BMJ Open

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Journal:	*BMJ Open*
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

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

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Running title: Boron citrate and obesity

Word Count: 2638

Number of Figures: 1

Number of Tables: 1

Abstract

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

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

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

Trial registration number: IRCT20220806055624N1.

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

Introduction

Obesity, which typically refers to excess body fat, has emerged as a major public health issue. Obesity is associated with numerous comorbidities, including hypertension, dyslipidemia, type 2 diabetes, cardiovascular disease, and nonalcoholic fatty liver disease [1]. Existing data show that the prevalence of obesity is rising [2]. In 2016, more than 20% of the world's population were obese [3]. In this way, Iran is not exempt from this issue. About 40.3% and 19.2% of Iranians were overweight or obese, respectively, based on the second national integrated micronutrient study conducted from 2011 to 2015 [4].

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

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

dose of boric acid reduced body weight [10] [11], findings that supported further investigation. Furthermore, a meta-analysis of animal studies documented that boron has weight-lowering effects [12]. Boron citrate (BC) could induce weight loss in obese patients through increasing energy metabolism, thermogenesis, lipolysis, and inhibition of adiposeness [12]. Additionally, supplementation with boron could decrease inflammatory cytokines in eight healthy males [13]. Given the role of BC in suppressing inflammation and inducing lipolysis, we hypothesized that administration of BC may reduce body weight and inflammatory markers and improve cardiometabolic factors in obese patients. Therefore, the purpose of the present study is to investigate the effects of BC supplementation on cardiometabolic factors, inflammatory biomarkers including, anthropometric measures, and body composition in obese patients.

Methods and design

Study design

This parallel-group, randomized, double-blind clinical trial will be evaluated the effects of BC supplementation on cardiometabolic factors, inflammatory biomarkers including tumor necrosis factor α (TNF-α), C-reactive protein (CRP), interleukin-6 (IL-6), and IL-10 levels, anthropometric measures, and body composition in obese patients. The trial was registered on the Iranian Registry of Clinical Trials website (http://www.irct.ir) at 2022-08-31 with code number of IRCT20220806055624N1. Moreover, the Ethics Committee of Research Vice-Chancellor of Tabriz University of Medical Sciences ethically approved the study (identifier: IR.TBZMED.REC.1401.350). This trial will be conducted at Tabriz University of Medical Sciences, Tabriz, Iran. Written informed consent will be obtained from the volunteers at the beginning of the study by researchers. The Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT 2013 checklist) was used to develop the protocol for this study. The time schedule of the trial and the progress of the study along with the registration of volunteers, allocation, intervention, and review are displayed in **Table 1** and **Figure 1.**

Study participants and enrolment

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

Inclusion criteria

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

Exclusion criteria

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

Sample size calculation

We calculated the required sample size based on data from a previous study [14] by considering plasma CRP level as a key dependent variable, type I error of 0.05, and study power of 80%. Based on the suggested formula for parallel clinical trials, we reached the sample size of 24 patients in each group. Taking into account a possible drop-out rate of 20%, 30 patients will be enrolled in each group.

Randomization

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

Study interventions

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

Study outcomes

Primary and secondary outcomes

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

circumference (HC), waist-to-hip ratio (WHR), and waist-to-height ratio (WHtR)), and body composition (total body fat mass (FM) and total body fat-free mass (FFM)). Changes in nutritional status (amount of energy intake and macronutrients), glycemic parameters (fasting blood sugar (FBS), fasting blood insulin (FBI), hemostatic model assessment of insulin resistance (HOMA-IR), and the quantitative insulin sensitivity check index (QUICKI)), lipid profile (triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C)), and blood pressure are regarded as secondary outcomes.

Measurements and assessments

Clinical assessments

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

Blood pressure

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

Assessment of physical activity and dietary intake

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

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

Assessment of appetite sensations

Appetite sensations (hunger, satiety, fullness, desire to consume something sweet, fatty, or salty) of the patients will be measured using a visual analogue scale (VAS) at the beginning and end of the study after overnight fasting [16]

Anthropometric measurements

Data on anthropometric measurements will be collected at baseline and end of the trial. The body weight will be recorded in a fasted state, without footwear, and wearing light clothing to an accuracy of 100 g. Height will be measured using a stadiometer (Seca, Hamburg, Germany) without shoes at a standing position to the nearest 0.1 cm accuracy. WC is defined as the smallest horizontal girth between the costal and iliac crests, measured to the nearest 0.1 cm with a non-stretchable measuring tape. The hip circumference (HC) will be measured at the widest point above the great trochanters. Then, WHR will be calculated. BMI is calculated by dividing weight in kilograms by the square of height in meters. Body composition including FM and FFM will be measured using bioelectrical impedance analysis (MC-780; Tanita, Amsterdam, The Netherlands).

Blood sampling and biochemical measurements

The blood samples (10 ml) will be taken following a 12-h overnight fasting at pre-and post-intervention phases. The blood samples will be kept inside the gel tube for 20 minutes for better precipitation and will be centrifuged at 3000 rpm for 7 minutes to extract serum samples. The serum levels of FBS, TC, TG, and HDL-C will be measured instantly on fresh blood samples by enzymatic methods using colorimetric technique, by commercial kits (Pars-Azmoon Co., Tehran, Iran). LDL-C will be calculated by the Friedewald equation [17]. The enzyme-linked immunosorbent assay (ELISA) method will be applied to measure FBI using

commercial kits (Monobind, Lake Forest, CA, USA). Serum levels of inflammatory factors (CRP, TNF-α, IL-10, and IL-6) will be assessed using ELISA kits. The following formula will be applied to determine HOMA-IR and QUICKI indexes.

HOMA1-IR = (fasting insulin (μIU/ml) × fasting glucose (mg/dl))/405 [18]

QUICKI = 1/ (log (insulin, U ml^{-1}) + log (FBS, mg dl^{-1})

Adherence to the intervention

To assess adherence to the intervention, participants will be asked to document their daily usage of BC supplements on a checklist provided by the researchers. To enhance compliance and prevent participants from forgetting to take supplements, investigators will send daily text messages to participants' cell phones as reminders. In addition, adherence to the intervention will be evaluated by counting the returned capsules at the midpoint and end of the experiment.

Statistical analysis

Analyses will follow the intention-to-treat (ITT) convention. Missing values will be imputed by multiple imputation procedures. The normality of the variables' distribution will be assessed using the Shapiro–Wilk test. The findings will be presented as mean (SD) for numerical data, frequency (percentage) for categorical variables, and median (25th, 75th) for values with skewed distribution. The independent samples t-test and Mann-Whitney U test will be applied to compare quantitative variables with normal and skewed distribution between the two groups, respectively. To do a within-group comparison, we will use the paired-sample t-test and Wilcoxon signed-rank test for values with normal and skewed distribution, respectively. We will use the Chi-square test and Fisher's exact test to examine differences in qualitative variables between the two groups. To estimate the intervention effect for all primary and secondary outcomes, an analysis of covariance (ANCOVA) will be used. In this analysis, we will include baseline measurements as a covariate to adjust for potential differences between

treatment groups at baseline. Statistical analysis will be carried out using SPSS, version 23. $P<0.05$ will be considered statistically significant.

Adverse effects, safety, and data monitoring

Although the values of Recommended Daily Intake (RDA), Estimated Average Requirement (EAR), and Adequate Intake (AI) for boron have not been reported, the tolerable Upper Level (UL) in adults is 20 mg/day [19]. After BC supplementation, patients will be required to report any potential side effects. In case of side effects, more information is required to make a decision for excluding the participants from the study. In such conditions, unbinding is permissible based on the Medical Ethics Committee criteria. Two supervisors from a Data Monitoring Committee (DMC) will track the findings of the RCT. The DMC is independent of the study organizers (sponsor and trial investigators). Moreover, the report of any adverse effects will be sent to the Ethics Committee of Tabriz University of Medical Sciences. One of the investigators will check the coding, security, and storage of the data. In addition, he/she will assess the data entry and data values twice.

Patient and public involvement

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

Discussion

Obesity is a chronic and progressive disease that affects approximately 650 million adults worldwide. It is associated with multiple coexisting conditions and complications and imposes a considerable burden on the healthcare system [20 21]. In adults, the first-line treatment for obesity is typically lifestyle therapy, which often provides poor responses [22 23]. Therefore, finding novel treatment options that can be used as adjuncts to lifestyle therapy in obese adults is of interest.

There is growing evidence that boron plays a multitude of crucial roles in animal and human health. Several experimental research concluded that boron can reduce weight and may even enhance obesity-related markers [12]. It has also been found that boron decreases FBI, FBS, and pancreatic beta cell stress [24 25]. Finding from a study demonstrated that oral administration of boric acid reduced the serum levels of TC and LDL-C and increased HDL-C levels in diabetic rats [26]. In a clinical trial in which healthy women consumed diets containing 10 mg more boron than their usual diet for one month, a significant reduction was observed in serum levels of TC, LDL-C, very low-density lipoprotein cholesterol (VLDL-C), and TG. Moreover, the body weight, body FM, and BMI of the women decreased significantly [27]. Overall, findings from animal and human studies provide supportive evidence for the beneficial effects of boron on metabolic parameters. To our knowledge, available investigations regarding the effects of BC supplementation on features of obesity in humans are limited. The finding from this study will provide clinical evidence on the effectiveness of BC supplementation in obese patients.

Strengths and limitations: This is the first randomized controlled clinical trial that will investigate the effects of BC supplementation in obese patients. Using stratified block randomization, patients will be matched based on a number of characteristics that may impact the final results. Several outcomes, including anthropometric and biochemical indicators, will be examined at the study baseline and endpoint. Moreover, dietary intake, physical activity level, and compliance to the intervention will be assessed. Several limitations to this study should be considered. Given the evaluation of compliance in the current study, low adherence to intervention might be undetectable. Moreover, the serum levels of boron will not be measured to examine the compliance of study participants due to financial constraints. A single dose of BC will be used in this study, therefore, we cannot explore dose-response effects. As different doses may have different effects, dose-finding trials are needed to identify the lowest safe and effective dose. The 3-month intervention period may not be long enough to see a

beneficial effect on secondary outcomes. Obese individuals will be recruited in the study, which may represent a subpopulation that is more adherent to weight management interventions than the general population with obesity. Moreover, the efficacy of the same intervention in other metabolic diseases is not known. Future research should explore the effects of boron on such diseases.

Trial status

Recruitment for this trial has begun at June 2023 and is expected to be completed by December 2023.

Declarations

Ethics approval and consent to participate

This trial was approved by the Ethics Committee of Tabriz University of Medical Sciences (approval number: IR.TBZMED.REC.1401.350). Informed consent will be obtained from all study participants prior to the study onset.

Availability of data and materials

Not applicable

Competing interest

The authors declare no competing interests.

Funding

The study was financially supported by Endocrine Research Center of Tabriz University of Medical Sciences.

Author Contributions

Study design: AO, HT and MNK. Methodology: HT, MNK, SRN and ES. Statistical plan: AO, SN, and HT. Coordination of the study implementation: MNK, SRN and ES. Data collection: MNK, SRN and ES. Manuscript preparation: MNK and SRN. Review and editing: AO, HT, SN, and ES.

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

Acknowledgements

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

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Legend to figure(s)

Figure 1: Study flow diagram

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	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*

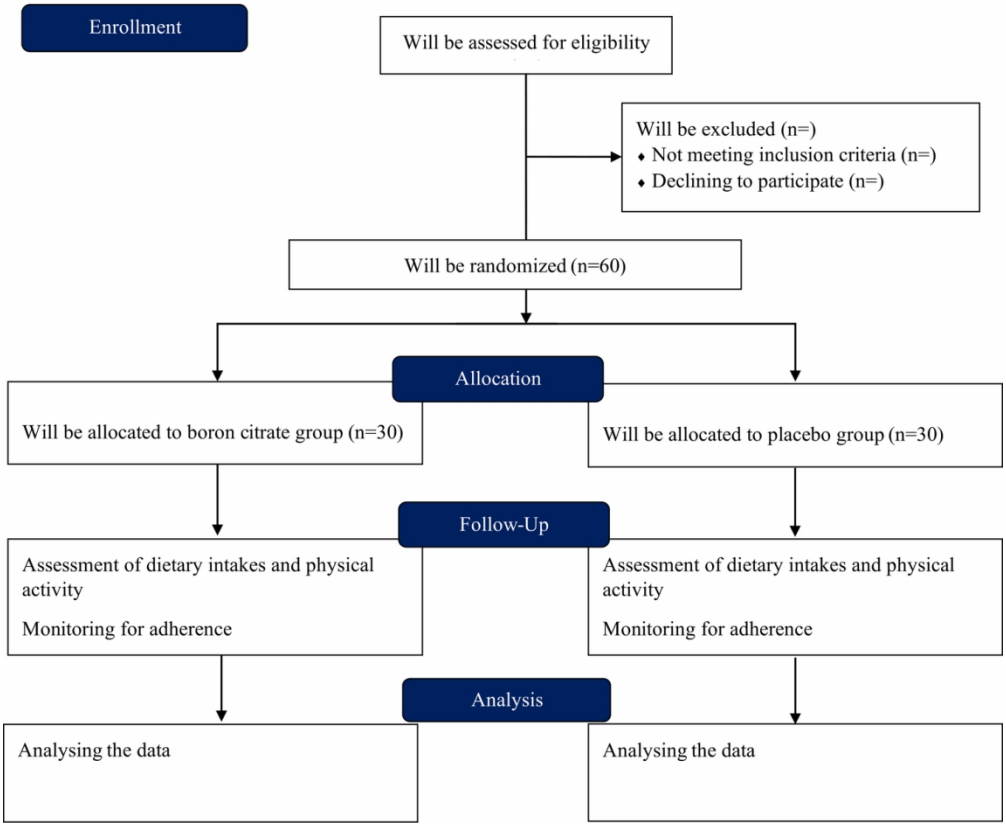

Figure 1: Study flow diagram

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
Administrative information			
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

	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	9
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	6
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	6,7
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)	Table 1 and page 6
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	10
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	5
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
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	6

Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	6
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	6
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	6
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	6
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	7,8
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	10
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	10

Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	9,10
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	9,10
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	9,10
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	10
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	10
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	10
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	7,8
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	4,12

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

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.

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:	*BMJ Open*
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
Primary Subject Heading:	Nutrition and metabolism
Secondary Subject Heading:	Nutrition and metabolism
Keywords:	Obesity, Randomized Controlled Trial, NUTRITION & DIETETICS

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

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Running title: Boron citrate and obesity

Word Count: 3170

Number of Figures: 1

Abstract

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

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

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

Trial registration number: IRCT20220806055624N1.

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

Strengths and limitations of the study:

* This is the first randomized controlled clinical trial that will investigate the effects of boron citrate supplementation in obese patients.

* Several outcomes, including anthropometric and biochemical indicators, will be examined at the study baseline and endpoint.

* Given the evaluation of compliance in the current study, low adherence to intervention might be undetectable.

* A single dose of boron citrate will be used in this study, therefore, we cannot explore dose-response effects.

* Obese individuals will be recruited in the study, which may represent a subpopulation that is more adherent to weight management interventions than the general population with obesity.

Introduction

Obesity, which typically refers to excess body fat, has emerged as a major public health issue. Obesity is associated with numerous comorbidities, including hypertension, dyslipidemia, type 2 diabetes, cardiovascular disease, and nonalcoholic fatty liver disease.(1) Existing data show that the prevalence of obesity is rising.(2) In 2016, more than 20% of the world's population were obese.(3) In this way, Iran is not exempt from this issue. About 40.3% and 19.2% of Iranians were overweight or obese, respectively, based on the second national integrated micronutrient study conducted from 2011 to 2015.(4)

Obesity is a complicated multifactorial disease in which genetic, metabolic, and environmental factors are thought to play a major role in its development. Moreover, several lines of evidence suggest that low-grade, long-standing adipose tissue inflammation is strongly associated with insulin resistance and excess body fat mass.(5) The excessive adipose tissue stimulates the secretion of inflammatory mediators and consequently leads to metabolic disorders.(6) Lifestyle interventions (diet and exercise) have long been known as the cornerstone of weight management.(7, 8) Additionally, clinical guidelines recommend adjunctive anti-obesity medications, but their usage is severely constrained by their unfavorable side effects.(9) Therefore, novel anti-obesity and anti-inflammatory approaches focusing on the control of lipid metabolism to limit fat production and storage with minimal side effects have recently attracted substantial attention.(10)

Elemental boron has been known to be an essential micronutrient for plants since the 1920s. Yet, boron looks favorable not only for plant cells but also for the majority of animal and human cells. Boron has been linked to bone development and maintenance, cognitive function, steroid hormone regulation, and immune response.(11) Boron deficiency has been documented to have major effects on mammalian metabolic and physiological systems (lipid, bone, mineral, endocrine function, and energy metabolism).(10) In previous experimental studies, a low oral

dose of boric acid reduced body weight,(12, 13) findings that supported further investigation. Furthermore, a meta-analysis of animal studies documented that boron has weight-lowering effects.(14) Boron citrate (BC) could induce weight loss in obese patients through increasing energy metabolism, thermogenesis, lipolysis, and inhibition of adiposeness.(14) Findings from a prospective randomized controlled trial study showed that supplementation with different doses of calcium fructoborate lowered inflammatory biomarkers including C-reactive protein (CRP), fibrinogen, and erythrocyte sedimentation rate (ESR) in individuals with primary osteoarthritis.(15) Additionally, supplementation with boron was able to decrease inflammatory cytokines in eight healthy males.(16)

Given the role of BC in suppressing inflammation and inducing lipolysis, we hypothesized that administration of BC may reduce body weight and inflammatory markers and improve cardiometabolic factors in obese patients. Therefore, the purpose of the present study is to investigate the effects of BC supplementation on cardiometabolic factors, inflammatory biomarkers, anthropometric measures, and body composition in obese patients.

Methods and design

Study design

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

beginning of the study by researchers. The Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT 2013 checklist) was used to develop the protocol for this study. The time schedule of the trial and the progress of the study along with the registration of volunteers, allocation, intervention, and review are displayed in **Supplementary Table 1** and **Figure 1.**

Study participants and enrolment

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

Inclusion criteria

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

Exclusion criteria

Individuals who are professional athletes, smokers, those consuming alcohol (consuming more than 14 units of alcohol per week for women and more than 21 units per week for men), taking chemical or herbal drugs for weight loss, hepatotoxic drugs such as phenytoin, tamoxifen, lithium, anti-hypertensive drugs, lipid-lowering drugs (statins), insulin-sensitive drugs, corticosteroid, and non-steroidal anti-inflammatory drugs, antibiotics, supplements affecting liver enzyme levels or any supplements over the past three months will not be included. We also will not include individuals who are pregnant, lactating, menopause, those who have undergone infertility treatment, and those who use birth control pills and estrogen. Individuals who have some pathologic conditions such as cardiovascular, liver, thyroid, parathyroid, kidney, gastrointestinal, and known autoimmune diseases as well as suffering

from polycystic ovary syndrome, cancer, severe infection, or inflammatory disease will be excluded. In addition, those who underwent weight loss surgery in the last year or strict weight loss diets in the last three months will not be included in the current study. If a participant becomes pregnant during the course of the research, consumes alcohol and/or tobacco, or utilizes multivitamin-mineral supplements, he or she will be excluded.

Sample size calculation

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

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

n = sample size for each group.

σ = The variance (SD) for mean CRP, which was considered as 41.5 (the average SDs reported for CRP level in the control and intervention groups of Akbari et al study (17)

$\mu 1$ = mean difference for CRP level in the intervention group (we considered it as 57.9 mg/l based on the study of Akbari et al study.(17)

$\mu 2$ = mean difference for CRP level in the control group (which was considered as 34.1 mg/l based on the study of Akbari et al study.(17)

Overall, using this formula and assuming a 20% drop-out rate in each group, we will require a sample size of 30 subjects in each group.

Randomization

To stratify individuals into distinct strata and blocks, stratified block randomization will be implemented based on age (18-40 vs. 40-60 years) and gender (male vs. female). For each individual placed in a given stratum, a matched individual will be considered based on these variables in the same stratum. As a result, two participants with similar characteristics (for age and gender) will be placed in the same stratum. Finally, a research assistant will randomize each stratum into either the BC or placebo groups based on a predefined computer-generated number with a 1:1 allocation that was concealed using serially numbered, opaque, sealed envelopes. Participants and researchers will be blinded to randomization and allocation until the end of the study. The randomization list will be provided by the pharmacist of the research center at the end of the study.

Study interventions

Sixty obese patients will be randomly assigned in a 1:1 allocation ratio to receive either a BC capsule (containing 10 mg of boron) or a matched placebo capsule (containing 10 mg of maltodextrin) once daily before lunch for 12 weeks. Thirty capsules of BC or placebos will be delivered to patients every 30 days (at the study baseline, day 30, and day 60). Patients in study groups will be asked to consume BC or placebo capsules 30 minutes before lunch or dinner. Patients will be asked to give back the empty pockets at the end of the study. Following ethical considerations, the researchers will give physical activity and dietary advice to enhance the lifestyles of patients in both groups. The participants will be requested to follow general healthy eating recommendations, including changing cooking methods to healthier ways and limiting fast foods, saturated fats, high-fat foods, sugar, sweets, and sugar-sweetened beverages.

Study outcomes

Primary and secondary outcomes

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

circumference (HC), waist-to-hip ratio (WHR), and waist-to-height ratio (WHtR)). Changes in body composition (total body fat mass (FM) and total body fat-free mass (FFM)), glycemic parameters (fasting blood sugar (FBS), fasting blood insulin (FBI), hemostatic model assessment of insulin resistance (HOMA-IR), and the quantitative insulin sensitivity check index (QUICKI)), lipid profile (triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C)), and blood pressure are regarded as secondary outcomes.

Measurements and assessments

Clinical assessments

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

Blood pressure

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

Assessment of physical activity and dietary intake

To ensure that participants' physical activity does not alter throughout the trial, we will monitor their activity. Already validated in Iran,(19) the International Physical Activity Questionnaire-Short Form (IPAQ-SF) will be used to estimate the physical activity level at the beginning and end of the study. According to the IPAQ scoring system, physical activity levels will be classified as low, moderate, and high activity. To assess the dietary intake of each participant, a 3-day food record (2 days of the week and 1 day of the weekend) will be filled at the beginning and end of the study. The 3-day food record booklet will be designed to be

entirely self-administered. It will be contained instructions for recording the day, time, place, type of meal (breakfast, lunch, dinner, and snack), name of the food, ingredients of mixed dishes and recipes, food preparation methods, name of the brand/company, and estimated portion size and any leftovers of all food and drink consumed. The portion sizes of consumed foods will be converted to grams per day using household measures.(20) Then, all gram values of food items will be entered into Nutritionist IV software (First Databank, San Bruno, CA, USA), to obtain daily intake of energy and all nutrients.

Assessment of appetite sensations

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

Anthropometric measurements

Data on anthropometric measurements will be collected at baseline and end of the trial. The body weight will be recorded in a fasted state, without footwear, and wearing light clothing to an accuracy of 100 g. Height will be measured using a stadiometer (Seca, Hamburg, Germany) without shoes at a standing position to the nearest 0.1 cm accuracy. WC will be measured as the smallest horizontal circumference between the costal and iliac crests using a non-stretchable measuring tape with an accuracy of 0.1 cm. The hip circumference (HC) will be measured at the widest point above the great trochanters. Then, WHR will be calculated. BMI is calculated by dividing weight in kilograms by the square of height in meters. Body composition including FM and FFM will be measured using Tanita MC780 multi-frequency segmental bioelectrical impedance analysis applying 3 different frequencies (5kHz/ 50kHz/ 250kHz).

Blood sampling and biochemical measurements

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

better precipitation and will be centrifuged at 3000 rpm for 7 minutes to extract serum samples. The serum levels of FBS, TC, TG, and HDL-C will be measured instantly on fresh blood samples by enzymatic methods using colorimetric technique, by commercial kits (Pars-Azmoon Co., Tehran, Iran). LDL-C will be calculated by the Friedewald equation.(22) The enzyme-linked immunosorbent assay (ELISA) method will be applied to measure FBI using commercial kits (Monobind, Lake Forest, CA, USA). Serum levels of inflammatory factors (CRP, TNF-α, IL-10, and IL-6) will be assessed using ELISA kits. The following formula will be applied to determine HOMA-IR and QUICKI indexes.

HOMA1-IR = (fasting insulin (μIU/ml) × fasting glucose (mg/dl))/405(23)

QUICKI = 1/ (log (insulin, U ml^{-1}) + log (FBS, mg dl^{-1})

Adherence to the intervention

To assess adherence to the intervention, participants will be asked to document their daily usage of BC supplements on a checklist provided by the researchers. To enhance compliance and prevent participants from forgetting to take supplements, investigators will send daily text messages to participants' cell phones as reminders. In addition, adherence to the intervention will be evaluated by counting the returned capsules at the midpoint and end of the experiment.

Statistical analysis

Analyses will follow the intention-to-treat (ITT) convention. Multiple imputation with chained equations will be used to assign any missing values.(24) The Shapiro–Wilk normality test will be used to assess distributions of continuous variables for normality, and natural logarithm transformations of skewed variables will be applied before analyses. The findings will be presented as mean (SD) for numerical data, frequency (percentage) for categorical variables, and median (25th, 75th) for values with skewed distribution. The independent samples t-test and Mann-Whitney U test will be applied to compare quantitative variables with normal and skewed distribution between the two groups, respectively. To do a within-group comparison,

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

Adverse effects, safety, and data monitoring

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

Patient and public involvement

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

Discussion

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

and pancreatic beta cell stress.(26, 27) Finding from a study demonstrated that oral administration of boric acid reduced the serum levels of TC and LDL-C and increased HDL-C levels in diabetic rats.(28) In a clinical trial in which healthy women consumed diets containing 10 mg more boron than their usual diet for one month, a significant reduction was observed in serum levels of TC, LDL-C, very low-density lipoprotein cholesterol (VLDL-C), and TG. Moreover, the body weight, body FM, and BMI of the women decreased significantly.(29) In COVID-19 patients, supplementation with BC alone or in combination with oleoylethanolamide resulted in a significant decrease in serum lactate dehydrogenase and ESR levels.(17) In an experimental study, supplementation with boron compounds reduced lipid peroxidation, and enhanced the antioxidant defense mechanism in rats.(30) Moreover, a study documented that boron could effectively ameliorate oxidative stress, inflammation, and biochemical parameters in rats treated with acrylamide.(31) Concerning the biological mechanisms, the anti-inflammatory effects of boro are thought to be mediated through inhibition of the oxidative process by activating scavenging cells like leukocytes and neutrophils.(32) Boron also enhances the inhibition of free radicals by increasing the level of antioxidant enzymes (superoxide dismutase, catalase, and glutathione peroxidase) in blood and cells.(33) Boron significantly improves magnesium absorption. It has been shown that in magnesium deficiency, the level of proinflammatory cytokines increases.(33) Magnesium also plays an important role in carbohydrate metabolism; its deficiency provokes and worsens insulin resistance.(33) Overall, findings from animal and human studies provide supportive evidence for the beneficial effects of boron on metabolic and inflammatory parameters. To our knowledge, available investigations regarding the effects of BC supplementation on features of obesity in humans are limited. The findings from this study will provide clinical evidence on the effectiveness of BC supplementation in obese patients. The trial results will be disseminated in peer-reviewed scientific and clinical journals.

Strengths and limitations: This is the first randomized controlled clinical trial that will investigate the effects of BC supplementation in obese patients. Using stratified block randomization, patients will be matched based on a number of characteristics that may impact the final results. Several outcomes, including anthropometric and biochemical indicators, will be examined at the study baseline and endpoint. Moreover, dietary intake, physical activity level, and compliance to the intervention will be assessed. Several limitations to this study should be considered. Given the evaluation of compliance in the current study, low adherence to intervention might be undetectable. Moreover, the serum levels of boron will not be measured to examine the compliance of study participants due to financial constraints. A single dose of BC will be used in this study, therefore, we cannot explore dose-response effects. As different doses may have different effects, dose-finding trials are needed to identify the lowest safe and effective dose. The 3-month intervention period may not be long enough to see a beneficial effect on secondary outcomes. Obese individuals will be recruited in the study, which may represent a subpopulation that is more adherent to weight management interventions than the general population with obesity. Moreover, the efficacy of the same intervention in other metabolic diseases is not known. Future research should explore the effects of boron on such diseases.

Trial status

Recruitment for this trial has begun at June 2023 and is expected to be completed by December 2023.

Declarations

Ethics approval and consent to participate

This trial was approved by the Ethics Committee of Tabriz University of Medical Sciences (approval number: IR.TBZMED.REC.1401.350). Informed consent will be obtained from all study participants prior to the study onset.

Availability of data and materials

Not applicable

Competing interest

The authors declare no competing interests.

Funding

The study was financially supported by Endocrine Research Center of Tabriz University of Medical Sciences (grant number: 70107).

Author Contributions

Study design: AO, HT and MNK. Methodology: HT, MNK, SRN and ES. Statistical plan: AO, SN, and HT. Coordination of the study implementation: MNK, SRN and ES. Data collection: MNK, SRN and ES. Manuscript preparation: MNK and SRN. Review and editing: AO, HT, SN, and ES.

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

Acknowledgements

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

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Legend to figure(s)

Figure 1: Study flow diagram

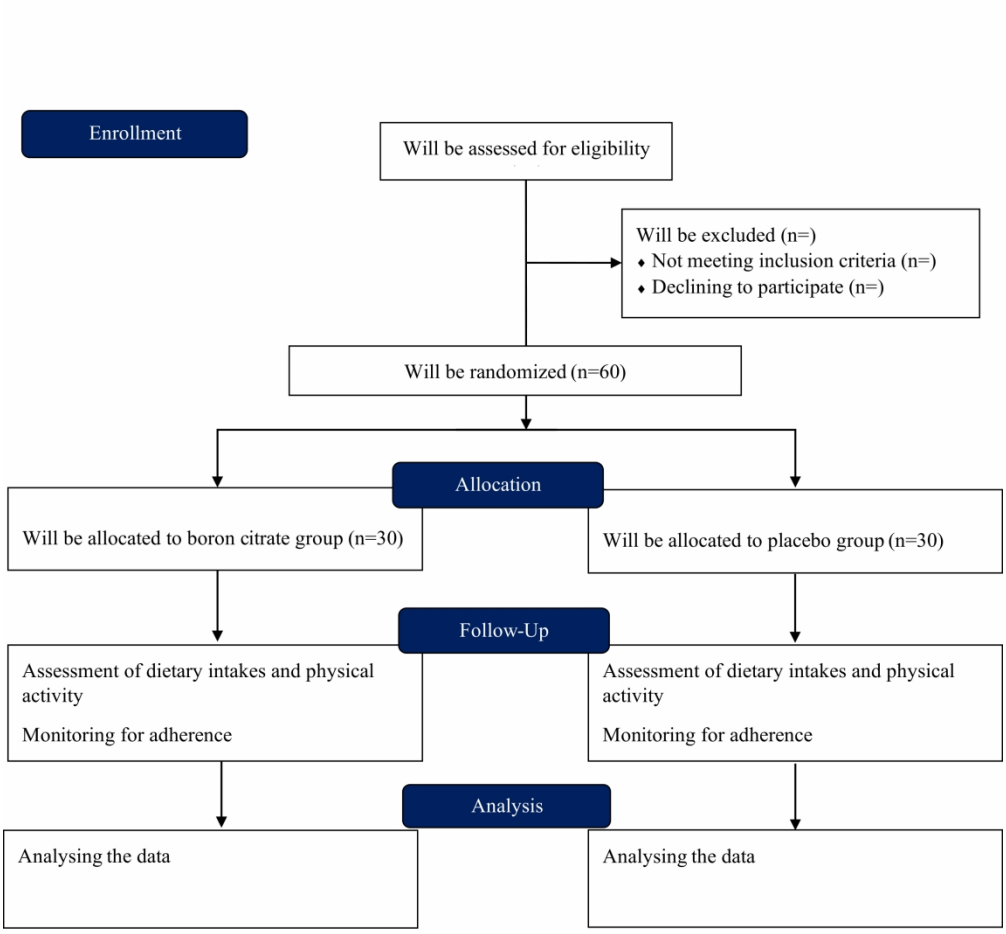

Figure 1: Study flow diagram

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	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*

"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

best of my ability. I will inform the participant of any changes in possible risks and benefits during the study or information that will depend on the participant's willingness to continue treatment in this study.

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

Section/item	Item No	Description	Addressed on page number
Administrative information			
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

	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	9
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	6
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	6,7
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)	Table 1 and page 6
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	10
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	5
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
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	6

Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	6
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	6
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	6
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	6
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	7,8
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	10
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	10

Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	9,10
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	9,10
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	9,10
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	10
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	10
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	10
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	7,8
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	4,12

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

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.