed-effects analysis due to missing samples (see *Results* for final sample size), with time (pre vs. post intervention), group (MYC vs. CON) and prandial status (PA vs. PP) as fixed effects, and expressed as mean ± SEM. *A*: main effects of prandial status ($P < 0.05$) and time ($P < 0.05$). Significant group-by-time interaction effect ($P < 0.01$), denoted by $\underline{*}P < 0.001$ significantly greater post vs. pre intervention in CON, $\underline{\dagger}P < 0.05$ significantly lower in MYC vs. CON post intervention, with PA and PP pooled. *B*: main effects of prandial status ($P < 0.001$), group ($P < 0.05$) and time ($P < 0.01$). Significant prandial status-by-time interaction effect ($P < 0.05$; post hoc differences not shown). Significant group-by-time interaction effect ($P < 0.05$), denoted by $\underline{*}P < 0.001$ significantly greater post vs. pre intervention in CON, $\underline{\dagger}P < 0.001$ significantly lower in MYC vs. CON post intervention, with PA and PP pooled.

samples and 5 postprandial samples, so data reflect $n = 29$ and $n = 38$, respectively. Blood c-peptide concentrations (Fig. 4B) were lower in MYC compared with CON (group effect; $P < 0.05$, $r = 0.32$). Blood c-peptide concentrations increased over the 4-week dietary intervention period (time effect $P < 0.01$, $r = 0.39$) in CON (45 ± 9 %), but did not change in MYC (group-by-time interaction; $P < 0.05$, $r = 0.36$), such that concentrations were lower post-intervention in MYC vs. CON (779 ± 76 vs. 1064 ± 86 pmol·L^{-1}, respectively, with postabsorptive and postprandial pooled; $P < 0.001$). Blood c-peptide concentrations were greater in the postprandial compared with. postabsorptive state (prandial effect; $P < 0.001$, $r = 0.52$), pre- (51 ± 12 %) and post- (85 ± 16 %; prandial-by-time interaction; $P < 0.05$, $r = 0.33$) intervention, regardless of group (prandial-by-group interaction; $P > 0.05$, $r = 0.23$). Post hoc testing revealed that although postabsorptive c-peptide concentrations did not change pre-to-post intervention ($P > 0.05$), postprandial c-peptide concentrations were 56 ± 15 % greater post intervention vs. pre across both CON and MYC groups (post hoc $P < 0.001$).

4. Discussion

The present study demonstrates that a remotely conducted four-week dietary intervention involving the home-delivery of mycoprotein-containing food products to be consumed daily reduced total circulating cholesterol (TC) concentrations in overweight, hypercholesterolaemic adults. This cholesterol lowering effect was characterized by parallel reductions in low-density lipoprotein cholesterol (LDL-c) and non-high-density lipoprotein cholesterol (non-HDL-c), and decreased postabsorptive and postprandial glycaemia and circulating c-peptide concentrations following the intervention period compared with the control group.

Prior work from us [20] and others [21,22,31] has reliably demonstrated that 1–8 weeks of daily mycoprotein consumption, largely under strictly-controlled laboratory conditions, reduces circulating concentrations of TC, LDL-c, and other cholesterol moieties, by 10–20 % [20–23,31]. Whilst this shows promise, the additional variability that may be introduced when translating these findings to a home setting away from the laboratory (where all food can be controlled, activity monitored, alcohol consumption/caffeine consumption regulated or prohibited, etc.) has the potential to compromise the overall effectiveness of such interventions. Moreover, the success of such strategies is dependent on practicality and compliance [19], which is influenced by several personal, socioeconomic and geographic factors [17–19]. Here, we applied an intervention that provided a comparable quantity of mycoprotein to our recent work [20], and whilst we aimed to avoid weight loss, forewent any further laboratory restrictions (to the extent of employing novel, home-based fingertip blood sampling collections) in order to maximize ecological validity. To this end, we have successfully translated prior findings to a home-based intervention, by reporting that 4 weeks of mycoprotein consumption reduced serum TC concentrations by ~6 % (corresponding to a ~0.3 mmol·L^{-1} decrease) compared with a control group where no change was observed. This was achieved with subjects reporting consumption of ~82 % of the products provided, making the daily mycoprotein intake similar to our previous work using both healthy young (18–40 years) [20] and older (55–75 years) participants [25]. We therefore support and extend this previous work to show that mycoprotein containing products can readily be incorporated into omnivorous (or plant-based) diets as a feasible and efficacious cholesterol lowering dietary intervention.

In addition to reductions in TC, we observed that LDL-c and non-HDL-c (of which LDL-c is a component) concentrations decreased by ~10 and ~6 %, respectively (corresponding to a ~0.3 mmol·L^{-1} decrease in both cholesterol moieties). Meta-analyses of more wholesale dietary changes (i.e. Mediterranean [14] or vegan diets [16]) indicate TC and LDL-c reductions of ~0.2 mmol·L^{-1} after at least 12 weeks of intervention. Moreover, typical doses of atorvastatin generally yield variable 0.3–1.3 mmol·L^{-1} reductions in both TC and LDL-c after 12 months [32–34]. Therefore, the present reduction over only 4 weeks is notable and warrants further attention, as data from meta-analyses of pharmaceutical trials supports a linear relationship with extent of LDL-c reduction and lowering of CVD risk, corresponding to a ~10 % decrease in major CVD events (i.e. myocardial infarction, or fatal or non-fatal stroke) per 1 mmol·L^{-1} reduction in LDL-c [35,36]. Whilst the time-course of LDL-c reduction with mycoprotein is limited to one trial, whereby ~130 g/day mycoprotein decreased LDL-c by ~0.7 and ~0.8 mmol·L^{-1} after 4 and 8 weeks [22], there is clear potential for future work to examine whether this results in reduced incidence of major CVD events following longer intervention periods. However, it is worth noting that both TC:HDL-c and LDL-c:HDL-c ratios (representing other robust disease risk biomarkers [37,38]) remained unchanged. This is in keeping with our previous work [20] where all cholesterol variants reduced, thus leaving ratios unchanged, and suggests either decreased synthesis or increased clearance of all cholesterol types.

Whilst we did not set out to identify the mechanism by which cholesterol moieties may change, here we report that changes in body mass were comparable between mycoprotein and control conditions (Table 1). Moreover, despite participants in the mycoprotein group consuming ~2g less saturated fat per day from the foods provided versus the control group, total daily saturated fat intake remained unchanged (Table 4). Moreover, despite the mycoprotein intervention modestly reducing dietary cholesterol intake, support for a direct effect of this on circulating cholesterol concentrations within the literature is lacking [39,40]. Rather, mycoprotein likely reduces circulating cholesterol concentrations through increasing fibre intake (and/or composition), as we [20,41] and others [21,22] have previously discussed at length. Indeed, daily fibre intake represented the only dietary variable to change with mycoprotein (with intakes of energy, macronutrients, and most lipid subclasses equivocal between groups; Tables 2 and 3), increasing by ~13 g to achieve a daily intake of ~35 g·d^{-1} (Fig. 5), thereby meeting (and/or surpassing) the RDV for fibre. In line, a meta-analysis of dietary fibre interventions revealed similar reductions in TC and LDL-c to those we observed, by ~0.2 mmol·L^{-1} [42–44], which manifests with much smaller [42], or a complete absence of [44], changes in HDL-c concentration, again consistent with the present findings (Fig. 3B). Although this suggests that interventions increasing fibre intake *per se* may be efficacious, the cholesterol lowering effect may be attributable to β-glucan specifically [42–44], which is predominant in cereal grains including oats and barley, and comprises two-thirds of the fibre within mycoprotein (with the remainder being chitin). From a mechanistic standpoint, inhibition of cholesterol biosynthesis would be a prime candidate, given intestinal fermentation of mycoprotein (and its isolated fibre) produces the short chain fatty acids (SCFAs) acetate, propionate and butyrate [45]. Increasing propionate availability has been shown to inhibit incorporation of acetate into cholesterol [46]. Consistent with this, we recently observed an accumulation of plasma acetate concentrations following 1 week of mycoprotein consumption [20].

Elevated (postabsorptive and/or postprandial) plasma glucose and impaired insulin sensitivity is also considered a major risk factor for CVD [1,47]. As such, we determined blood glucose and c-peptide concentrations in the postabsorptive state and in response to the participants' habitual breakfast (postprandial) before and after the dietary intervention. Blood glucose and c-peptide

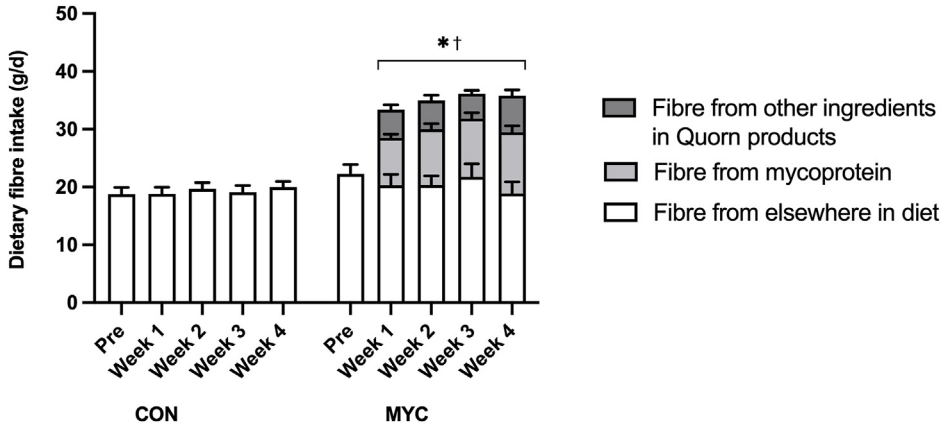

Fig. 5. Total dietary fibre intake pre and each week during a 4 week dietary intervention period with meat and fish control products (CON; $n = 39$) or a protein matched quantity of mycoprotein products (MYC; $n = 33$). Data analysed by two-way analysis of variance (ANOVA) and expressed as mean ± SEM. Main effects of time ($P < 0.001$) and group ($P < 0.001$). Significant interaction effect ($P < 0.001$), denoted by *$P < 0.001$ all timepoints significantly greater than pre in MYC, †$P < 0.001$ greater in MYC than corresponding values in CON. Shading for illustrative purposes representing fibre from mycoprotein (light grey), fibre from other sources in the mycoprotein products provided (dark grey), and fibre from elsewhere in the diet (white).

concentrations were ~13 % and ~27 % lower, respectively, in the mycoprotein compared with the control group following the intervention period. This is in keeping with our previous findings which implied that fasting blood glucose concentrations decreased in healthy individuals after 1 week of consuming mycoprotein-containing food products [20]. Although a mechanism is not apparent from the present study, SCFAs (e.g. from intestinal fibre fermentation) have also been hypothesized to improve insulin sensitivity and reduce hepatic glucose production, likely via AMPK-activated mechanisms [48]. In keeping with this, previous work has also shown that mycoprotein ingestion as part of a single mixed meal (providing 5–6 g fibre) reduces postprandial insulin concentrations by ~9–13 % [49,50], which is accompanied by a reduction in postprandial glucose concentration by ~9 % in some [50], albeit not all [49] studies. With gastric emptying rates being similar [49], the data collectively imply that mycoprotein may improve peripheral insulin sensitivity, which has been shown previously with other high fibre containing foods [51], and represents a promising area for future research.

We acknowledge that this study is not without limitations. Whilst the blood sampling kits were administered to maximize ecological validity and reduce any need for face-to-face contact, factors including differing ability to collect blood samples and varying transit times through the postal service may conceivably introduce additional variation. However, the effect on the interpretation of our results is likely negligible, as the cholesterol-lowering effect of mycoprotein, reported here under a free-living setting, has previously been reported by us and others when blood was obtained in a laboratory setting [20–22,31]. Secondly, the study took place during the UK's SARS CoV2 pandemic and associated social restrictions, and several recent reviews have addressed the associated deleterious effects on metabolic health, both as a direct result of infection (which we did not identify in our subjects while enrolled) [52], and indirectly following radical changes to (and restrictions on) lifestyle behaviours [53,54]. Indeed, this may explain the rise in blood glucose and c-peptide concentrations over the intervention period in the control group (Fig. 4). Nonetheless, should this have influenced the present study, it would appear the mycoprotein intervention could feasibly protect against worsening of metabolic health induced by detrimental lifestyle changes, which should be followed up by future investigation.

In conclusion, these data show a successful translation of tightly controlled nutritional intervention laboratory studies to a relatively innocuous dietary intervention applied remotely within the community. Namely, we demonstrate that intervention with mycoprotein-containing food products is a feasible and efficacious strategy to reduce circulating cholesterol, blood glucose and c-peptide concentrations in adults at increased risk of cardiovascular disease.

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Author contributions

GFP, RRI, HR, EJD, TJAF, FBS and BTW designed the study. GFP generated randomization sequence, recruited participants, organized and carried out data collection. GFP performed the statistical analyses. GFP and BTW interpreted the primary data. GFP drafted and BTW edited and revised the manuscript. All authors approved the final version.

Data availability statement

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

Conflicts of interest

GFP, RRI, FBS and BTW are employees of the University of Exeter. HR, HET and TJAF are employees of Marlow Foods. Aside from those mentioned above, the authors declare that there are no conflicts of interest.

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References

[1] Roth GA, Mensah GA, Johnson CO, Addolorato G, Ammirati E, Baddour LM, et al. Global burden of cardiovascular diseases and risk factors, 1990-2019: update from the GBD 2019 study. J Am Coll Cardiol 2020;76(25):2982–3021. https://doi.org/10.1016/j.jacc.2020.11.010.

[2] Wilson PW, Meigs JB. Cardiometabolic risk: a Framingham perspective. Int J Obes (Lond). 2008;32(Suppl 2):S17–20. https://doi.org/10.1038/ijo.2008.30.

[3] Mach F, Baigent C, Catapano AL, Koskinas KC, Casula M, Badimon L, et al. 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. Eur Heart J 2020;41(1):111–88. https://doi.org/10.1093/eurheartj/ehz455.

[4] Zomer E, Gurusamy K, Leach R, Trimmer C, Lobstein T, Morris S, et al. Interventions that cause weight loss and the impact on cardiovascular risk factors: a systematic review and meta-analysis. Obes Rev 2016;17(10):1001–11. https://doi.org/10.1111/obr.12433.

[5] Blair SN. Physical inactivity: the biggest public health problem of the 21st century. Br J Sports Med 2009;43(1):1–2.

[6] Yokose C, McCormick N, Rai SK, Lu N, Curhan G, Schwarzfuchs D, et al. Effects of low-fat, mediterranean, or low-carbohydrate weight loss diets on serum urate and cardiometabolic risk factors: a secondary analysis of the dietary intervention randomized controlled trial (DIRECT). Diabetes Care 2020;43(11):2812–20. https://doi.org/10.2337/dc20-1002.

[7] Jarbol DE, Larsen PV, Gyrd-Hansen D, Sondergaard J, Brandt C, Leppin A, et al. Determinants of preferences for lifestyle changes versus medication and beliefs in ability to maintain lifestyle changes. A population-based survey. Prev Med Rep 2017;6:66–73. https://doi.org/10.1016/j.pmedr.2017.02.010.

[8] Horne R, Weinman J. Patients' beliefs about prescribed medicines and their role in adherence to treatment in chronic physical illness. J Psychosom Res 1999;47(6):555–67. https://doi.org/10.1016/s0022-3999(99)00057-4.

[9] Nordmann AJ, Nordmann A, Briel M, Keller U, Yancy Jr WS, Brehm BJ, et al. Effects of low-carbohydrate vs low-fat diets on weight loss and cardiovascular risk factors: a meta-analysis of randomized controlled trials. Arch Intern Med 2006;166(3):285–93. https://doi.org/10.1001/archinte.166.3.285.

[10] Headland ML, Clifton PM, Keogh JB. Effect of intermittent compared to continuous energy restriction on weight loss and weight maintenance after 12 months in healthy overweight or obese adults. Int J Obes 2019;43(10):2028–36. https://doi.org/10.1038/s41366-018-0247-2.

[11] Anderson JW, Konz EC, Frederich RC, Wood CL. Long-term weight-loss maintenance: a meta-analysis of US studies. Am J Clin Nutr 2001;74(5):579–84. https://doi.org/10.1093/ajcn/74.5.579.

[12] Threapleton DE, Greenwood DC, Evans CE, Cleghorn CL, Nykjaer C, Woodhead C, et al. Dietary fibre intake and risk of cardiovascular disease: systematic review and meta-analysis. BMJ 2013;347:f6879. https://doi.org/10.1136/bmj.f6879.

[13] Butler T, Paterson K, Stanford D, Moore S, West E, Kausar N, et al. Joint BACPR/BDA/PHNSG statement on nutrition and cardiovascular health post-COVID-19 pandemic. Br J Cardiol 2020;27(79). https://doi.org/10.5837/bjc.2020.029.

[14] Rees K, Takeda A, Martin N, Ellis L, Wijesekara D, Vepa A, et al. Mediterranean-style diet for the primary and secondary prevention of cardiovascular disease. Cochrane Database Syst Rev 2019;3(3):CD009825. https://doi.org/10.1002/14651858.CD009825.pub3.

[15] Sofi F, Dinu M, Pagliai G, Cesari F, Gori AM, Sereni A, et al. Low-calorie vegetarian versus mediterranean diets for reducing body weight and improving cardiovascular risk profile: CARDIVEG study (cardiovascular prevention with vegetarian diet). Circulation 2018;137(11):1103–13. https://doi.org/10.1161/CIRCULATIONAHA.117.030088.

[16] Rees K, Al-Khudairy L, Takeda A, Stranges S. Vegan dietary pattern for the primary and secondary prevention of cardiovascular diseases. Cochrane Database Syst Rev 2021;2(2):CD013501. https://doi.org/10.1002/14651858.CD013501.pub2.

[17] Tong TYN, Imamura F, Monsivais P, Brage S, Griffin SJ, Wareham NJ, et al. Dietary cost associated with adherence to the Mediterranean diet, and its variation by socio-economic factors in the UK Fenland Study. Br J Nutr 2018;119(6):685–94. https://doi.org/10.1017/S0007114517003993.

[18] Mattavelli E, Olmastroni E, Bonofiglio D, Catapano AL, Baragetti A, Magni P. Adherence to the mediterranean diet: impact of geographical location of the observations. Nutrients 2022;14(10). https://doi.org/10.3390/nu14102040.

[19] Freedhoff Y, Hall KD. Weight loss diet studies: we need help not hype. Lancet 2016;388(10047):849–51. https://doi.org/10.1016/S0140-6736(16)31338-1.

[20] Coelho MOC, Monteyne AJ, Dirks ML, TJA Finnigan, Stephens FB, Wall BT. Daily mycoprotein consumption for 1 week does not affect insulin sensitivity or glycaemic control but modulates the plasma lipidome in healthy adults: a randomised controlled trial. Br J Nutr 2021;125(2):147–60. https://doi.org/10.1017/S0007114520002524.

[21] Turnbull WH, Leeds AR, Edwards GD. Effect of mycoprotein on blood lipids. Am J Clin Nutr 1990;52(4):646–50. https://doi.org/10.1093/ajcn/52.4.646.

[22] Turnbull WH, Leeds AR, Edwards DG. Mycoprotein reduces blood lipids in free-living subjects. Am J Clin Nutr 1992;55(2):415–9. https://doi.org/10.1093/ajcn/55.2.415.

[23] Shahid M, Gaines A, Coyle D, Alessandrini R, Finnigan T, Frost G, et al. The effect of mycoprotein intake on biomarkers of human health: a systematic review and meta-analysis. Am J Clin Nutr 2023;118(1):141–50. https://doi.org/10.1016/j.ajcnut.2023.03.019.

[24] SACN. Carbohydrates and Health report. London: TSO; 2015. p. 384.

[25] Monteyne AJ, Dunlop MV, Machin DJ, Coelho MOC, Pavis GF, Porter C, et al. A mycoprotein-based high-protein vegan diet supports equivalent daily myofibrillar protein synthesis rates compared with an isonitrogenous omnivorous diet in older adults: a randomised controlled trial. Br J Nutr 2021;126(5):674–84. https://doi.org/10.1017/S0007114520004481.

[26] Ashwell M, Gunn P, Gibson S. Waist-to-height ratio is a better screening tool than waist circumference and BMI for adult cardiometabolic risk factors: systematic review and meta-analysis. Obes Rev 2012;13(3):275–86. https://doi.org/10.1111/j.1467-789X.2011.00952.x.

[27] Guenther PM, DeMaio TJ, Ingwersen LA, Berlin M. The multiple-pass approach for the 24-h recall in the continuing survey of food intakes by individuals, 1994-1996. Am J Clin Nutr 1997;65(4):1316.

[28] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18(6):499–502.

[29] World Health Organisation fact sheets. Obesity and overweight. 2021 [22 February 2023]. Available from: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight.

[30] NHS health A to Z - high cholesterol - cholesterol levels. 2022 [22 February 2023]. Available from: https://www.nhs.uk/conditions/high-cholesterol/cholesterol-levels/.

[31] Udall JN, Lo CW, Young VR, Scrimshaw NS. The tolerance and nutritional value of two microfungal foods in human subjects. Am J Clin Nutr 1984;40(2):285–92. https://doi.org/10.1093/ajcn/40.2.285.

[32] Sola S, Mir MQ, Lerakis S, Tandon N, Khan BV. Atorvastatin improves left ventricular systolic function and serum markers of inflammation in non-ischemic heart failure. J Am Coll Cardiol 2006;47(2):332–7. https://doi.org/10.1016/j.jacc.2005.06.088.

[33] Shukla A, Sharma MK, Jain A, Goel PK. Prevention of atherosclerosis progression using atorvastatin in normolipidemic coronary artery disease patients–a controlled randomized trial. Indian Heart J 2005;57(6):675–80.

[34] Sever PS, Dahlof B, Poulter NR, Wedel H, Beevers G, Caulfield M, et al. Prevention of coronary and stroke events with atorvastatin in hypertensive patients who have average or lower-than-average cholesterol concentrations, in the Anglo-Scandinavian Cardiac Outcomes Trial–Lipid Lowering Arm (ASCOT-LLA): a multicentre randomised controlled trial. Lancet 2003;361(9364):1149–58. https://doi.org/10.1016/S0140-6736(03)12948-0.

[35] Law MR, Wald NJ, Rudnicka AR. Quantifying effect of statins on low density lipoprotein cholesterol, ischaemic heart disease, and stroke: systematic review and meta-analysis. BMJ 2003;326(7404):1423. https://doi.org/10.1136/bmj.326.7404.1423.

[36] Baigent C, Keech A, Kearney PM, Blackwell L, Buck G, Pollicino C, et al. Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. Lancet 2005;366(9493):1267–78. https://doi.org/10.1016/S0140-6736(05)67394-1.

[37] Ingelsson E, Schaefer EJ, Contois JH, McNamara JR, Sullivan L, Keyes MJ, et al. Clinical utility of different lipid measures for prediction of coronary heart disease in men and women. JAMA 2007;298(7):776–85. https://doi.org/10.1001/jama.298.7.776.

[38] Kinosian B, Glick H, Garland G. Cholesterol and coronary heart disease: predicting risks by levels and ratios. Ann Intern Med 1994;121(9):641–7. https://doi.org/10.7326/0003-4819-121-9-199411010-00002.

[39] Eckel RH, Jakicic JM, Ard JD, de Jesus JM, Houston Miller N, Hubbard VS, et al. 2013 AHA/ACC guideline on lifestyle management to reduce cardiovascular risk: a report of the American college of cardiology/American heart association task force on practice guidelines. Circulation 2014;129(25 Suppl 2):S76–99. https://doi.org/10.1161/01.cir.0000437740.48606.d1.

[40] Carson JAS, Lichtenstein AH, Anderson CAM, Appel LJ, Kris-Etherton PM, Meyer KA, et al. Dietary cholesterol and cardiovascular risk: a science advisory from the American heart association. Circulation 2020;141(3):e39–53. https://doi.org/10.1161/CIR.0000000000000743.

[41] Coelho MOC, Monteyne AJ, Dunlop MV, Harris HC, Morrison DJ, Stephens FB, et al. Mycoprotein as a possible alternative source of dietary protein to support muscle and metabolic health. Nutr Rev 2020;78(6):486–97. https://doi.org/10.1093/nutrit/nuz077.

[42] Hartley L, May MD, Loveman E, Colquitt JL, Rees K. Dietary fibre for the primary prevention of cardiovascular disease. Cochrane Database Syst Rev 2016;2016(1):CD011472. https://doi.org/10.1002/14651858.CD011472.pub2.

[43] Ho HV, Sievenpiper JL, Zurbau A, Blanco Mejia S, Jovanovski E, Au-Yeung F, et al. The effect of oat beta-glucan on LDL-cholesterol, non-HDL-cholesterol and apoB for CVD risk reduction: a systematic review and meta-analysis of randomised-controlled trials. Br J Nutr 2016;116(8):1369–82. https://doi.org/10.1017/S000711451600341X.

[44] Theuwissen E, Mensink RP. Water-soluble dietary fibers and cardiovascular disease. Physiol Behav 2008;94(2):285–92. https://doi.org/10.1016/j.physbeh.2008.01.001.

[45] Harris HC, Edwards CA, Morrison DJ. Short chain fatty acid production from mycoprotein and mycoprotein fibre in an in vitro fermentation model. Nutrients 2019;11(4). https://doi.org/10.3390/nu11040800.
[46] Wolever TM, Spadafora PJ, Cunnane SC, Pencharz PB. Propionate inhibits incorporation of colonic [1,2-13C]acetate into plasma lipids in humans. Am J Clin Nutr 1995;61(6):1241–7. https://doi.org/10.1093/ajcn/61.6.1241.
[47] Collaborators GBDRF. Global burden of 87 risk factors in 204 countries and territories, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. Lancet 2020;396(10258):1223–49. https://doi.org/10.1016/S0140-6736(20)30752-2.
[48] Hu GX, Chen GR, Xu H, Ge RS, Lin J. Activation of the AMP activated protein kinase by short-chain fatty acids is the main mechanism underlying the beneficial effect of a high fiber diet on the metabolic syndrome. Med Hypotheses 2010;74(1):123–6. https://doi.org/10.1016/j.mehy.2009.07.022.
[49] Bottin JH, Swann JR, Cropp E, Chambers ES, Ford HE, Ghatei MA, et al. 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. Br J Nutr 2016;116(2):360–74. https://doi.org/10.1017/S0007114516001872.
[50] Turnbull WH, Ward T. Mycoprotein reduces glycemia and insulinemia when taken with an oral-glucose-tolerance test. Am J Clin Nutr 1995;61(1):135–40. https://doi.org/10.1093/ajcn/61.1.135.
[51] Schenk S, Davidson CJ, Zderic TW, Byerley LO, Coyle EF. Different glycemic indexes of breakfast cereals are not due to glucose entry into blood but to glucose removal by tissue. Am J Clin Nutr 2003;78(4):742–8. https://doi.org/10.1093/ajcn/78.4.742.
[52] Stefan N, Birkenfeld AL, Schulze MB. Global pandemics interconnected - obesity, impaired metabolic health and COVID-19. Nat Rev Endocrinol 2021;17(3):135–49. https://doi.org/10.1038/s41574-020-00462-1.
[53] Antwi J, Appiah B, Oluwakuse B, Abu BAZ. The nutrition-COVID-19 interplay: a review. Curr Nutr Rep 2021;10(4):364–74. https://doi.org/10.1007/s13668-021-00380-2.
[54] King AJ, Burke LM, Halson SL, Hawley JA. The challenge of maintaining metabolic health during a global pandemic. Sports Med 2020;50(7):1233–41. https://doi.org/10.1007/s40279-020-01295-8.