

Effects of mycoprotein on glycaemic control and energy intake in humans: a systematic review

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Abstract

Mycoprotein is a food high in both dietary fibre and non-animal-derived protein. Global mycoprotein consumption is increasing, although its effect on human health has not yet been systematically reviewed. This study aims to systematically review the effects of mycoprotein on glycaemic control and energy intake in humans. A literature search of randomised controlled trials was performed in PubMed, Embase, Web of Science, Google Scholar and hand search. A total of twenty-one studies were identified of which only five studies, totalling 122 participants, met the inclusion criteria. All five studies were acute studies of which one reported outcomes on glycaemia and insulinaemia, two reported on energy intake and two reported on all of these outcomes. Data were extracted, and risk-of-bias assessment was then conducted. The results did not show a clear effect of acute mycoprotein on blood glucose levels, but it showed a decrease in insulin levels. Acute mycoprotein intake also showed to decrease energy intake at an *ad libitum* meal and post-24 h in healthy lean, overweight and obese humans. In conclusion, the acute ingestion of mycoprotein reduces energy intake and insulinaemia, whereas its impact on glycaemia is currently unclear. However, evidence comes from a very limited number of heterogeneous studies. Further well-controlled studies are needed to elucidate the short- and long-term effects of mycoprotein intake on glycaemic control and energy intake, as well as the mechanisms underpinning these effects.

Key words: Mycoprotein: Glycaemic control: Energy intake: Systematic reviews

Non-communicable diseases are responsible for 71% of annual global deaths⁽¹⁾. The most important risk factors for non-communicable diseases are elevated blood glucose (hyperglycaemia) and excessive body weight⁽²⁾. Postprandial hyperglycaemia is defined as blood glucose levels ≥ 7.8 mmol/l (considered as impaired glucose tolerance) or ≥ 11.1 mmol/l (considered as diabetes) 180 min after the consumption of a 75 g glucose load⁽³⁾. Excessive body weight results from an imbalance between energy intake and energy expenditure, and it is diagnosed using BMI of which values ≥ 25 –29.9 or ≥ 30 kg/m² are categorised as ‘overweight’ or ‘obese’, respectively⁽⁴⁾. The aetiology of hyperglycaemia and increased body weight is multi-factorial, with lifestyle (e.g. diet and exercise) being a significant contributing factor to both⁽⁵⁾.

Dietary interventions are therefore employed as a cornerstone strategy for the prevention and management of both hyperglycaemia and increased body weight⁽⁶⁾. Of relevance, dietary fibre has been shown to positively modulate both blood

glucose levels and energy homeostasis via a myriad of mechanisms. For example, soluble fibres can delay gastric emptying, glucose absorption in the small intestine and starch degradation; all of which contribute to decreasing blood glucose levels. Also, soluble fibres have been shown to stimulate the secretion of hormones such as glucagon-like peptide 1 (GLP-1) which acts as an incretin (stimulating the secretion of insulin) and promotes pancreatic β -cell growth⁽⁷⁾. In contrast, insoluble fibres interact with host digestive enzymes to attenuate digestible polysaccharide hydrolysis and consequently dampen the glycaemic response⁽⁸⁾. Furthermore, insoluble fibres can be fermented by the gut microbiota to produce bioactive metabolites – such as the SCFA acetate, butyrate and propionate⁽⁹⁾ – which can stimulate incretin effects in the peripheral circulation⁽¹⁰⁾.

With regard to energy homeostasis, dietary fibre has been shown to reduce energy intake via various mechanisms⁽¹¹⁾. Increased bulk and viscosity⁽¹²⁾, increased gastric distension⁽¹³⁾ and decreased gastric emptying⁽¹⁴⁾ have all been implicated in fibre-induced satiety. Additionally, the release of the anorexigenic

Abbreviations: GLP-1, glucagon-like peptide 1; PYY, peptide tyrosine tyrosine; RCT, randomised controlled trial.

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hormones GLP-1 and peptide tyrosine tyrosine (PYY) following fibre ingestion (due to mechanisms such as gut-derived SCFA production⁽¹⁵⁾) may also be a key driver of this effect. Overall, these processes result in a decrease in appetite and energy intake which can ultimately facilitate body weight loss.

Dietary protein has also been shown to positively influence blood glucose levels⁽¹⁶⁾ through mechanisms that are not yet fully defined. Putative mechanisms include the effects of incretin secretion (e.g. GLP-1 and gastric inhibitory peptide⁽¹⁷⁾), as well as the insulin secretagogue effects of amino acids⁽¹⁸⁾. Moreover, dietary protein can increase satiety and suppress energy intake, possibly via its effect on gluconeogenesis⁽¹⁹⁾, diet-induced thermogenesis⁽²⁰⁾ and/or release of anorexigenic hormones from the gastrointestinal tract⁽²¹⁾. Therefore, foods high in both dietary fibre and protein may modulate blood glucose levels, appetite and consequently energy intake to a greater extent than foods that contain either fibre or protein alone.

Mycoprotein is the name given to the biomass of fungal filaments produced following the continuous fermentation of a glucose substrate by the microscopic fungus *Fusarium venenatum*⁽²²⁾. Owing to its fungal nature, mycoprotein is high in both dietary fibre (6 g/100 g wet weight; composed of 1/3 chitin and 2/3 β -glucan) and non-animal-derived protein (11 g/100 g wet weight) (Table 1; Fig. 1). Mycoprotein constitutes an ingredient which is processed into foods that resemble animal-derived meat products (e.g. nuggets, mince, fish fingers) and sold under the brand name of QuornTM. QuornTM foods have similar organoleptic properties to meat (taste and appearance)

Table 1. Macronutrient composition of 100 g of mycoprotein (wet weight)

	Amount per 100 g as wet weight (as consumed)
Energy (kJ)	356
Carbohydrates (g)	9
Sugars (g)	0
Fat (g)	3
Of which saturates (g)	0.7
Protein (g)	11
Fibre (g)	6

and contain all nine essential amino acids, making it a popular meat substitute.

Mycoprotein is commercially available in the USA, Europe, Asia and Australia and becoming increasingly incorporated into the diet of many populations⁽²²⁾. Mycoprotein is, therefore, a significant source of dietary fibre and non-animal-derived protein for numerous consumers worldwide. As such, and as mycoprotein consumption continues to increase, it is of growing interest to understand its role in nutrition and health. Randomised controlled trials (RCT) have been conducted to investigate the effects of mycoprotein on blood glucose and energy intake in healthy humans. However, to the best of our knowledge, such work has not been systematically reviewed, appraised and synthesised to provide a consensus on the role of mycoprotein in glycaemic control and energy intake regulation. In this paper, we aim to systematically review the RCT investigating the effects of mycoprotein on glycaemic control and energy intake in humans.

Methods

A systematic review of peer-reviewed literature published up to the 3 November 2019 was performed. The systematic review was conducted, in adherence to the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidelines⁽²⁶⁾. The protocol for this review has been registered in PROSPERO 2018 (CRD42018114566) (online Supplementary Table S1) and is available from https://www.crd.york.ac.uk/PROSPERO/display_record.php?RecordID=114566.

Eligibility criteria

The PICOS (patients, intervention, comparator, outcome, study design) criteria were used to establish study eligibility and focus the research question (Table 2).

Search strategy

The conducted search strategy was performed within the PubMed, Embase, Web of Science and Google Scholar database. The search used a combination of terms of both title headings, keywords or free-text words detailed in online Supplementary Table S2, with the English language and a date cut-off of

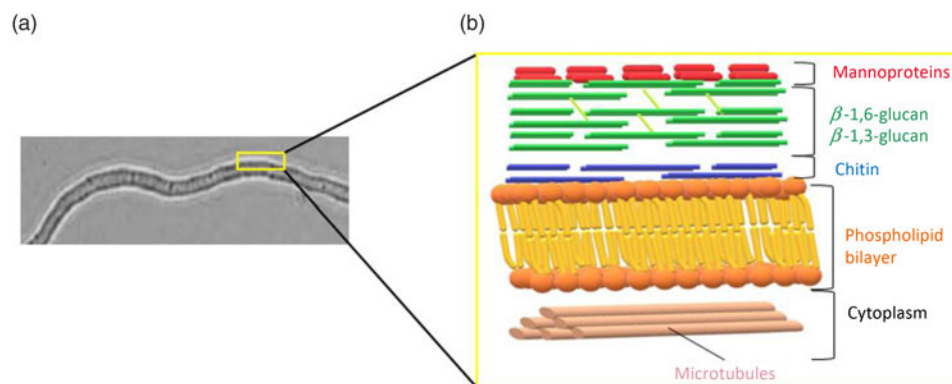


Fig. 1. (a) Fungal filament of *Fusarium venenatum* and (b) simplified schematic representation of the fungal cell wall (constituted by mannoproteins – constituting the protein source, β -1,6-glucan, β -1,3-glucans and chitin – constituting the fibre sources), phospholipid bilayer and cytoplasm including microtubules⁽²³⁾ – protein source. Figure adapted from Wiebe and Fesel & Zuccaro^(24,25).

Table 2. PICOS (patients, intervention, comparator, outcome, study design) criteria for inclusion and exclusion of studies

Criteria	Inclusion	Exclusion
Participants	Humans	Animals
Intervention	Studies assessing mycoprotein intake in any form	No mycoprotein or mycoprotein mixed with other sources of fibre
Comparator		No comparators were excluded
Outcome	Studies that contain a quantifiable measure of glucose, insulin appetite and/or energy intake	Studies that do not contain a quantifiable measure of the outcomes of interest
Study design	Study designs which generate empirical data from randomised controlled trial. Only results analysed statistically will be included	Reviews, conference abstracts, dissertation abstracts, lectures, information pieces and corrigendums were not included Studies were limited to English language up to 3 November 2019

3 November 2019. The following PubMed search algorithm was used: (mycoprotein[All Fields] OR myco-protein[All Fields] OR fungi-derived protein[All Fields] OR quorn[All Fields]) AND (glucose[MeSH Terms] OR A1c[All Fields] OR glycated-haemoglobin[All Fields] OR hba1c [All Fields] OR haemoglobin a [All Fields] OR dysglycemia [All Fields] OR dysglycaemia [All Fields] OR glycaemia[All Fields] OR glycemia [All Fields] OR glycaemic[All Fields] OR glyceimic[All Fields] OR insulin*[All Fields] OR insulin [All Fields] OR fasting [All Fields] OR postprandial [All Fields] OR homeostatic model assessment [All Fields] OR oral glucose tolerance test [All Fields] OR oral glucose tolerance test [All Fields] OR appetite [All Fields] OR energy intake [All Fields] OR food intake [All Fields] OR food behaviour [All Fields] OR food behavior [All Fields] OR eating behaviour [All Fields] OR eating behavior [All Fields] OR hunger [All Fields] OR food consumption [All Fields]).

Also, manual searches of bibliographies of original research and previous reviews were performed, to identify additional relevant articles.

Study selection

All articles identified by the search strategy were reviewed by two reviewers independently (A. C.-M. and A. M. L.). Title and abstract of each group of articles were screened by A. C.-M. and A. M. L. Post-screening, full-text articles indicated as potentially relevant were retrieved and study eligibility was assessed. Study eligibility was conducted, through assessing the title, abstract and full text against the defined inclusion and exclusion criteria (see Table 2). For both screening and assessment of study eligibility, all unclear eligibility of papers was resolved by consulting a third party (E. S. C.). Identified duplicates were removed.

Data extraction and management

Articles deemed eligible for inclusion were assigned to subject categories, reflective of the two outcomes of interest (glycaemic control and energy intake), dependent on the outcome of the article reported. Articles could be assigned to more than one subject category if they reported outcomes relevant to two of the subject categories. Tabulated summaries were derived from the extracted data of articles assigned to the subject category (Tables 3 and 4). The tabulated summary for studies assessing mycoprotein intake on glycaemic control, energy intake and appetite contained the following descriptors:

- Reference – presents the bibliographic reference of the study.
- Participants – presents the characteristics of the population (sample size (*n*), sex, age, BMI, healthy and smoker status).
- Study information – presents the study design (randomised, controlled, blinded, crossover), the test food (form, quantity of mycoprotein, control type and amount of carbohydrate given with the meal) and the protocol of the study.
- Outcome – presents the result of the outcome of interest in the study. For energy intake and appetite, the methods of assessment are described in each.

Risk-of-bias assessment

Studies were assessed for risk of bias by two independent reviewers (A. C. M. and A. M. L.) following the Cochrane Risk of Bias Assessment Tool. The five methodological features assessed were selection, performance, detection, attrition and reporting bias. Studies were classified as ‘high risk’ if they contained methodological flaws that may have influenced the results, ‘low risk’ if the flaw was not deemed to have affected the results and ‘unclear risk’ if not enough information was provided to perform a judgement. Disagreements in the classification were resolved by consulting a third party (E. S. C.).

Data analysis

Demographic data and described outcome values were reported as mean values and standard deviations.

Glycaemic control was defined as blood glucose and insulin AUC or incremental AUC. In addition, for one study⁽²⁹⁾, mean glucose levels (mmol/l) were reported at fasting (0 h), early (0–2 h) and late (2–4 h) postprandial stages. Energy intake was defined as acute energy intake (≤5 h post-ingestion) which included *ad libitum* meal and as short-term energy intake (>5 h post-ingestion) which included food diaries. Energy intake at *ad libitum* was assessed via buffet meal, macronutrient self-selection paradigm and universal eating monitor, and energy intake post-visit was assessed using self-reported food diaries. Appetite was measured via subjective appetite feelings scoring (mm) using a visual analogue scale. When possible, all outcomes were calculated as the percentage of change compared with control.

A statistical meta-analysis of the results was not possible due to the heterogeneity in the reported glycaemic outcomes and the insufficient power of studies assessing the energy intake outcomes measurement. Therefore, for all outcomes, a systematic, narrative approach was conducted according to the Cochrane

Table 3. Summary of the acute randomised trials on glycaemic control (Numbers and percentages; mean values and standard deviations)

References	Participants		Study information			Outcome	
	n	Male/ female	Characteristics (age/BMI/ health/smoking status)	Study design	Test food (form/quantity of mycoprotein/ control type/CHO)	Protocol	Glycaemic and insulinaemic response
(27)	19	4/15	Mean: 22.8 (SD 3.55) years/ mean: 22.74 (SD 2.59) kg/m ² /healthy/not reported	RCT, single- blinded, crossover	Drink/20 g dry weight/energy and macronutrient-matched (except fibre) soya-based protein drink/75 g CHO	Participant fasted overnight. Blood sampling at fasting, 30, 60, 120 min relative to test food	Glucose AUC _{0-60 min} : 9 % decrease with mycoprotein (<i>P</i> < 0.03) Glucose AUC _{0-120 min} : NSSD Insulin AUC _{0-60 min} : 20 % decrease with mycoprotein (<i>P</i> < 0.002) Insulin AUC _{0-120 min} : 13 % decrease with mycoprotein (<i>P</i> < 0.05)
(28)	36	19/17	Mean: 33 (SD 14) years/ mean: 28.1 (SD 2.3) kg/m ² / healthy/non-smokers	RCT, single- blinded, crossover	Solid/44, 88 and 132 g wet weight/energy and macronutrient-matched (except fibre) chicken (22, 44 and 66 g)/25- 30 g CHO	Participant had a standard evening meal, 12 h overnight fast, asked to refrain from alcohol and avoid any strenuous exercise 24 h prior to the study visit Test meal followed by an <i>ad libitum</i> meal at 180 min Blood sampling at -15, 0, 15, 30, 45, 60, 90, 120, 150 and 180 min relative to test food	Glucose iAUC _{0-180 min} : NSSD with 44, 88 and 132 g of mycoprotein v. matched controls Insulin iAUC _{0-180 min} : 9 % (<i>P</i> = 0.024), 12 % (<i>P</i> = 0.001) and 21 % (<i>P</i> < 0.001) decrease with 44, 88 and 132 g of mycoprotein, respectively, v. matched controls
(29)	12	12/0	Mean: 28 (SD 6.92) years/ mean: 26 (SD 3.46) kg/m ² / healthy/non-smokers	RCT, single- blinded, crossover	Drink/20 g (mass-matched), 40 g (protein- matched), 60 g and 80 g wet weight/not energy or other macronutrient-matched milk protein (20 g)/protein:CHO ratio of 23 (in 20 g of milk) and of 4.5 (in 20, 40, 60 and 80 g of mycoprotein)	Participant fasted. Abstained from physical activity and alcohol 48 h prior to the study visit. Blood sampling at -5, 15, 30, 45, 60, 90, 120, 150, 180, 210 and 240 min relative to test food	Mean glucose (mmol/l) (0-120 min): NSSD with any test drink v. fasting levels Mean glucose (mmol/l) (120-240 min): 5 % decrease with 20 g of milk and 4 % reduction with 20 and 40 g of mycoprotein v. fasting levels (<i>P</i> < 0.05) 2 % decrease with 20 g of milk, 20, 40 and 60 g of mycoprotein v. glucose (0- 120 min) (<i>P</i> < 0.05) Insulin AUC _{0-240 min} : 53 % decrease with 20 g of mycoprotein v. mass-matched milk (20 g) (<i>P</i> < 0.05). NSSD with 40 g of mycoprotein v. protein-matched milk (20 g). 70, 77 and 80 % increase with 40, 60 and 80 g of mycoprotein v. 20 g mycoprotein (<i>P</i> < 0.05). NSSD with 40 v. 60 and 80 g and 60 v. 80 g of mycoprotein

CHO, carbohydrate; RCT, randomised controlled trial; NSSD, no statistically significant difference; iAUC, incremental AUC.
*Glucose and insulin AUC are expressed as a percentage change from control food. Age and BMI expressed as mean values and standard deviations.

Table 4. Summary of the acute randomised trials on energy intake and appetite*
(Numbers and percentages; mean values and standard deviations)

References	Participants		Study information			Outcomes				
	<i>n</i>	Male/ female	Characteristics (age, BMI, health and smoking status)	Study design	Test food (form/quantity of mycoprotein/control type/CHO)	Protocol	Energy intake assessment method	Effect on energy intake	Appetite assessment method	Effect on appetite subjective feelings
(30)	13	0/13	Mean: 24.8 (SD 7.9) years, mean: 22.25 (SD 2.50) kg/m ² , healthy, one smoker	RCT, crossover, not blinded	Solid/130 g wet weight/energy and macronutrient-matched (except from fibre) chicken/77–80 g CHO	Participant fasted from midnight. No breakfast was given Test food served at lunchtime	Weighed food diary using digital food balances recorded for 3 d (the day before, on the day, day after study visit)	24 % decrease on the day ($P < 0.01$) and by 16 % on the day after ($P < 0.05$) with mycoprotein	VAS (100 mm) right after the meal and at 60, 120 and 180 min	Decrease by 25 % in the desire to eat at 180 min ($P < 0.05$) and by 39 % in prospective consumption feeling at 180 min ($P < 0.01$) with mycoprotein NSSD in hunger or fullness feelings with mycoprotein
(31)	42	0/42	Mean: 30.14 (SD 9.59) years, mean: 27.42 (SD 1.62) kg/m ² , healthy, non-smokers	RCT, crossover†	Solid/44.3 g wet weight/energy and macronutrient-matched chicken (except fibre) and tofu/63.6–65.3 g CHO	Participant fasted overnight for 12 h and deprived of exercise and alcohol 24 h prior to the study visit Standard breakfast served in the study visit. 4 h later a 220 g pasta meal with test food is served At 20 min later, an <i>ad libitum</i> meal of sandwiches is served and asked to consume as much as wished. 4.5 h later, an <i>ad libitum</i> dinner is served using MSSP	<i>Ad libitum</i> meal at lunch (20 min post-test food ingestion) using UEM <i>Ad libitum</i> meal at dinner (4.5 h post-test food ingestion) using MSSP	12 % decreased intake of total grams of food, energy and all macronutrient at <i>ad libitum</i> , v. chicken, ($P < 0.05$), and similarly to tofu Mycoprotein did not affect the duration of food intake NSSD in energy intake with mycoprotein, chicken or tofu ($P = 0.33$) 18 % (+427 kJ) increase in high fat/high sugar foods with mycoprotein v. tofu ($P < 0.01$), and similarly to chicken (–84 kJ) ($P < 0.01$)	VAS (100 mm) before and after the three meals and at 30, 60, 90, 120, 180 and 240 min after lunch	SSD in time effect. NSSD in treatment effect. SSD in interaction time × treatment for hunger NSSD for subjective appetite AUC

Table 4. (Continued)

References	Participants			Study information			Outcomes			
	<i>n</i>	Male/ female	Characteristics (age, BMI, health and smoking status)	Study design	Test food (form/quantity of mycoprotein/control type/CHO)	Protocol	Energy intake assessment method	Effect on energy intake	Appetite assessment method	Effect on appetite subjective feelings
(28)	36	19/17	Mean: 33 (SD 14) years, mean: 28.1 (SD 2.3) kg/m ² , healthy, non-smokers	RCT, single-blinded, crossover	Solid/44, 88, 132 g wet weight/energy and macronutrient-matched (except fibre) chicken (22, 44 and 66 g)/25–30 g CHO	Participant had a standard evening meal, 12 h overnight fast, asked to refrain from alcohol and avoid any strenuous exercise 24 h prior to the study visit Test food followed by an <i>ad libitum</i> meal at 180 min	<i>Ad libitum</i> meal served at 180 min 3-d food diary (results reported for energy intake over 24 h overall)	8% (–201 kJ) decrease in energy intake with 132 g of mycoprotein v. matched chicken ($P \leq 0.01$). NSSD with 44 or 88 g of mycoprotein v. matched chicken 13% (–1042 kJ) decrease in energy intake with 44 g ($P = 0.047$) and 132 g (almost SSD, $P = 0.083$). NSSD with 88 g of mycoprotein	VAS (100 mm) at –15, 0, 15, 30, 45, 60, 90, 120, 150 and 180 min relative to test food	22% increase in fullness score at 150 and 180 min with 132 g of mycoprotein ($P \leq 0.05$). NSSD in fullness with 44 and 88 g of mycoprotein NSSD in hunger, desire to eat and prospective food intake scores with 44, 88 and 132 g of mycoprotein
(29)	12	12/0	Mean: 28 (SD 6.92) years, mean: 26 (SD 3.46) kg/m ² , healthy, non-smokers	RCT, single-blinded, crossover	Drink/20 g (mass-matched), 40 g (protein-matched), 60 g and 80 g wet weight/not energy or other macronutrient-matched milk protein (20 g)/protein:CHO ratio of 23 (in 20 g of milk) and of 4.5 (in 20, 40, 60 and 80 g of mycoprotein)	Participant fasted. Abstained from physical activity and alcohol 48 h prior to the study visit Test food served	N/A	N/A	VAS (100 mm) at –15, 30, 60, 90, 120, 150, 180, 210 and 240 min relative to test food	From 0 to 120 min, decrease of appetite with 20 g of milk and 20, 40, 60 and 80 g of mycoprotein ($P < 0.05$) compared with baseline levels From 120 to 240 min, increase of appetite with 20 g of milk, 20, 40 and 60 g of mycoprotein ($P < 0.05$) compared with baseline levels NSSD in appetite with 80 g of mycoprotein compared with baseline from 120 to 240 min Statistical analysis against milk protein and other doses of mycoprotein N/A

CHO, carbohydrate; RCT, randomised controlled trial; VAS, visual analogue scale; NSSD, no statistically significant difference; MSSP, macronutrient self-selection paradigm; UEM, universal eating monitor; SSD, statistically significantly different; N/A, not applicable.
* VAS score is expressed as mm. Energy intakes at *ad libitum* and post-24 h are expressed as percentage change in kJ from control food. Age and BMI are expressed as mean values and standard deviations.

† In Williamson *et al.*⁽³¹⁾, blinding is not mentioned, although authors report that meals were prepared to be as equal as possible in palatability and appearance.

handbook for systematic review of interventions⁽³²⁾. Results were synthesised according to outcome measure.

Results

Overview of studies identified

The conducted search strategy yielded a total of twenty-three potentially relevant articles. Of which, eleven duplicates were identified and removed. Of the remaining twelve potentially relevant articles, six were excluded post-abstract screening as they were a review (*n* 1), European Food Safety Authority report (*n* 1), conference paper (*n* 2), an *in vitro* study (*n* 1) and irrelevant outcome measure (*n* 1). A total of six potentially relevant full-text articles were therefore assessed for eligibility, during which one was excluded for not meeting the defined inclusion and exclusion criteria: not being an RCT (*n* 1). A total of five articles were deemed eligible for inclusion and therefore included in the final synthesis (see Fig. 2). All of these studies were of an acute nature (measurements were performed for a minimum of 180 min to a maximum of 72 h post-mycoprotein ingestion).

Risk of bias

Individual studies were judged as having a high, low or unclear risk of bias for the five methodological features measured (see online Supplementary Table S3).

For studies assessing glycaemic control, for the methodological features of random sequence generation (selection bias), the majority of the studies (66%) were judged as having an unclear risk of bias, whereas the remaining 33% was given a low risk of bias. For the allocation concealment (selection bias), all studies (100%) had an unclear risk of bias. For the method of blinding (performance bias), 100% of the studies had a low risk of bias. For the blinding of the outcome assessment (detection bias), 100% of the studies had an unclear risk of bias. For the attrition bias, all studies (100%) had a high risk of bias. Selective reporting bias was unclear for all studies (100%).

For studies assessing energy intake, all studies (100%) were judged as having an unclear risk for both selection bias (random sequence generation and allocation concealment), a low (33%), high (33%) and unclear (33%) risk was given for both performance bias and detection bias, a high risk was given for attrition bias to all studies (100%) and an unclear risk for selective reporting bias was given for all studies (100%).

For studies assessing both outcomes (glycaemic control and energy intake), in the methodological features for selection bias (random sequence generation), half of the studies (50%) were judged as having a low and unclear risk of bias. For the selection bias (allocation concealment), all studies (100%) had an unclear risk of bias. For the performance bias, all of the studies (100%) had a low risk of bias. For the detection bias, 100% of the studies had an unclear risk of bias. For the attrition bias, all studies

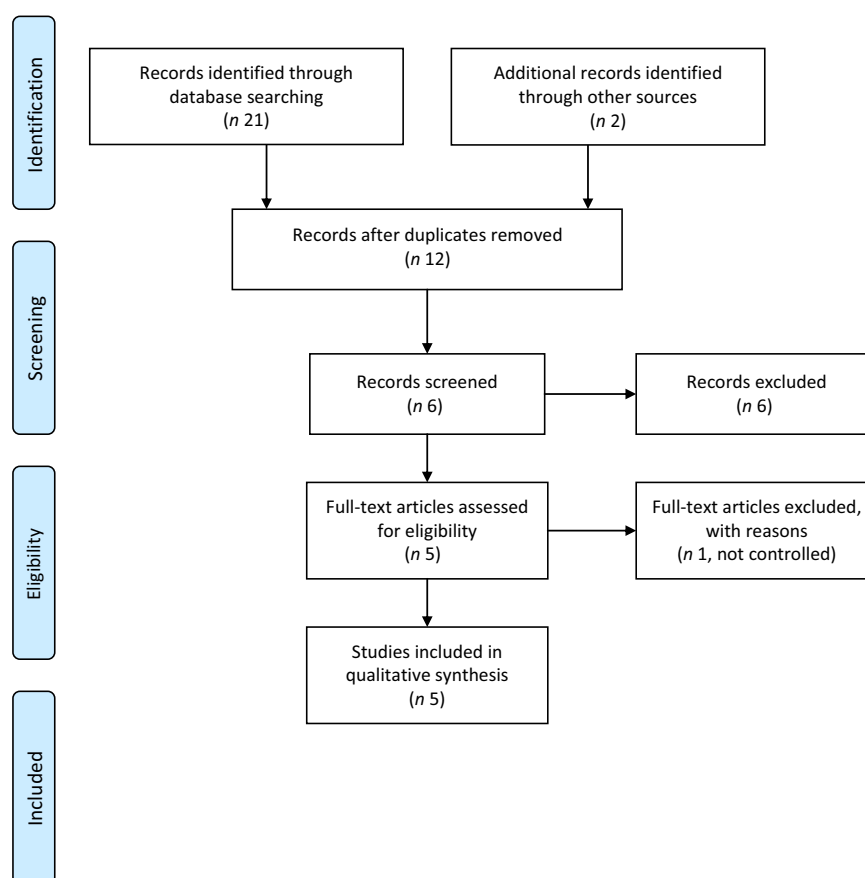


Fig. 2. Preferred Reporting Items for Systematic Reviews and Meta-analysis flow diagram of the literature search and screening process.

(100%) were given a high risk of bias. Selective reporting bias was unclear for all studies (100%).

Studies on glycaemic control

Table 3 shows the effect of mycoprotein on glycaemic control. In total, three randomised controlled and crossover trials assessed glycaemic control. Two studies^(28,29) were conducted in healthy (no chronic disease such as type 2 diabetes), overweight (BMI 25–29.9 kg/m²) and obese (BMI ≥ 30 kg/m²) adults and one study⁽²⁷⁾ with healthy lean (BMI 18.5–24.9 kg/m²) adults. Two studies were conducted using mycoprotein as liquid^(27,29), whereas one used mycoprotein as solid⁽²⁸⁾. The amounts of mycoprotein ranged from 20 to 132 g. The controls used were chicken⁽²⁸⁾, soya-based protein⁽²⁷⁾ and milk protein⁽²⁹⁾ drink. All three studies assessed insulin AUC and two studies assessed glucose AUC^(27,28). One study assessed mean glucose levels at fasting, early (0–2 h) and late (2–4 h) postprandial state⁽²⁹⁾.

Studies on energy intake and appetite

Table 4 shows the effect of mycoprotein on energy intake and appetite. In total, four randomised controlled and crossover trials assessed energy intake and appetite. All studies were conducted in healthy humans of which three studies^(28,29,31) included adults with a BMI ≥ 25 kg/m² and one study⁽³⁰⁾ with a BMI < 25 kg/m². Three studies were conducted using solid mycoprotein^(28,30,31) and one as liquid⁽²⁹⁾. The amounts used ranged from 44 to 132 g of mycoprotein. The controls used were chicken in two studies^(28,30), milk protein in one study⁽²⁹⁾ and both chicken and tofu in one study⁽³¹⁾. Two studies^(28,31) measured acute energy intake (<5 h post-ingestion) via *ad libitum* intake, of which one study used universal eating monitor and macronutrient self-selection paradigm⁽³¹⁾ and one study using a buffet meal⁽²⁸⁾. Two studies measured energy intake post-24 h using 3-d food diaries^(28,30). All four studies explored appetite feelings using subjective appetite scoring via visual analogue scale rating some of the following items: appetite, fullness, prospective food intake, hunger and desire to eat^(28–31). All studies assessed feelings of nausea and eating behaviour questionnaires were completed by participants in three studies^(28,30,31).

Discussion

Low intake of total dietary fibre increases the relative risk of developing non-communicable diseases such as type 2 diabetes⁽³³⁾, and high intake of animal-derived protein is also associated with type 2 diabetes risk⁽³⁴⁾. Therefore, strategies to increase dietary fibre and reduce animal-derived protein are of growing interest. Mycoprotein constitutes a food rich in both dietary fibre and non-animal-derived protein which is being increasingly consumed in the diet of many cultures; however, its effects on blood glucose levels and energy intake have not yet been fully established. This is the first systematic review of RCT investigating the effects of mycoprotein on glycaemia, insulinaemia and energy intake in humans. Overall, five RCT evaluating the effects of mycoprotein on glycaemia, energy intake/appetite or both were identified.

Effect of mycoprotein on glycaemic control

Three studies reported the effects of mycoprotein on glycaemia and insulinaemia (Table 3), of which one was performed in healthy lean adults⁽²⁷⁾ and two in healthy overweight and obese^(28,29).

Glycaemia

Blood glucose AUC following the intake of a 20 g mycoprotein drink showed a reduction in the first 60 min but not overall (AUC_{0–120 min}) in healthy lean adults, compared with energy and macronutrient-matched soya-based protein drink⁽²⁷⁾. A similar experiment in healthy overweight and obese adults showed no differences in blood glucose incremental AUC_{0–180} following the intake of mycoprotein meal at any dose (44, 88 and 132 g) compared with an energy and macronutrient-matched chicken meal⁽²⁸⁾. These similar effects on blood glucose are interesting as both studies are conducted in different populations (lean *v.* overweight and obese) with varying doses of mycoprotein. When different doses of mycoprotein drink are compared over time, mean glucose levels over the first interval (0–120 min) are unchanged relative to baseline levels but are decreased in the second interval (120–240 min) relative to the first interval (0–120 min) with 20, 40 and 60 g of mycoprotein. However, this decrease during the second interval (relative to both baseline and the first interval) is also observed following the ingestion of 20 g of milk protein drink⁽²⁹⁾.

Insulinaemia

Insulin AUC was decreased with a drink containing 20 g of mycoprotein at the first 60 min but not overall (AUC_{0–120 min}) in healthy lean individuals compared with a soya-based protein drink⁽²⁷⁾. A similar effect in insulin incremental AUC_{0–180} was shown in overweight and obese individuals when 44, 88 and 132 g of mycoprotein were compared with an energy and macronutrient-matched chicken⁽²⁸⁾. These findings, coupled with the observations on blood glucose, suggest that mycoprotein may increase peripheral insulin sensitivity. This is supported by the work of Bottin and colleagues who reported an increased Matsuda index (a measure of insulin sensitivity with higher values indicating improved sensitivity) and reduced insulinogenic index (a measure of insulin output with lower values indicating reduced output) of mycoprotein compared with intake of energy and macronutrient-matched chicken⁽²⁸⁾. This study is of particular importance as it was performed in overweight and obese individuals, a population that is at greater risk of developing non-communicable diseases in which hyperglycaemia and hyperinsulinaemia are implicated, such as type 2 diabetes⁽³⁵⁾ and CVD⁽³⁶⁾.

The effect of increasing doses (20, 40, 60 and 80 g) of mycoprotein on insulin AUC_{0–240} was compared with a milk protein drink, in which 40 g of mycoprotein drink (containing 18 g of protein) induced a similar insulin AUC_{0–240} response to 20 g of the milk protein drink (containing 16 g of protein), suggesting that these protein sources produce similar effects on insulinaemia when matched for protein content. Doses of 40, 60 and 80 g of mycoprotein increased insulin AUC_{0–240} in a dose-dependent manner, in which these three doses were

significantly different from 20 g of mycoprotein. This response is expected as the insulinotropic effect of dietary protein is proportional to the dose⁽³⁷⁾.

In summary, the effects of mycoprotein on glycaemia are unclear. However, the data suggest that the acute intake of mycoprotein may decrease insulin output in healthy lean and overweight adults. Such conclusions, however, are limited, given they are drawn from a limited number (n 3) of highly heterogeneous acute studies. Further understanding and validation of the acute effects of mycoprotein on glycaemia coupled with translation into real-world settings via well-controlled studies investigating chronic effects are required.

Possible mechanisms underlying the effect of mycoprotein on glycaemic control

The mechanisms involved in the effects of mycoprotein on glycaemia and insulinaemia may be due to a combination of different factors, acting independently or in a concerted manner.

A delay in gastric emptying is often attributed to the reduction in blood glucose observed following protein⁽¹⁷⁾ and fibre⁽³⁸⁾ ingestion. Nevertheless, 132 g of mycoprotein showed no effect on gastric emptying compared with energy and macronutrient-matched chicken⁽²⁸⁾. It should, however, be noted that the methodology (paracetamol) employed in this study to measure gastric emptying when solid foods are consumed is not considered suitable. This is because paracetamol follows a liquid-phase gastric emptying⁽³⁹⁾, and liquid and solid phases empty at different rates and in patterns⁽⁴⁰⁾; therefore, a better methodology should be employed to measure gastric emptying in solid mycoprotein. Thus, an effect of mycoprotein on gastric emptying cannot yet be ruled out.

It is possible that characteristics inherent to mycoprotein (e.g. hyphae, protein and fibre composition) modify the rate of gastric emptying. It has been hypothesised that mycoprotein could delay gastric emptying via the conversion of chitin to its soluble form (namely chitosan) via alkaline deacetylation, although this remains speculative as this process has only been demonstrated *in vitro*⁽⁴¹⁾. Other mechanisms, such as the secretion of GLP-1 and PYY (two hormones that are involved in gastric emptying via activation of the ileal brake), are also possible. However, Bottin *et al.* showed no significant differences in neither GLP-1 nor PYY following 132 g of acute mycoprotein relative to energy and macronutrient-matched chicken⁽²⁸⁾.

Lastly, the protein characteristics of mycoprotein may be responsible – at least in part – for its effect on postprandial insulin concentrations. Mycoprotein possesses a lower insulinotropic amino acid profile as well as a lower 120-min postprandial amino acid appearance relative to protein-matched milk⁽²⁹⁾ and therefore may explain the reduction in insulin output reported in the literature^(27,28).

In summary, the mechanisms underpinning the blood glucose and insulin response of mycoprotein intake are largely unclear. Mechanisms such as altered gastric emptying and the secretion of gastrointestinal hormones warrant further investigation.

Effect of mycoprotein on energy intake

Four studies have reported the acute effects of mycoprotein on energy intake (Table 4) in healthy lean, overweight and obese

humans, with two studies observing a decrease in energy intake at an *ad libitum* meal^(28,31), two in post-24 h energy intake^(28,30) and one in energy intake during the day⁽³⁰⁾. All studies assessed participant's eating behaviour at screening to exclude abnormal responses.

Acute energy intake

Acute energy intake (≤ 5 h post-ingestion) at a single *ad libitum* meal was measured in healthy overweight and obese individuals following mycoprotein ingestion and consistently showed an overall reduction relative to energy and macronutrient-matched protein sources.

In healthy overweight and obese individuals, a reduction of *ad libitum* energy intake by 12 % (167 kJ) was achieved 20 min following 44.3 g of mycoprotein ingestion compared with energy and macronutrient-matched chicken, and similarly to energy and macronutrient-matched tofu⁽³¹⁾. In this study, a second *ad libitum* meal served 4.5 h post-ingestion showed no differences in energy intake with mycoprotein compared with energy and macronutrient-matched chicken and tofu. This suggests that intake of mycoprotein did not induce a compensatory response in energy intake at 4.5 h despite a reduced ingestion post-20 min. Interestingly, food intake using the macronutrient self-selection paradigm showed that mycoprotein promoted an increased intake of high fat/high sugar foods compared with energy and macronutrient-matched tofu⁽³¹⁾. The magnitude of this effect was however not reported by the authors, and therefore, the potential implications of this finding are difficult to evaluate.

In a more recent study with overweight and obese individuals⁽²⁸⁾, Bottin *et al.* tested the effect of 44 and 88 g of mycoprotein on energy intake at an *ad libitum* meal 180 min post-ingestion relative to energy and macronutrient-matched chicken, reporting no differences between protein sources. However, when 132 g of mycoprotein was tested, an 8 % (201 kJ) reduction in *ad libitum* energy intake was observed when compared with energy and macronutrient-matched chicken.

The differences in the *ad libitum* energy intake reduction observed between the two studies could be related to differences in the mycoprotein dose and inter-meal interval employed (i.e. the time between protein ingestion and presentation of the *ad libitum* meal). However, the small number of studies and the heterogeneous methods employed prevents any definitive conclusions from being made.

Short-term energy intake

Short-term (> 5 h post-ingestion) energy intake was assessed in both healthy lean⁽³⁰⁾, overweight and obese population⁽²⁸⁾, showing an overall decrease in energy intake relative to other energy and macronutrient-matched protein sources. In healthy lean individuals, 130 g of mycoprotein reduced energy intake by 24 % (987 kJ) on the day and by 16 % (1180 kJ) on the next day, relative to energy and macronutrient-matched chicken⁽³⁰⁾. This suggests that mycoprotein did not only induce a compensatory eating response on the day after but also decreased energy intake during the time measured (2 d). Foods rich in dietary fibre and/or protein have been shown to acutely and

chronically decrease energy intake^(11,42–44). Considering the literature on other foods high in fibre and/or protein, and the acute data on mycoprotein, we could hypothesise that chronic intake of mycoprotein results in a net reduction in energy intake, although this remains to be investigated.

In a separate study, healthy overweight and obese people showed a smaller decrease in energy intake (13%; 1042 kJ) on the next day following 44 g of mycoprotein compared with energy and protein-matched chicken, even though 88 and 132 g of mycoprotein did not significantly decrease energy intake⁽²⁸⁾. This difference in energy intake is possibly due to the heterogeneity of the studies (i.e. different BMI, proportion males/females that could have affected appetite feelings due to menses) or due to the reporting method used in one study⁽²⁸⁾ using self-reported food diaries (*v.* weighted food diaries using digital scales) whose validity has been questioned⁽⁴⁵⁾.

In summary, the limited data suggest that acute mycoprotein may reduce energy intake at an *ad libitum* meal and over the short-term (>5 h post-ingestion) in healthy lean, overweight and obese individuals. Such conclusions, however, are limited, given they are drawn from a limited number (*n* 4) of highly heterogeneous acute studies. Further understanding and validation of the acute effects of mycoprotein on energy intake coupled with translation into real-world settings via well-controlled studies investigating the chronic effects are required.

Possible mechanisms underlying the effect of mycoprotein on energy intake

Different mechanisms, acting separately or together, may have been involved in the effect of mycoprotein on energy intake.

Subjective appetite has been demonstrated to correlate with subsequent energy intake⁽⁴⁶⁾. Mycoprotein showed a decrease in energy intake at an *ad libitum* meal following a decrease in subjective appetite^(28,29). Furthermore, this reduction in subjective appetite and energy intake may be mediated by changes in gastrointestinal hormones related to satiety. Despite mycoprotein showing no differences in GLP-1 and PYY concentrations compared with energy and macronutrient-matched chicken⁽²⁸⁾, the role of other appetite-regulating gut hormones (e.g. cholecystokinin or ghrelin) has not been investigated. Metabolomics analysis also indicates that mycoprotein intake increases the presence of guanidine acetic acid and β -hydroxybutyrate, which are known to suppress appetite via direct interaction with the hypothalamus^(28,47–49). Additionally, while a reduction in gastric emptying and/or transit time correlates with appetite⁽¹⁴⁾, the effect of mycoprotein on gastric emptying is unclear. At present, only one study has measured gastric emptying following mycoprotein ingestion, showing no effect compared with energy and macronutrient-matched chicken⁽²⁸⁾. However, as previously mentioned, the paracetamol method is not an appropriate method to measure gastric emptying in solid foods. In support of this, the presence of insoluble fibre within mycoprotein (of which certain types can increase gut transit time⁽⁵⁰⁾), as well as data showing a steady plasma amino acid appearance following mycoprotein intake⁽²⁹⁾ (compared with an acute appearance with protein-matched milk protein), suggests that mycoprotein may decrease the rate of gastric emptying.

The effects of mycoprotein on short-term (>5 h post-ingestion) energy reduction could be in part due to fibre fermentation by the resident gut microbiota and the subsequent production of the SCFA acetate, butyrate and propionate. A recent study using *in vitro* batch culture fermentation with human faeces showed that mycoprotein fibre increased the production of propionate, without affecting acetate or butyrate levels⁽⁵¹⁾. Propionate has been shown to increase GLP-1 and PYY production and to decrease acute energy intake in overweight humans⁽⁵²⁾. Therefore, it is plausible that the intake of mycoprotein in humans may have stimulated similar pathways to induce positive effects on appetite and ultimately energy intake.

Although these are all conceivable possibilities, the evidence is limited and hence further research is needed to uncover the mechanisms relating to mycoprotein-induced decreases in subjective appetite, acute and short-term energy intake.

Conclusion

Mycoprotein is a food high in both dietary fibre and non-animal-derived protein whose consumption is increasing across the world. This systematic review reports that the acute effects of mycoprotein on glycaemia are currently unclear, but likely decrease insulinaemia and energy intake (at an *ad libitum* meal and post-24 h) in healthy lean and overweight and obese humans acutely. The mechanisms underpinning these effects are not yet elucidated and should be further explored. In addition to clarifying the acute effects of mycoprotein on glycaemia, the translation of these findings into real-world settings via well-controlled chronic investigations is a logical next step.

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Supplementary material

For supplementary material referred to in this article, please visit <https://doi.org/10.1017/S0007114520000756>

References

- World Health Organization (2018) *Noncommunicable Diseases Country Profiles 2018*. WHO Global Report. Geneva: WHO.
- Lim SS, Vos T, Flaxman AD, *et al.* (2012) A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* **380**, 2224–2260.
- World Health Organization (2006) *Definition and Diagnosis of Diabetes Mellitus and Intermediate Hyperglycaemia: Report of a WHO/IDF Consultation*. WHO Global Report. Geneva: WHO.
- Bray GA (1978) Definition, measurement, and classification of the syndromes of obesity. *Int J Obes* **2**, 99–112.
- Murea M, Ma L & Freedman BI (2012) Genetic and environmental factors associated with type 2 diabetes and diabetic vascular complications. *Rev Diabet Stud* **9**, 6.
- Ajala O, English P & Pinkney J (2013) Systematic review and meta-analysis of different dietary approaches to the management of type 2 diabetes. *Am J Clin Nutr* **97**, 505–516.
- Chutkan R, Fahey G, Wright WL, *et al.* (2012) Viscous versus nonviscous soluble fiber supplements: mechanisms and evidence for fiber-specific health benefits. *J Am Assoc Nurse Pract* **24**, 476–487.
- Dhital S, Gidley MJ & Warren FJ (2015) Inhibition of α -amylase activity by cellulose: kinetic analysis and nutritional implications. *Carbohydr Polym* **123**, 305–312.
- 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. *Appl Physiol Nutr Metab* **35**, 9–16.
- Wong JM, De Souza R, Kendall CW, *et al.* (2006) Colonic health: fermentation and short chain fatty acids. *J Clin Gastroenterol* **40**, 235–243.
- Wanders AJ, van den Borne JJ, de Graaf C, *et al.* (2011) Effects of dietary fibre on subjective appetite, energy intake and body weight: a systematic review of randomized controlled trials. *Obes Rev* **12**, 724–739.
- Slavin J & Green H (2007) Dietary fibre and satiety. *Nutr Bull* **32**, 32–42.
- De Graaf C, Blom WA, Smeets PA, *et al.* (2004) Biomarkers of satiation and satiety. *Am J Clin Nutr* **79**, 946–961.
- Bergmann J, Chassany O, Petit A, *et al.* (1992) Correlation between echographic gastric emptying and appetite: influence of psyllium. *Gut* **33**, 1042–1043.
- Byrne C, Chambers E, Morrison D, *et al.* (2015) The role of short chain fatty acids in appetite regulation and energy homeostasis. *Int J Obes* **39**, 1331.
- Nuttall FQ, Mooradian AD, Gannon MC, *et al.* (1984) Effect of protein ingestion on the glucose and insulin response to a standardized oral glucose load. *Diabetes Care* **7**, 465–470.
- Ma J, Stevens JE, Cukier K, *et al.* (2009) Effects of a protein pre-load on gastric emptying, glycemia, and gut hormones after a carbohydrate meal in diet-controlled type 2 diabetes. *Diabetes Care* **32**, 1600–1602.
- Lindgren O, Pacini G, Tura A, *et al.* (2015) Incretin effect after oral amino acid ingestion in humans. *J Clin Endocrinol Metab* **100**, 1172–6.
- Mithieux G, Misery P, Magnan C, *et al.* (2005) Portal sensing of intestinal gluconeogenesis is a mechanistic link in the diminution of food intake induced by diet protein. *Cell Metab* **2**, 321–329.
- Rampone A & Reynolds P (1991) Food intake regulation by diet-induced thermogenesis. *Med Hypotheses* **34**, 7–12.
- Belza A, Ritz C, Sørensen MQ, *et al.* (2013) Contribution of gastroenteropancreatic appetite hormones to protein-induced satiety. *Am J Clin Nutr* **97**, 980–989.
- Finnigan TJA, Needham L & Abbott CM (2016) Mycoprotein: a healthy new protein with a low environmental impact. In *Sustainable Proteins*, pp. 301–326 [S Nadathur, JPD Wanasundara and L Scanlin, editors]. Cambridge, MA: Academic Press.
- Riquelme M, Aguirre J, Bartnicki-García S, *et al.* (2018) Fungal morphogenesis, from the polarized growth of hyphae to complex reproduction and infection structures. *Microbiol Mol Biol Rev* **82**, e00068–00017.
- Wiebe M (2002) Myco-protein from *Fusarium venenatum*: a well-established product for human consumption. *Appl Microbiol Biotechnol* **58**, 421–427.
- Fesel PH & Zuccaro A (2016) β -glucan: crucial component of the fungal cell wall and elusive MAMP in plants. *Fungal Genet Biol* **90**, 53–60.
- Moher D, Liberati A, Tetzlaff J, *et al.* (2009) Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Ann Intern Med* **151**, 264–269.
- Turnbull WH & Ward T (1995) Mycoprotein reduces glycemia and insulinemia when taken with an oral-glucose-tolerance test. *Am J Clin Nutr* **61**, 135–140.
- 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. *Br J Nutr* **116**, 360–374.
- 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. *Br J Nutr* **118**, 673–685.
- Turnbull WH, Walton J & Leeds AR (1993) Acute effects of mycoprotein on subsequent energy intake and appetite variables. *Am J Clin Nutr* **58**, 507–512.
- Williamson DA, Geiselman PJ, Lovejoy J, *et al.* (2006) Effects of consuming mycoprotein, tofu or chicken upon subsequent eating behaviour, hunger and safety. *Appetite* **46**, 41–48.
- Higgins JP (2008) *Cochrane Handbook for Systematic Reviews of Interventions* version 5.0. 1. The Cochrane Collaboration. <http://www.cochrane-handbook.org>.
- Reynolds A, Mann J, Cummings J, *et al.* (2019) Carbohydrate quality and human health: a series of systematic reviews and meta-analyses. *Lancet* **393**, 434–445.
- Sluijs I, Beulens JW, Spijkerman AM, *et al.* (2010) Dietary intake of total, animal, and vegetable protein and risk of type 2 diabetes in the European Prospective Investigation into Cancer and Nutrition (EPIC)-NL study. *Diabetes Care* **33**, 43–48.
- Zheng Y, Ley SH & Hu FB (2018) Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. *Nature Reviews Endocrinology* **14**, 88.
- Coutinho M, Gerstein HC, Wang Y, *et al.* (1999) The relationship between glucose and incident cardiovascular events. A meta-regression analysis of published data from 20 studies of 95,783 individuals followed for 12.4 years. *Diabetes Care* **22**, 233–240.
- Gunnerud U, Östman E & Björck I (2013) Effects of whey proteins on glycaemia and insulinaemia to an oral glucose

- load in healthy adults; a dose–response study. *Eur J Clin Nutr* **67**, 749.
38. Jenkins DJ, Jenkins AL (1985) Dietary fiber and the glycemic response. *Proc Soc Exp Biol Med* **180**, 422–431.
 39. Wagner JG & Nelson E (1964) Kinetic analysis of blood levels and urinary excretion in the absorptive phase after single doses of drug. *J Pharm Sci* **53**, 1392–1403.
 40. Horowitz M, Collins PJ & Shearman DJ (1985) Disorders of gastric emptying in humans and the use of radionuclide techniques. *Arch Intern Med* **145**, 1467–1472.
 41. Turnbull WH (1998) Myco-protein as a functional food: effects on lipemia, glycemia and appetite variables. *16th International Congress of Nutrition*, pp. 249–251.
 42. Halton TL & Hu FB (2004) The effects of high protein diets on thermogenesis, satiety and weight loss: a critical review. *J Am Coll Nutr* **23**, 373–385.
 43. Lluch A, Hanet-Geisen N, Salah S, *et al.* (2010) Short-term appetite-reducing effects of a low-fat dairy product enriched with protein and fibre. *Food Qual Preference* **21**, 402–409.
 44. Lee YP, Mori TA, Sipsas S, *et al.* (2006) Lupin-enriched bread increases satiety and reduces energy intake acutely. *Am J Clin Nutr* **84**, 975–980.
 45. Wolper C, Heshka S & Heymsfield SB (1995) Measuring food intake: an overview. In *Handbook of Assessment Methods for Eating Behaviors and Weight-related Problems: Measures, Theory, and Research*, pp. 215–240 [DB Allison, editor]. Thousand Oaks, CA: Sage Publications, Inc.
 46. Parker BA, Sturm K, MacIntosh C, *et al.* (2004) Relation between food intake and visual analogue scale ratings of appetite and other sensations in healthy older and young subjects. *Eur J Clin Nutr* **58**, 212.
 47. Jordi J, Herzog B, Camargo SM, *et al.* (2013) Specific amino acids inhibit food intake via the area postrema or vagal afferents. *J Physiol* **591**, 5611–5621.
 48. Alamshah A, McGavigan AK, Spreckley E, *et al.* (2016) L-Arginine promotes gut hormone release and reduces food intake in rodents. *Diabetes Obes Metab* **18**, 508–518.
 49. Stubbs BJ, Cox PJ, Evans RD, *et al.* (2018) A ketone ester drink lowers human ghrelin and appetite. *Obesity* **26**, 269–273.
 50. Burkitt DP, Walker A & Painter NS (1972) Effect of dietary fibre on stools and transit-times, and its role in the causation of disease. *Lancet* **300**, 1408–1411.
 51. 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.
 52. Chambers ES, Viardot A, Psichas A, *et al.* (2015) Effects of targeted delivery of propionate to the human colon on appetite regulation, body weight maintenance and adiposity in overweight adults. *Gut* **64**, 1744–1754.