

# BMJ Open

BMJ Open is committed to open peer review. As part of this commitment we make the peer review history of every article we publish publicly available.

When an article is published we post the peer reviewers' comments and the authors' responses online. We also post the versions of the paper that were used during peer review. These are the versions that the peer review comments apply to.

The versions of the paper that follow are the versions that were submitted during the peer review process. They are not the versions of record or the final published versions. They should not be cited or distributed as the published version of this manuscript.

BMJ Open is an open access journal and the full, final, typeset and author-corrected version of record of the manuscript is available on our site with no access controls, subscription charges or pay-per-view fees (<http://bmjopen.bmj.com>).

If you have any questions on BMJ Open's open peer review process please email [info.bmjopen@bmj.com](mailto:info.bmjopen@bmj.com)

# BMJ Open

## Effects of boron citrate supplementation on cardiometabolic factors, inflammatory biomarkers, nutritional status, and anthropometric measures in obese patients: study protocol for a randomized double-blind clinical trial

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2023-075941
Article Type:	Protocol
Date Submitted by the Author:	23-May-2023
Complete List of Authors:	Naemi, Mohammad; Tabriz University of Medical Sciences naghshi, sina; Tabriz University of Medical Sciences Rostami, Somaye; Tabriz University of Medical Sciences Safaei, Ehsan; Tabriz University of Medical Sciences Tutunchi, Helda; Tabriz University of Medical Sciences Ostadrahimi, Alireza; Tabriz University of Medical Sciences, Department of Clinical Nutrition
Keywords:	Obesity, Randomized Controlled Trial, NUTRITION & DIETETICS

SCHOLARONE™  
Manuscripts

1  
2  
3  
4  
5  
6  
7  
8  
9

## Effects of boron citrate supplementation on cardiometabolic factors, inflammatory biomarkers, nutritional status, and anthropometric measures in obese patients: study protocol for a randomized double-blind clinical trial

10 Mohammad Naemi Kermanshahi<sup>1</sup>, Sina Naghshi<sup>1</sup>, Somaye Rostami<sup>1</sup>, Ehsan Safaei<sup>1</sup>, Helda Tutunchi<sup>2\*</sup>, Alireza Ostadrahimi<sup>3\*</sup>

14 <sup>1</sup>Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran. Email: [mohammadnaemi732@yahoo.com](mailto:mohammadnaemi732@yahoo.com), [naghshi\\_sina@yahoo.com](mailto:naghshi_sina@yahoo.com), [somyrostammi96@gmail.com](mailto:somyrostammi96@gmail.com)

18 <sup>2</sup> Endocrine Research Center, Tabriz University of Medical Sciences, Tabriz, Iran. Email: [helda.nutrition@gmail.com](mailto:helda.nutrition@gmail.com)

21 <sup>3</sup> Nutrition Research Center, Department of Clinical Nutrition, School of Nutrition and Food Science, Tabriz University of Medical Sciences, Tabriz, Iran. Email: [ostadrahimi@tbzmed.ac.ir](mailto:ostadrahimi@tbzmed.ac.ir)

28  
29

### \*Corresponding to:

30 Helda Tutunchi. Ph.D.

31 Endocrine Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.

32 Email: [helda.nutrition@gmail.com](mailto:helda.nutrition@gmail.com)

35 Alireza Ostadrahimi. Ph.D.

36 Department of Clinical Nutrition, School of Nutrition and Food Sciences, Tabriz University of Medical Sciences, Tabriz, Iran.

37 Email: [ostadrahimi@tbzmed.ac.ir](mailto:ostadrahimi@tbzmed.ac.ir)

42  
43 Running title: Boron citrate and obesity

44  
45 Word Count: 2638

46  
47 Number of Figures: 1

48  
49 Number of Tables: 1

## 1 **Abstract**

2 **Introduction:** Obesity is a chronic disease with serious health consequences, but weight loss  
3 is difficult to maintain through lifestyle intervention alone. The efficacy and safety of boron  
4 citrate (BC), a novel therapeutic approach, in patients with obesity are not known. The current  
5 trial will take place to determine the effects of BC supplementation on cardiometabolic factors,  
6 inflammatory biomarkers, nutritional status, anthropometric measures, and body composition  
7 in obese patients.

8 **Methods and analysis:** This double-blind, placebo-controlled, randomized clinical trial (RCT)  
9 will involve 60 eligible obese participants aged 18 to 60 years old. Subjects will randomly be  
10 allocated to receive either BC capsules (containing 10 mg of boron) in the intervention group  
11 or placebo capsules (containing 10 mg of maltodextrin) in the placebo group for 12 weeks.  
12 Cardiometabolic factors, inflammatory biomarkers including tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ),  
13 C-reactive protein (CRP), interleukin-6 (IL-6), and IL-10 levels, anthropometric measures, and  
14 body composition will be assessed at baseline and end of the intervention. Statistical analysis  
15 will be carried out using SPSS, version 23 and  $p < 0.05$  will be considered statistically  
16 significant.

17 **Ethics and dissemination:** This trial was approved by the Ethics Committee of Tabriz  
18 University of Medical Sciences (approval number: IR.TBZMED.REC.1401.350).

19 **Trial registration number:** IRCT20220806055624N1.

20 **Key words:** Boron citrate, Inflammation, Obesity, Randomized controlled trial

21

## 22 Introduction

23 Obesity, which typically refers to excess body fat, has emerged as a major public health issue.

24 Obesity is associated with numerous comorbidities, including hypertension, dyslipidemia, type  
25 2 diabetes, cardiovascular disease, and nonalcoholic fatty liver disease <sup>1</sup>. Existing data show  
26 that the prevalence of obesity is rising <sup>2</sup>. In 2016, more than 20% of the world's population  
27 were obese <sup>3</sup>. In this way, Iran is not exempt from this issue. About 40.3% and 19.2% of  
28 Iranians were overweight or obese, respectively, based on the second national integrated  
29 micronutrient study conducted from 2011 to 2015 <sup>4</sup>.

30 Obesity is a complicated multifactorial disease in which genetic, metabolic, and environmental  
31 factors are thought to play a major role in the development of this disease. Moreover, several  
32 lines of evidence suggest that low-grade, long-standing adipose tissue inflammation is strongly  
33 associated with insulin resistance and excess body fat mass <sup>5</sup>. The excessive adipose tissue  
34 stimulates the secretion of inflammatory mediators and consequently leads to metabolic  
35 disorders <sup>6</sup>. Lifestyle interventions (diet and exercise) have long been known as the cornerstone  
36 of weight management. Additionally, clinical guidelines recommend adjunctive anti-obesity  
37 medications, but their usage is severely constrained by their unfavorable side effects <sup>7</sup>.  
38 Therefore, novel anti-obesity and anti-inflammatory approaches focusing on the control of lipid  
39 metabolism to limit fat production and storage with minimal side effects have recently attracted  
40 substantial attention <sup>8</sup>.

41 Elemental boron has been known to be an essential micronutrient for plants since the 1920s.  
42 Yet, boron looks favorable not only for plant cells but also for the majority of animal and  
43 human cells. Boron has been linked to bone development and maintenance, cognitive function,  
44 steroid hormone regulation, and immune response <sup>9</sup>. Boron deficiency has been documented to  
45 have major effects on mammalian metabolic and physiological systems (lipid, bone, mineral,  
46 endocrine function, and energy metabolism) <sup>8</sup>. In previous experimental studies, a low oral

1  
2  
3 47 dose of boric acid reduced body weight <sup>10 11</sup>, findings that supported further investigation.  
4  
5 48 Furthermore, a meta-analysis of animal studies documented that boron has weight-lowering  
6  
7 49 effects <sup>12</sup>. Boron citrate (BC) could induce weight loss in obese patients through increasing  
8  
9 50 energy metabolism, thermogenesis, lipolysis, and inhibition of adiposeness <sup>12</sup>. Additionally,  
10  
11 51 supplementation with boron could decrease inflammatory cytokines in eight healthy males <sup>13</sup>.  
12  
13 52 Given the role of BC in suppressing inflammation and inducing lipolysis, we hypothesized that  
14  
15 53 administration of BC may reduce body weight and inflammatory markers and improve  
16  
17 54 cardiometabolic factors in obese patients. Therefore, the purpose of the present study is to  
18  
19 55 investigate the effects of BC supplementation on cardiometabolic factors, inflammatory  
20  
21 56 biomarkers including, anthropometric measures, and body composition in obese patients.  
22  
23  
24  
25

## 26 57 **Methods and design**

### 28 58 **Study design**

30  
31 59 This parallel-group, randomized, double-blind clinical trial will be evaluated the effects of  
32  
33 60 BC supplementation on cardiometabolic factors, inflammatory biomarkers including tumor  
34  
35 61 necrosis factor  $\alpha$  (TNF- $\alpha$ ), C-reactive protein (CRP), interleukin-6 (IL-6), and IL-10 levels,  
36  
37 62 anthropometric measures, and body composition in obese patients. The trial was registered on  
38  
39 63 the Iranian Registry of Clinical Trials website (<http://www.irct.ir>) at 2022-08-31 with code  
40  
41 64 number of IRCT20220806055624N1. Moreover, the Ethics Committee of Research Vice-  
42  
43 65 Chancellor of Tabriz University of Medical Sciences ethically approved the study (identifier:  
44  
45 66 IR.TBZMED.REC.1401.350). This trial will be conducted at Tabriz University of Medical  
46  
47 67 Sciences, Tabriz, Iran. Written informed consent will be obtained from the volunteers at the  
48  
49 68 beginning of the study by researchers. The Standard Protocol Items: Recommendations for  
50  
51 69 Interventional Trials (SPIRIT 2013 checklist) was used to develop the protocol for this study.  
52  
53 70 The time schedule of the trial and the progress of the study along with the registration of  
54  
55 71 volunteers, allocation, intervention, and review are displayed in **Table 1** and **Figure 1**.  
56  
57  
58  
59  
60

## 72 **Study participants and enrolment**

73 The participants will be recruited through advertisements and referrals from physicians and  
74 families. Individuals who are wishing to participate in the present study will participate in  
75 this research project after the initial interview by the researchers and considering the defined  
76 inclusion and exclusion criteria. All participants will be assigned a visit time, and their blood  
77 samples will be taken following the visit.

### 78 **Inclusion criteria**

79 In the present study, we will recruit adults aged between 18-60 years and with a body-mass  
80 index (BMI) of 30 to 40 kg/m<sup>2</sup>.

### 81 **Exclusion criteria**

82 Individuals who are professional athletes, smokers, those consuming alcohol, taking chemical  
83 or herbal drugs for weight loss, hepatotoxic drugs such as phenytoin, tamoxifen, lithium, anti-  
84 hypertensive drugs, lipid-lowering drugs (statins), insulin-sensitive drugs, corticosteroid, and  
85 non-steroidal anti-inflammatory drugs, antibiotics, supplements affecting liver enzyme levels  
86 or any supplements over the past three months will not be included. We also will not include  
87 individuals who are pregnant, lactating, menopause, those who have undergone infertility  
88 treatment, and those who use birth control pills and estrogen. Individuals who have some  
89 pathologic conditions such as cardiovascular, liver, thyroid, parathyroid, kidney,  
90 gastrointestinal, and known autoimmune diseases as well as suffering from polycystic ovary  
91 syndrome, cancer, severe infection, or inflammatory disease will be excluded. In addition,  
92 those who underwent weight loss surgery in the last year or strict weight loss diets in the last  
93 three months will not be included in the current study. If a subject becomes pregnant during  
94 the course of the research, consumes alcohol and/or tobacco, or utilizes multivitamin-mineral  
95 supplements, he or she will be excluded.

## 96 **Sample size calculation**

1  
2  
3 97 We calculated the required sample size based on data from a previous study<sup>14</sup> by considering  
4  
5 98 plasma CRP level as a key dependent variable, type I error of 0.05, and study power of 80%.  
6  
7 99 Based on the suggested formula for parallel clinical trials, we reached the sample size of 24  
8  
9  
10 100 patients in each group. Taking into account a possible drop-out rate of 20%, 30 patients will  
11  
12 101 be enrolled in each group.

## 102 **Randomization**

103 To stratify individuals into distinct strata and blocks, stratified block randomization will be  
104 implemented based on age (18-40 vs. 40-60 years) and gender (male vs. female). For each  
105 individual placed in a given stratum, a matched individual will be considered based on these  
106 variables in the same stratum. As a result, two participants with similar characteristics (for  
107 age and gender) will be placed in the same stratum. Finally, each stratum will be randomly  
108 allocated to the BC or placebo groups using Random Allocation Software (RAS). Participants  
109 and researchers will be blinded to randomization and allocation until the end of the study.  
110 The randomization list will be provided by the pharmacist of the research center at the end of  
111 the study.

## 112 **Study interventions**

113 Sixty obese patients will be randomly assigned in a 1:1 allocation ratio to receive either a BC  
114 capsule (containing 10 mg of boron) or a matched placebo capsule (containing 10 mg of  
115 maltodextrin) once daily before lunch for 12 weeks. Following ethical considerations, the  
116 researchers will give physical activity and dietary advice to enhance the lifestyles of patients  
117 in both groups.

## 118 **Study outcomes**

### 119 **Primary and secondary outcomes**

120 The primary outcomes of this study are changes in inflammatory factors (CRP, TNF- $\alpha$ , IL-10,  
121 and IL-6), anthropometric indicators (weight, BMI, waist circumference (WC), hip



1  
2  
3 122 circumference (HC), waist-to-hip ratio (WHR), and waist-to-height ratio (WHtR)), and body  
4  
5 123 composition (total body fat mass (FM) and total body fat-free mass (FFM)). Changes in  
6  
7  
8 124 nutritional status (amount of energy intake and macronutrients), glycemic parameters (fasting  
9  
10 125 blood sugar (FBS), fasting blood insulin (FBI), hemostatic model assessment of insulin  
11  
12 126 resistance (HOMA-IR), and the quantitative insulin sensitivity check index (QUICKI)), lipid  
13  
14 127 profile (triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-  
15  
16  
17 128 C), and low-density lipoprotein cholesterol (LDL-C)), and blood pressure are regarded as  
18  
19 129 secondary outcomes.

## 130 **Measurements and assessments**

### 131 **Clinical assessments**

132 At the onset of the study, all patients will be asked about their medical history, smoking  
133 history, underlying diseases, hormonal problems, alcohol consumption, supplement and drug  
134 use history, and demographic characteristics (age, gender, education level, employment  
135 status, marital status, and contact number).

### 136 **Blood pressure**

137 Systolic and diastolic blood pressures will be measured in a seated posture twice with a 15-  
138 minute interval at the right arm. Before measurement, patients will be required to rest for five  
139 minutes. The mean of two measurements will be used in the analyses.

### 140 **Assessment of physical activity and dietary intake**

141 To ensure that participants' physical activity does not alter throughout the trial, we will  
142 monitor their activity. Already validated in Iran <sup>15</sup>, the International Physical Activity  
143 Questionnaire-Short Form (IPAQ-SF) will be used to estimate the physical activity level.  
144 According to the IPAQ scoring system, physical activity levels will be classified as low,  
145 moderate, and high activity. To assess the dietary intake of each participant, a 3-day food  
146 record (2 days of the week and 1 day of the weekend) will be filled at the beginning and end

1  
2  
3 147 of the study. Data on food intake will be analyzed by Nutritionist IV software (First  
4  
5 148 Databank, San Bruno, CA, USA) modified for Iranian foods.

### 8 149 **Assessment of appetite sensations**

9  
10 150 Appetite sensations (hunger, satiety, fullness, desire to consume something sweet, fatty, or  
11  
12 151 salty) of the patients will be measured using a visual analogue scale (VAS) at the beginning  
13  
14 152 and end of the study after overnight fasting <sup>16</sup>

### 17 153 **Anthropometric measurements**

18  
19 154 Data on anthropometric measurements will be collected at baseline and end of the trial. The  
20  
21 155 body weight will be recorded in a fasted state, without footwear, and wearing light clothing to  
22  
23 156 an accuracy of 100 g. Height will be measured using a stadiometer (Seca, Hamburg,  
24  
25 157 Germany) without shoes at a standing position to the nearest 0.1 cm accuracy. WC is defined  
26  
27 158 as the smallest horizontal girth between the costal and iliac crests, measured to the nearest 0.1  
28  
29 159 cm with a non-stretchable measuring tape. The hip circumference (HC) will be measured at  
30  
31 160 the widest point above the great trochanters. Then, WHR will be calculated. BMI is  
32  
33 161 calculated by dividing weight in kilograms by the square of height in meters. Body  
34  
35 162 composition including FM and FFM will be measured using bioelectrical impedance analysis  
36  
37 163 (MC-780; Tanita, Amsterdam, The Netherlands).

### 42 164 **Blood sampling and biochemical measurements**

43  
44 165 The blood samples (10 ml) will be taken following a 12-h overnight fasting at pre-and post-  
45  
46 166 intervention phases. The blood samples will be kept inside the gel tube for 20 minutes for  
47  
48 167 better precipitation and will be centrifuged at 3000 rpm for 7 minutes to extract serum  
49  
50 168 samples. The serum levels of FBS, TC, TG, and HDL-C will be measured instantly on fresh  
51  
52 169 blood samples by enzymatic methods using colorimetric technique, by commercial kits (Pars-  
53  
54 170 Azmoon Co., Tehran, Iran). LDL-C will be calculated by the Friedewald equation <sup>17</sup>. The  
55  
56 171 enzyme-linked immunosorbent assay (ELISA) method will be applied to measure FBI using  
57  
58  
59  
60

1  
2  
3 172 commercial kits (Monobind, Lake Forest, CA, USA). Serum levels of inflammatory factors  
4  
5 173 (CRP, TNF- $\alpha$ , IL-10, and IL-6) will be assessed using ELISA kits. The following formula  
6  
7  
8 174 will be applied to determine HOMA-IR and QUICKI indexes.

9  
10 175  $HOMA1-IR = (\text{fasting insulin } (\mu\text{IU/ml}) \times \text{fasting glucose (mg/dl)})/405$  <sup>18</sup>

11  
12 176  $QUICKI = 1/(\log(\text{insulin, U ml}^{-1}) + \log(\text{FBS, mg dl}^{-1}))$

### 14 15 177 **Adherence to the intervention**

16  
17 178 To assess adherence to the intervention, participants will be asked to document their daily usage  
18  
19 179 of BC supplements on a checklist provided by the researchers. To enhance compliance and  
20  
21 180 prevent participants from forgetting to take supplements, investigators will send daily text  
22  
23 181 messages to participants' cell phones as reminders. In addition, adherence to the intervention  
24  
25 182 will be evaluated by counting the returned capsules at the midpoint and end of the experiment.

### 26 27 183 **Statistical analysis**

28  
29 184 Analyses will follow the intention-to-treat (ITT) convention. Missing values will be imputed  
30  
31 185 by multiple imputation procedures. The normality of the variables' distribution will be assessed  
32  
33 186 using the Shapiro–Wilk test. The findings will be presented as mean (SD) for numerical data,  
34  
35 187 frequency (percentage) for categorical variables, and median (25th, 75th) for values with  
36  
37 188 skewed distribution. The independent samples t-test and Mann-Whitney U test will be applied  
38  
39 189 to compare quantitative variables with normal and skewed distribution between the two groups,  
40  
41 190 respectively. To do a within-group comparison, we will use the paired-sample t-test and  
42  
43 191 Wilcoxon signed-rank test for values with normal and skewed distribution, respectively. We  
44  
45 192 will use the Chi-square test and Fisher's exact test to examine differences in qualitative  
46  
47 193 variables between the two groups. To estimate the intervention effect for all primary and  
48  
49 194 secondary outcomes, an analysis of covariance (ANCOVA) will be used. In this analysis, we  
50  
51 195 will include baseline measurements as a covariate to adjust for potential differences between  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 196 treatment groups at baseline. Statistical analysis will be carried out using SPSS, version 23.  
4  
5 197  $P < 0.05$  will be considered statistically significant.  
6  
7

### 8 198 **Adverse effects, safety, and data monitoring**

9  
10 199 Although the values of Recommended Daily Intake (RDA), Estimated Average Requirement  
11  
12 200 (EAR), and Adequate Intake (AI) for boron have not been reported, the tolerable Upper Level  
13  
14 201 (UL) in adults is 20 mg/day<sup>19</sup>. After BC supplementation, patients will be required to report  
15  
16 202 any potential side effects. In case of side effects, more information is required to make a  
17  
18 203 decision for excluding the participants from the study. In such conditions, unbinding is  
19  
20 204 permissible based on the Medical Ethics Committee criteria. Two supervisors from a Data  
21  
22 205 Monitoring Committee (DMC) will track the findings of the RCT. The DMC is independent of  
23  
24 206 the study organizers (sponsor and trial investigators). Moreover, the report of any adverse  
25  
26 207 effects will be sent to the Ethics Committee of Tabriz University of Medical Sciences. One of  
27  
28 208 the investigators will check the coding, security, and storage of the data. In addition, he/she  
29  
30 209 will assess the data entry and data values twice.  
31  
32  
33  
34

### 35 210 **Patient and public involvement**

36  
37 211 Patients and/or the public were not involved in the design, conduct reporting or dissemination  
38  
39 212 plans of this study.  
40  
41

### 42 213 **Discussion**

43  
44 214 Obesity is a chronic and progressive disease that affects approximately 650 million adults  
45  
46 215 worldwide. It is associated with multiple coexisting conditions and complications and imposes  
47  
48 216 a considerable burden on the healthcare system<sup>20 21</sup>. In adults, the first-line treatment for  
49  
50 217 obesity is typically lifestyle therapy, which often provides poor responses<sup>22 23</sup>. Therefore,  
51  
52 218 finding novel treatment options that can be used as adjuncts to lifestyle therapy in obese adults  
53  
54 219 is of interest.  
55  
56  
57  
58  
59  
60

1  
2  
3 220 There is growing evidence that boron plays a multitude of crucial roles in animal and human  
4  
5 221 health. Several experimental research concluded that boron can reduce weight and may even  
6  
7  
8 222 enhance obesity-related markers<sup>12</sup>. It has also been found that boron decreases FBI, FBS, and  
9  
10 223 pancreatic beta cell stress<sup>24 25</sup>. Finding from a study demonstrated that oral administration of  
11  
12 224 boric acid reduced the serum levels of TC and LDL-C and increased HDL-C levels in diabetic  
13  
14 225 rats<sup>26</sup>. In a clinical trial in which healthy women consumed diets containing 10 mg more boron  
15  
16 226 than their usual diet for one month, a significant reduction was observed in serum levels of TC,  
17  
18 227 LDL-C, very low-density lipoprotein cholesterol (VLDL-C), and TG. Moreover, the body  
19  
20 228 weight, body FM, and BMI of the women decreased significantly<sup>27</sup>. Overall, findings from  
21  
22 229 animal and human studies provide supportive evidence for the beneficial effects of boron on  
23  
24 230 metabolic parameters. To our knowledge, available investigations regarding the effects of BC  
25  
26 231 supplementation on features of obesity in humans are limited. The finding from this study will  
27  
28 232 provide clinical evidence on the effectiveness of BC supplementation in obese patients.

29  
30  
31  
32  
33 233 **Strengths and limitations:** This is the first randomized controlled clinical trial that will  
34  
35 234 investigate the effects of BC supplementation in obese patients. Using stratified block  
36  
37 235 randomization, patients will be matched based on a number of characteristics that may impact  
38  
39 236 the final results. Several outcomes, including anthropometric and biochemical indicators, will  
40  
41 237 be examined at the study baseline and endpoint. Moreover, dietary intake, physical activity  
42  
43 238 level, and compliance to the intervention will be assessed. Several limitations to this study  
44  
45 239 should be considered. Given the evaluation of compliance in the current study, low adherence  
46  
47 240 to intervention might be undetectable. Moreover, the serum levels of boron will not be  
48  
49 241 measured to examine the compliance of study participants due to financial constraints. A single  
50  
51 242 dose of BC will be used in this study, therefore, we cannot explore dose-response effects. As  
52  
53 243 different doses may have different effects, dose-finding trials are needed to identify the lowest  
54  
55 244 safe and effective dose. The 3-month intervention period may not be long enough to see a  
56  
57  
58  
59  
60

1  
2  
3 245 beneficial effect on secondary outcomes. Obese individuals will be recruited in the study,  
4  
5 246 which may represent a subpopulation that is more adherent to weight management  
6  
7 247 interventions than the general population with obesity. Moreover, the efficacy of the same  
8  
9 248 intervention in other metabolic diseases is not known. Future research should explore the  
10  
11 249 effects of boron on such diseases.  
12  
13

#### 14 250 **Trial status**

15 251  
16 252 Recruitment for this trial has begun at June 2023 and is expected to be completed by December  
17  
18 253 2023.  
19  
20

#### 21 254 **Declarations**

22 255

#### 23 256 **Ethics approval and consent to participate**

24  
25  
26 257 This trial was approved by the Ethics Committee of Tabriz University of Medical Sciences  
27  
28 258 (approval number: IR.TBZMED.REC.1401.350). Informed consent will be obtained from all  
29  
30 259 study participants prior to the study onset.  
31  
32

#### 33 260 **Availability of data and materials**

34 261

35 262 Not applicable  
36

#### 37 263 **Competing interest**

38  
39 264 The authors declare no competing interests.  
40  
41

#### 42 265 **Funding**

43 266

44 267 The study was financially supported by Endocrine Research Center of Tabriz University of  
45  
46 268 Medical Sciences.  
47  
48

#### 49 269 **Author Contributions**

50  
51 270 Study design: AO, HT and MNK. Methodology: HT, MNK, SRN and ES. Statistical plan: AO,  
52  
53 271 SN, and HT. Coordination of the study implementation: MNK, SRN and ES. Data collection:  
54  
55 272 MNK, SRN and ES. Manuscript preparation: MNK and SRN. Review and editing: AO, HT,  
56  
57 273 SN, and ES.  
58  
59  
60

274 All named authors have read and approved the final manuscript, adhere to the authorship  
275 guidelines of journal and have agreed to publication.

## 276 Acknowledgements

277  
278 We would like to appreciate the cooperation of the Clinical Research Development Unit of  
279 Imam Reza General Hospital, Tabriz, Iran in conducting this research. We sincerely thank the  
280 patients who participated in the present study.

## 281 References

- 282  
283 1. Flegal KM, Carroll MD, Ogden CL, et al. Prevalence and trends in obesity among US adults,  
284 1999-2000. *Jama* 2002;288(14):1723-7. doi: 10.1001/jama.288.14.1723 [published  
285 Online First: 2002/10/09]
- 286 2. Aekplakorn W, Inthawong R, Kessomboon P, et al. Prevalence and trends of obesity and  
287 association with socioeconomic status in Thai adults: National Health Examination  
288 Surveys, 1991-2009. *Journal of obesity* 2014;2014:410259. doi: 10.1155/2014/410259  
289 [published Online First: 2014/04/24]
- 290 3. Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to  
291 2016: a pooled analysis of 2416 population-based measurement studies in 128·9 million  
292 children, adolescents, and adults. *Lancet (London, England)* 2017;390(10113):2627-  
293 42. doi: 10.1016/s0140-6736(17)32129-3 [published Online First: 2017/10/17]
- 294 4. Pouraram H, Djazayeri A, Mohammad K, et al. Second National Integrated Micronutrient  
295 Survey in Iran: Study Design and Preliminary Findings. *Archives of Iranian medicine*  
296 2018;21(4):137-44. [published Online First: 2018/04/26]
- 297 5. Burhans MS, Hagman DK, Kuzma JN, et al. Contribution of Adipose Tissue Inflammation  
298 to the Development of Type 2 Diabetes Mellitus. *Comprehensive Physiology*  
299 2018;9(1):1-58. doi: 10.1002/cphy.c170040 [published Online First: 2018/12/15]
- 300 6. Ellulu MS, Patimah I, Khaza'ai H, et al. Obesity and inflammation: the linking mechanism  
301 and the complications. *Archives of medical science : AMS* 2017;13(4):851-63. doi:  
302 10.5114/aoms.2016.58928 [published Online First: 2017/07/20]
- 303 7. Wang L, Yu CC, Li J, et al. Mechanism of Action of Acupuncture in Obesity: A Perspective  
304 From the Hypothalamus. *Frontiers in endocrinology* 2021;12:632324. doi:  
305 10.3389/fendo.2021.632324 [published Online First: 2021/04/20]
- 306 8. Abdik EA, Abdik H, Taşlı PN, et al. Suppressive Role of Boron on Adipogenic  
307 Differentiation and Fat Deposition in Human Mesenchymal Stem Cells. *Biological*  
308 *trace element research* 2019;188(2):384-92. doi: 10.1007/s12011-018-1428-5  
309 [published Online First: 2018/07/08]
- 310 9. Nielsen FH, Meacham SL. Growing evidence for human health benefits of boron. *Journal*  
311 *of Evidence-Based Complementary & Alternative Medicine* 2011;16(3):169-80.
- 312 10. Aysan E, Sahin F, Telci D, et al. Mechanism of body weight reducing effect of oral boric  
313 Acid intake. *International journal of endocrinology* 2013;2013:914651. doi:  
314 10.1155/2013/914651 [published Online First: 2013/07/19]
- 315 11. Basoglu A, Baspınar N, Tenori L, et al. Effects of Boron Supplementation on Peripartum  
316 Dairy Cows' Health. *Biological trace element research* 2017;179(2):218-25. doi:  
317 10.1007/s12011-017-0971-9 [published Online First: 2017/02/24]

- 1  
2  
3 318 12. Farrin N, Rezazadeh L, Pourmoradian S, et al. Boron compound administration; A novel  
4 319 agent in weight management: A systematic review and meta- analysis of animal studies.  
5 320 *Journal of trace elements in medicine and biology : organ of the Society for Minerals  
6 321 and Trace Elements (GMS)* 2022;72:126969. doi: 10.1016/j.jtemb.2022.126969  
7 322 [published Online First: 2022/03/18]
- 8 323 13. Naghii MR, Mofid M, Asgari AR, et al. Comparative effects of daily and weekly boron  
9 324 supplementation on plasma steroid hormones and proinflammatory cytokines. *Journal  
10 325 of trace elements in medicine and biology : organ of the Society for Minerals and Trace  
11 326 Elements (GMS)* 2011;25(1):54-8. doi: 10.1016/j.jtemb.2010.10.001 [published Online  
12 327 First: 2010/12/07]
- 13 328 14. Akbari N, Ostadrahimi A, Tutunchi H, et al. Possible therapeutic effects of boron citrate  
14 329 and oleoylethanolamide supplementation in patients with COVID-19: A pilot  
15 330 randomized, double-blind, clinical trial. *Journal of trace elements in medicine and  
16 331 biology : organ of the Society for Minerals and Trace Elements (GMS)*  
17 332 2022;71:126945. doi: 10.1016/j.jtemb.2022.126945 [published Online First:  
18 333 2022/02/21]
- 19 334 15. Moghaddam MHB, Aghdam F, Asghari Jafarabadi M, et al. The Iranian Version of  
20 335 International Physical Activity Questionnaire (IPAQ) in Iran: Content and Construct  
21 336 Validity, Factor Structure, Internal Consistency and Stability. *World Applied Sciences  
22 337 Journal* 2012;18:1073-80. doi: 10.5829/idosi.wasj.2012.18.08.754
- 23 338 16. Flint A, Raben A, Blundell JE, et al. Reproducibility, power and validity of visual analogue  
24 339 scales in assessment of appetite sensations in single test meal studies. *International  
25 340 journal of obesity and related metabolic disorders : journal of the International  
26 341 Association for the Study of Obesity* 2000;24(1):38-48. doi: 10.1038/sj.ijo.0801083  
27 342 [published Online First: 2000/03/07]
- 28 343 17. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density  
29 344 lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge.  
30 345 *Clinical chemistry* 1972;18(6):499-502. [published Online First: 1972/06/01]
- 31 346 18. Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin  
32 347 resistance and beta-cell function from fasting plasma glucose and insulin concentrations  
33 348 in man. *Diabetologia* 1985;28(7):412-9. doi: 10.1007/bf00280883 [published Online  
34 349 First: 1985/07/01]
- 35 350 19. Kuru R, Yilmaz S, Balan G, et al. Boron-rich diet may regulate blood lipid profile and  
36 351 prevent obesity: A non-drug and self-controlled clinical trial. *Journal of Trace Elements  
37 352 in Medicine and Biology* 2019;54:191-98.
- 38 353 20. Okunogbe A, Nugent R, Spencer G, et al. Economic impacts of overweight and obesity:  
39 354 current and future estimates for eight countries. *BMJ Global Health*  
40 355 2021;6(10):e006351. doi: 10.1136/bmjgh-2021-006351
- 41 356 21. Global BMIMC, Di Angelantonio E, Bhupathiraju Sh N, et al. Body-mass index and all-  
42 357 cause mortality: individual-participant-data meta-analysis of 239 prospective studies in  
43 358 four continents. *Lancet (London, England)* 2016;388(10046):776-86. doi:  
44 359 10.1016/s0140-6736(16)30175-1 [published Online First: 2016/07/18]
- 45 360 22. Aronne LJ, Hall KD, J MJ, et al. Describing the Weight-Reduced State: Physiology,  
46 361 Behavior, and Interventions. *Obesity (Silver Spring, Md)* 2021;29 Suppl 1(Suppl 1):S9-  
47 362 s24. doi: 10.1002/oby.23086 [published Online First: 2021/03/25]
- 48 363 23. Schwartz MW, Seeley RJ, Zeltser LM, et al. Obesity Pathogenesis: An Endocrine Society  
49 364 Scientific Statement. *Endocrine reviews* 2017;38(4):267-96. doi: 10.1210/er.2017-  
50 365 00111 [published Online First: 2017/09/14]



- 1  
2  
3 366 24. Hunt CD. Regulation of enzymatic activity: one possible role of dietary boron in higher  
4 367 animals and humans. *Biological trace element research* 1998;66(1-3):205-25. doi:  
5 368 10.1007/bf02783139 [published Online First: 1999/03/02]  
6  
7 369 25. Kucukkurt I, Akbel E, Karabag F, et al. The effects of dietary boron compounds in  
8 370 supplemented diet on hormonal activity and some biochemical parameters in rats.  
9 371 *Toxicology and industrial health* 2015;31(3):255-60. doi: 10.1177/0748233712469648  
10 372 [published Online First: 2013/01/08]  
11 373 26. Cakir S, Eren M, Senturk M, et al. The Effect of Boron on Some Biochemical Parameters  
12 374 in Experimental Diabetic Rats. *Biological trace element research* 2018;184(1):165-72.  
13 375 doi: 10.1007/s12011-017-1182-0 [published Online First: 2017/10/12]  
14 376 27. Kuru R, Yilmaz S, Balan G, et al. Boron-rich diet may regulate blood lipid profile and  
15 377 prevent obesity: A non-drug and self-controlled clinical trial. *Journal of trace elements*  
16 378 *in medicine and biology : organ of the Society for Minerals and Trace Elements (GMS)*  
17 379 2019;54:191-98. doi: 10.1016/j.jtemb.2019.04.021 [published Online First:  
18 380 2019/05/22]  
19  
20  
21 381  
22  
23  
24 382

**Legend to figure(s)**

25 383 **Figure 1:** Study flow diagram  
26  
27 384  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

**Table 1:** Timeline of the trial

Explanation of the trial activities	Time (months)															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Material preparation	*															
Recruitment		*	*	*	*	*	*									
Clinical assessments at baseline		*	*	*	*	*	*									
Nutritional assessments at baseline		*	*	*	*	*	*									
Biochemical assessments at baseline		*	*	*	*	*	*									
Intervention								*	*	*	*	*				
Clinical assessments after intervention													*	*		
Nutritional assessments after intervention													*	*		
Biochemical assessments after intervention													*	*		
Data analysis															*	
Writing the final report of the trial																*
The expected time	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*

bmjopen-2023-075941 on 10 December 2023. Downloaded from <http://bmjopen.bmj.com/> on August 25, 2024 by guest. Protected by copyright.

For peer review only

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

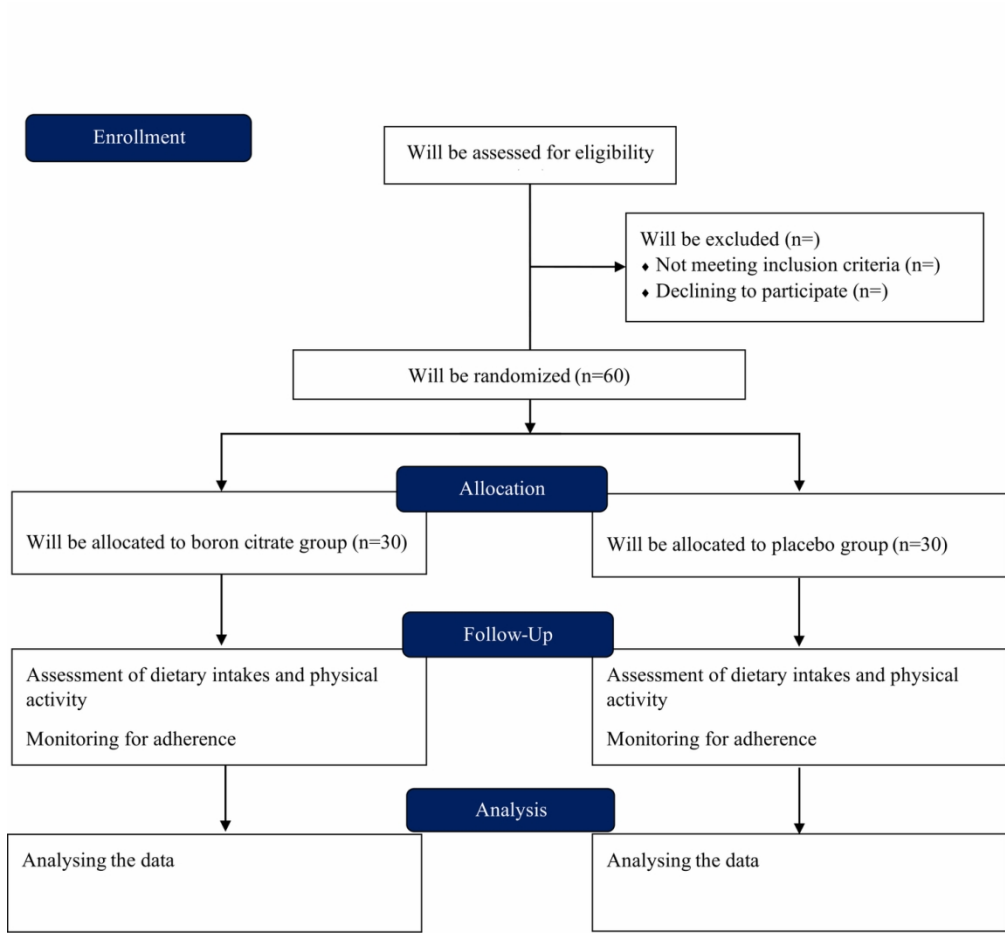


Figure 1: Study flow diagram

126x118mm (300 x 300 DPI)



SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents\*

Section/item	Item No	Description	Addressed on page number
<b>Administrative information</b>			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	2,4
	2b	All items from the World Health Organization Trial Registration Data Set, Iranian Registry of Clinical Trials Registration Data Set, In abstract and methods	2,4
Protocol version	3	Date and version identifier.	2
Funding	4	Sources and types of financial, material, and other support	12
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1,12
	5b	Name and contact information for the trial sponsor	12
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	12

1				
2		5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	12
3				
4				
5				
6				
7				
8	<b>Introduction</b>			
9				
10	Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3,4
11				
12				
13				
14		6b	Explanation for choice of comparators	4
15				
16	Objectives	7	Specific objectives or hypotheses	4
17				
18	Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	4
19				
20				
21				
22	<b>Methods: Participants, interventions, and outcomes</b>			
23				
24	Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	4
25				
26				
27				
28	Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	5
29				
30				
31				
32				
33	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	6
34				
35				
36		11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	5,9
37				
38				
39				
40				
41				
42				
43				
44				
45				
46				

1			
2		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)
3			9
4			
5			
6		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial
7			6
8			
9			
10	Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended
11			6,7
12			
13			
14			
15			
16			
17			
18	Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)
19			Table 1 and page 6
20			
21			
22			
23	Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations
24			10
25			
26			
27	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size
28			5
29			

### Methods: Assignment of interventions (for controlled trials)

#### Allocation:

32			
33			
34	Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions
35			6
36			
37			
38			
39			
40			
41			
42			
43			
44			
45			
46			

1				
2	Allocation	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered,	6
3	concealment		opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	
4	mechanism			
5				
6				
7				
8	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to	6
9			interventions	
10				
11	Blinding	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome	6
12	(masking)		assessors, data analysts), and how	
13				
14				
15				
16		17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a	6
17			participant's allocated intervention during the trial	
18				
19				
20	<b>Methods: Data collection, management, and analysis</b>			
21				
22	Data collection	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related	7,8
23	methods		processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of	
24			study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known.	
25			Reference to where data collection forms can be found, if not in the protocol	
26				
27				
28				
29				
30		18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be	10
31			collected for participants who discontinue or deviate from intervention protocols	
32				
33				
34				
35	Data	19	Plans for data entry, coding, security, and storage, including any related processes to promote data	10
36	management		quality (eg, double data entry; range checks for data values). Reference to where details of data	
37			management procedures can be found, if not in the protocol	
38				
39				
40				
41				
42				
43				
44				
45				
46				

1			
2			
3	Statistical	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the 9,10
4	methods		statistical analysis plan can be found, if not in the protocol
5			
6			
7		20b	Methods for any additional analyses (eg, subgroup and adjusted analyses) 9,10
8			
9			
10		20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any 9,10
11			statistical methods to handle missing data (eg, multiple imputation)
12			
13			
14			
15	<b>Methods: Monitoring</b>		
16			
17	Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of 10
18			whether it is independent from the sponsor and competing interests; and reference to where further details
19			about its charter can be found, if not in the protocol.
20			Alternatively, an explanation of why a DMC is not needed
21			
22		21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim 10
23			results and make the final decision to terminate the trial
24			
25			
26			
27	Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse 10
28			events and other unintended effects of trial interventions or trial conduct
29			
30			
31			
32	Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent 7,8
33			from investigators and the sponsor
34			
35			
36			
37	<b>Ethics and dissemination</b>		
38			
39	Research ethics	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval 4,12
40	approval		
41			
42			
43			
44			
45			
46			



1				
2				
3	Protocol	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes,	4
4	amendments		analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals,	
5			regulators)	
6				
7				
8	Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorized	4
9			surrogates, and how (see Item 32)	
10				
11				
12		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary	NA
13			studies, if applicable	
14				
15	Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained	12
16			in order to protect confidentiality before, during, and after the trial	
17				
18				
19	Declaration of	28	Financial and other competing interests for principal investigators for the overall trial and each study site	12
20	interests			
21				
22				
23	Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements	12
24			that limit such access for investigators	
25				
26				
27	Ancillary and	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm	10
28	post-trial		from trial participation	
29	-----			
30	Dissemination	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals,	12
31	policy		the public, and other relevant groups (eg, via publication, reporting in results databases, or other data	
32			sharing arrangements), including any publication restrictions	
33				
34				
35				
36		31b	Authorship eligibility guidelines and any intended use of professional writers	12
37				
38				
39		31c	Plans, if any, for granting public access to the full protocol, participant- level dataset, and statistical code	12
40				

## Appendices

Informed consent materials	32	Model consent form and other related documentation given to participants and authorized surrogates	-
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA

✓ Diagnosis of IBS disease is based on the Rome IV criteria and there is no need to take blood tests.

\*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "[Attribution-NonCommercial-NoDerivs 3.0 Unported](https://creativecommons.org/licenses/by-nc-nd/3.0/)" license.

For peer review only

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46

# BMJ Open

## Effects of boron citrate supplementation on cardiometabolic factors, inflammatory biomarkers, and anthropometric measures in obese patients: study protocol for a randomized double-blind clinical trial

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2023-075941.R1
Article Type:	Protocol
Date Submitted by the Author:	28-Oct-2023
Complete List of Authors:	Naemi, Mohammad; Tabriz University of Medical Sciences naghshi, sina; Tabriz University of Medical Sciences Rostami, Somaye; Tabriz University of Medical Sciences Safaei, Ehsan; Tabriz University of Medical Sciences Tutunchi, Helda; Tabriz University of Medical Sciences Ostadrahimi, Alireza; Tabriz University of Medical Sciences, Department of Clinical Nutrition
<b>Primary Subject Heading</b>:	Nutrition and metabolism
Secondary Subject Heading:	Nutrition and metabolism
Keywords:	Obesity, Randomized Controlled Trial, NUTRITION & DIETETICS

SCHOLARONE™  
Manuscripts

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

## Effects of boron citrate supplementation on cardiometabolic factors, inflammatory biomarkers, and anthropometric measures in obese patients: study protocol for a randomized double-blind clinical trial

Mohammad Naemi Kermanshahi<sup>1</sup>, Sina Naghshi<sup>1</sup>, Somaye Rostami<sup>1</sup>, Ehsan Safaei<sup>1</sup>, Helda Tutunchi<sup>2\*</sup>, Alireza Ostadrahimi<sup>3\*</sup>

<sup>1</sup>*Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran.*

<sup>2</sup>*Endocrine Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.*

<sup>3</sup>*Nutrition Research Center, Department of Clinical Nutrition, School of Nutrition and Food Science, Tabriz University of Medical Sciences, Tabriz, Iran.*

### \*Corresponding to:

Helda Tutunchi. Ph.D.

Endocrine Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.

Email: [helda.nutrition@gmail.com](mailto:helda.nutrition@gmail.com)

Alireza Ostadrahimi. Ph.D.

Department of Clinical Nutrition, School of Nutrition and Food Sciences, Tabriz University of Medical Sciences, Tabriz, Iran.

Email: [ostadrahimi@tbzmed.ac.ir](mailto:ostadrahimi@tbzmed.ac.ir)

Running title: Boron citrate and obesity

Word Count: 3170

Number of Figures: 1

## 1 **Abstract**

2 **Introduction:** Obesity is a chronic disease with serious health consequences, but weight loss  
3 is difficult to maintain through lifestyle intervention alone. The efficacy and safety of boron  
4 citrate (BC), a novel therapeutic approach, in patients with obesity are not known. The current  
5 trial will take place to determine the effects of BC supplementation on cardiometabolic factors,  
6 inflammatory biomarkers, nutritional status, anthropometric measures, and body composition  
7 in obese patients.

8 **Methods and analysis:** This double-blind, placebo-controlled, randomized clinical trial will  
9 involve 60 eligible obese participants aged 18 to 60 years old. Participants will randomly be  
10 allocated to receive either BC capsules (containing 10 mg of boron) in the intervention group  
11 or placebo capsules (containing 10 mg of maltodextrin) in the placebo group for 12 weeks.  
12 Moreover, physical activity and dietary recommendations will be provided for both groups. To assess  
13 the nutritional status of participants, a 3-day food record (2 days of the week and 1 day of the  
14 weekend) will be filled. Cardiometabolic factors, inflammatory biomarkers including tumor  
15 necrosis factor  $\alpha$ , C-reactive protein, interleukin-6, and interleukin-10 levels, anthropometric  
16 measures, and body composition will be assessed at baseline and end of the intervention. The  
17 findings of this study will provide evidence for the effectiveness of BC in the management of  
18 obesity.

19 **Ethics and dissemination:** There are so far no reported adverse effects associated with the use  
20 of boron. This trial was approved by the Ethics Committee of Tabriz University of Medical  
21 Sciences (approval number: IR.TBZMED.REC.1401.350). Positive as well as negative  
22 findings will be published in peer-reviewed journals.

23 **Trial registration number:** IRCT20220806055624N1.

24 **Key words:** Boron citrate, Inflammation, Obesity, Randomized controlled trial

1  
2  
3 25 **Strengths and limitations of the study:**  
4

5 26 \* This is the first randomized controlled clinical trial that will investigate the effects of boron  
6  
7  
8 27 citrate supplementation in obese patients.  
9

10 28 \* Several outcomes, including anthropometric and biochemical indicators, will be examined at  
11  
12 29 the study baseline and endpoint.  
13

14 30 \* Given the evaluation of compliance in the current study, low adherence to intervention might  
15  
16  
17 31 be undetectable.  
18

19 32 \* A single dose of boron citrate will be used in this study, therefore, we cannot explore dose-  
20  
21 33 response effects.  
22

23 34 \* Obese individuals will be recruited in the study, which may represent a subpopulation that is  
24  
25  
26 35 more adherent to weight management interventions than the general population with obesity.  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

## 36 **Introduction**

37 Obesity, which typically refers to excess body fat, has emerged as a major public health issue.  
38 Obesity is associated with numerous comorbidities, including hypertension, dyslipidemia, type  
39 2 diabetes, cardiovascular disease, and nonalcoholic fatty liver disease.(1) Existing data show  
40 that the prevalence of obesity is rising.(2) In 2016, more than 20% of the world's population  
41 were obese.(3) In this way, Iran is not exempt from this issue. About 40.3% and 19.2% of  
42 Iranians were overweight or obese, respectively, based on the second national integrated  
43 micronutrient study conducted from 2011 to 2015.(4)  
44 Obesity is a complicated multifactorial disease in which genetic, metabolic, and environmental  
45 factors are thought to play a major role in its development. Moreover, several lines of evidence  
46 suggest that low-grade, long-standing adipose tissue inflammation is strongly associated with  
47 insulin resistance and excess body fat mass.(5) The excessive adipose tissue stimulates the  
48 secretion of inflammatory mediators and consequently leads to metabolic disorders.(6)  
49 Lifestyle interventions (diet and exercise) have long been known as the cornerstone of weight  
50 management.(7, 8) Additionally, clinical guidelines recommend adjunctive anti-obesity  
51 medications, but their usage is severely constrained by their unfavorable side effects.(9)  
52 Therefore, novel anti-obesity and anti-inflammatory approaches focusing on the control of lipid  
53 metabolism to limit fat production and storage with minimal side effects have recently attracted  
54 substantial attention.(10)  
55 Elemental boron has been known to be an essential micronutrient for plants since the 1920s.  
56 Yet, boron looks favorable not only for plant cells but also for the majority of animal and  
57 human cells. Boron has been linked to bone development and maintenance, cognitive function,  
58 steroid hormone regulation, and immune response.(11) Boron deficiency has been documented  
59 to have major effects on mammalian metabolic and physiological systems (lipid, bone, mineral,  
60 endocrine function, and energy metabolism).(10) In previous experimental studies, a low oral

1  
2  
3 61 dose of boric acid reduced body weight,(12, 13) findings that supported further investigation.  
4  
5 62 Furthermore, a meta-analysis of animal studies documented that boron has weight-lowering  
6  
7 63 effects.(14) Boron citrate (BC) could induce weight loss in obese patients through increasing  
8  
9 64 energy metabolism, thermogenesis, lipolysis, and inhibition of adiposeness.(14) Findings from  
10  
11 65 a prospective randomized controlled trial study showed that supplementation with different  
12  
13 66 doses of calcium fructoborate lowered inflammatory biomarkers including C-reactive protein  
14  
15 67 (CRP), fibrinogen, and erythrocyte sedimentation rate (ESR) in individuals with primary  
16  
17 68 osteoarthritis.(15) Additionally, supplementation with boron was able to decrease  
18  
19 69 inflammatory cytokines in eight healthy males.(16)  
20  
21  
22  
23  
24 70 Given the role of BC in suppressing inflammation and inducing lipolysis, we hypothesized that  
25  
26 71 administration of BC may reduce body weight and inflammatory markers and improve  
27  
28 72 cardiometabolic factors in obese patients. Therefore, the purpose of the present study is to  
29  
30 73 investigate the effects of BC supplementation on cardiometabolic factors, inflammatory  
31  
32 74 biomarkers, anthropometric measures, and body composition in obese patients.  
33  
34

## 75 **Methods and design**

### 76 **Study design**

77 This parallel-group, randomized, double-blind clinical trial will be evaluated the effects of  
78 BC supplementation on cardiometabolic factors, and inflammatory biomarkers including  
79 tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), CRP, interleukin-6 (IL-6), and IL-10 levels, anthropometric  
80 measures, and body composition in obese patients. The trial was registered on the Iranian  
81 Registry of Clinical Trials website (<http://www.irct.ir>) at 2022-08-31 with code number of  
82 IRCT20220806055624N1. Moreover, the Ethics Committee of Research Vice-Chancellor of  
83 Tabriz University of Medical Sciences ethically approved the study (identifier:  
84 IR.TBZMED.REC.1401.350). This trial will be conducted at Tabriz University of Medical  
85 Sciences, Tabriz, Iran. Written informed consent will be obtained from the volunteers at the  
60



1  
2  
3 86 beginning of the study by researchers. The Standard Protocol Items: Recommendations for  
4  
5 87 Interventional Trials (SPIRIT 2013 checklist) was used to develop the protocol for this study.  
6  
7  
8 88 The time schedule of the trial and the progress of the study along with the registration of  
9  
10 89 volunteers, allocation, intervention, and review are displayed in **Supplementary Table 1** and  
11  
12 90 **Figure 1**.

### 91 **Study participants and enrolment**

16  
17 92 The participants will be recruited through advertisements and referrals from physicians and  
18  
19 93 families. Individuals who want to participate in the present study will participate in this  
20  
21 94 research project after the initial interview by the researchers and considering the defined  
22  
23 95 inclusion and exclusion criteria. All participants will be assigned a visit time, and their blood  
24  
25 96 samples will be taken following the visit.

### 28 97 **Inclusion criteria**

30  
31 98 In the present study, we will recruit adults aged between 18-60 years and with a body-mass  
32  
33 99 index (BMI) of 30 to 40 kg/m<sup>2</sup>.

### 35 100 **Exclusion criteria**

37 101 Individuals who are professional athletes, smokers, those consuming alcohol (consuming  
38  
39 102 more than 14 units of alcohol per week for women and more than 21 units per week for men),  
40  
41 103 taking chemical or herbal drugs for weight loss, hepatotoxic drugs such as phenytoin,  
42  
43 104 tamoxifen, lithium, anti-hypertensive drugs, lipid-lowering drugs (statins), insulin-sensitive  
44  
45 105 drugs, corticosteroid, and non-steroidal anti-inflammatory drugs, antibiotics, supplements  
46  
47 106 affecting liver enzyme levels or any supplements over the past three months will not be  
48  
49 107 included. We also will not include individuals who are pregnant, lactating, menopause, those  
50  
51 108 who have undergone infertility treatment, and those who use birth control pills and estrogen.  
52  
53 109 Individuals who have some pathologic conditions such as cardiovascular, liver, thyroid,  
54  
55 110 parathyroid, kidney, gastrointestinal, and known autoimmune diseases as well as suffering  
56  
57  
58  
59  
60

1  
2  
3 111 from polycystic ovary syndrome, cancer, severe infection, or inflammatory disease will be  
4  
5 112 excluded. In addition, those who underwent weight loss surgery in the last year or strict  
6  
7 113 weight loss diets in the last three months will not be included in the current study. If a  
8  
9 114 participant becomes pregnant during the course of the research, consumes alcohol  
10  
11 115 and/or tobacco, or utilizes multivitamin-mineral supplements, he or she will be excluded.  
12  
13

### 14 116 **Sample size calculation**

16 117 We estimated sample size taking into consideration all primary outcome measures and all  
17  
18 118 yielded similar sample sizes. For example, here we calculated the sample size based on plasma  
19  
20 119 CRP level. Using a type I error of 5% ( $\alpha = 0.05$ ) and a type II error of 20% ( $\beta = 0.20$ , power =  
21  
22 120 80%), the sample size in this study was calculated using the following formula, which has been  
23  
24 121 proposed for parallel clinical trials:  
25  
26 122

$$n = \frac{[(Z_{1-\frac{\alpha}{2}} + Z_{1-\beta})^2 \times \sigma^2]}{(\mu_1 - \mu_2)^2}$$

27  
28  
29  
30  
31  
32 123  
33  
34  
35 124  $n$  = sample size for each group.

36  
37  
38 125  $\sigma$  = The variance (SD) for mean CRP, which was considered as 41.5 (the average SDs reported  
39  
40 126 for CRP level in the control and intervention groups of Akbari et al study (17)

41  
42 127  $\mu_1$  = mean difference for CRP level in the intervention group (we considered it as 57.9 mg/l  
43  
44 128 based on the study of Akbari et al study.(17)

45  
46 129  $\mu_2$  = mean difference for CRP level in the control group (which was considered as 34.1 mg/l  
47  
48 130 based on the study of Akbari et al study.(17)

49  
50  
51 131 Overall, using this formula and assuming a 20% drop-out rate in each group, we will require a  
52  
53 132 sample size of 30 subjects in each group.

### 54 133 **Randomization**

1  
2  
3 134 To stratify individuals into distinct strata and blocks, stratified block randomization will be  
4  
5 135 implemented based on age (18-40 vs. 40-60 years) and gender (male vs. female). For each  
6  
7 136 individual placed in a given stratum, a matched individual will be considered based on these  
8  
9  
10 137 variables in the same stratum. As a result, two participants with similar characteristics (for  
11  
12 138 age and gender) will be placed in the same stratum. Finally, a research assistant will  
13  
14 139 randomize each stratum into either the BC or placebo groups based on a predefined  
15  
16  
17 140 computer-generated number with a 1:1 allocation that was concealed using serially  
18  
19 141 numbered, opaque, sealed envelopes. Participants and researchers will be blinded to  
20  
21 142 randomization and allocation until the end of the study. The randomization list will be  
22  
23  
24 143 provided by the pharmacist of the research center at the end of the study.

#### 25 26 144 **Study interventions**

27  
28 145 Sixty obese patients will be randomly assigned in a 1:1 allocation ratio to receive either a BC  
29  
30 146 capsule (containing 10 mg of boron) or a matched placebo capsule (containing 10 mg of  
31  
32 147 maltodextrin) once daily before lunch for 12 weeks. Thirty capsules of BC or placebos will be  
33  
34 148 delivered to patients every 30 days (at the study baseline, day 30, and day 60). Patients in study  
35  
36 149 groups will be asked to consume BC or placebo capsules 30 minutes before lunch or dinner.  
37  
38 150 Patients will be asked to give back the empty pockets at the end of the study. Following ethical  
39  
40 151 considerations, the researchers will give physical activity and dietary advice to enhance the  
41  
42 152 lifestyles of patients in both groups. The participants will be requested to follow general healthy  
43  
44 153 eating recommendations, including changing cooking methods to healthier ways and limiting  
45  
46 154 fast foods, saturated fats, high-fat foods, sugar, sweets, and sugar-sweetened beverages.

#### 47 48 49 50 51 155 **Study outcomes**

##### 52 53 156 **Primary and secondary outcomes**

54  
55  
56 157 The primary outcomes of this study are changes in inflammatory factors (CRP, TNF- $\alpha$ , IL-10,  
57  
58 158 and IL-6), anthropometric indicators (weight, BMI, waist circumference (WC), hip  
59  
60

1  
2  
3 159 circumference (HC), waist-to-hip ratio (WHR), and waist-to-height ratio (WHtR)). Changes in  
4  
5 160 body composition (total body fat mass (FM) and total body fat-free mass (FFM)), glycemic  
6  
7  
8 161 parameters (fasting blood sugar (FBS), fasting blood insulin (FBI), hemostatic model  
9  
10 162 assessment of insulin resistance (HOMA-IR), and the quantitative insulin sensitivity check  
11  
12 163 index (QUICKI)), lipid profile (triglyceride (TG), total cholesterol (TC), high-density  
13  
14 164 lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C)), and blood  
15  
16  
17 165 pressure are regarded as secondary outcomes.

## 19 166 **Measurements and assessments**

### 21 167 **Clinical assessments**

23  
24 168 At the onset of the study, all patients will be asked about their medical history, smoking  
25  
26 169 history, underlying diseases, hormonal problems, alcohol consumption, supplement and drug  
27  
28 170 use history, and demographic characteristics (age, gender, education level, employment  
29  
30  
31 171 status, and marital status).

### 33 172 **Blood pressure**

35 173 Systolic and diastolic blood pressures will be measured in a seated posture twice with a 15-  
36  
37 174 minute interval at the right arm. Before measurement, patients will be required to rest for five  
38  
39 175 minutes. The mean of two measurements will be used in the analyses.(18)

### 42 176 **Assessment of physical activity and dietary intake**

44 177 To ensure that participants' physical activity does not alter throughout the trial, we will  
45  
46 178 monitor their activity. Already validated in Iran,(19) the International Physical Activity  
47  
48 179 Questionnaire-Short Form (IPAQ-SF) will be used to estimate the physical activity level at  
49  
50 180 the beginning and end of the study. According to the IPAQ scoring system, physical activity  
51  
52 181 levels will be classified as low, moderate, and high activity. To assess the dietary intake of  
53  
54 182 each participant, a 3-day food record (2 days of the week and 1 day of the weekend) will be  
55  
56 183 filled at the beginning and end of the study. The 3-day food record booklet will be designed to be  
57  
58  
59  
60

1  
2  
3 184 entirely self-administered. It will be contained instructions for recording the day, time, place, type of  
4  
5 185 meal (breakfast, lunch, dinner, and snack), name of the food, ingredients of mixed dishes and recipes,  
6  
7 186 food preparation methods, name of the brand/company, and estimated portion size and any leftovers  
8  
9 187 of all food and drink consumed. The portion sizes of consumed foods will be converted to grams per  
10  
11 188 day using household measures.(20) Then, all gram values of food items will be entered into  
12  
13  
14 189 Nutritionist IV software (First Databank, San Bruno, CA, USA), to obtain daily intake of energy  
15  
16 190 and all nutrients.

### 18 191 **Assessment of appetite sensations**

19 192 Appetite sensations (hunger, satiety, fullness, desire to consume something sweet, fatty, or  
20  
21 193 salty) of the patients will be measured using a visual analogue scale (VAS) at the beginning  
22  
23 194 and end of the study after overnight fasting.(21)

### 27 195 **Anthropometric measurements**

28  
29  
30 196 Data on anthropometric measurements will be collected at baseline and end of the trial. The  
31  
32 197 body weight will be recorded in a fasted state, without footwear, and wearing light clothing to  
33  
34 198 an accuracy of 100 g. Height will be measured using a stadiometer (Seca, Hamburg,  
35  
36 199 Germany) without shoes at a standing position to the nearest 0.1 cm accuracy. WC will be  
37  
38 200 measured as the smallest horizontal circumference between the costal and iliac crests using a  
39  
40 201 non-stretchable measuring tape with an accuracy of 0.1 cm. The hip circumference (HC) will  
41  
42 202 be measured at the widest point above the great trochanters. Then, WHR will be calculated.  
43  
44 203 BMI is calculated by dividing weight in kilograms by the square of height in meters. Body  
45  
46 204 composition including FM and FFM will be measured using Tanita MC780 multi-frequency  
47  
48 205 segmental bioelectrical impedance analysis applying 3 different frequencies (5kHz/ 50kHz/  
49  
50 206 250kHz).

### 55 207 **Blood sampling and biochemical measurements**

56  
57 208 The blood samples (10 ml) will be taken following a 12-h overnight fasting at pre-and post-  
58  
59 209 intervention phases. The blood samples will be kept inside the gel tube for 20 minutes for

1  
2  
3 210 better precipitation and will be centrifuged at 3000 rpm for 7 minutes to extract serum  
4  
5 211 samples. The serum levels of FBS, TC, TG, and HDL-C will be measured instantly on fresh  
6  
7 212 blood samples by enzymatic methods using colorimetric technique, by commercial kits (Pars-  
8  
9 213 Azmoon Co., Tehran, Iran). LDL-C will be calculated by the Friedewald equation.(22) The  
10  
11 214 enzyme-linked immunosorbent assay (ELISA) method will be applied to measure FBI using  
12  
13 215 commercial kits (Monobind, Lake Forest, CA, USA). Serum levels of inflammatory factors  
14  
15 216 (CRP, TNF- $\alpha$ , IL-10, and IL-6) will be assessed using ELISA kits. The following formula  
16  
17 217 will be applied to determine HOMA-IR and QUICKI indexes.

18  
19  
20  
21  
22 218  $HOMA1-IR = (\text{fasting insulin } (\mu\text{IU/ml}) \times \text{fasting glucose (mg/dl)})/405(23)$

23  
24 219  $QUICKI = 1/(\log(\text{insulin, U ml}^{-1}) + \log(\text{FBS, mg dl}^{-1}))$

#### 25 26 220 **Adherence to the intervention**

27  
28 221 To assess adherence to the intervention, participants will be asked to document their daily usage  
29  
30 222 of BC supplements on a checklist provided by the researchers. To enhance compliance and  
31  
32 223 prevent participants from forgetting to take supplements, investigators will send daily text  
33  
34 224 messages to participants' cell phones as reminders. In addition, adherence to the intervention  
35  
36 225 will be evaluated by counting the returned capsules at the midpoint and end of the experiment.

#### 37 38 226 **Statistical analysis**

39  
40  
41  
42 227 Analyses will follow the intention-to-treat (ITT) convention. Multiple imputation with chained  
43  
44 228 equations will be used to assign any missing values.(24) The Shapiro–Wilk normality test will  
45  
46 229 be used to assess distributions of continuous variables for normality, and natural logarithm  
47  
48 230 transformations of skewed variables will be applied before analyses. The findings will be  
49  
50 231 presented as mean (SD) for numerical data, frequency (percentage) for categorical variables,  
51  
52 232 and median (25th, 75th) for values with skewed distribution. The independent samples t-test  
53  
54 233 and Mann-Whitney U test will be applied to compare quantitative variables with normal and  
55  
56 234 skewed distribution between the two groups, respectively. To do a within-group comparison,  
57  
58  
59  
60

235 we will use the paired-sample t-test and Wilcoxon signed-rank test for values with normal and  
236 skewed distribution, respectively. We will use the Chi-square test and Fisher's exact test to  
237 examine differences in qualitative variables between the two groups. To estimate the  
238 intervention effect for all primary and secondary outcomes, an analysis of covariance  
239 (ANCOVA) will be used. In this analysis, we will include baseline measurements as a covariate  
240 to adjust for potential differences between treatment groups at baseline. Statistical analysis will  
241 be carried out using SPSS, version 23.  $P < 0.05$  will be considered statistically significant.

#### 242 **Adverse effects, safety, and data monitoring**

243 Although the values of Recommended Daily Intake (RDA), Estimated Average Requirement  
244 (EAR), and Adequate Intake (AI) for boron have not been reported, the tolerable Upper Level  
245 (UL) in adults is 20 mg/day.<sup>(25)</sup> All participants will be instructed about potential adverse  
246 effects. Each participant will be asked to document all the symptoms that they experienced  
247 during the study period, whether related to boron or not. In case of side effects, more  
248 information is required to make a decision for excluding the participants from the study. In  
249 such conditions, unbinding is permissible based on the Medical Ethics Committee criteria. The  
250 report of any adverse effects will be sent to the Ethics Committee of Tabriz University of  
251 Medical Sciences. One of the investigators will check the coding, security, and storage of the  
252 data. In addition, he/she will assess the data entry and data values twice.

#### 253 **Patient and public involvement**

254 Patients and/or the public were not involved in the design, conduct reporting or dissemination  
255 plans of this study.

#### 256 **Discussion**

257 There is growing evidence that boron plays a multitude of crucial roles in animal and human  
258 health. Several experimental research concluded that boron can reduce weight and may even  
259 enhance obesity-related markers.<sup>(14)</sup> It has also been found that boron decreases FBI, FBS,

1  
2  
3 260 and pancreatic beta cell stress.(26, 27) Finding from a study demonstrated that oral  
4  
5 261 administration of boric acid reduced the serum levels of TC and LDL-C and increased HDL-C  
6  
7 262 levels in diabetic rats.(28) In a clinical trial in which healthy women consumed diets containing  
8  
9  
10 263 10 mg more boron than their usual diet for one month, a significant reduction was observed in  
11  
12 264 serum levels of TC, LDL-C, very low-density lipoprotein cholesterol (VLDL-C), and TG.  
13  
14 265 Moreover, the body weight, body FM, and BMI of the women decreased significantly.(29) In  
15  
16 266 COVID-19 patients, supplementation with BC alone or in combination with  
17  
18 267 oleoylethanolamide resulted in a significant decrease in serum lactate dehydrogenase and ESR  
19  
20 268 levels.(17) In an experimental study, supplementation with boron compounds reduced lipid  
21  
22 269 peroxidation, and enhanced the antioxidant defense mechanism in rats.(30) Moreover, a study  
23  
24 270 documented that boron could effectively ameliorate oxidative stress, inflammation, and  
25  
26 271 biochemical parameters in rats treated with acrylamide.(31) Concerning the biological  
27  
28 272 mechanisms, the anti-inflammatory effects of boro are thought to be mediated through  
29  
30 273 inhibition of the oxidative process by activating scavenging cells like leukocytes and  
31  
32 274 neutrophils.(32) Boron also enhances the inhibition of free radicals by increasing the level of  
33  
34 275 antioxidant enzymes (superoxide dismutase, catalase, and glutathione peroxidase) in blood and  
35  
36 276 cells.(33) Boron significantly improves magnesium absorption. It has been shown that in  
37  
38 277 magnesium deficiency, the level of proinflammatory cytokines increases.(33) Magnesium also  
39  
40 278 plays an important role in carbohydrate metabolism; its deficiency provokes and worsens  
41  
42 279 insulin resistance.(33) Overall, findings from animal and human studies provide supportive  
43  
44 280 evidence for the beneficial effects of boron on metabolic and inflammatory parameters. To our  
45  
46 281 knowledge, available investigations regarding the effects of BC supplementation on features  
47  
48 282 of obesity in humans are limited. The findings from this study will provide clinical evidence  
49  
50 283 on the effectiveness of BC supplementation in obese patients. The trial results will be  
51  
52 284 disseminated in peer-reviewed scientific and clinical journals.  
53  
54  
55  
56  
57  
58  
59  
60



1  
2  
3 285 **Strengths and limitations:** This is the first randomized controlled clinical trial that will  
4  
5 286 investigate the effects of BC supplementation in obese patients. Using stratified block  
6  
7 287 randomization, patients will be matched based on a number of characteristics that may impact  
8  
9 288 the final results. Several outcomes, including anthropometric and biochemical indicators, will  
10  
11 289 be examined at the study baseline and endpoint. Moreover, dietary intake, physical activity  
12  
13 290 level, and compliance to the intervention will be assessed. Several limitations to this study  
14  
15 291 should be considered. Given the evaluation of compliance in the current study, low adherence  
16  
17 292 to intervention might be undetectable. Moreover, the serum levels of boron will not be  
18  
19 293 measured to examine the compliance of study participants due to financial constraints. A single  
20  
21 294 dose of BC will be used in this study, therefore, we cannot explore dose-response effects. As  
22  
23 295 different doses may have different effects, dose-finding trials are needed to identify the lowest  
24  
25 296 safe and effective dose. The 3-month intervention period may not be long enough to see a  
26  
27 297 beneficial effect on secondary outcomes. Obese individuals will be recruited in the study,  
28  
29 298 which may represent a subpopulation that is more adherent to weight management  
30  
31 299 interventions than the general population with obesity. Moreover, the efficacy of the same  
32  
33 300 intervention in other metabolic diseases is not known. Future research should explore the  
34  
35 301 effects of boron on such diseases.  
36  
37  
38  
39  
40  
41

#### 302 **Trial status**

42  
43 303  
44 304 Recruitment for this trial has begun at June 2023 and is expected to be completed by December  
45  
46 305 2023.  
47  
48

#### 49 306 **Declarations**

#### 50 307 51 308 **Ethics approval and consent to participate**

52  
53 309 This trial was approved by the Ethics Committee of Tabriz University of Medical Sciences  
54  
55 310 (approval number: IR.TBZMED.REC.1401.350). Informed consent will be obtained from all  
56  
57 311 study participants prior to the study onset.  
58  
59  
60

1  
2  
3 **312 Availability of data and materials**

4 313

5 314 Not applicable

7  
8 **315 Competing interest**

9  
10 316 The authors declare no competing interests.

11  
12 **317 Funding**

13 318

14 319 The study was financially supported by Endocrine Research Center of Tabriz University of

15  
16  
17 320 Medical Sciences (grant number: 70107).

18  
19 **321 Author Contributions**

20  
21 322 Study design: AO, HT and MNK. Methodology: HT, MNK, SRN and ES. Statistical plan: AO,

22  
23 323 SN, and HT. Coordination of the study implementation: MNK, SRN and ES. Data collection:

24  
25 324 MNK, SRN and ES. Manuscript preparation: MNK and SRN. Review and editing: AO, HT,

26  
27 325 SN, and ES.

28  
29 326 All named authors have read and approved the final manuscript, adhere to the authorship

30  
31 327 guidelines of journal and have agreed to publication.

32  
33  
34  
35 **328 Acknowledgements**

36 329

37 330 We would like to appreciate the cooperation of the Clinical Research Development Unit of

38  
39  
40 **331 Imam Reza General Hospital, Tabriz, Iran in conducting** this research. We sincerely thank the

41  
42 332 patients who participated in the present study.

43  
44  
45 **333 References**

46 334

47 335 1. Flegal KM, Carroll MD, Ogden CL, Johnson CL. Prevalence and trends in obesity  
48 336 among US adults, 1999-2000. *Jama*. 2002;288(14):1723-7.

49 337 2. Aekplakorn W, Inthawong R, Kessomboon P, Sangthong R, Chariyalertsak S,  
50 338 Putwatana P, et al. Prevalence and trends of obesity and association with socioeconomic status  
51 339 in Thai adults: National Health Examination Surveys, 1991-2009. *Journal of obesity*.  
52 340 2014;2014:410259.

53  
54 341 3. Worldwide trends in body-mass index, underweight, overweight, and obesity from  
55 342 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128·9  
56 343 million children, adolescents, and adults. *Lancet (London, England)*. 2017;390(10113):2627-  
57 344 42.

- 345 4. Pouraram H, Djazayeri A, Mohammad K, Parsaeian M, Abdollahi Z, Dorosty Motlagh  
346 A, et al. Second National Integrated Micronutrient Survey in Iran: Study Design and  
347 Preliminary Findings. *Archives of Iranian medicine*. 2018;21(4):137-44.
- 348 5. Burhans MS, Hagman DK, Kuzma JN, Schmidt KA, Kratz M. Contribution of Adipose  
349 Tissue Inflammation to the Development of Type 2 Diabetes Mellitus. *Comprehensive  
350 Physiology*. 2018;9(1):1-58.
- 351 6. Ellulu MS, Patimah I, Khaza'ai H, Rahmat A, Abed Y. Obesity and inflammation: the  
352 linking mechanism and the complications. *Archives of medical science : AMS*.  
353 2017;13(4):851-63.
- 354 7. Bazshahi E, Sheikhhossein F, Amini MR, Shab-Bidar S. The association of dietary  
355 energy density and the risk of obesity, type 2 diabetes and metabolic syndrome: A systematic  
356 review and meta-analysis of observational studies. *International journal of clinical practice*.  
357 2021;75(10):e14291.
- 358 8. Shahavandi M, Amini MR, Shahinfar H, Shab-Bidar S. Major dietary patterns and  
359 predicted cardiovascular disease risk in an Iranian adult population. *Nutrition and health*.  
360 2021;27(1):27-37.
- 361 9. Wang L, Yu CC, Li J, Tian Q, Du YJ. Mechanism of Action of Acupuncture in Obesity:  
362 A Perspective From the Hypothalamus. *Frontiers in endocrinology*. 2021;12:632324.
- 363 10. Abdik EA, Abdik H, Taşlı PN, Deniz AAH, Şahin F. Suppressive Role of Boron on  
364 Adipogenic Differentiation and Fat Deposition in Human Mesenchymal Stem Cells. *Biological  
365 trace element research*. 2019;188(2):384-92.
- 366 11. Nielsen FH, Meacham SL. Growing evidence for human health benefits of boron.  
367 *Journal of Evidence-Based Complementary & Alternative Medicine*. 2011;16(3):169-80.
- 368 12. Aysan E, Sahin F, Telci D, Erdem M, Muslumanoğlu M, Yardımcı E, et al. Mechanism  
369 of body weight reducing effect of oral boric Acid intake. *International journal of  
370 endocrinology*. 2013;2013:914651.
- 371 13. Basoglu A, Baspinar N, Tenori L, Vignoli A, Gulersoy E. Effects of Boron  
372 Supplementation on Peripartum Dairy Cows' Health. *Biological trace element research*.  
373 2017;179(2):218-25.
- 374 14. Farrin N, Rezazadeh L, Pourmoradian S, Attari VE, Tutunchi H, Zarezadeh M, et al.  
375 Boron compound administration; A novel agent in weight management: A systematic review  
376 and meta- analysis of animal studies. *Journal of trace elements in medicine and biology : organ  
377 of the Society for Minerals and Trace Elements (GMS)*. 2022;72:126969.
- 378 15. Scorei R, Mitrut P, Petrisor I, Scorei I. A double-blind, placebo-controlled pilot study  
379 to evaluate the effect of calcium fructoborate on systemic inflammation and dyslipidemia  
380 markers for middle-aged people with primary osteoarthritis. *Biological trace element research*.  
381 2011;144(1-3):253-63.
- 382 16. Naghii MR, Mofid M, Asgari AR, Hedayati M, Daneshpour MS. Comparative effects  
383 of daily and weekly boron supplementation on plasma steroid hormones and proinflammatory  
384 cytokines. *Journal of trace elements in medicine and biology : organ of the Society for Minerals  
385 and Trace Elements (GMS)*. 2011;25(1):54-8.
- 386 17. Akbari N, Ostadrahimi A, Tutunchi H, Pourmoradian S, Farrin N, Najafipour F, et al.  
387 Possible therapeutic effects of boron citrate and oleoylethanolamide supplementation in  
388 patients with COVID-19: A pilot randomized, double-blind, clinical trial. *Journal of trace  
389 elements in medicine and biology : organ of the Society for Minerals and Trace Elements  
390 (GMS)*. 2022;71:126945.
- 391 18. Ogedegbe G, Pickering T. Principles and techniques of blood pressure measurement.  
392 *Cardiology clinics*. 2010;28(4):571-86.
- 393 19. Moghaddam MHB, Aghdam F, Asghari Jafarabadi M, Allahverdipour H, Nikookheslat  
394 S, Safarpour S. The Iranian Version of International Physical Activity Questionnaire (IPAQ)

- 395 in Iran: Content and Construct Validity, Factor Structure, Internal Consistency and Stability.  
396 World Applied Sciences Journal. 2012;18:1073-80.
- 397 20. Ghaffarpour M, Houshiar-Rad A, Kianfar H. The manual for household measures,  
398 cooking yields factors and edible portion of foods. Tehran: Nashre Olume Keshavarzy.  
399 1999;7(213):42-58.
- 400 21. Flint A, Raben A, Blundell JE, Astrup A. Reproducibility, power and validity of visual  
401 analogue scales in assessment of appetite sensations in single test meal studies. International  
402 journal of obesity and related metabolic disorders : journal of the International Association for  
403 the Study of Obesity. 2000;24(1):38-48.
- 404 22. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-  
405 density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge.  
406 Clinical chemistry. 1972;18(6):499-502.
- 407 23. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC.  
408 Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma  
409 glucose and insulin concentrations in man. Diabetologia. 1985;28(7):412-9.
- 410 24. Azur MJ, Stuart EA, Frangakis C, Leaf PJ. Multiple imputation by chained equations:  
411 what is it and how does it work? International journal of methods in psychiatric research.  
412 2011;20(1):40-9.
- 413 25. Kuru R, Yilmaz S, Balan G, Tuzuner BA, Tasli PN, Akyuz S, et al. Boron-rich diet  
414 may regulate blood lipid profile and prevent obesity: A non-drug and self-controlled clinical  
415 trial. Journal of Trace Elements in Medicine and Biology. 2019;54:191-8.
- 416 26. Hunt CD. Regulation of enzymatic activity: one possible role of dietary boron in higher  
417 animals and humans. Biological trace element research. 1998;66(1-3):205-25.
- 418 27. Kucukkurt I, Akbel E, Karabag F, Ince S. The effects of dietary boron compounds in  
419 supplemented diet on hormonal activity and some biochemical parameters in rats. Toxicology  
420 and industrial health. 2015;31(3):255-60.
- 421 28. Cakir S, Eren M, Senturk M, Sarica ZS. The Effect of Boron on Some Biochemical  
422 Parameters in Experimental Diabetic Rats. Biological trace element research.  
423 2018;184(1):165-72.
- 424 29. Kuru R, Yilmaz S, Balan G, Tuzuner BA, Tasli PN, Akyuz S, et al. Boron-rich diet  
425 may regulate blood lipid profile and prevent obesity: A non-drug and self-controlled clinical  
426 trial. Journal of trace elements in medicine and biology : organ of the Society for Minerals and  
427 Trace Elements (GMS). 2019;54:191-8.
- 428 30. Ince S, Kucukkurt I, Cigerci IH, Fatih Fidan A, Eryavuz A. The effects of dietary boric  
429 acid and borax supplementation on lipid peroxidation, antioxidant activity, and DNA damage  
430 in rats. Journal of trace elements in medicine and biology : organ of the Society for Minerals  
431 and Trace Elements (GMS). 2010;24(3):161-4.
- 432 31. Acaroz U, Ince S, Arslan-Acaroz D, Gurler Z, Kucukkurt I, Demirel HH, et al. The  
433 ameliorative effects of boron against acrylamide-induced oxidative stress, inflammatory  
434 response, and metabolic changes in rats. Food and chemical toxicology : an international  
435 journal published for the British Industrial Biological Research Association. 2018;118:745-52.
- 436 32. Scorei RI, Ciofrangeanu C, Ion R, Cimpean A, Galateanu B, Mitran V, et al. In vitro  
437 effects of calcium fructoborate upon production of inflammatory mediators by LPS-stimulated  
438 RAW 264.7 macrophages. Biological trace element research. 2010;135(1-3):334-44.
- 439 33. Pizzorno L. Nothing Boring About Boron. Integrative medicine (Encinitas, Calif).  
440 2015;14(4):35-48.

1  
2  
3 443

**Legend to figure(s)**

4  
5 444 **Figure 1: Study flow diagram**

6  
7 445  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

For peer review only

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

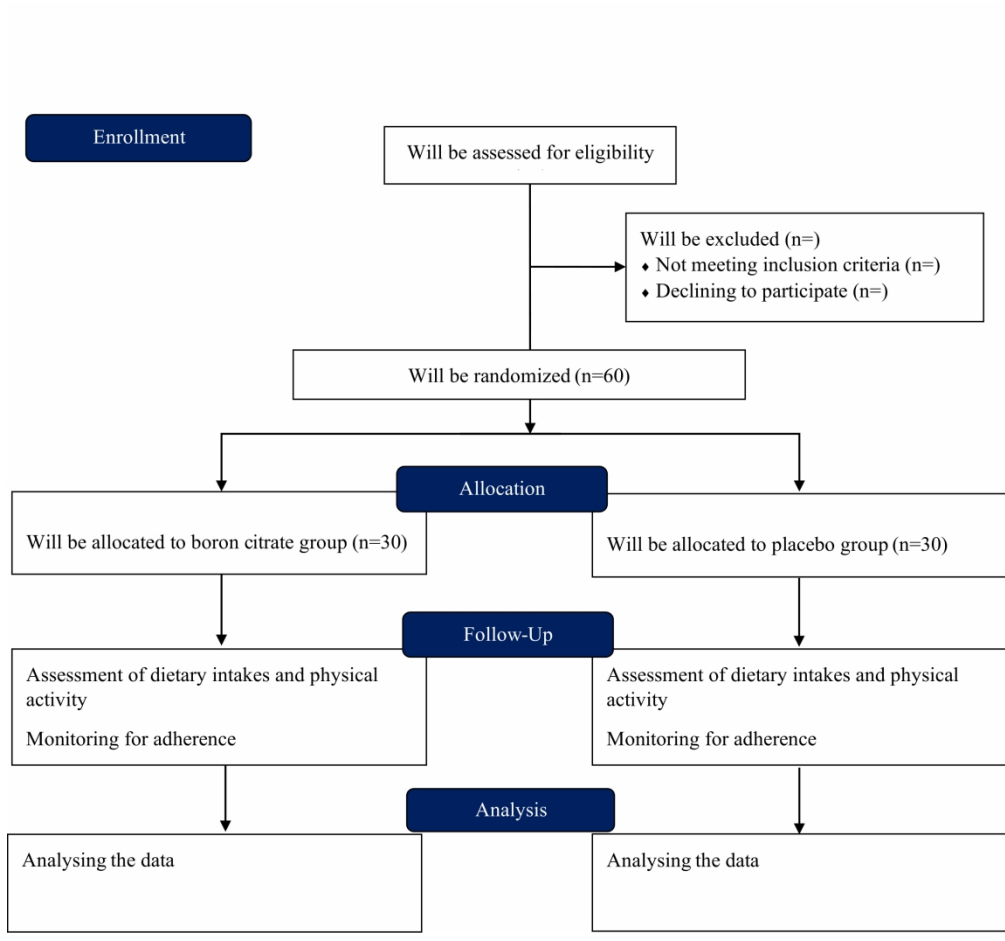


Figure 1: Study flow diagram  
127x118mm (500 x 500 DPI)

**Supplementary Table 1: Timeline of the trial**

Explanation of the trial activities	Time (months)															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Material preparation	*															
Recruitment		*	*	*	*	*	*									
Clinical assessments at baseline		*	*	*	*	*	*									
Nutritional assessments at baseline		*	*	*	*	*	*									
Biochemical assessments at baseline		*	*	*	*	*	*									
Intervention								*	*	*	*	*				
Clinical assessments after intervention													*	*		
Nutritional assessments after intervention													*	*		
Biochemical assessments after intervention													*	*		
Data analysis															*	
Writing the final report of the trial																*
The expected time	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*

bmjopen-2023-075941 on 10 December 2023. Downloaded from <http://bmjopen.bmj.com/> on August 25, 2024 by guest. Protected by copyright.

## "Consent Form"

I ..... hereby agree to participate in a research project entitled "Effects of boron citrate supplementation on cardiometabolic factors, inflammatory biomarkers, nutritional status, and anthropometric measures in obese patients: study protocol for a randomized double-blind clinical trial" under the supervision of Dr. Helda Tutunchi.

It was explained to me about the effect boron citrate supplementation on cardiometabolic factors, inflammatory biomarkers, nutritional status, and anthropometric measures will be studied.

In this research, I will answer the questions about my characteristics and dietary intakes, and blood sample will be taken from me at the beginning and end of the intervention. The present study is designed to be 12 weeks. During the research period, I will be consumed boron citrate supplements during the intervention.

My name and all information that is taken from me will be remained confidential (in writing) and the research results will be published as the general answer of the studied group and the individual results will be presented without mentioning names.

The researcher has answered all my questions, so I agree to participate in this research. By mentioning this, this agreement will not prevent legal actions - in case of illegal action or inhumane method.

**Name and surname of the person being studied:**

**Study address:**

**Date and signature of the participant:**

Statement of the research officer: I have informed the participant about the nature of the above plan process and the treatment used and the possible risks. I have answered all questions to the



1  
2  
3 best of my ability. I will inform the participant of any changes in possible risks and benefits  
4  
5 during the study or information that will depend on the participant's willingness to continue  
6  
7 treatment in this study.  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

For peer review only



SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents\*

Section/item	Item No	Description	Addressed on page number
<b>Administrative information</b>			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	2,4
	2b	All items from the World Health Organization Trial Registration Data Set, Iranian Registry of Clinical Trials Registration Data Set, In abstract and methods	2,4
Protocol version	3	Date and version identifier.	2
Funding	4	Sources and types of financial, material, and other support	12
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1,12
	5b	Name and contact information for the trial sponsor	12
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	12

	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	12
--	----	--	----

## Introduction

Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3,4
	6b	Explanation for choice of comparators	4
Objectives	7	Specific objectives or hypotheses	4
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	4

## Methods: Participants, interventions, and outcomes

Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	4
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	5
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	6
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	5,9

1			
2		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)
3			9
4			
5			
6		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial
7			6
8			
9			
10	Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended
11			6,7
12			
13			
14			
15			
16			
17			
18	Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)
19			Table 1 and page 6
20			
21			
22			
23	Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations
24			10
25			
26			
27	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size
28			5
29			

### Methods: Assignment of interventions (for controlled trials)

#### Allocation:

34	Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions
35			6
36			
37			
38			
39			
40			
41			
42			
43			
44			
45			
46			

1			
2	Allocation	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered,
3	concealment		opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned
4			
5	mechanism		
6			
7			
8	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to
9			interventions
10			
11	Blinding	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome
12	(masking)		assessors, data analysts), and how
13			
14			
15			
16		17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a
17			participant's allocated intervention during the trial
18			
19			
20	<b>Methods: Data collection, management, and analysis</b>		
21			
22	Data collection	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related
23	methods		processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of
24			study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known.
25			Reference to where data collection forms can be found, if not in the protocol
26			
27			
28			
29			
30		18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be
31			collected for participants who discontinue or deviate from intervention protocols
32			
33			
34			
35	Data	19	Plans for data entry, coding, security, and storage, including any related processes to promote data
36	management		quality (eg, double data entry; range checks for data values). Reference to where details of data
37			management procedures can be found, if not in the protocol
38			
39			
40			
41			
42			
43			
44			
45			
46			

1			
2			
3	Statistical	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the
4	methods		statistical analysis plan can be found, if not in the protocol
5			
6			
7		20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)
8			
9			
10		20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any
11			statistical methods to handle missing data (eg, multiple imputation)
12			
13			
14			
15	<b>Methods: Monitoring</b>		
16			
17	Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of
18			whether it is independent from the sponsor and competing interests; and reference to where further details
19			about its charter can be found, if not in the protocol.
20			Alternatively, an explanation of why a DMC is not needed
21			
22		21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim
23			results and make the final decision to terminate the trial
24			
25			
26			
27	Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse
28			events and other unintended effects of trial interventions or trial conduct
29			
30			
31			
32	Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent
33			from investigators and the sponsor
34			
35			
36			
37	<b>Ethics and dissemination</b>		
38			
39	Research ethics	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval
40	approval		
41			
42			
43			
44			
45			
46			

1				
2				
3	Protocol	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes,	4
4	amendments		analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals,	
5			regulators)	
6				
7				
8	Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorized	4
9			surrogates, and how (see Item 32)	
10				
11				
12		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary	NA
13			studies, if applicable	
14				
15	Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained	12
16			in order to protect confidentiality before, during, and after the trial	
17				
18				
19	Declaration of	28	Financial and other competing interests for principal investigators for the overall trial and each study site	12
20	interests			
21				
22				
23	Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements	12
24			that limit such access for investigators	
25				
26				
27	Ancillary and	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm	10
28	post-trial		from trial participation	
29	-----			
30	Dissemination	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals,	12
31	policy		the public, and other relevant groups (eg, via publication, reporting in results databases, or other data	
32			sharing arrangements), including any publication restrictions	
33				
34				
35				
36		31b	Authorship eligibility guidelines and any intended use of professional writers	12
37				
38				
39		31c	Plans, if any, for granting public access to the full protocol, participant- level dataset, and statistical code	12
40				

## Appendices

Informed consent materials	32	Model consent form and other related documentation given to participants and authorized surrogates	-
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA

✓ Diagnosis of IBS disease is based on the Rome IV criteria and there is no need to take blood tests.

\*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "[Attribution-NonCommercial-NoDerivs 3.0 Unported](https://creativecommons.org/licenses/by-nc-nd/3.0/)" license.