BMJ Open

Impact of Botanical Fermented Foods on Metabolic Biomarkers and Gut Microbiota in Adults with Metabolic Syndrome and Type 2 Diabetes: A Systematic Review Protocol

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-029242
Article Type:	Protocol
Date Submitted by the Author:	20-Jan-2019
Complete List of Authors:	Chan, Miin; University of Melbourne, School of Agriculture and Food Baxter, Helen; Austin Health, Clinical Library Larsen, Nadja; Kobenhavns Universitet, Department of Food Science Jespersen, Lene; Kobenhavns Universitet, Department of Food Science Ekinci , Elif ; University of Melbourne Department of Medicine Austin Health, Howell, Kate; University of Melbourne, School of Agriculture and Food
Keywords:	General diabetes < DIABETES & ENDOCRINOLOGY, Microbiology < BASIC SCIENCES, NUTRITION & DIETETICS

Impact of Botanical Fermented Foods on Metabolic Biomarkers and Gut Microbiota in Adults with Metabolic Syndrome and Type 2 Diabetes: A Systematic Review Protocol

Miin Chan, Helen Baxter, Nadja Larsen, Lene Jespersen, Elif I. I. Ekinci, Kate Howell

CORRESPONDING AUTHOR

Kate Howell

School of Agriculture and Food, Faculty of Veterinary and Agricultural Sciences

Level 1, Room 133, FVAS Building

University of Melbourne, Melbourne, VIC, Australia

ORCID ID: https://orcid.org/0000-0001-6498-0472

Email: khowell@unimelb.edu.au Phone: +613 9035 3119

Miin Chan

School of Agriculture and Food, Faculty of Veterinary and Agricultural Sciences

University of Melbourne, Melbourne, VIC, Australia

ORCID ID: https://orcid.org/0000-0002-4695-7057

Email: ktmchan@unimelb.edu.au Mobile: +61 490 367 714

Helen Baxter

Austin Health Sciences Library

Austin Hospital, Melbourne, VIC, Australia

ORCID ID: https://orcid.org/0000-0001-6579-8584

Email: Helen.BAXTER@austin.org.au Phone: +613 9496 6949

Nadja Larsen

Department of Food Science, Faculty of Science

University of Copenhagen, Frederiksberg, Denmark

ORCID ID: https://orcid.org/0000-0002-5899-4883

Email: nf@food.ku.dk Phone: +45 353 33235

Lene Jespersen

Department of Food Science, Faculty of Science

University of Copenhagen, Frederiksberg, Denmark

ORCID ID: https://orcid.org/0000-0002-2555-5948

Email: lj@food.ku.dk Phone: +45 3533 3230

Elif I. I. Ekinci

Department of Endocrinology, Austin Health, Melbourne, VIC, Australia

Department of Medicine, Austin Health, University of Melbourne, Melbourne, VIC, Australia

ORCID ID: https://orcid.org/0000-0003-2372-395X

Email: elif.ekinci@unimelb.edu.au Phone: +613 9496 2250

Word Count: 1736

ABSTRACT

Introduction: Dysfunctional gut microbiota is a common finding in patients with Metabolic Syndrome (MetS) and Type 2 Diabetes Mellitus (T2DM). Recent clinical trials have assessed whether botanical fermented foods (BFF) have beneficial effects on metabolic biomarkers, inflammatory markers and gut microbiota. The aim of this review is to critically evaluate all randomised controlled trials of BFF for evidence of impact on the outcome measures of these disease states.

Methods and analysis: Four electronic databases (Embase, MEDLINE, CENTRAL and Google Scholar) as well as the grey literature will be searched from inception to present without language or publication status restrictions applied. Eligible randomised controlled trials (RCT) which have enrolled adult participants with T2DM, any MetS components or combinations of these components, treated prophylactically or therapeutically with any botanical fermented food intervention, compared with a control group (no intervention, placebo or active control) will be assessed. Primary outcomes are related to the target conditions, including metabolic biomarkers, inflammatory markers and gut microbiota composition/ function. Using Covidence, two independent investigators will conduct title and abstract screening, followed by full-text screening to identify appropriate studies. Methodological quality of the trials will be assessed using the Cochrane risk of bias (ROB) assessment tool. Findings will be summarised with a narrative analysis of the differences between included studies. The review will follow the Preferred Reporting Items for Systematic Reviews and Meta-Analyses reporting guidelines for systematic review protocols, PRISMA-P. A meta-analysis will be conducted if sufficient data is obtained.

Ethics and dissemination: Ethical approval is not required as primary data will not be collected. Results will be disseminated through peer-reviewed publication, conference presentations and press.

Systematic Review Registration: PROSPERO 2018 CRD42018117766

Keywords: metabolic syndrome, type 2 diabetes, hypertension, cardiovascular disease, obesity, inflammation, gut microbiota, fermented food, clinical trial

ARTICLE SUMMARY

Strengths and limitations of this study

• Although there have been recent reviews (2017, 2018) of the health benefits of fermented foods, no systematic review of BFF for adults with T2DM, MetS and its components has been undertaken.

• As evidence mounts that gut-mediated chronic inflammation is a major driver of increasingly prevalent non-communicable metabolic diseases, the results of this systematic review may assist in the creation of cheap, dietary, functional therapies for the prevention and management of these conditions.

• Our comprehensive, objective review also aims to determine the research gaps and further questions to be addressed in this area of study and its results will assist in the design and execution of appropriate clinical intervention trials.

• Despite a robust, systematic methodology, this review is limited to peer-reviewed literature exploring BFF for identified patient populations, and as such may not capture all available evidence.

• Significant heterogeneity is anticipated in this systematic review due to the vast differences between BFF (ingredients, bioactive compounds, involved microorganisms, production techniques), trial administration issues and the variety of outcome measures.

1. INTRODUCTION

Diet alters the structure and activity of human gut microbiota,[1] with direct effects on host health.[2] Shifts in gut microbiota have been linked to host metabolism dysfunction and low-grade chronic inflammation; these disorders of metabolism are implicated in the development of obesity, Metabolic Syndrome (MetS) and Type 2 Diabetes Mellitus (T2DM).[3-5] MetS affects more than 25% of all adults globally;[6] T2DM is the world's most prevalent endocrine disorder.[5] As such, cheap, effective dietary therapies are of great interest to researchers, clinicians and government bodies.

Fermented products of plant origin, or botanical fermented foods (BFF), are a microbially-diverse part of global traditional diets.[7] These indigenous traditional fermented foods (e.g. kimchi, sauerkraut, tibicos, tempeh, miso, kombucha, natto and fermented olives), as well as newer functional fermented products such as red yeast rice and functional kimchi, have been recognised as having beneficial effects on human health.[8, 9] The diversity of such ferments and their ingredients means they are abundant in microbiota-accessible carbohydrates, food-associated microorganisms such as lactic acid bacteria, bacterial components and metabolites, bioactive compounds such as polyphenols, vitamins and minerals.[9, 10] Besides the basic nutritional properties of BFF ingredients, fermentation itself may confer additional health benefits through interactions between the host and consumed live microorganisms (probiotics), or through the ingestion of food-associated microbe-produced metabolites (biogenics) and other products of fermentation.[11] These include secondary phytochemicals, bioactive peptides and other compounds which have been shown to affect blood pressure, immune responses, antioxidant activity, insulin sensitivity, fasting and postprandial blood glucose.[12] The action of human gut microbiota on fermented food components in the intestinal lumen also produces health-promoting compounds, such as fermentation of microbiota-accessible carbohydrates into short chain fatty acids.[8] Though

relatively sparse compared to studies on fermented dairy products for human health, recent clinical trials of BFF support their role in the prevention and treatment of non-communicable chronic diseases,[9] including obesity, prediabetes, type 2 diabetes, lipid dysfunction, cardiovascular disease, inflammatory bowel disorders and mental health disorders.[8]

Although a recent review of BFF in relation to non-communicable diseases has been conducted,[9] as well as others critically reviewing the health benefits of fermented foods,[8] no systematic review of the impact of BFF on MetS and T2DM has been undertaken. The aim is to systematically review randomised controlled trials for evidence of the impact of BFF compared to control on gut microbiota and metabolic biomarkers in adult human subjects suffering from components of MetS or T2DM. Analysis of the pooled data from these trials will elucidate the overall effect.

2. METHODS

2.1 Eligibility Criteria

2.1.1 Types of studies

All human randomised controlled trials in any language will be included.

2.1.2 Type of participants

This review will include trials with adult participants suffering from T2DM, or any MetS components or combinations of these components (e.g. obesity, hypertension, lipid dysfunction, glucose intolerance/ prediabetes, Non-Alcoholic Fatty Liver Disease). Participants in the sample will have been randomly allocated into intervention (BFF) and placebo, no intervention or active control groups.

2.1.3 Type of interventions

This review will include studies that evaluate traditional BFF (e.g. kimchi, sauerkraut, tibicos, tempeh, kombucha), as well as modern functional BFF (e.g. functional kimchi, red yeast rice) made

with specific microbial strains or additional beneficial ingredients. These plant-derived interventions may contain any concentration of any types of live microorganisms, measured in colony forming units (CFU); BFF interventions without live microorganisms at time of consumption will also be included. Studies that utilise BFF as the sole intervention or as an adjuvant dietary therapy (e.g. as part of a whole diet intervention) will be considered. Coffee, tea, chocolate, beer, wine and other high alcoholic beverages will not be included.

2.1.4 Type of outcome measures

We will search for all published quantitative research based on one or more included outcome measures. Outcome measures will be related to the target conditions, including, but not limited to changes in:

- weight as measured via waist circumference, body mass index and weight
- blood pressure (diastolic and systolic)
- lipid profile (fasting serum total cholesterol, high density lipoprotein, low density lipoprotein, triglycerides, free fatty acids)
- glucose metabolism (glycated haemoglobin, fasting plasma glucose, serum C-peptide, serum insulin)
- inflammatory markers (fasting serum high sensitivity C reactive protein, interleukin-6, interleukin-1B, tumour necrosis factor alpha)
- gut microbiota composition and metabolites (faecal metabolome, ribosomal RNA sequencing)

Other outcomes include liver markers (fasting serum aspartate aminotransferase, alanine aminotransferase), quality of life, mental health scales and adverse events.

2.2 Search methods for study identification

2.2.1 Data searches

The following four electronic databases will be searched from inception to present: Embase via

Ovid, MEDLINE via Ovid, and Cochrane CENTRAL and Google Scholar (first 200 relevancy ranked results). Reference lists in identified articles and reviews, as well as studies that cited these articles, will be searched with Scopus. We will also search the grey literature via trials registries and conference papers. When a study has unreported data, authors will be contacted for further information.

2.2.2 Search strategy

The search strategy will combine subject heading terms and text words for BFF (e.g. fermented food, fermentation, red yeast rice) and subject heading terms and text words to capture MetS or T2DM (e.g. metabolic syndrome, obesity, hypertension, blood pressure, diabetes, prediabetes, hyperlipidaemia, microbiota, dysbiosis, inflammation/inflammatory). To retrieve randomised controlled trials the Cochrane Highly Sensitive Search Strategy for MEDLINE will be used. No date or language limits will be applied. The MEDLINE draft search strategy is included as Table 1.

Table 1: Draft search strategy for MEDLINE

1. randomized controlled trial.pt.

2. controlled clinical trial.pt.

3. (randomized or randomised).ab.

4. placebo.ab.

5. drug therapy.fs.

6. randomly.ab.

7. trial.ab.

8. groups.ab.

9. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8

10. exp animals/ not humans.sh.

11. 9 not 10

12. Metabolic Syndrome/

13. diabetes mellitus, type 2/ or diabetes mellitus, lipoatrophic/

14. Hypertension/

15. Insulin Resistance/

16. INSULIN/

17. Blood Glucose/

18. blood pressure/

19. cholesterol, HDL/

20. cholesterol, LDL/

21. Non-alcoholic Fatty Liver Disease/

22. Dyslipidemias/

23. PREDIABETIC STATE/

24. obesity/ or obesity, abdominal/ or obesity, morbid/

25. overweight/

26. 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25

27. (metabolic syndrome* or metabolic disorder* or MetS or dyslipidemia* or dysglycemia* or hypertension or diabetes or prediabetes or neo diabetic or obesity or overweight or insulin or hyperlipidemia* or lipid or blood pressure or NAFLD or non-alcoholic fatty liver or microbiota or

microbiome or microflora or flora or intestinal or dysbiosis or inflamm*).mp.

28. 26 or 27

29. FERMENTATION/

30. Fermented Foods/

31. (monascus or monacolin or red yeast rice or Korean diet).mp.

32. (fermented or fermentation).mp.

33. 29 or 30 or 31 or 32

34. 28 and 33

35. 11 and 34

2.3 Selection of studies

One author (MC) and a clinical librarian (HB) will develop and execute a strategic search strategy following PRISMA guidelines. Two authors (MC and NL) will independently select articles to include by screening titles and abstracts, followed by full text assessment according to eligibility criteria. Duplicates will be removed and reasons for study exclusion will be recorded. Final eligibility will be determined through agreement between the two reviewers; resolution to any disagreements will be achieved through discussion. Authors of trials will be contacted for clarification when necessary. All processes and data will be recorded using Covidence software.

2.4 Data extraction and management

Using Covidence, two authors (MC and NL) will extract and manage the following data from eligible publications: study design, BFF type and dosage, duration of intervention, sample size, population, subjects' characteristics (age, sex, body mass index, symptom types), baseline metabolic biomarkers/ gut microbiota profile, medication use, adverse events, treatment outcomes and other

information. If reported data are insufficient, the authors of these studies will be contacted. Any disagreements will be resolved through discussion between the two authors.

2.5 Risk of bias assessment

All included studies will be qualified using the Cochrane Collaboration's tool for risk of bias (ROB) assessment.[13] Domains will include random sequence generation, allocation sequence concealment, participant and outcome assessor blinding, incomplete outcome data, selective outcome reporting and other sources of bias. Each domain ROB will be classified as low, high or unclear risk.

2.6 Data synthesis

If sufficient studies for each outcome are identified, meta-analysis will be conducted. We will assume risk ratio-derived summary estimates for dichotomous outcomes, and mean difference for continuous outcomes. Adoption of a random effect model will be considered for predicted clinical heterogeneity of BFF types. Expected inconsistencies across studies will require the use of I 2 statistics and Galbraith plots;[13] substantial heterogeneity is considered at a 50% cut off point. Depending on number of retrieved studies and their sample size, subgroup analyses will be stratified according to participant disease category, type of BFF and control intervention.

Covidence will be used to create a summary of findings table. If more than 10 studies are identified, potential publication and small sample bias will be assessed with funnel plots and Egger's test.[14] We will strive to identify possible causes of asymmetry, such as poor methodology or inappropriate effect measures.

If insufficient RCTs are available for meta-analysis, we will complete a descriptive narrative review, summarising the study characteristics and BFF effectiveness based on the specific results of the included studies. Subgroup analysis will also be conducted in this context.

2.7 Grading Evidence Quality

Quality of evidence for all included outcomes will be assessed using Grading of Recommendations Assessment, Development and Evaluation working group methodology.[15] Domains to be assessed: risk of bias, consistency, precision, directness, publication bias and any additional points; classification will be into four levels (high, moderate, low or very low).

2.8 Registration

To report this protocol, we used the Preferred Reporting Items for Systematic Reviews and Meta Analyses reporting guideline extension for systematic review protocols (PRISMA-P).[16] The PRISMA-P checklist for this protocol is available (online Supplementary File 1). Methodology is informed by the Cochrane Handbook for Systematic Reviews of Interventions. A standard version of the protocol has been registered with the International Prospective Register of Systematic Reviews (PROSPERO) http://www.crd.york.ac.uk/PROSPERO/display_record.php?ID=CRD42018117766, and will be updated as necessary.

2.8 Ethics and Dissemination

Formal ethical approval is not required as no individualised data will be used; as such no privacy issues are apparent. Review findings will be disseminated through peer-reviewed publications (print and online) and conference presentations.

2.9 Patient and Public Involvement

No patients or public will be involved in this systematic review protocol.

3. DISCUSSION

As far as can be established, no systematic review of clinical studies focused on BFF for MetS and T2DM has been conducted. When completed, this review will provide a summary of current evidence and identify further gaps in the research. This information will inform our interventional trial design, as well as researchers, clinicians, government food policy bodies, food companies, patients and consumers.

REFERENCES

1 David LA, Maurice CF, Carmody RN, et al. Diet rapidly and reproducibly alters the human gut microbiome. *Nature* 2014;505(7484):559-563. doi:10.1038/nature12820

2 Clemente JC, Ursell LK, Parfrey LW, et al. The impact of gut microbiota on human health: an integrative view. *Cell* 2012;148(6):1258-70. doi:10.1016/j.cell.2012.01.035

3 Everard A, Cani PD. Diabetes, obesity and gut microbiota. *Best Pract Res Clin Gastroenterol* 2013;27:73–83. doi:10.1016/j.bpg.2013.03.007

4 Wilson K, Situ C. Systematic Review on Effects of Diet on Gut Microbiota in Relation to Metabolic Syndromes. *J Clin Nutr Metab* 2017;1(2). doi:10.4172/jcnm1000111

5 Qin J, Li Y, Cai Z, et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature* 2012;490:55-60. doi:10.1038/nature11450

6 O'Neill S, O'Driscoll L. Metabolic syndrome: a closer look at the growing epidemic and its associated pathologies. *Obes Rev* 2015;16(1):1-12. doi:10.1111/obr.12229

7 Hutkins RW. Microbiology and Technology of Fermented Foods. In: Introduction. Hoboken, NJ: Wiley-Blackwell 2008:3-14.

8 Marco ML, Heeney D, Binda S, et al. Health benefits of fermented foods: microbiota and beyond. *Curr Opin Biotechnol* 2017;44:94-102. doi:10.1016/j.copbio.2016.11.010

9 Gille D, Schmid A, Walther B, et al. Fermented food and non-communicable chronic diseases: a review. *Nutrients* 2018;10(4):448. doi:10.3390/nu10040448

10 Tamang JP. Fermented Foods and Beverages of the World. In: JP Tamang, K Kailasapathy, eds. Diversity of fermented foods. New York, NY: CRC Press, Taylor and Francis Group 2010:41-84.

11 Wilburn JR, Ryan EP. Fermented foods in health and disease prevention. In: Frias J, Martinez-Villaluenga C, Penas E, eds. Fermented foods in health promotion and disease prevention: An overview. Amsterdam, The Netherlands: Elsevier 2017:23-47.

12 Selhub EM, Logan AC, Bested AC. Fermented foods, microbiota, and mental health: Ancient practice meets nutritional psychiatry. *J Physiol Anthropol* 2014;33(1):2. doi:10.1186/1880-6805-33-2

13 Higgins JPT, Altman DG, Sterne JAC. Cochrane Handbook for Systematic Reviews of Interventions. In: Higgins JPT, Green S, eds. Chapter 8: Assessing risk of bias in included studies. Chichester, UK: John Wiley & Sons 2008:187-237.

14 Egger M, Davey Smith G, Schneider M, et al. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997;315:629-634. doi:10.1136/bmj.315.7109.629

15 Guyatt GH, Oxman AD, Vist GE, et al. GRADE: an emerging consensus on rating quality of

evidence and strength of recommendations. *BMJ* 2008;336:924-6. doi:10.1136/bmj.39489.470347.AD

16 Moher D, Shamseer L, Clarke M, et al. Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) 2015 statement. *Syst Rev* 2015;4(1):1. doi:10.1186/2046-4053-4-1

FOOTNOTES

- **Contributors:** MC, EE and KH conceived the study. MC developed the criteria, performed the preliminary literature searches and wrote this review protocol, with assistance from NL. HB and MC designed and wrote the search strategy. KH, NM, EE, HB and LJ supervised, advised on protocol design and revised the manuscript. All authors read and approved the final manuscript and order of authorship.
- **Funding:** This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors. This protocol and the systematic review are part of a scholarship-funded PhD project at the University of Melbourne, Australia.
- **Competing interests:** None declared.
- **Patient consent:** Not required.
- **Ethics approval:** Not required as data is not individualised, and no privacy will be involved.
- **Provenance and peer review:** Not commissioned; externally peer reviewed.
- **Data sharing statement:** All data relevant to this protocol is included within the article.

Reporting checklist for protocol of a systematic review.

Based on the PRISMA-P guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the PRISMA-P reporting guidelines, and cite them as:

Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart LA. Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) 2015 statement. Syst Rev. 2015;4(1):1.

		Reporting Item	Page Number
Identification	#1a	Identify the report as a protocol of a systematic review	1
Update	#1b	If the protocol is for an update of a previous systematic review, identify as such	n/a
	#2	If registered, provide the name of the registry (such as PROSPERO) and registration number	3
Contact	#3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	1,2
Contribution	#3b	Describe contributions of protocol authors and identify the guarantor of the review	15
	#4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important	12

		protocol amendments	
Sources	#5a	Indicate sources of financial or other support for the review	15
Sponsor	#5b	Provide name for the review funder and / or sponsor	15
Role of sponsor or funder	#5c	Describe roles of funder(s), sponsor(s), and / or institution(s), if any, in developing the protocol	15
Rationale	#6	Describe the rationale for the review in the context of what is already known	3,4
Objectives	#7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	6
Eligibility criteria	#8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review	6
Information sources	#9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage	7
Search strategy	#10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	8
Study records - data management	#11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	10
Study records - selection process	#11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	10
Study records - data collection process	#11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	10
Data items	#12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	6

Outcomes and prioritization	#13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	7
Risk of bias in individual studies	#14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	11
Data synthesis	#15a	Describe criteria under which study data will be quantitatively synthesised	11
	#15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I2, Kendall's τ)	11
	#15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	11
	#15d	If quantitative synthesis is not appropriate, describe the type of summary planned	11
Meta-bias(es)	#16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	11
Confidence in cumulative evidence	#17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	12

BMJ Open

Impact of Botanical Fermented Foods on Metabolic Biomarkers and Gut Microbiota in Adults with Metabolic Syndrome and Type 2 Diabetes: A Systematic Review Protocol

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-029242.R1
Article Type:	Protocol
Date Submitted by the Author:	27-Jun-2019
Complete List of Authors:	Chan, Miin; University of Melbourne, School of Agriculture and Food Baxter, Helen; Austin Health, Clinical Library Larsen, Nadja; Kobenhavns Universitet, Department of Food Science Jespersen, Lene; Kobenhavns Universitet, Department of Food Science Ekinci , Elif ; University of Melbourne Department of Medicine Austin Health, Howell, Kate; University of Melbourne, School of Agriculture and Food
Primary Subject Heading:	Nutrition and metabolism
Secondary Subject Heading:	Diabetes and endocrinology
Keywords:	General diabetes < DIABETES & ENDOCRINOLOGY, Microbiology < BASIC SCIENCES, NUTRITION & DIETETICS

Impact of Botanical Fermented Foods on Metabolic Biomarkers and Gut Microbiota in Adults with Metabolic Syndrome and Type 2 Diabetes: A Systematic Review Protocol

Miin Chan, Helen Baxter, Nadja Larsen, Lene Jespersen, Elif I. I. Ekinci, Kate Howell

CORRESPONDING AUTHOR

Kate Howell

School of Agriculture and Food, Faculty of Veterinary and Agricultural Sciences

Level 1, Room 133, FVAS Building

University of Melbourne, Melbourne, VIC, Australia

ORCID ID: https://orcid.org/0000-0001-6498-0472

Email: khowell@unimelb.edu.au Phone: +613 9035 3119

Miin Chan

School of Agriculture and Food, Faculty of Veterinary and Agricultural Sciences

University of Melbourne, Melbourne, VIC, Australia

ORCID ID: https://orcid.org/0000-0002-4695-7057

Email: ktmchan@unimelb.edu.au Mobile: +61 490 367 714

Helen Baxter

Austin Health Sciences Library

Austin Hospital, Melbourne, VIC, Australia

ORCID ID: https://orcid.org/0000-0001-6579-8584

Email: Helen.BAXTER@austin.org.au Phone: +613 9496 6949

Nadja Larsen

Department of Food Science, Faculty of Science

University of Copenhagen, Frederiksberg, Denmark

ORCID ID: https://orcid.org/0000-0002-5899-4883

Email: nf@food.ku.dk Phone: +45 353 33235

Lene Jespersen

Department of Food Science, Faculty of Science

University of Copenhagen, Frederiksberg, Denmark

ORCID ID: https://orcid.org/0000-0002-2555-5948

Email: lj@food.ku.dk Phone: +45 3533 3230

Elif I. I. Ekinci

Department of Endocrinology, Austin Health, Melbourne, VIC, Australia

Department of Medicine, Austin Health, University of Melbourne, Melbourne, VIC, Australia

ORCID ID: https://orcid.org/0000-0003-2372-395X

Email: elif.ekinci@unimelb.edu.au Phone: +613 9496 2250

ABSTRACT

Introduction: Dysfunctional gut microbiota is a common finding in patients with Metabolic Syndrome (MetS) and Type 2 Diabetes Mellitus (T2DM). Recent clinical trials have assessed whether botanical fermented foods (BFF) have beneficial effects on metabolic biomarkers, inflammatory markers and gut microbiota. The aim of this review is to critically evaluate all randomised controlled trials of BFF for evidence of impact on the outcome measures of these disease states.

Methods and analysis: Four electronic databases (Embase, MEDLINE, CENTRAL and Google Scholar) as well as the grey literature will be searched from inception to present without language or publication status restrictions applied. Eligible randomised controlled trials (RCT) which have enrolled adult participants with T2DM, any MetS components or combinations of these components, treated prophylactically or therapeutically with any botanical fermented food intervention, compared with a control group (no intervention, placebo or active control) will be assessed. Primary outcomes are related to the target conditions, including metabolic biomarkers, inflammatory markers and gut microbiota composition/ function. Using Covidence, two independent investigators will conduct title and abstract screening, followed by full-text screening to identify appropriate studies. Methodological quality of the trials will be assessed using the Cochrane risk of bias (ROB) assessment tool. Findings will be summarised with a narrative synthesis of the differences between included studies. A meta-analysis will be conducted if sufficient data is obtained.

Ethics and dissemination: Ethical approval is not required as primary data will not be collected. Results will be disseminated through peer-reviewed publication, conference presentations and press.

Systematic Review Registration: PROSPERO 2018 CRD42018117766

Keywords: metabolic syndrome, type 2 diabetes, hypertension, cardiovascular disease, obesity, inflammation, gut microbiota, fermented food, clinical trial

ARTICLE SUMMARY

Strengths and limitations of this study

• This is the first systematic review and meta-analysis assessing the effectiveness of BFFs for adults with T2DM, MetS and its components.

• To ensure highest quality scientific data, the conduct of this review will adhere to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement.

• Robust systematic methodology used to identify quality evidence will assist in elucidating whether BFFs are effective prevention and management tools for non-communicable metabolic diseases.

• This systematic review and meta-analysis will have inherent limitations related to included studies such as risk of bias, methodological inconsistencies and incomplete outcome data.

• Result interpretation may be affected by significant heterogeneity due to the vast differences between BFFs, trial administration issues and the variety of outcome measures.

1. INTRODUCTION

Diet alters the structure and activity of human gut microbiota,[1] with direct effects on host health.[2] Shifts in gut microbiota have been linked to host metabolism dysfunction and low-grade chronic inflammation; these disorders of metabolism are implicated in the development of obesity, Metabolic Syndrome (MetS) and Type 2 Diabetes Mellitus (T2DM).[3-5] MetS affects more than 25% of all adults globally;[6] T2DM is the world's most prevalent endocrine disorder.[5] As such, cheap, effective dietary therapies are of great interest to researchers, clinicians and government bodies.

Fermented products of plant origin, or botanical fermented foods (BFF), are a microbially-diverse part of global traditional diets.[7] These indigenous traditional fermented foods (e.g. kimchi,

sauerkraut, tibicos, tempeh, miso, kombucha, natto and fermented olives), as well as newer functional fermented products such as red yeast rice and functional kimchi, have been recognised as having beneficial effects on human health.[8, 9] The diversity of such ferments and their ingredients means they are abundant in microbiota-accessible carbohydrates, food-associated microorganisms such as lactic acid bacteria, bacterial components and metabolites, bioactive compounds such as polyphenols, vitamins and minerals.[9, 10] Besides the basic nutritional properties of BFF ingredients, fermentation itself may confer additional health benefits through interactions between the host and consumed live microorganisms (probiotics), or through the ingestion of food-associated microbe-produced metabolites (biogenics) and other products of fermentation.[11] These include secondary phytochemicals, bioactive peptides and other compounds which have been shown to affect blood pressure, immune responses, antioxidant activity, insulin sensitivity, fasting and postprandial blood glucose.[12] The action of human gut microbiota on fermented food components in the intestinal lumen also produces health-promoting compounds, such as fermentation of microbiota-accessible carbohydrates into short chain fatty acids.[8] Though relatively sparse compared to studies on fermented dairy products for human health, recent clinical trials of BFF support their role in the prevention and treatment of non-communicable chronic diseases,[9] including obesity, prediabetes, type 2 diabetes, lipid dysfunction, cardiovascular disease, inflammatory bowel disorders and mental health disorders.[8]

Although a recent review of BFF in relation to non-communicable diseases has been conducted,[9] as well as others critically reviewing the health benefits of fermented foods,[8] no systematic review of the impact of BFF on MetS and T2DM has been undertaken. The aim is to systematically review randomised controlled trials for evidence of the impact of BFF compared to control on gut microbiota and metabolic biomarkers in adult human subjects suffering from components of MetS or T2DM. If sufficient homogeneous studies are identified, meta-analysis of the pooled data from these trials will elucidate the overall effect.

2. METHODS

2.1 Eligibility Criteria

The PICOS acronym[13] was used in the determination of the inclusion and exclusion criteria for this review. We will select RCTs (S) investigating the impact of BFFs (I), compared with placebo, no intervention or active controls (C), on adults (P) with T2DM or MetS components, or any combination of these components (O). Inclusion and exclusion criteria are shown in Table 1.

Table 1 Inclusion and exclusion criteria

Criteria	Inclusion criteria	Exclusion criteria
Population	Adults over 18 years old. Diagnosed with T2DM, or suffering from any MetS components or combinations of these components.	Children. Non-T2DM individuals. Healthy subjects not suffering from any MetS components.
Intervention	BFFs and beverages. May contain any concentration of any types of live microorganisms, or no live microorganisms at time of consumption. Sole intervention.	Single compound extracts. BFFs mixed with non-fermented ingredients. BFFs as part of whole diet interventions. Coffee, tea, chocolate, beer, wine, high alcoholic beverages.
Comparator	Placebo, no intervention or active control groups.	Any other type of intervention or comparison.
Outcome	Related to target conditions (T2DM, MetS). Changes in anthropometric measurements, blood pressure, lipid profile, glucose metabolism/ glycaemic control, inflammatory markers, gut microbiota composition and metabolites.Others: liver markers, quality of life, mental health scales, adverse events.	Not related to target conditions.
Study design	All clinical randomised controlled trials.	All other study designs.
Language	All languages.	None.
Setting	All settings.	None.

2.1.1 Types of studies

All human randomised controlled trials in any language will be included.

2.1.2 Type of participants

This review will include trials with adult participants suffering from T2DM, or any MetS components or combinations of these components (e.g. obesity, hypertension, lipid dysfunction,

glucose intolerance/ prediabetes, Non-Alcoholic Fatty Liver Disease).

2.1.3 Type of interventions and comparators

This review will include studies that evaluate traditional BFF (e.g. kimchi, sauerkraut, tibicos, tempeh, kombucha), as well as modern functional BFF (e.g. functional kimchi, red yeast rice) made with specific microbial strains or additional beneficial ingredients. These plant-derived interventions may contain any concentration of any types of live microorganisms, measured in colony forming units (CFU); BFF interventions without live microorganisms at time of consumption will also be included. Studies that utilise BFF as the sole intervention will be considered. Single compound extracts, BFFs mixed with non-fermented ingredients and BFFs as part of whole diet interventions will be excluded. Coffee, tea, chocolate, beer, wine and other high alcoholic beverages will not be included.

Participants in the sample will have been randomly allocated into intervention (BFF) and placebo, no intervention or active control groups.

2.1.4 Type of outcome measures

We will search for all published quantitative research based on one or more included outcome measures. Outcome measures will be related to the target conditions, including, but not limited to changes in:

- weight as measured via waist circumference, body mass index and weight
- blood pressure (diastolic and systolic)
- lipid profile (fasting serum total cholesterol, high density lipoprotein, low density lipoprotein, triglycerides, free fatty acids)

- glucose metabolism (glycated haemoglobin, fasting plasma glucose, serum C-peptide, serum insulin)

- inflammatory markers (fasting serum high sensitivity C reactive protein, interleukin-6, interleukin-1B, tumour necrosis factor alpha)

- gut microbiota composition and metabolites (faecal metabolome, ribosomal RNA sequencing)

Other outcomes include liver markers (fasting serum aspartate aminotransferase, alanine aminotransferase), quality of life, mental health scales and adverse events.

2.2 Search methods for study identification

2.2.1 Data searches

The following four electronic databases will be searched from inception to present: Embase via Ovid, MEDLINE via Ovid, and Cochrane CENTRAL and Google Scholar (first 200 relevancy ranked results). Reference lists in identified articles and reviews, as well as studies that cited these articles, will be searched with Scopus. We will also search the grey literature via trials registries and conference papers. When a study has unreported data, authors will be contacted for further information.

2.2.2 Search strategy

The search strategy will combine subject heading terms and text words for BFF (e.g. fermented food, fermentation, red yeast rice) and subject heading terms and text words to capture MetS or T2DM (e.g. metabolic syndrome, obesity, hypertension, blood pressure, diabetes, prediabetes, hyperlipidaemia, microbiota, dysbiosis, inflammation/inflammatory). To retrieve randomised controlled trials the Cochrane Highly Sensitive Search Strategy for MEDLINE will be used. No date or language limits will be applied. The MEDLINE draft search strategy is included as Table 2.

Table 2 Draft search strategy for MEDLINE

1. randomized controlled trial.pt.
2. controlled clinical trial.pt.
3. (randomized or randomised).ab.
4. placebo.ab.
5. drug therapy.fs.
6. randomly.ab.
7. trial.ab.
8. groups.ab.
9. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8
10. exp animals/ not humans.sh.
11. 9 not 10
12. Metabolic Syndrome/
13. diabetes mellitus, type 2/ or diabetes mellitus, lipoatrophic/
14. Hypertension/
15. Insulin Resistance/
16. INSULIN/
17. Blood Glucose/
18. blood pressure/
19. cholesterol, HDL/
20. cholesterol, LDL/
21. Non-alcoholic Fatty Liver Disease/
22. Dyslipidemias/
23. PREDIABETIC STATE/
24. obesity/ or obesity, abdominal/ or obesity, morbid/
25. overweight/
26. 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25
27. (metabolic syndrome* or metabolic disorder* or MetS or dyslipidemia* or dysglycemia* or hypertension or diabetes or prediabetes or neo diabetic or obesity or overweight or insulin or hyperlipidemia* or lipid or blood pressure or NAFLD or non-alcoholic fatty liver or microbiota or microbiome or microflora or flora or intestinal or dysbiosis or inflamm*).mp.
28. 26 or 27
29. FERMENTATION/
30. Fermented Foods/
31. (monascus or monacolin or red yeast rice or Korean diet).mp.
32. (fermented or fermentation).mp.

33. 29 or 30 or 31 or 32

34. 28 and 33

35. 11 and 34

2.3 Selection of studies

One author (MC) and a clinical librarian (HB) will develop and execute a strategic search strategy following PRISMA guidelines. Two authors (MC and NL) will independently select articles to include by screening titles and abstracts, followed by full text assessment according to eligibility criteria. Duplicates will be removed and reasons for study exclusion will be recorded. Final eligibility will be determined through agreement between the two reviewers; resolution to any disagreements will be achieved through discussion. Authors of trials will be contacted for clarification when necessary. All processes and data will be recorded using Covidence software.

2.4 Data extraction and management

Using Covidence, two authors (MC and NL) will extract and manage the following data from eligible publications: study design, BFF type and dosage, duration of intervention, sample size, population, subjects' characteristics (age, sex, body mass index, symptom types), baseline metabolic biomarkers/ gut microbiota profile, medication use, adverse events, treatment outcomes and other information. If reported data are insufficient, the authors of these studies will be contacted. Any disagreements will be resolved through discussion between the two authors.

2.5 Risk of bias assessment

All included studies will be qualified using the Cochrane Collaboration's tool for risk of bias (ROB) assessment.[14] Domains will include random sequence generation, allocation sequence concealment, participant and outcome assessor blinding, incomplete outcome data, selective outcome reporting and other sources of bias. Each domain ROB will be classified as low, high or unclear risk.

2.6 Data synthesis

If sufficient RCTs with robust heterogeneous pooled data for each outcome are identified, meta-analysis will be conducted. We will assume risk ratio-derived summary estimates for dichotomous outcomes, and mean difference for continuous outcomes. Adoption of a random effect model will be considered for predicted clinical heterogeneity of BFF types. Expected inconsistencies across studies will require the use of I2 statistics and Galbraith plots;[14] substantial heterogeneity is considered at a 50% cut off point. Depending on number of retrieved studies and their sample size, subgroup analyses will be stratified according to participant disease category, type of BFF and control intervention.

Covidence will be used to create a summary of findings table. If more than 10 studies are identified, potential publication and small sample bias will be assessed with funnel plots and Egger's test.[15] We will strive to identify possible causes of asymmetry, such as poor methodology or inappropriate effect measures.

If insufficient RCTs are available for meta-analysis, we will complete a narrative synthesis of included studies, summarising the study characteristics and BFF effectiveness based on the specific results of the included studies. Subgroup analysis will also be conducted in this context.

2.7 Grading Evidence Quality

Quality of evidence for all included outcomes will be assessed using Grading of Recommendations Assessment, Development and Evaluation working group methodology.[16] Domains to be assessed: risk of bias, consistency, precision, directness, publication bias and any additional points; classification will be into four levels (high, moderate, low or very low).

2.8 Registration

To report this protocol, we used the Preferred Reporting Items for Systematic Reviews and Meta Analyses reporting guideline extension for systematic review protocols (PRISMA-P).[17] The PRISMA-P checklist for this protocol is available (online Supplementary File 1). Methodology is informed by the Cochrane Handbook for Systematic Reviews of Interventions. A standard version of the protocol has been registered with the International Prospective Register of Systematic Reviews (PROSPERO) http://www.crd.york.ac.uk/PROSPERO/display_record.php?ID=CRD42018117766, and will be updated as necessary.

2.8 Ethics and Dissemination

Formal ethical approval is not required as no individualised data will be used; as such no privacy issues are apparent. Review findings will be disseminated through peer-reviewed publications (print and online) and conference presentations.

2.9 Patient and Public Involvement

No patients or public will be involved in this systematic review protocol.

3. DISCUSSION

As far as can be established, no systematic review of clinical studies focused on BFF for MetS and T2DM has been conducted. When completed, this review will provide a summary of current evidence and identify further gaps in the research. The findings have the potential to influence clinical management of these increasingly prevalent non-communicable metabolic diseases, as well as contribute to the inclusion of BFFs in global food guides. The results of this review will also inform our interventional trial design.

REFERENCES

1 David LA, Maurice CF, Carmody RN, et al. Diet rapidly and reproducibly alters the human gut microbiome. *Nature* 2014;505(7484):559-563. doi:10.1038/nature12820

2 Clemente JC, Ursell LK, Parfrey LW, et al. The impact of gut microbiota on human health: an integrative view. *Cell* 2012;148(6):1258-70. doi:10.1016/j.cell.2012.01.035

3 Everard A, Cani PD. Diabetes, obesity and gut microbiota. *Best Pract Res Clin Gastroenterol* 2013;27:73–83. doi:10.1016/j.bpg.2013.03.007

4 Wilson K, Situ C. Systematic Review on Effects of Diet on Gut Microbiota in Relation to Metabolic Syndromes. *J Clin Nutr Metab* 2017;1(2). doi:10.4172/jcnm1000111

5 Qin J, Li Y, Cai Z, et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature* 2012;490:55-60. doi:10.1038/nature11450

6 O'Neill S, O'Driscoll L. Metabolic syndrome: a closer look at the growing epidemic and its associated pathologies. *Obes Rev* 2015;16(1):1-12. doi:10.1111/obr.12229

7 Hutkins RW. Microbiology and Technology of Fermented Foods. In: Introduction. Hoboken, NJ: Wiley-Blackwell 2008:3-14.

8 Marco ML, Heeney D, Binda S, et al. Health benefits of fermented foods: microbiota and beyond. *Curr Opin Biotechnol* 2017;44:94-102. doi:10.1016/j.copbio.2016.11.010

9 Gille D, Schmid A, Walther B, et al. Fermented food and non-communicable chronic diseases: a

review. *Nutrients* 2018;10(4):448. doi:10.3390/nu10040448

10 Tamang JP. Fermented Foods and Beverages of the World. In: JP Tamang, K Kailasapathy, eds. Diversity of fermented foods. New York, NY: CRC Press, Taylor and Francis Group 2010:41-84.

11 Wilburn JR, Ryan EP. Fermented foods in health and disease prevention. In: Frias J, Martinez-Villaluenga C, Penas E, eds. Fermented foods in health promotion and disease prevention: An overview. Amsterdam, The Netherlands: Elsevier 2017:23-47.

12 Selhub EM, Logan AC, Bested AC. Fermented foods, microbiota, and mental health: Ancient practice meets nutritional psychiatry. *J Physiol Anthropol* 2014;33(1):2. doi:10.1186/1880-6805-33-2

13 Higgins JPT, Green S. Cochrane Handbook for Systematic Reviews of Interventions, Version 5.1.0. The Cochrane Collaboration. 2013.

14 Higgins JPT, Altman DG, Sterne JAC. Cochrane Handbook for Systematic Reviews of Interventions. In: Higgins JPT, Green S, eds. Chapter 8: Assessing risk of bias in included studies. Chichester, UK: John Wiley & Sons 2008:187-237.

15 Egger M, Davey Smith G, Schneider M, et al. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997;315:629-634. doi:10.1136/bmj.315.7109.629

16 Guyatt GH, Oxman AD, Vist GE, et al. GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. *BMJ* 2008;336:924-6. doi:10.1136/bmj.39489.470347.AD

17 Moher D, Shamseer L, Clarke M, et al. Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) 2015 statement. *Syst Rev* 2015;4(1):1. doi:10.1186/2046-4053-4-1

FOOTNOTES

- **Contributors:** MC, EE and KH conceived the study. MC developed the criteria, performed the preliminary literature searches and wrote this review protocol, with assistance from NL. HB and MC designed and wrote the search strategy. KH, EE, HB and LJ supervised, advised on protocol design and revised the manuscript. All authors read and approved the final manuscript and order of authorship.
- **Funding:** This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors. This protocol and the systematic review are part of a scholarship-funded PhD project at the University of Melbourne, Australia.
- **Competing interests:** None declared.
- **Patient consent:** Not required.
- **Ethics approval:** Not required as data is not individualised, and no privacy will be involved.
- **Provenance and peer review:** Not commissioned; externally peer reviewed.
- **Data sharing statement:** All data relevant to this protocol is included within the article.

Reporting checklist for protocol of a systematic review.

Based on the PRISMA-P guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the PRISMA-P reporting guidelines, and cite them as:

Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart LA. Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) 2015 statement. Syst Rev. 2015;4(1):1.

		Reporting Item	Page Number
Identification	#1a	Identify the report as a protocol of a systematic review	1
Update	#1b	If the protocol is for an update of a previous systematic review, identify as such	n/a
	#2	If registered, provide the name of the registry (such as PROSPERO) and registration number	3
Contact	#3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	1,2
Contribution	#3b	Describe contributions of protocol authors and identify the guarantor of the review	15
	#4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important	12

		protocol amendments	
Sources	#5a	Indicate sources of financial or other support for the review	15
Sponsor	#5b	Provide name for the review funder and / or sponsor	15
Role of sponsor or funder	#5c	Describe roles of funder(s), sponsor(s), and / or institution(s), if any, in developing the protocol	15
Rationale	#6	Describe the rationale for the review in the context of what is already known	3,4
Objectives	#7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	6
Eligibility criteria	#8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review	6
Information sources	#9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage	7
Search strategy	#10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	8
Study records - data management	#11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	10
Study records - selection process	#11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	10
Study records - data collection process	#11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	10
Data items	#12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	6

Outcomes and prioritization	#13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	7
Risk of bias in individual studies	#14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	11
Data synthesis	#15a	Describe criteria under which study data will be quantitatively synthesised	11
	#15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I2, Kendall's τ)	11
	#15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	11
	#15d	If quantitative synthesis is not appropriate, describe the type of summary planned	11
Meta-bias(es)	#16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	11
Confidence in cumulative evidence	#17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	12