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# BMJ Open

## Intestinal barrier function in obese patients with or without metabolic syndrome: A systematic review protocol

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Keywords:	General endocrinology < DIABETES & ENDOCRINOLOGY, GASTROENTEROLOGY, CLINICAL PHYSIOLOGY

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3 1 **Intestinal barrier function in obese patients with or without metabolic syndrome:**  
4  
5 2 **A systematic review protocol**  
6

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28 13 **Abstract**

29  
30 14 **Introduction:** Intestinal barrier function (IBF) is dependent on the structure and  
31 15 function of intestinal epithelial cells and paracellular pathway. The derangement of  
32 16 IBF can originate from conditions involving local and systemic chronic inflammation  
33 17 and metabolic diseases such as obesity and metabolic disorders. The aim of this paper  
34 18 was to describe a systematic review protocol with studies that determine the  
35 19 relationship between the intestinal barrier function, in obese patients with or without  
36 20 metabolic syndrome. **Methods and analysis:** This protocol is guided by the Preferred  
37 21 Reporting Items for Systematic Reviews and Meta-Analyses Protocols (PRISMAP).  
38 22 The databases to be searched are PubMed, EMBASE, Scopus, ScienceDirect, and Web  
39 23 of Science. The systematic review will include original articles with adults and  
40 24 elderlies, obese with or without the diagnosis of metabolic syndrome, that address the  
41 25 intestinal barrier function in this population. Two independent reviewers will perform  
42 26 study selection, data extraction, and methodological quality assessment. Results  
43 27 corresponding to the analysis of intestinal barrier function between the studied groups  
44 28 will be described and will consider the difference in means and p values. Heterogeneity  
45 29 between study results will be assessed using a standard chi-squared test with a  
46 30 significance level of less than 0.05. The present protocol will assist in producing a  
47 31 systematic review that addresses if obesity or obesity associated with metabolic

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3 32 syndrome alters intestinal barrier function. **Ethics and dissemination:** No ethical  
4 33 statement will be required. The results will be disseminated through a peer-reviewed  
5 34 publication and conference presentations.

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8 35 **Trial registration number:** International Prospective Register for Systematic  
9 36 Reviews (PROSPERO) number CRD42020178658

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11 37 **Keywords:** Intestinal barrier function, obesity, metabolic syndrome, systematic  
12 38 review

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16 39 **Strengths and limitations of this study:**

- 17  
18 40 • This study will focus in clinical research instead of the majority that focus in  
19 41 animal models researches;  
20  
21 42 • It will bring evidence of the most used *in vivo* tests to assess intestinal barrier  
22 43 function and integrity;  
23  
24 44 • In this study, obese with or without metabolic syndrome will be included;  
25  
26 45 • The scarcity of researches with elderlies and the methodological quality of studies  
27 46 may be the main limitations of the study.

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29  
30 47 **1. Introduction**

31  
32 48 The incidence of obesity and metabolic syndrome has risen significantly worldwide  
33 49 over the last decades and reach epidemic proportions affecting all ages and  
34 50 socioeconomic groups.<sup>1 2</sup> Some evidence supports a causal pathway between diet, gut  
35 51 microbiota, intestinal barrier function and metabolic dysfunction.<sup>3-5</sup> Most of this  
36 52 knowledge is based on animal studies, where the link between alterations in the gut  
37 53 microbiota and, more recently, changes in intestinal barrier function was shown.<sup>6</sup>

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43 54 The intestinal barrier is a complex multilayer system, consisting of an external  
44 55 physical barrier and an inner functional immunological barrier. The interaction of these  
45 56 two barriers enables the maintenance of equilibrated intestinal barrier function.<sup>7</sup> It  
46 57 prevents against loss of water and electrolytes and entry of antigens and microorganisms  
47 58 into the body while allowing the exchange of molecules between host and environment  
48 59 and the absorption of nutrients from the diet.<sup>8</sup>

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54 60 Many factors can alter intestinal barrier function such as gut microbiota  
55 61 modifications, mucus layer alterations, and epithelial damage, resulting in translocation  
56 62 of luminal content to the inner layers of the intestinal epithelial cells.<sup>9 10</sup> Evidence  
57 63 obtained in animal models as well as in humans is accumulating supporting a role of

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3 64 alterations of intestinal barrier function in many conditions, which include intestinal  
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5 65 disorders such as malnutrition, diarrheal diseases, environmental enteric disease (EED),  
6  
7 66 inflammatory bowel disease, irritable bowel syndrome, hepatic fibrosis, inflammation,  
8  
9 67 sepsis and pancreatitis, but also obesity and metabolic syndrome.<sup>11-14</sup>

10  
11 68 Intestinal barrier function and integrity can be measured in different ways. The  
12  
13 69 techniques used for IBF and integrity assessment vary depending on the setting (*in vitro*  
14  
15 70 versus *in vivo* measurements), the species (human or animals), the marker molecules used  
16  
17 71 (ions, carbohydrates of different sizes, macromolecules and antigens, bacterial products  
18  
19 72 and bacteria), and the compartments used for measurement of the marker molecules  
20  
21 73 (peripheral blood, portal vein blood or urine).<sup>15 16</sup>

22  
23 74 *In vivo* assessment of intestinal barrier absorption, damage and permeability in  
24  
25 75 humans are currently possible by using intestinal barrier function biomarkers and assays.  
26  
27 76 One of the most used assays is the lactulose:mannitol test, a quantitative non-invasive test  
28  
29 77 that directly measures the ability of two non-metabolized sugar molecules, lactulose and  
30  
31 78 mannitol, to permeate the intestinal mucosa.<sup>17</sup> Lactulose (L), a disaccharide, is absorbed  
32  
33 79 through cell junctions or epithelial cell turnover or damage, while mannitol (M), a  
34  
35 80 monosaccharide, is absorbed most across the epithelial cell membranes.<sup>18</sup> Once absorbed,  
36  
37 81 these sugars are excreted unmetabolized in the urine. Elevated lactulose to mannitol ratio  
38  
39 82 is an indicator of intestinal barrier dysfunction.<sup>10</sup>

40  
41 83 Despite the test's immense potentials, its application in clinical research remains  
42  
43 84 limited due to variations in the methodologies such as study population, sugar solution  
44  
45 85 formulation and administration, urine collection time, and assay method to measure  
46  
47 86 lactulose and mannitol between studies.<sup>19</sup> These variations restrict the clinical sensibility  
48  
49 87 and accuracy of the lactulose:mannitol test, for example, the relationship between  
50  
51 88 intestinal barrier function, integrity and inflammatory outcomes in diseases such as  
52  
53 89 obesity and metabolic syndrome.

54  
55 90 The aim of this paper is to describe a systematic review protocol with studies that  
56  
57 91 determine the relationship between the intestinal barrier function, in obese patients with  
58  
59 92 or without metabolic syndrome. In addition, the systematic review will evaluate  
60  
93 methodologies used in the studies regarding intestinal barrier function biomarkers and  
94 assays methods. This review protocol will address if obesity or obesity associated with  
95 metabolic syndrome alters intestinal barrier function and integrity.

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## 96 2. Methods

### 97 2.1 Protocol and registration

98 This protocol has been prepared according to the guidelines described in Preferred  
99 Reporting Items for Systematic Reviews and Meta-Analyses Protocols (PRISMA-P).<sup>20</sup> A  
100 27-item checklist was used to improve the quality of the systematic review data. The  
101 protocol was registered with the International Prospective Register of Systematic  
102 Reviews (PROSPERO) on July 10, 2020 (CRD42020178658) and is available at:  
103 [https://www.crd.york.ac.uk/prospero/display\\_record.php?ID=CRD42020178658](https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42020178658).

### 104 2.2 Eligibility criteria

105 The search will be performed using journal articles that enhance methodological  
106 transparency. In this sense, the search will be elaborated and implemented before study  
107 selection, according to the PRISMA-P checklist as guidance. Additionally, journal  
108 articles that meet eligibility criteria using the Population, Intervention, Comparison,  
109 Outcome and Study design (PICOS) strategy will be included to ensure the systematic  
110 search of available literature.

#### 111 2.2.1 Inclusion criteria

112 The review will include original articles with adults and elderlies, obese with or  
113 without the diagnosis of metabolic syndrome [according to modified National Cholesterol  
114 Education Program (NCEP) criteria is the presence of 3 or more of the following  
115 components: (1) waist circumference more than 90 cm in men or 80 cm in women; (2)  
116 triacylglycerols equal to or more than 150 mg/dL; (3) HDL-c less than 40mg/dL in men  
117 or 50 mg/dL in women; (4) blood pressure equal to or more than 130/85 mgHg and (5)  
118 fasting glucose between 100 and 125 mg/dL)]<sup>21</sup>, studies regarding the intestinal barrier  
119 function in this population, studies evaluating the intestinal epithelial cells integrity from  
120 obese patients with or without the diagnosis of metabolic syndrome.

#### 121 2.2.2 Exclusion criteria

122 Review articles, case reports, comments, editorials, letters to the editor, theses,  
123 conference proceedings, studies with animals or cell models, studies with children,  
124 studies with adults and/or elderlies that have other metabolic diseases, studies that did not  
125 evaluate the intestinal barrier function.

### 126 2.3 Information sources and literature search

1  
2  
3 127 To identify the studies to be included in the systematic review, search strategies  
4  
5 128 will be developed based on keywords indexed in the Medical Subject Headings (MeSH).  
6  
7 129 The descriptors used will be related to intestinal barrier function, obesity, and metabolic  
8  
9 130 syndrome, such as anthropometric data, biochemical analysis, intestinal permeability and  
10  
11 131 integrity methodologies and assays. These descriptors will be accompanied by Boolean  
12  
13 132 operators “AND” and “OR”.

13  
14 133 Two reviewers will independently conduct sensitive search for eligible systematic  
15  
16 134 reviews through the electronic databases PubMed Database, Embase Database, Cochrane  
17  
18 135 Library, Scopus, Web of Science (WOS) and Science Direct. Initial searches will test  
19  
20 136 preliminary equations with the prospect of applying highly sensitive search strategies.  
21  
22 137 Articles will be imported into Mendeley reference manager (1.19.4) and duplicates will  
23  
24 138 be deleted. Initial screening of studies will be based on the information contained in their  
25  
26 139 title, keywords and abstracts, following the eligibility criteria. When the reviewers  
27  
28 140 disagree, the article will be re-evaluated and, if the disagreement persists, a third reviewer  
29  
30 141 will make a final decision. Full-paper screening will be conducted by the same  
31  
32 142 independent investigators. The references of the included articles will also be reviewed  
33  
34 143 to identify those potentially eligible studies not found in the database search, considered  
35  
36 144 as manual search.

#### 35 145 2.4 Data extraction

36  
37 146 For data extraction, two independent Microsoft Excel spreadsheets will be  
38  
39 147 elaborated for two reviewers to summarize the data from the included studies. The  
40  
41 148 following information will be extracted and entered in the spreadsheet: first author; year  
42  
43 149 and language of publication; the country where the study was conducted; characteristics  
44  
45 150 of the population (Metabolic Syndrome presence, age, gender, health conditions, total  
46  
47 151 sample size, chronic diseases); methods to evaluate the intestinal barrier function; effects  
48  
49 152 of obesity with or without metabolic syndrome in the intestinal barrier function;  
50  
51 153 description of results and conclusions that are relevant to the overview; key findings;  
52  
53 154 reported limitations.

#### 53 155 2.5 Methodological quality assessment

54  
55 156 Assessment of methodological quality and risk of bias in the studies with case-  
56  
57 157 control design will be performed using Newcastle-Ottawa Scale, which includes eight  
58  
59 158 items related to selection, comparison, and outcome.  
60



## 159 2.6 Data analysis and synthesis

160 The systematic review will describe the relevant information of the included  
161 studies. Results corresponding to the analysis of intestinal barrier permeability between  
162 the studied groups will be described and will consider the difference in means and p  
163 values. Comparative analyses performed between the cases (obese with or without  
164 metabolic syndrome) and controls (healthy groups) will be presented. Heterogeneity  
165 between study results will be assessed using a standard chi-squared test with a  
166 significance level of 0.05.

## 167 3. Discussion

168 Obesity has become a global epidemic and is a substantial threat to patients and  
169 healthcare systems because of related morbidity and costs.<sup>22</sup> Metabolic and  
170 cardiovascular complications are a major obesity-associated burden, with critical roles  
171 for insulin resistance, type 2 diabetes and atherosclerosis.<sup>23</sup> Given the increasing  
172 prevalence of obesity worldwide, it is necessary to identify individuals with or without  
173 metabolic syndrome as a clinical priority.

174 Evidence has proposed the potential role of the gut microbiota as a pathogenic  
175 factor affecting host metabolic balance and disorders.<sup>1</sup> Gut microbiota seems to exert a  
176 great variety of functional properties impacting human physiology and pathology:  
177 modulation of host nutrition and energy harvest by the production of vitamins and  
178 fermentation of food components indigestible by the host; influence on intestinal  
179 epithelial homeostasis; intestinal barrier function; development of host immune system;  
180 protection against pathogens; drug metabolism.<sup>6</sup>

181 Animal models and some human studies are accumulating to support alterations  
182 of the intestinal barrier function in a vast array of conditions, which include obesity and  
183 metabolic syndrome.<sup>24</sup> Given the importance of the intestinal barrier function and  
184 integrity, understanding what can disrupt it and cause the loss of function and integrity  
185 are necessary. Even though no final conclusions exist, it is more evident that besides  
186 nutrients acting as down-regulators of tight junctions or as histone deacetylase (HDAC)  
187 inhibitors, also viral infections, toxins, hypoperfusion of the gut play a role.<sup>25 26</sup> Lifestyle  
188 factors such as living place, physical activity, dietary patterns and drug usage seem to  
189 play an important role as well, and they offer new approaches for improving gut barrier  
190 function.<sup>26</sup>

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3 191 In this perspective, this systematic review will evaluate studies with obese patients  
4  
5 192 with or without metabolic syndrome, focusing on the analysis of their intestinal barrier  
6  
7 193 function. This review will also generate evidence for the use of lactulose:mannitol test  
8  
9 194 for the diagnosis of *in vivo* intestinal barrier function and integrity.

10  
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12  
13 196 of Federal University of Ceará and Postgraduated Program in Nutrition of Federal  
14  
15 197 University of Rio Grande do Norte.

16 198 **Contributors:** MDB, CHMT, SCVCL and BLLM conceived the idea, planned and  
17  
18 199 designed the study protocol. MDB and CHMT wrote the first draft; SCVCL and BLLM  
19  
20 200 planned the data extraction and statistical analysis; AAML and BLLM provided critical  
21  
22 201 insights. All authors have approved and contributed to the final written manuscript.

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26 204 **Competing interests:** None declared.

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Based on the PRISMA-P guidelines.

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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.

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Reporting Item			Page Number
<b>Title</b>			
Identification	<a href="#">#1a</a>	Identify the report as a protocol of a systematic review	1
Update	<a href="#">#1b</a>	If the protocol is for an update of a previous systematic review, identify as such	1
<b>Registration</b>			
	<a href="#">#2</a>	If registered, provide the name of the registry (such as PROSPERO) and registration number	1
<b>Authors</b>			
Contact	<a href="#">#3a</a>	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	1
Contribution	<a href="#">#3b</a>	Describe contributions of protocol authors and identify the guarantor of the review	7

## Amendments

1	<a href="#">#4</a>	If the protocol represents an amendment of a previously completed or	1
2		published protocol, identify as such and list changes; otherwise, state	
3		plan for documenting important protocol amendments	
4			
5			
6	<b>Support</b>		
7			
8	Sources	<a href="#">#5a</a> Indicate sources of financial or other support for the review	7
9			
10	Sponsor	<a href="#">#5b</a> Provide name for the review funder and / or sponsor	7
11			
12	Role of sponsor or	<a href="#">#5c</a> Describe roles of funder(s), sponsor(s), and / or institution(s), if any,	7
13	funder	in developing the protocol	
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15			
16	<b>Introduction</b>		
17			
18	Rationale	<a href="#">#6</a> Describe the rationale for the review in the context of what is already	2
19		known	
20	Objectives	<a href="#">#7</a> Provide an explicit statement of the question(s) the review will	3
21		address with reference to participants, interventions, comparators, and	
22		outcomes (PICO)	
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28	<b>Methods</b>		
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30	Eligibility criteria	<a href="#">#8</a> Specify the study characteristics (such as PICO, study design, setting,	4
31		time frame) and report characteristics (such as years considered,	
32		language, publication status) to be used as criteria for eligibility for	
33		the review	
34			
35			
36			
37	Information sources	<a href="#">#9</a> Describe all intended information sources (such as electronic	4
38		databases, contact with study authors, trial registers or other grey	
39		literature sources) with planned dates of coverage	
40			
41			
42			
43	Search strategy	<a href="#">#10</a> Present draft of search strategy to be used for at least one electronic	4, 5
44		database, including planned limits, such that it could be repeated	
45			
46			
47	Study records - data	<a href="#">#11a</a> Describe the mechanism(s) that will be used to manage records and	5
48	management	data throughout the review	
49			
50	Study records -	<a href="#">#11b</a> State the process that will be used for selecting studies (such as two	5
51	selection process	independent reviewers) through each phase of the review (that is,	
52		screening, eligibility and inclusion in meta-analysis)	
53			
54			
55	Study records - data	<a href="#">#11c</a> Describe planned method of extracting data from reports (such as	5
56	collection process	piloting forms, done independently, in duplicate), any processes for	
57			
58			
59			
60			

		obtaining and confirming data from investigators	
1			
2			
3	Data items	<a href="#">#12</a> List and define all variables for which data will be sought (such as	4,5
4		PICO items, funding sources), any pre-planned data assumptions and	
5		simplifications	
6			
7			
8	Outcomes and	<a href="#">#13</a> List and define all outcomes for which data will be sought, including	4,5
9	prioritization	prioritization of main and additional outcomes, with rationale	
10			
11	Risk of bias in	<a href="#">#14</a> Describe anticipated methods for assessing risk of bias of individual	5
12	individual studies	studies, including whether this will be done at the outcome or study	
13		level, or both; state how this information will be used in data synthesis	
14			
15			
16			
17	Data synthesis	<a href="#">#15a</a> Describe criteria under which study data will be quantitatively	5
18		synthesised	
19			
20			
21	Data synthesis	<a href="#">#15b</a> If data are appropriate for quantitative synthesis, describe planned	5
22		summary measures, methods of handling data and methods of	
23		combining data from studies, including any planned exploration of	
24		consistency (such as I <sup>2</sup> , Kendall's $\tau$ )	
25			
26			
27	Data synthesis	<a href="#">#15c</a> Describe any proposed additional analyses (such as sensitivity or	5
28		subgroup analyses, meta-regression)	
29			
30			
31	Data synthesis	<a href="#">#15d</a> If quantitative synthesis is not appropriate, describe the type of	5
32		summary planned	
33			
34			
35	Meta-bias(es)	<a href="#">#16</a> Specify any planned assessment of meta-bias(es) (such as publication	5
36		bias across studies, selective reporting within studies)	
37			
38			
39	Confidence in	<a href="#">#17</a> Describe how the strength of the body of evidence will be assessed	5
40	cumulative	(such as GRADE)	
41	evidence		
42			
43			

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# BMJ Open

## Intestinal barrier function in obesity with or without metabolic syndrome: A systematic review protocol

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Keywords:	General endocrinology < DIABETES & ENDOCRINOLOGY, GASTROENTEROLOGY, CLINICAL PHYSIOLOGY

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3 1 **Intestinal barrier function in obesity with or without metabolic syndrome: A**  
4 **systematic review protocol**  
5 2

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25  
26  
27  
28 13 **Abstract**

29  
30 14 **Introduction:** Intestinal barrier function is dependent on the structure and function of  
31  
32 15 intestinal epithelial cells and paracellular pathway. The derangement of the intestinal  
33  
34 16 barrier function can originate from conditions involving local and systemic chronic  
35  
36 17 inflammation and metabolic diseases such as obesity and metabolic disorders. This  
37  
38 18 study aims to describe a systematic review protocol investigating if obesity with or  
39  
40 19 without metabolic syndrome is associated with an altered intestinal barrier function.

41 20 **Methods and analysis:** This protocol is guided by the Preferred Reporting Items for  
42  
43 21 Systematic Reviews and Meta-Analyzes Protocols (PRISMAP). The databases to be  
44  
45 22 searched are PubMed, EMBASE, Scopus, ScienceDirect, and Web of Science. The  
46  
47 23 systematic review will include original articles with adults and elderlies, who present  
48  
49 24 obesity with or without metabolic syndrome, that address the intestinal barrier  
50  
51 25 function. Two independent reviewers will perform study selection, data extraction, and  
52  
53 26 methodological quality assessment. Key information will be tabulated and a narrative  
54  
55 27 synthesis will be conducted. The GRADE framework will be used to assess the quality  
56  
57 28 of evidence concerning the associations between intestinal barrier function and obesity  
58  
59 29 with or without metabolic syndrome. The present protocol will assist in producing a  
60  
30 systematic review that addresses if obesity with or without metabolic syndrome alters  
31  
32 intestinal barrier function. **Ethics and dissemination:** No ethical statement will be

1  
2  
3 32 required. The results will be disseminated through a peer-reviewed publication and  
4  
5 33 conference presentations.

6 34 **Trial registration number:** International Prospective Register for Systematic  
7  
8 35 Reviews (PROSPERO) number CRD42020178658

9  
10 36 **Keywords:** Intestinal barrier function, obesity, metabolic syndrome, systematic  
11  
12 37 review

13  
14 38 **Strengths and limitations of this study:**

- 15  
16 39 • This study will focus in clinical research instead of the majority that focus in  
17  
18 40 animal models researches;  
19  
20 41 • It will bring evidence of the most used *in vivo* tests to assess intestinal barrier  
21  
22 42 function and integrity;  
23  
24 43 • In this study, individuals with obesity with or without metabolic syndrome will  
25  
26 44 be included;  
27  
28 45 • The scarcity of researches with elderlies and the methodological quality of studies  
29  
30 46 may be the main limitations of the study.

31  
32 48 **1. Introduction**

33  
34 49 The incidence of obesity and metabolic syndrome has risen significantly worldwide  
35  
36 50 over the last decades and reach epidemic proportions affecting all ages and  
37  
38 51 socioeconomic groups.<sup>1 2</sup> Some evidence supports a causal pathway between diet, gut  
39  
40 52 microbiota, intestinal barrier function and metabolic dysfunction.<sup>3-5</sup> Most of this  
41  
42 53 knowledge is based on animal studies, where the link between alterations in the gut  
43  
44 54 microbiota and in intestinal barrier function was shown.<sup>6 7</sup>

45  
46 55 The intestinal barrier is a complex multilayer system, consisting of an external  
47  
48 56 physical barrier and an inner functional immunological barrier. The interaction of these  
49  
50 57 two barriers enables the maintenance of equilibrated intestinal barrier function.<sup>8</sup> It  
51  
52 58 prevents against loss of water and electrolytes and entry of antigens and microorganisms  
53  
54 59 into the body while allowing the exchange of molecules between host and environment  
55  
56 60 and the absorption of nutrients from the diet.<sup>9</sup>

57  
58 61 Many factors can alter intestinal barrier function such as gut microbiota  
59  
60 62 modifications, mucus layer alterations, and epithelial damage, resulting in translocation  
61  
62 63 of luminal content to the inner layers of the intestinal epithelial cells.<sup>10 11</sup> Evidence

1  
2  
3 64 obtained in animal models as well as in humans is accumulating supporting a role of  
4 65 alterations of intestinal barrier function in many conditions, which include intestinal  
5 66 disorders such as malnutrition, diarrheal diseases, environmental enteric disease (EED),  
6 67 inflammatory bowel disease, irritable bowel syndrome, hepatic fibrosis, inflammation,  
7 68 sepsis and pancreatitis, but also obesity and metabolic syndrome.<sup>12-15</sup>

11  
12 69 Intestinal barrier function and integrity can be measured in different ways. The  
13 70 techniques used for this assessment vary depending on the setting (*in vitro* versus *in vivo*  
14 71 measurements), the species (human or animals), the marker molecules used (ions,  
15 72 carbohydrates of different sizes, macromolecules and antigens, bacterial products and  
16 73 bacteria), and the compartments used for measurement of the marker molecules  
17 74 (peripheral blood, portal vein blood or urine).<sup>16 17</sup> Each method is specific for a certain  
18 75 section of the gastrointestinal tract and measures different functional aspects of epithelial  
19 76 integrity of the intestine.

23  
24  
25  
26  
27 77 *In vivo* assessment of intestinal barrier absorption, damage and permeability in  
28 78 humans are currently possible by using intestinal barrier function biomarkers and assays.  
29 79 One of the most used assays is the oral Lactulose:Mannitol permeability test, a  
30 80 quantitative non-invasive test that directly measures the ability of two non-metabolized  
31 81 sugar molecules, lactulose and mannitol, to permeate the intestinal mucosa.<sup>18</sup> Lactulose  
32 82 (L), a disaccharide, is absorbed through cell junctions or epithelial cell turnover or  
33 83 damage, while mannitol (M), a monosaccharide, is absorbed most across the epithelial  
34 84 cell membranes.<sup>19</sup> Once absorbed, these sugars are excreted unmetabolized in the urine,  
35 85 and the sugar excretion is determined by chromatography. Elevated lactulose to mannitol  
36 86 ratio is an indicator of intestinal barrier dysfunction.<sup>11</sup> Other sugar probes used to evaluate  
37 87 the intestinal barrier function include sucralose, rhamnose, sucrose, and these are also  
38 88 measured in the urine after an oral dose.<sup>20</sup> The extent of sucrose absorption and  
39 89 subsequently excretion correlate with gastroduodenal permeation.<sup>3</sup> Sucralose is resistant  
40 90 to bacterial utilization in the colon and therefore has been used for measuring colonic  
41 91 permeability.<sup>21</sup> Rhamnose is used as a marker for small bowel permeability.<sup>22</sup> Sometimes,  
42 92 all these saccharides markers are used together to appraise pan-gastrointestinal  
43 93 permeability.<sup>3</sup>

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56  
57 94 The human protein zonulin is the main physiological modulator of tight junctions  
58 95 (TJs) in the intestinal epithelial layer that increases intestinal permeability in small  
59 96 intestine by inducing the opening of TJ and also participates in intestinal innate

1  
2  
3 97 immunity.<sup>23</sup> Circulating zonulin in serum is considered as a useful marker of intestinal  
4  
5 98 barrier integrity and is measured using enzyme-linked immunosorbent assay. <sup>24</sup> In  
6  
7 99 humans, it has been validated using lactulose/mannitol tests, being serum zonulin strongly  
8  
9 100 correlated with the lactulose/mannitol ratio.<sup>25</sup>

10  
11 101 Despite the tests immense potentials, application in clinical research remains limited  
12  
13 102 due to variations in the methodologies such as study population, sugar solution  
14  
15 103 formulation and administration, urine collection time, assay method and sensitivity.<sup>26</sup>  
16  
17 104 These variations restrict the clinical sensibility and accuracy of the diagnostic tests of  
18  
19 105 intestinal permeability, for example, the relationship between intestinal barrier function,  
20  
21 106 integrity and inflammatory outcomes in diseases such as obesity and metabolic syndrome.

22  
23 107 Animal models have shown that communication between the gut-adipose tissue and  
24  
25 108 the gut-brain is essential for maintaining energy balance, and this communication is  
26  
27 109 impaired during obesity and type 2 diabetes.<sup>27</sup> In this context, metabolic endotoxemia,  
28  
29 110 characterized by an increase in lipopolysaccharides in plasma, was identified as one of  
30  
31 111 the main factors that lead to the development of metabolic inflammation and insulin  
32  
33 112 resistance. Increasing evidence supports that the intestinal microflora is responsible for  
34  
35 113 the development of a low-grade inflammation that generates dysfunctions in the intestinal  
36  
37 114 barrier, increases its permeability, and allows a consequent endotoxemia.<sup>28</sup>

38  
39 115 Although these findings are well delineated for animal models, few studies in humans  
40  
41 116 have been performed.<sup>29</sup> A study compared two groups of women with and without  
42  
43 117 obesity, assessing intestinal permeability by urinary lactulose/mannitol ratio. Although  
44  
45 118 both sugars' urinary excretions were higher in women with obesity, a statistically  
46  
47 119 significant difference in the lactulose/mannitol ratio was not found between the studied  
48  
49 120 groups. Nevertheless, a higher lactulose/mannitol ratio was associated with higher  
50  
51 121 homeostatic model assessment (HOMA), insulin and LDL/HDL concentrations, and  
52  
53 122 lower HDL concentrations.<sup>30</sup> Thus, the intestinal barrier function might be associated  
54  
55 123 with obesity and metabolic syndrome.

56  
57 124 This study aims to describe a systematic review protocol investigating if obesity with  
58  
59 125 or without metabolic syndrome is associated with an altered intestinal barrier function. In  
60  
126 addition, the systematic review will evaluate methodologies used in the studies regarding  
127 intestinal barrier function biomarkers and assays methods. This review protocol will  
128 address if obesity or obesity associated with metabolic syndrome alters intestinal barrier  
129 function and integrity.

## 130 2. Methods

### 131 2.1 Patient and public involvement

132 No patients involved.

### 133 2.2 Protocol and registration

134 This protocol has been prepared according to the guidelines described in Preferred  
135 Reporting Items for Systematic Reviews and Meta-Analyses Protocols (PRISMA-P).<sup>31</sup> A  
136 27-item checklist was used to improve the quality of the systematic review data. The  
137 protocol was registered with the International Prospective Register of Systematic  
138 Reviews (PROSPERO) on July 10, 2020 (CRD42020178658) and is available at:  
139 [https://www.crd.york.ac.uk/prospero/display\\_record.php?ID=CRD42020178658](https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42020178658).

### 140 2.3 Eligibility criteria

141 Observational studies, published in scientific journals, will be included in the  
142 review. The guiding question in order to ensure the systematic search of available  
143 literature is ‘*Is there alteration of intestinal barrier function in individuals with obesity  
144 with or without metabolic syndrome?*’. Thus, studies that have addressed as population  
145 individuals with obesity with or without metabolic syndrome assessing intestinal  
146 permeability as a variable will be included in the systematic review.

#### 147 2.3.1 Inclusion criteria

148 The review will include original articles studying adults and elderlies with obesity  
149 with or without metabolic syndrome [according to modified National Cholesterol  
150 Education Program (NCEP) criteria is the presence of 3 or more of the following  
151 components: (1) waist circumference more than 90 cm in men or 80 cm in women; (2)  
152 triacylglycerols equal to or more than 150 mg/dL; (3) HDL-c less than 40mg/dL in men  
153 or 50 mg/dL in women; (4) blood pressure equal to or more than 130/85 mgHg and (5)  
154 fasting glucose between 100 and 125 mg/dL)]<sup>32</sup>, studies regarding the intestinal barrier  
155 function in this population and studies evaluating the intestinal epithelial cells integrity  
156 from individuals with obesity with or without metabolic syndrome.

157 All diagnostic test for intestinal barrier function will be considered for the  
158 systematic review (urinary measurement of orally-administrated sugar probe molecules  
159 and assays that use zonulin as a marker for intestinal permeability).

#### 160 2.3.2 Exclusion criteria

1  
2  
3 161 Review articles, case reports, comments, editorials, letters to the editor, theses,  
4  
5 162 conference proceedings, studies with animals or cell models, studies with children,  
6  
7 163 studies with adults and/or elderly that have other metabolic diseases, studies that did not  
8  
9 164 evaluate the intestinal barrier function.

#### 10 165 2.4 Information sources and literature search

11  
12 166 The search will be elaborated and implemented according to the PRISMA-P  
13  
14 167 checklist as guidance. Search strategies will be developed based on keywords indexed in  
15  
16 168 the Medical Subject Headings (MeSH) to identify the studies to be included in the  
17  
18 169 systematic review. The descriptors used will be related to intestinal barrier function,  
19  
20 170 obesity, and metabolic syndrome, such as anthropometric data, biochemical analysis,  
21  
22 171 intestinal permeability and integrity methodologies and assays. These descriptors will be  
23  
24 172 accompanied by Boolean operators “AND” and “OR”.

25 173 Two reviewers will independently conduct sensitive search for eligible studies  
26  
27 174 through the electronic databases PubMed Database, Embase Database, Cochrane Library,  
28  
29 175 Scopus, Web of Science (WOS) and Science Direct. In order to reflect the latest data, a  
30  
31 176 search of the literature from the last 15 years (2006 to 2021) will be performed. Articles  
32  
33 177 will be imported into Mendeley reference manager (1.19.4) and duplicates will be deleted.  
34  
35 178 Initial screening of studies will be based on the information in their title, keywords and  
36  
37 179 abstracts, following the eligibility criteria. When the reviewers disagree, the article will  
38  
39 180 be re-evaluated and, if the disagreement persists, a third reviewer will make a final  
40  
41 181 decision. Full-paper screening will be conducted by the same independent investigators.  
42  
43 182 The references of the included articles will also be reviewed to identify those potentially  
44  
45 183 eligible studies not found in the database search, considered as manual search.

#### 46 184 2.5 Data extraction

47 185 For data extraction, two independent Microsoft Excel spreadsheets will be  
48  
49 186 elaborated for two reviewers to summarize the data from the included studies. The  
50  
51 187 following information will be extracted and entered in the spreadsheet: first author; year  
52  
53 188 and language of publication; the country where the study was conducted; characteristics  
54  
55 189 of the population (Metabolic Syndrome presence, age, gender, health conditions, total  
56  
57 190 sample size, chronic diseases); methods to evaluate the intestinal barrier function; effects  
58  
59 191 of obesity with or without metabolic syndrome in the intestinal barrier function;  
60  
192 description of results and conclusions that are relevant to the overview; key findings;  
193 reported limitations.

## 194 2.6 Methodological quality assessment

195 Assessment of methodological quality and risk of bias in the studies with case-  
196 control design will be performed using the adapted Newcastle-Ottawa Scale<sup>33</sup>, in which  
197 studies that receive at least five stars (maximum of eight) will be classified as good quality  
198 studies. Two independent reviewers will assess the methodological quality of eligible  
199 studies. These independent reviewers will score the selected studies, and a third reviewer  
200 will resolve any disagreement.

## 201 2.7 Data analysis and synthesis

202 The systematic review will describe the relevant information of the included  
203 studies. Key information on characteristics, methods, results and quality scores of  
204 included studies will be tabulated. Following this, a narrative synthesis will be conducted.

205 Firstly, in the narrative review, the number of studies to be included in the  
206 synthesis will be reported and characteristics of each study will be described as well the  
207 location, kind and study population. Secondly, the narrative synthesis will report and  
208 discuss the methods used to evaluate the intestinal permeability and the relevant data.  
209 Also, the quality of the methods used will be discussed based on the related and observed  
210 study limitations. Finally, the observation of altered intestinal barrier function in obesity  
211 with or without metabolic syndrome will be explored and similarities and differences of  
212 findings will be reported.

213 The best-evidence synthesis will be guaranteed, and the risk of bias due to  
214 selective publication will be controlled by following the steps described above and  
215 assessing the quality of the evidence. The GRADE framework will be used to assess the  
216 quality of evidence concerning the association between intestinal barrier function  
217 alteration in obesity with or without metabolic syndrome. GRADE ranks the evidence as  
218 high (when there is strong certainty that the association is close to the estimated);  
219 moderate (when there is moderate certainty in the estimated association); low (when  
220 certainty in association is limited); and very low (when certainty in the association  
221 estimate is very limited owing to a significant degree of uncertainty in the findings).<sup>34</sup>

## 222 3. Discussion

223 Obesity has become a global epidemic and is a substantial threat to patients and  
224 healthcare systems because of related morbidity and costs.<sup>35</sup> Metabolic and

1  
2  
3 225 cardiovascular complications are a major obesity-associated burden, with critical roles  
4  
5 226 for insulin resistance, type 2 diabetes and atherosclerosis.<sup>36</sup> Given the increasing  
6  
7 227 prevalence of obesity worldwide, it is necessary to identify individuals with or without  
8  
9 228 metabolic syndrome as a clinical priority.

10  
11 229 Evidence has proposed the potential role of the gut microbiota as a pathogenic  
12  
13 230 factor affecting host metabolic balance and disorders.<sup>1</sup> Gut microbiota seems to exert a  
14  
15 231 great variety of functional properties impacting human physiology and pathology:  
16  
17 232 modulation of host nutrition and energy harvest by the production of vitamins and  
18  
19 233 fermentation of food components indigestible by the host; influence on intestinal  
20  
21 234 epithelial homeostasis; intestinal barrier function; development of host immune system;  
22  
23 235 protection against pathogens; drug metabolism.<sup>7</sup>

23  
24 236 Animal models and some human studies are accumulating to support alterations  
25  
26 237 of the intestinal barrier function in a vast array of conditions, which include obesity and  
27  
28 238 metabolic syndrome.<sup>37</sup> Given the importance of the intestinal barrier function and  
29  
30 239 integrity, understanding what can disrupt it and cause the loss of function and integrity  
31  
32 240 are necessary. Even though no final conclusions exist, it is more evident that besides  
33  
34 241 nutrients acting as down-regulators of tight junctions or as histone deacetylase (HDAC)  
35  
36 242 inhibitors, also viral infections, toxins, hypoperfusion of the gut play a role. <sup>38 39</sup> Lifestyle  
37  
38 243 factors such as living place, physical activity, dietary patterns and drug usage seem to  
39  
40 244 play an important role as well, and they offer new approaches for improving gut barrier  
41  
42 245 function.<sup>39</sup>

41  
42 246 In this perspective, this systematic review will address studies that evaluated  
43  
44 247 individuals with obesity with or without metabolic syndrome, focusing on the analysis of  
45  
46 248 their intestinal barrier function. This review will also generate evidence for the use of  
47  
48 249 different tests for the diagnosis of *in vivo* intestinal barrier function and integrity.

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52  
53 252 University of Rio Grande do Norte.

54 253 **Contributors:** MDB, CHMT, SCVCL and BLLM conceived the idea, planned and  
55  
56 254 designed the study protocol. MDB and CHMT wrote the first draft; SCVCL and BLLM  
57  
58 255 planned the data extraction and statistical analysis; AAML and BLLM provided critical  
59  
60 256 insights. All authors have approved and contributed to the final written manuscript.



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# 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-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.

		Page
	Reporting Item	Number
<b>Title</b>		
Identification	<a href="#">#1a</a> Identify the report as a protocol of a systematic review	1
Update	<a href="#">#1b</a> If the protocol is for an update of a previous systematic review, identify as such	1

1 **Registration**

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4 [#2](#) If registered, provide the name of the registry (such as 2

5 PROSPERO) and registration number

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10 **Authors**

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13 **Contact** [#3a](#) Provide name, institutional affiliation, e-mail address of all 1

14 protocol authors; provide physical mailing address of

15 corresponding author

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20 **Contribution** [#3b](#) Describe contributions of protocol authors and identify the 8

21 guarantor of the review

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26 **Amendments**

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29 [#4](#) If the protocol represents an amendment of a previously 1

30 completed or published protocol, identify as such and list

31 changes; otherwise, state plan for documenting important

32 protocol amendments

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39 **Support**

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42 **Sources** [#5a](#) Indicate sources of financial or other support for the review 8

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45 **Sponsor** [#5b](#) Provide name for the review funder and / or sponsor 8

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48 **Role of sponsor or** [#5c](#) Describe roles of funder(s), sponsor(s), and / or 8

49 funder

50 institution(s), if any, in developing the protocol

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53 **Introduction**

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1	Rationale	<a href="#">#6</a>	Describe the rationale for the review in the context of what is	2
2			already known	
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6	Objectives	<a href="#">#7</a>	Provide an explicit statement of the question(s) the review	4
7			will address with reference to participants, interventions,	
8			comparators, and outcomes (PICO)	
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14	<b>Methods</b>			
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17	Eligibility criteria	<a href="#">#8</a>	Specify the study characteristics (such as PICO, study	5
18			design, setting, time frame) and report characteristics (such	
19			as years considered, language, publication status) to be	
20			used as criteria for eligibility for the review	
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27	Information	<a href="#">#9</a>	Describe all intended information sources (such as	6
28			electronic databases, contact with study authors, trial	
29	sources		registers or other grey literature sources) with planned dates	
30			of coverage	
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37	Search strategy	<a href="#">#10</a>	Present draft of search strategy to be used for at least one	6
38			electronic database, including planned limits, such that it	
39			could be repeated	
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45	Study records -	<a href="#">#11a</a>	Describe the mechanism(s) that will be used to manage	6
46			records and data throughout the review	
47	data management			
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50	Study records -	<a href="#">#11b</a>	State the process that will be used for selecting studies	6,7
51			(such as two independent reviewers) through each phase of	
52	selection process		the review (that is, screening, eligibility and inclusion in	
53			meta-analysis)	
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1	Study records -	<a href="#">#11c</a>	Describe planned method of extracting data from reports	6
2				
3	data collection		(such as piloting forms, done independently, in duplicate),	
4				
5	process		any processes for obtaining and confirming data from	
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7			investigators	
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11	Data items	<a href="#">#12</a>	List and define all variables for which data will be sought	5
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13			(such as PICO items, funding sources), any pre-planned	
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15			data assumptions and simplifications	
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18	Outcomes and	<a href="#">#13</a>	List and define all outcomes for which data will be sought,	
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20	prioritization		including prioritization of main and additional outcomes, with	
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22			rationale	
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26	Risk of bias in	<a href="#">#14</a>	Describe anticipated methods for assessing risk of bias of	7
27				
28	individual studies		individual studies, including whether this will be done at the	
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30			outcome or study level, or both; state how this information	
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32			will be used in data synthesis	
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36	Data synthesis	<a href="#">#15a</a>	Describe criteria under which study data will be	
37				
38			quantitatively synthesized	
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42	Data synthesis	<a href="#">#15b</a>	If data are appropriate for quantitative synthesis, describe	
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44			planned summary measures, methods of handling data and	
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46			methods of combining data from studies, including any	
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48			planned exploration of consistency (such as I <sup>2</sup> , Kendall's $\tau$ )	
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51	Data synthesis	<a href="#">#15c</a>	Describe any proposed additional analyses (such as	
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53			sensitivity or subgroup analyses, meta-regression)	
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1	Data synthesis	<a href="#">#15d</a>	If quantitative synthesis is not appropriate, describe the type	7
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3			of summary planned	
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6	Meta-bias(es)	<a href="#">#16</a>	Specify any planned assessment of meta-bias(es) (such as	
7			publication bias across studies, selective reporting within	
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9			studies)	
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14	Confidence in	<a href="#">#17</a>	Describe how the strength of the body of evidence will be	7
15	cumulative		assessed (such as GRADE)	
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17	evidence			
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24 tool made by the [EQUATOR Network](#) in collaboration with [Penelope.ai](#)  
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