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Effect of oral trehalose supplementation on inflammation and wound healing in patients with peri-trochanteric fractures: study protocol for a randomised clinical trial

Reza Zandi,[1,2] Alireza Tavallaei Zavareh,[3] Amir Mehrvar,[2] Mohammad-Reza Jowshan,[4] Amirhossein Sahebkar,[5,6] Bahareh Nikooyeh,[7] Hoda Zahedi ,[8,9] Shahin Talebi[2]

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For numbered affiliations see end of article.

Correspondence to
Dr Hoda Zahedi; hoda.s.zahedi@gmail.com and Dr Shahin Talebi; sh.talebi1365@gmail.com

ABSTRACT

Introduction Peri-trochanteric fractures, common among the elderly with osteoporosis, pose significant morbidity and mortality risks. These fractures are increasing due to the ageing population, with Nordic countries seeing a high incidence. They present challenges for orthopaedic surgeons and can lead to functional decline and high medical costs. Hip fractures have severe consequences, including pain, immobility and increased mortality. Managing wound care is complex, involving various healing phases. Trehalose, a disaccharide with antioxidant and anti-inflammatory properties, has shown promise in wound healing and other health conditions. Studies suggest its potential benefits in reducing inflammation and aiding wound healing in peri-trochanteric fracture patients, but more research is needed to confirm its clinical effectiveness in humans.

Methods and analysis The study is a double-blind, placebo-controlled randomised clinical trial aiming to evaluate the effect of trehalose consumption on patients with peri-trochanteric fractures. The study will include 64 patients meeting specific inclusion criteria and will assess inflammatory markers and wound healing at different time points. Patients will be divided into two groups, one receiving trehalose and the other receiving a placebo for 12 weeks. Various measurements and assessments will be conducted, including biochemical assays, wound assessments, anthropometric measurements and dietary intake evaluations. Data analysis will be performed using SPSS software, and statistical tests will be used to compare outcomes between the intervention and control groups.

Ethics and dissemination The Ethics Committee of Shahid Beheshti University of Medical Sciences (IR.SBMU.RETECH.REC.1403.191).

Trial registration number Iranian Registry of Clinical Trials. IRCT20180404039188N5. URL of trial registry record: https://irct.behdasht.gov.ir/trial/77572. Registration date: 7 July 2024.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This is the first study about the effectiveness of trehalose on wound healing and inflammation in humans.
⇒ Studies are lacking regarding the effects of trehalose on inflammation factors.
⇒ The possibility of the patient not using trehalose regularly due to the long duration of the intervention.

INTRODUCTION

Peri-trochanteric fractures are prevalent among the elderly population, particularly those with osteoporosis,[1] and serve as a significant contributor to morbidity and mortality.[2–4] These fractures, a predominant type of lower limb fracture, are on the rise due to the expanding elderly demographic.[1 3] In Nordic countries, peri-trochanteric fractures make up nearly half of all hip fractures, highlighting the importance of addressing this issue in the context of increasing incidences of hip fractures.[5] Peri-trochanteric fractures, characterised by varying fracture patterns involving the trochanter, present a considerable challenge for orthopaedic surgeons.[6] Statistics indicate that approximately 30% of individuals who sustain a hip fracture will pass away within the subsequent year,[7] with many others facing substantial declines in functionality.[8–11]

The financial implications of treating hip fractures are also noteworthy, with the average cost for direct medical expenses totalling around $40 000 in the first year.[12] Hip fractures can have severe consequences for older adults, leading to pain, immobility and a spectrum of complications from delirium to functional impairment and mortality.[13] Despite recent studies suggesting a potential stabilisation or decrease in hip fracture occurrences,[14] there remains a concern that

the global incidence of hip fractures may rise due to the ageing demographic, necessitating further preventive measures.[15]

Managing wound care presents a significant clinical challenge, involving an intricate series of steps including haemostasis, inflammation, angiogenesis, proliferation, connective tissue restructuring and restoration of wound strength.[16] In cases where any of these healing phases are not fully executed, the healing process may be obstructed, leading to delayed tissue recovery and the potential development of chronic non-healing wounds.[17] Effective management of postoperative wounds in the community is crucial to avoid possible complications like surgical site infections and wound dehiscence.[18] Prolonged inflammation can disrupt the normal course of wound healing and bone healing, particularly complicating the recovery of diabetic wounds.[19 20] Therefore, medications with anti-inflammatory characteristics are considered optimal for addressing diabetic wound healing.[21]

Trehalose, an endogenous nonreducing disaccharide composed of two glucose molecules joined by covalent bonds, is biosynthesised by various organisms but not by vertebrates.[22] There is evidence for its antioxidant,[23] anti-inflammatory,[24] autophagy-enhancing,[25] and wound healing properties[26] with therapeutic potential for several common health problems, including cardiometabolic disorders[27] and neurodegenerative diseases.[28] There are in vivo[29] and in vitro[24 30] studies on the effect of trehalose on wound healing and inflammation. In healthy subjects, oral trehalose ingestion resulted in lower blood glucose, and insulin levels, as well as lower active gastric inhibitory peptide levels compared with glucose ingestion.[31] Others reported that daily consumption of 3.3 g of trehalose improved glucose tolerance in nondiabetic individuals with higher postprandial glucose levels.[32] Previous studies demonstrated that trehalose reduces serum tumour necrosis factor alpha (TNF-α) and prevents mortality.[33]

Overall, these clinical data together with the established immunological profile of the molecule seem promising for the potential use of trehalose in reducing inflammation and wound healing. Since it is possible to significantly contribute to the treatment process of patients with peri-trochanteric fracture by controlling the inflammation and wound caused by surgery, this study was designed. However, currently, no information has been published regarding the clinical effectiveness of this compound in humans. Therefore, this study aims to demonstrate the concept and investigate the potential impact of trehalose in humans, for the first time, in reducing inflammation and wound healing in patients with fractures in the peri-trochanteric region.

Rationale

Peri-trochanteric fractures are among the most prevalent types of injuries that typically necessitate surgical intervention.[34] Given their associated high rates of morbidity and mortality, hip fractures have emerged as a significant public health concern globally. Recently, there has been increased interest in the connection between inflammatory markers and patient outcomes.[35] Furthermore, skin injuries or wounds resulting from surgical procedures can cause significant disability and distress, presenting a considerable challenge to healthcare systems worldwide.[36] Numerous studies have demonstrated the positive anti-inflammatory and wound-healing properties of trehalose in various conditions. However, no clinical trial studies have been performed on patients, especially peri-trochanteric patients.[24 26] We hypothesised that trehalose could reduce inflammation and enhance wound healing. Thus, this study was developed to evaluate the effectiveness of trehalose administration in patients with peri-trochanteric fractures.

METHODS AND ANALYSIS

Study design

A double-blind, placebo-controlled randomised clinical trial with an intervention period of 12 weeks will be conducted in this study as presented in figure 1. The trial was registered at the Iranian Registry of Clinical Trials at https://irct.behdasht.gov.ir (Identifier: IRCT20180404039188N5). In this article, we will use the SPIRIT to present both the findings and specifics of the study protocol, such as the timing of participant enrolment, interventions and assessments.[37]

Study participants

Participants aged 18 to 60 years with peri-trochanteric hip fractures admitted to the Talaghani Hospital's orthopaedic department will be eligible for inclusion. The subjects must satisfy all inclusion criteria and no exclusion criteria presented in box 1 to be eligible for the trial. The study is a randomised controlled trial, and recruitment will begin in December 2024 and is expected to be completed approximately by the end of December 2025.

Sample size calculation

We determined the sample size using Power Analysis and Sample Size Software (PASS) 2021 software (V.21.0.3) for each group with a clinically important difference of 3 points on the change in inflammatory markers (specifically interleukin-6 (IL-6) levels, the primary outcome of the study),[38] assuming a SD of 3.13 points. We then used two-sample t-tests, assuming an equal variance of the difference between means, a power of 90% and a significance level of 5%. According to the above calculations, 24 people in each group (48 people in total) will be needed. This study will involve 32 people in each group (64 people in total), considering the possibility of 30% dropout and individual non-compliance. Therefore, the final sample size for the study is as follows:

1. Thirty-two patients with a per-trochanteric fracture will receive a trehalose powder supplement for 12 weeks which will be orally consumed dissolved in water after breakfast.

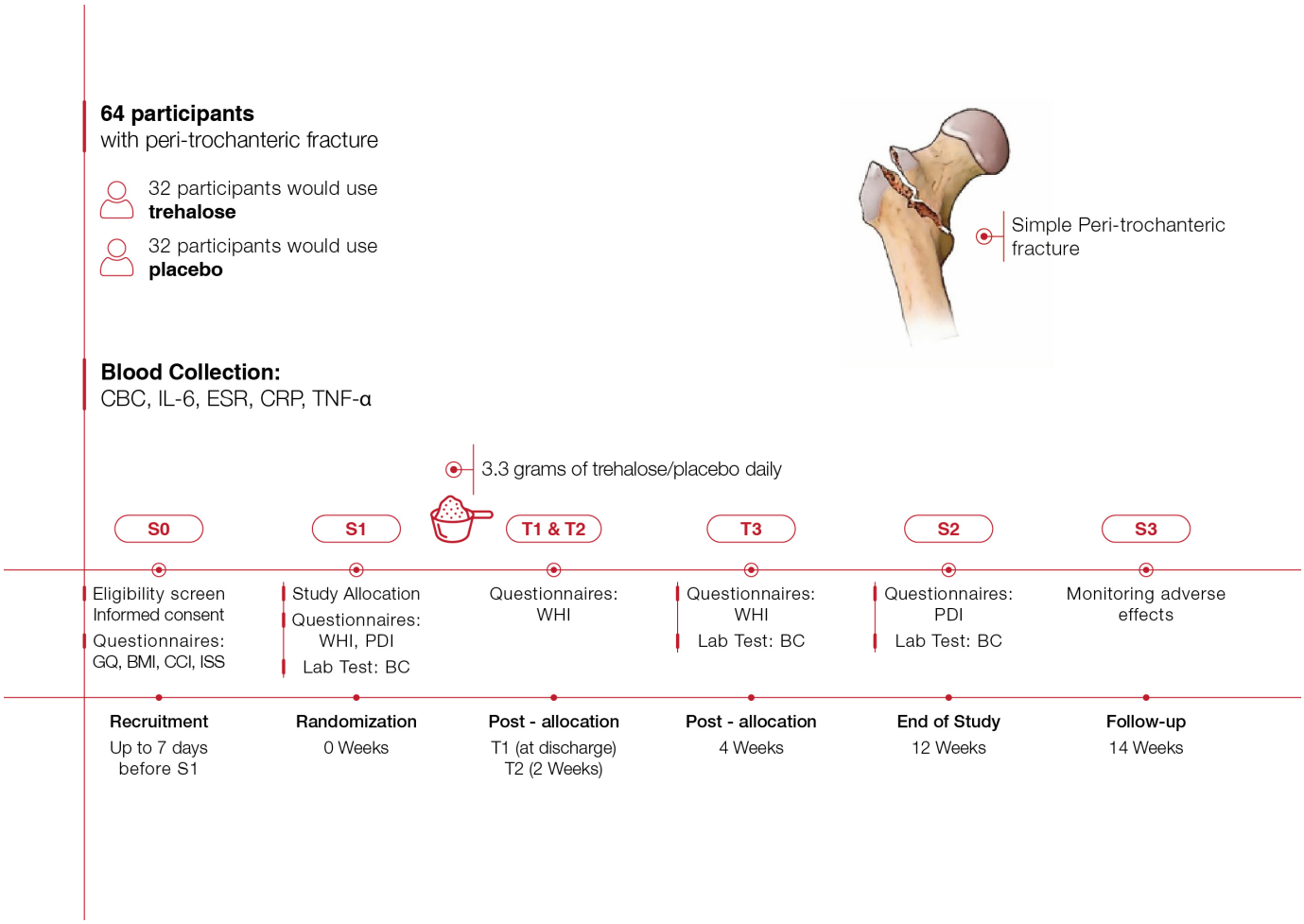

Figure 1 Overview of study timeline. BC, blood collection; BMI, body mass index; CBC, complete blood cells parameters; CCI, Charlson Comorbidity Index; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; GQ, general questionnaires; IL-6, interleukin-6; ISS, Injury Severity Score; PDI, patient's dietary intake, TNF-α, tumour necrosis factor-alpha; WHI, wound healing index,

2. Thirty-two patients with a peri-trochanteric fracture will receive a placebo powder supplement for 12 weeks which will be orally consumed dissolved in water after breakfast.

Randomisation

Randomisation in this study will be performed using the permuted block randomisation method through SPSS random number generator software. A qualified statistician not involved in the recruitment process will generate the allocation sequence. Patients will be randomly assigned to the intervention or placebo group using block sizes of four patients. For example, pairs of blocks have six possible sequence arrangements based on A (intervention group) and B (placebo group): AABB, ABAB, ABBA, BBAA, BAAB and BABA. The block size will be concealed from the study personnel to prevent prediction of group assignments.

To achieve random allocation, these sequences will be assigned to the third-party pharmacist, and based on these predetermined random blocks, patients will be sequentially allocated to either the intervention or placebo group. The randomisation codes will be stored in sealed, opaque envelopes and kept in a secure location accessible only to the designated third-party pharmacist. The allocation ratio will be 1:1 to ensure equal group sizes.

Intervention

The present study will be conducted as a randomised, double-blind, placebo-controlled clinical trial to determine the effect of trehalose consumption in patients with femoral fractures. A total of 64 patients from the orthopaedic department of Taleghani Hospital will be enrolled based on inclusion and exclusion criteria. These individuals will be randomly assigned to two groups receiving either trehalose or placebo, with 32 individuals in each group. The trehalose group will receive 3.3 g of trehalose powder daily which will be orally consumed dissolved in water, while the placebo group will receive 3.3 g of placebo powder (containing sucrose) daily which will be orally consumed dissolved in water for 12 weeks (less than two-thirds of a teaspoon) after breakfast for 12 weeks (received a box containing about 280 g). Throughout the

Box 1 The eligibility criteria,

Inclusion criteria

1) Age 18 to 60 years.
2) Admission to the orthopaedic department of Taleghani Hospital with a diagnosis of peri-trochanteric hip fracture.
3) Completion of an informed consent form by the patient or first-degree relatives of the patient.
4) Injury Severity Score ≤ 15 (mild to moderate).

Exclusion criteria

1) Pregnancy and lactation.
2) Septic patients who are not hemodynamically and metabolically stable.
3) History of any autoimmune diseases or disorders.
4) History of cancer.
5) Chemotherapy and radiotherapy within the past month.
6) Diabetes mellitus.
7) History of any liver failure.
8) History of HIV infection.
9) Body mass index ≥ 40 kg/m$^{2.}$
10) Unwillingness to participate in the study.
11) Significant change in the patient's treatment process or admission to the ICU.
12) Intolerance to trehalose.
13) The patient is nothing by mouth and unable to start oral feeding.
14) Development of infection during the recovery and improvement period.
15) Alcohol consumption.
16) Smoking.
17) Different and heavy physical activity programs.
18) Those who are on a specific diet throughout the study.
19) Weight changes (±3) during the study.
20) Have a history of taking antibiotics and non-steroidal anti-inflammatory drugs during the last month.

study period, weekly telephone contact will be made with the patients, reminding them of the supplement intake.

Observational methods, interviews, questionnaire completion and biochemical analysis methods will be used to collect data.

The study timetable included the following steps (table 1):

S0 (' recruitment', preferably using the list of eligible cohort participants): the assessment of inclusion and exclusion criteria study questionnaires completion and obtaining informed consent.

S1 (1 week after S0, 'randomization'): completing the demographic questionnaire, wound healing index (WHI), the patient's dietary intake (PDI), blood sampling, International Physical Activity Questionnaire (IPAQ) and delivery of trehalose or placebo for 12 weeks.

T1–T3 ('post-allocation'): personal supervision and completing WHI, daily telephone calls and messages to remind trehalose intake.

T3: completing PDI and lab tests.

S2 ('end of the study,' at the end of week 12): completing IPAQ, PDI and lab tests.

S3 ('follow-up,' week 14): contacting and informing the patients regarding the test results.

The patients in the intervention group received a 3.3 g serving/day of trehalose, while the control group used a 3.3 g serving/day of placebo, and they will be reminded to take trehalose daily by telephone.

Blinding

In our study, we will implement a double-blind, randomised design to enhance the validity of our findings and minimise bias. The blinding process will involve the same third-party pharmacist responsible for randomisation, who will be responsible for the packaging and labelling of the study products. Each package will be assigned a unique identification number, which will be systematically recorded in a secure database separate from the randomization codes.

This third party will maintain a confidential master list that correlates each identification number with its corresponding randomisation code, designating which package contains the placebo and which contains the trehalose supplement. This list will be stored in password-protected files in a secure location, separated from the sealed randomisation envelopes, and accessible only to the designated third-party pharmacist. This approach ensures that neither the participants, investigators, research assistants, statistician nor the personnel involved in administering or analysing the outcomes will have access to this information during the study period.

The confidentiality of the treatment assignments will be strictly maintained until study completion and database lock. The only circumstance under which this list may be disclosed is if a participant experiences severe adverse effects requiring immediate medical attention. In such cases, a formal unblinding procedure will be followed, requiring approval from the principal investigator, and all unblinding events will be thoroughly documented including the reason, date and personnel involved. This ethical safeguard allows for immediate medical intervention while maintaining the study's scientific integrity. Through this carefully structured blinding method, we aim to uphold the rigour and reliability of our research outcomes while ensuring participant safety throughout the study duration.

Adherence to the intervention

Adherence to the intervention will be monitored through regular phone calls and SMS reminders, occurring weekly throughout the 12-week intervention period. These communications will include encouragement and address any potential barriers participants may face regarding adherence.

In cases where adherence falls below 80% of the recommended supplement consumption), we will implement contingency plans, which include:

To promote adherence to the intervention, we will provide educational materials and one-on-one counselling during clinic follow-ups. These resources will emphasise the importance of adherence and address individual concerns. This approach aims to empower participants

Table 1 The schedule of enrolment, interventions and assessments

	Study period						
	Recruitment	Randomisation	Post-allocation			End of study	Follow-up
	S0 (up to 7 days before S1)	S1 (0 weeks)	T1 (at discharge)	T2 (suture removal)	T3 (4 weeks)	S2 (12 weeks)	S3 (14 weeks)
Enrolment							
Eligibility screen	X						
Informed consent	X						
Allocation		X					
Interventions		◆	———	———	———	◆	
Assessments							
BMI	X						
ISS	X						
CCI	X						
WHI		X	X	X	X		
PDI		X			X	X	
IPAQ		X				X	
Blood collection: CBC IL-6 ESR CRP TNF-α		X			X	X	
Face-to-face meetings	X	X	X	X	X	X	
reminder	The patients will be reminded to take trehalose daily by telephone						

BMI, body mass index; CBC, complete blood cell; CCI, Charlson Comorbidity Index; CRP, C-reactive protein; IL-6, interleukin-6; IPAQ, International Physical Activity Questionnaire; ISS, Injury Severity Score; PDI, patients' dietary intake; TNF-α, tumour necrosis factor-alpha; WHI, wound healing index.

and improve overall adherence to the study. We will systematically document the reasons for non-adherence, which will allow us to analyse patterns and potential barriers in our final analysis. By incorporating these strategies, we aim to minimise potential adherence issues and maintain the integrity of the study outcomes.

Manufacture of study supplements

The trehalose with a chemical formula of C12H22O11•2H2O, purity ≥99% and low metal ion content will be procured from Shaanxi Fruiterco Biotechnology Co., Ltd.

Adverse event reporting and management

Flushing, loose faeces that self-limit and momentary bloating are possible signs of mild to moderate gastrointestinal distress. These negative effects are generally indicative of consuming disaccharides. To avoid this, nutritional and clinical conditions will be evaluated regularly, and complete trehalose will not be served in a single meal. One strategy for managing eating intolerance is to temporarily restrict nutritional intake; if acute gastrointestinal issues do not resolve, another strategy is to think about using prokinetic medications.[39]

Outcome measures

The primary outcome measures comprised IL-6 and WHI. These markers were selected as primary outcomes due to their established role as key indicators of systemic inflammation and wound healing progression.[40 41] IL-6, as a principal pro-inflammatory cytokine, serves as a sensitive marker of the acute inflammatory response and has been strongly correlated with wound healing outcomes in previous studies.[42] The WHI provides a standardised, clinically validated measure of wound healing progression,[43] incorporating multiple aspects including wound size reduction, granulation tissue formation and epithelialisation.[43]

The secondary outcome measures were the levels of inflammatory markers, including erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), TNF-α and complete blood cells (CBC). Each of these markers provides unique and complementary information about the inflammatory and healing processes[44] : ESR and CRP offer insights into acute phase responses and systemic inflammation levels;[45] TNF-α, another crucial pro-inflammatory cytokine, helps evaluate the inflammatory cascade regulation;[46] and CBC parameters assist in monitoring the overall immune response and potential complications.[47] The combination of these primary and secondary outcomes allows for a comprehensive assessment of both local wound healing progression and systemic inflammatory responses.[48 49]

Measurements

The following indices will be recorded at the patient's admission stage: age, gender, height, weight, body mass index (BMI) and smoking status. The Injury Severity Score (ISS)[50] will be used to measure the extent of injury, which is a valid system that calculates the score based on the sum of the squares of the highest values from the Abbreviated

Table 2 Wound healing index

Healing index (two or more signs are present)	Tissue colour	Bleeding on palpation	Granulation tissue	Incision margin	Suppuration
1: very poor	≥ 50% of red gingiva	Yes	Yes	Not epithelised, with loss of epithelium beyond the incision margin	Yes
2: poor	≥ 50% of red gingiva	Yes	Yes	Not epithelised, with exposed connective tissue	No
3: good	25–50% of red gingiva	No	No	No exposed connective tissue	No
4: very good	< 25% of red gingiva	No	No	No exposed connective tissue	No
5: excellent	All pink tissues	No	No	No exposed connective tissue	No

Injury Scale[51] in three body regions that have received the most severe injuries. Additionally, patient comorbidities will be predicted based on the Charlson Comorbidity Index (CCI)[52] calculated using a questionnaire.

Venous blood samples were taken from the participants by qualified phlebotomists according to reliable protocols for the collection, storage and shipment of biological materials. A blood sample of 15 mL was drawn from each subject in both groups via brachial vein puncture. Biochemical assays including inflammatory factors such as IL-6, TNF-α, ESR and CRP and 5 cc of venous blood will be collected 3 times: 1, at the beginning of the study; 2, after 4 weeks; and 3, at the end of the study. Serum separation will be performed to evaluate the levels of the mentioned markers. The wound of the patient will be assessed at four stages: 1, the beginning of the study; 2, at discharge; 3, during suture removal; and 4, at the end of the first month (the patient's first visit to the clinic after the operation) and scored based on the WHI (table 2) criteria. Anthropometric measurements including height, weight and BMI for each patient will be measured at the beginning and the end of the study.

Height

Trotter and Gleser provided formulas for estimating stature from long bone lengths for different populations.[53]

An example equation for a white male might look like this:

Stature (cm)=73.570 + (2.970×Ulna length (cm))

And for a white female:

Stature (cm)=61.412 + (3.147×Ulna length (cm))

Weight

Weight will be measured using a bed scale (Balas company, Iran):

$$\mathrm{BMI} = \left(height\left(m\right)\right)^2 \times weight\left(Kg\right)$$

Physical activity

Physical activity level will be evaluated at baseline, during the 6th week and during the last week using the IPAQ, which has been validated for the Iranian population.[54] Physical activity level in terms of Metabolic Equivalent of Task hours per week (MET-h/wk) will be calculated according to the activity performed using the standard MET table and will be classified into three levels including low, moderate and high-intensity physical activity.

Laboratory factors

At the study baseline and after the intervention, 10 mL blood sample will be obtained, and serum will be isolated from the whole blood by centrifugation for 10 min at 3500 rpm. In order to detect inflammatory factors, after separating the serum from the blood sample, the serums will be maintained in a freezer at −80°C until the tests are completed. To evaluate inflammation factors including IL-6, TNF-α and quantitative CRP, blood samples will be analysed by using ELISA kits,[55 56] and to evaluate ESR, blood samples will be analysed by using Western green method.[57]

The PDI will be examined 3 times: 1, at the beginning of the study; 2, after 4 weeks; and 3, at the end of the study, which will be analysed using the Nutritionist-IV software programme (V.7.0; N-Squared Computing, Salem, OR, USA).

Statistical analysis

Statistical data were analysed using the SPSS software V.23. Normal quantitative data will be expressed as mean±SD, while qualitative data will be reported as frequency (percentage). The Kolmogorov-Smirnov test[58] will assess normality, with logarithmic transformation applied to non-normally distributed data as needed.

Data quality assessment and handling of inconsistencies will be performed prior to the main analysis. Outliers will be identified using both graphical methods (box plots, histogram) and statistical approaches (z-scores > ±3 SD, Cook's distance). Extreme outliers will be verified for data entry errors, and genuine outliers will be handled using robust statistical methods rather than automatic exclusion. Missing data patterns will be evaluated using Little's missing completely at random test to determine if data are missing completely at random. For missing data exceeding 5%, multiple imputation techniques using chained equations (MICE) will be employed, creating 20 imputed datasets. Sensitivity analyses will be conducted to compare results between complete case analysis and multiple imputation approaches.

To control for possible confounders such as age, gender, drug intake, smoking status, alcohol consumption, BMI, marital status, history of comorbidities, education and energy intake, we will use covariance analysis. Baseline characteristics of participants will be thoroughly assessed for significant differences between intervention and

control groups, using descriptive statistics for a comprehensive summary. The χ^2 test[59] will be used for qualitative data comparisons, and the independent t-test[60] will apply for quantitative data. For inter-group comparisons, analysis of covariance will compare means while adjusting for baseline values and any relevant confounding variables. Paired t-tests will be used for intragroup comparisons (pre and postintervention).

We will employ an intention-to-treat (ITT)[61] approach to include all participants in the initial analysis, minimising the biases associated with dropouts or non-compliance. To ensure robust handling of missing data in the ITT analysis, we will use both last observations carried forward and mixed models with repeated measures (MMRM) approaches. The MMRM analysis will be considered primary as it provides valid inference under the missing at random assumption. A secondary per-protocol analysis will be conducted for biochemical measurements, retaining participants who completed the minimum duration of intervention. Both crude and adjusted findings will be reported if significant differences are noted, with a significance level set at 0.05 for all tests. Standardised effect sizes with 95% CIs will be reported to aid in the interpretation of results.

DISCUSSION

The results of this double-blind, placebo-controlled randomised clinical trial offer valuable insights into the therapeutic potential of trehalose in enhancing wound healing and reducing inflammation in patients with peri-trochanteric fractures. The impact of trehalose on participants, as reflected through the assessment of inflammatory markers and wound healing over 12 weeks, contributes significantly to our understanding of its mechanisms and benefits in a clinical setting, especially considering the lack of prior information regarding its clinical effectiveness in humans with fractures in the peri-trochanteric region.

Peri-trochanteric fractures represent a substantial challenge in orthopaedic practice,[62] not only due to their immediate health implications but also owing to the long-term morbidity and increased mortality rates observed in patients, particularly among the elderly with underlying conditions such as osteoporosis.[63] The financial strain these injuries place on the healthcare system further underscores the need for effective interventions that can accelerate recovery, reduce complications and improve overall outcomes.[64]

The anti-inflammatory[24] and wound-healing properties[26] of trehalose, as observed in various organisms, have positioned this disaccharide as a promising candidate for addressing the complex cascade of events involved in wound healing. Mechanistically, the reduction of serum TNF-α levels and the enhancement of autophagy by trehalose are crucial aspects that likely contribute to its beneficial effects in human patients.[65] The ability of trehalose to modulate these processes, coupled with its favourable safety profile, makes it a compelling therapeutic option.

Analysing the study's findings, patients receiving trehalose exhibited a trend towards improved wound healing and a reduction in inflammatory markers compared with the placebo group. These results align with the hypothesised role of trehalose in modulating the inflammatory response and promoting healing processes, thus offering a vital addition to the existing therapeutic arsenal for managing peri-trochanteric fractures.

It is also noteworthy that the study's implications extend beyond the immediate context of bone fracture healing,[66] suggesting potential broader applications of trehalose in managing diabetic wounds and other conditions characterised by chronic inflammation and impaired healing.[21] However, while the reduction in markers such as TNF-α points to a systemic anti-inflammatory effect, the direct mechanisms by which trehalose exerts these effects in humans require further elucidation.[65]

The generalisability of this protocol requires consideration of several aspects. First, our focus on peri-trochanteric fractures in adults aged 18–60 years, while providing specific insights for this population, means that the findings may not directly translate to other fracture types (such as vertebral, tibial or upper extremity fractures) or different age groups (particularly elderly patients who comprise a significant portion of fracture cases). Furthermore, the exclusion of patients with common conditions such as diabetes may limit the protocol's applicability to the broader fracture patient population typically seen in clinical practice. Additionally, our single tertiary care centre setting and the monitoring through weekly telephone contact and standardised assessments require specific resources and staffing that may vary across institutions. The protocol's defined supplementation schedule (3.3 g daily trehalose after breakfast) has been designed to maximise compliance and standardisation in our setting.

The limitations of this protocol include its single-centre design and 14-week timeline, with a final follow-up at week 14. This relatively short follow-up period, while practical for initial safety and efficacy assessment, represents one of the protocol's main limitations. The bone healing and inflammatory processes often extend beyond this timeframe. Other limitations include the single-centre nature of the study and the specific supplementation protocol that may need adjustment in different healthcare settings.

In conclusion, this protocol outlines our approach to investigating trehalose's potential in enhancing wound healing and reducing inflammation in peri-trochanteric fractures. Future studies should consider: (1) including broader age ranges and different fracture types, (2) implementing multicenter designs to account for healthcare setting variations, (3) adapting the protocol for patients with common comorbidities and (4) extending follow-up periods to evaluate long-term outcomes. These modifications would help establish

the broader clinical utility of trehalose supplementation in fracture management across diverse patient populations.

Author affiliations
[1]Musculoskeletal Disorders Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2]Department of Orthopedics, Taleghani Hospital Research Development Committee, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[3]Student Research Committee, Department of Orthopedics, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[4]Department of Clinical Nutrition, School of Nutritional Sciences and Dietetics, Tehran University of Medical Sciences, Tehran, Iran
[5]Biotechnology Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran
[6]Applied Biomedical Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
[7]Department of Nutrition Research, National Nutrition and Food Technology Research Institute, Faculty of Nutrition Sciences and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[8]Department of Clinical Nutrition and Dietetics, National Nutrition and Food Technology Research Institute, Faculty of Nutrition Sciences and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[9]Hematopoietic Stem Cell Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

X Mohammad-Reza Jowshan @MrJowshan

Contributors Conception and design: ST and HZ. Software and formal analysis: MRJ and BN. Investigation and resources: ATZ, AS and HZ. Drafting the manuscript: MRJ and ATZ. Revising manuscript for intellectual content: HZ, MRJ and ST. Visualisation: ATZ. Supervision: ST and HZ. Project administration: ATZ, AM, RZ and HZ. Authors have read and agreed to the published version of the manuscript. HZ takes responsibility for the overall content as guarantor.

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ORCID iD
Hoda Zahedi http://orcid.org/0000-0003-4431-7459

REFERENCES

1 Dai J-Q, Jin D, Zhang C, *et al*. Radiologic predictor of intraoperative lateral wall fractures in treatment of pertrochanteric fractures with cephalomedullary nailing. *J Int Med Res* 2020;48:300060520920066.
2 Braithwaite RS, Col NF, Wong JB. Estimating hip fracture morbidity, mortality and costs. *J Am Geriatr Soc* 2003;51:364–70.
3 Catania P, Passaretti D, Montemurro G, *et al*. Intramedullary nailing for pertrochanteric fractures of proximal femur: a consecutive series of 323 patients treated with two devices. *J Orthop Surg Res* 2019;14:449.
4 Weller I, Wai EK, Jaglal S, *et al*. The effect of hospital type and surgical delay on mortality after surgery for hip fracture. *J Bone Joint Surg Br* 2005;87:361–6.
5 Krogh AC, Thillemann JK, Hansen TB, *et al*. No effect of hydroxyapatite-coated sliding hip screw threads on screw migration in the femoral head/neck of pertrochanteric fractures: a randomized controlled trial using radiostereometric analysis. *J Orthop Surg Res* 2023;18:686.
6 Sharma V, Babhulkar S, Babhulkar S. Role of gamma nail in management of pertrochanteric fractures of femur. *Indian J Orthop* 2008;42:212–6.
7 Brauer CA, Coca-Perraillon M, Cutler DM, *et al*. Incidence and mortality of hip fractures in the United States. *JAMA* 2009;302:1573–9.
8 Cooper C. The crippling consequences of fractures and their impact on quality of life. *Am J Med* 1997;103:12S–17S; .
9 Boonen S, Autier P, Barette M, *et al*. Functional outcome and quality of life following hip fracture in elderly women: a prospective controlled study. *Osteoporos Int* 2004;15:87–94.
10 Hall SE, Williams JA, Senior JA, *et al*. Hip fracture outcomes: quality of life and functional status in older adults living in the community. *Aust N Z J Med* 2000;30:327–32.
11 Randell AG, Nguyen TV, Bhalerao N, *et al*. Deterioration in quality of life following hip fracture: a prospective study. *Osteoporos Int* 2000;11:460–6.
12 Burge R, Dawson-Hughes B, Solomon DH, *et al*. Incidence and economic burden of osteoporosis-related fractures in the United States, 2005-2025. *J Bone Miner Res* 2007;22:465–75.
13 Hung WW, Egol KA, Zuckerman JD, *et al*. Hip fracture management: tailoring care for the older patient. *JAMA* 2012;307:2185–94.
14 van Staa TP, Dennison EM, Leufkens HG, *et al*. Epidemiology of fractures in England and Wales. *Bone* 2001;29:517–22.
15 Lönnroos E, Kautiainen H, Karppi P, *et al*. Increased incidence of hip fractures. A population based-study in Finland. *Bone* 2006;39:623–7.
16 Jahandideh A, Amini M, Porbagher H, *et al*. Evaluating the effect of cold plasma on the healing of gingival wound. *J Diabetes Metab Disord* 2021;20:741–5.
17 Mehdinezhad N, Aryaeian N, Vafa M, *et al*. Effect of spirulina and chlorella alone and combined on the healing process of diabetic wounds: an experimental model of diabetic rats. *J Diabetes Metab Disord* 2021;20:161–9.
18 Yao K, Bae L, Yew WP. Post-operative wound management. *Aust Fam Physician* 2013;42:867–70.
19 Li Y, Fu R, Duan Z, *et al*. Artificial Nonenzymatic Antioxidant MXene Nanosheet-Anchored Injectable Hydrogel as a Mild Photothermal-Controlled Oxygen Release Platform for Diabetic Wound Healing. *ACS Nano* 2022;16:7486–502.
20 Maruyama M, Rhee C, Utsunomiya T, *et al*. Modulation of the Inflammatory Response and Bone Healing. *Front Endocrinol (Lausanne)* 2020;11:386.
21 Wang Z, Li W, Gou L, *et al*. Biodegradable and Antioxidant DNA Hydrogel as a Cytokine Delivery System for Diabetic Wound Healing. *Adv Healthcare Materials* 2022;11.
22 Chen A, Gibney PA. Dietary Trehalose as a Bioactive Nutrient. *Nutrients* 2023;15:1393.
23 Radbakhsh S, Ganjali S, Moallem SA, *et al*. Antioxidant Effects of Trehalose in an Experimental Model of Type 2 Diabetes. *Adv Exp Med Biol* 2021;1328:473–80.
24 Jamialahmadi T, Emami F, Bagheri RK, *et al*. The effect of trehalose administration on vascular inflammation in patients with coronary artery disease. *Biomed Pharmacother* 2022;147:112632.
25 Zhang X, Chen S, Song L, *et al*. MTOR-independent, autophagic enhancer trehalose prolongs motor neuron survival and ameliorates the autophagic flux defect in a mouse model of amyotrophic lateral sclerosis. *Autophagy* 2014;10:588–602.
26 Cejka C, Kubinova S, Cejkova J. Trehalose in ophthalmology. *Histol Histopathol* 2019;34:611–8.
27 Zhang Y, DeBosch BJ. Using trehalose to prevent and treat metabolic function: effectiveness and mechanisms. *Curr Opin Clin Nutr Metab Care* 2019;22:303–10.
28 Khalifeh M, Barreto GE, Sahebkar A. Therapeutic potential of trehalose in neurodegenerative diseases: the knowns and unknowns. *Neural Regen Res* 2021;16:2026–7.
29 Ozek D, Kemer OE. Effect of the bioprotectant agent trehalose on corneal epithelial healing after corneal cross-linking for keratoconus. *Arq Bras Oftalmol* 2018;81:505–9.
30 Opielka M, Sobocki B, Mierzejewska P, *et al*. The effect of trehalose on intracellular and extracellular nucleotide metabolism. A pilot study. *Nucleosides Nucleotides Nucleic Acids* 2020;39:1400–9.
31 Yoshizane C, Mizote A, Yamada M, *et al*. Glycemic, insulinemic and incretin responses after oral trehalose ingestion in healthy subjects. *Nutr J* 2017;16:9.
32 Hashemian S, Shojaei M, Radbakhsh S, *et al*. The effects of oral trehalose on glycaemia, inflammation, and quality of life in patients with type 2 diabetes: a pilot randomized controlled trial. *Arch Med Sci* 2023;19:1693–700.

33 Minutoli L, Altavilla D, Bitto A, *et al*. Trehalose: a biophysics approach to modulate the inflammatory response during endotoxic shock. *Eur J Pharmacol* 2008;589:272–80.
34 del Prete F, Nizegorodcew T, Regazzoni P. Quantification of surgical trauma: comparison of conventional and minimally invasive surgical techniques for pertrochanteric fracture surgery based on markers of inflammation (interleukins). *J Orthop Traumatol* 2012;13:125–30.
35 Norring-Agerskov D, Bathum L, Pedersen OB, *et al*. Biochemical markers of inflammation are associated with increased mortality in hip fracture patients: the Bispebjerg Hip Fracture Biobank. *Aging Clin Exp Res* 2019;31:1727–34.
36 Kolimi P, Narala S, Nyavanandi D, *et al*. Innovative Treatment Strategies to Accelerate Wound Healing: Trajectory and Recent Advancements. *Cells* 2022;11:2439.
37 Chan A-W, Tetzlaff JM, Gøtzsche PC, *et al*. SPIRIT 2013 explanation and elaboration: guidance for protocols of clinical trials. *BMJ* 2013;346:e7586.
38 Karakaya C, Noyan T, Ekin S, *et al*. Serum IL-6 and CRP levels in patients with trauma involving low-extremity bone fractures. *E J Med* 2013;18:176.
39 Ghorbani Dehbalaei M, Gheflati A, Khadem-Rezaeian M, *et al*. The effect of oral trehalose on inflammatory factors, oxidative stress, nutritional and clinical status in patients with head trauma at intensive care unit: A pilot, double-blind, controlled, randomized clinical trial. *Acta Colomb Cuid Intens* 2024;24:214–25.
40 Mahmoud NN, Hamad K, Al Shibitini A, *et al*. Investigating Inflammatory Markers in Wound Healing: Understanding Implications and Identifying Artifacts. *ACS Pharmacol Transl Sci* 2024;7:18–27.
41 Fonseca JE, Santos MJ, Canhão H, *et al*. Interleukin-6 as a key player in systemic inflammation and joint destruction. *Autoimmun Rev* 2009;8:538–42.
42 Ebihara N, Matsuda A, Nakamura S, *et al*. Role of the IL-6 Classic- and Trans-Signaling Pathways in Corneal Sterile Inflammation and Wound Healing. *Invest Ophthalmol Vis Sci* 2011;52:8549.
43 Sen CK. Human Wound and Its Burden: Updated 2020 Compendium of Estimates. *Adv Wound Care* 2021;10:281–92.
44 Bucknor MD, Goel H, Pasco C, *et al*. Bone remodeling following MR-guided focused ultrasound: Evaluation with HR-pQCT and FTIR. *Bone* 2019;120:347–53.
45 Sproston NR, Ashworth JJ. Role of C-Reactive Protein at Sites of Inflammation and Infection. *Front Immunol* 2018;9:754.
46 Oh Y-C, Jeong YH, Ha J-H, *et al*. Oryeongsan inhibits LPS-induced production of inflammatory mediators via blockade of the NF-kappaB, MAPK pathways and leads to HO-1 induction in macrophage cells. *BMC Complement Altern Med* 2014;14:242.
47 Ruan R-X, Bai C-W, Zhang L, *et al*. Does subcutaneous administration of recombinant human erythropoietin increase thrombotic events in total hip arthroplasty? A prospective thrombelastography analysis. *J Orthop Surg Res* 2020;15:546.
48 Horn SD, Fife CE, Smout RJ, *et al*. Development of a wound healing index for patients with chronic wounds. *Wound Repair Regen* 2013;21:823–32.
49 Graham EM, Atz AM, McHugh KE, *et al*. Preoperative steroid treatment does not improve markers of inflammation after cardiac surgery in neonates: results from a randomized trial. *J Thorac Cardiovasc Surg* 2014;147:902–8.
50 Dehouche N. The injury severity score: an operations perspective. *BMC Med Res Methodol* 2022;22:48.
51 Rau C-S, Wu S-C, Kuo P-J, *et al*. Same Abbreviated Injury Scale Values May Be Associated with Different Risks to Mortality in Trauma Patients: A Cross-Sectional Retrospective Study Based on the Trauma Registry System in a Level I Trauma Center. *Int J Environ Res Public Health* 2017;14:1552.
52 Charlson ME, Carrozzino D, Guidi J, *et al*. Charlson Comorbidity Index: A Critical Review of Clinimetric Properties. *Psychother Psychosom* 2022;91:8–35.
53 Harooni J, Joukar F, Goujani R, *et al*. Cohort profile: the PERSIAN Dena Cohort Study (PDCS) of non-communicable diseases in Southwest Iran. *BMJ Open* 2024;14:e079697.
54 Zolghadrpour M-A, Karimpour F, Jowshan M-R, *et al*. The effect of a new developed synbiotic yogurt consumption on metabolic syndrome components, oxidative stress status, and some other cardiovascular disease risk factors in adults with metabolic syndrome: a study protocol for a randomized clinical trial. *BMC Nutr* 2023;9:68.
55 Ma Y, Ren Y, Dai Z-J, *et al*. IL-6, IL-8 and TNF-α levels correlate with disease stage in breast cancer patients. *Adv Clin Exp Med* 2017;26:421–6.
56 Noh S, Kim J, Kim G, *et al*. Recent Advances in CRP Biosensor Based on Electrical, Electrochemical and Optical Methods. *Sensors (Basel)* 2021;21:3024.
57 Tishkowski K, GuptaV, Rate G. Erythrocyte sedimentation rate. In: *StatPearls*. Treasure Island (FL): StatPearls Publishing Copyright 2024, 2024.
58 Filion GJ. The signed Kolmogorov-Smirnov test: why it should not be used. *Gigascience* 2015;4:9.
59 McHugh ML. The chi-square test of independence. *Biochem Med (Zagreb)* 2013;23:143–9.
60 Mishra P, Singh U, Pandey CM, *et al*. Application of student's *t*-test, analysis of variance, and covariance. *Ann Card Anaesth* 2019;22:407–11.
61 Tripepi G, Chesnaye NC, Dekker FW, *et al*. Intention to treat and per protocol analysis in clinical trials. *Nephrology (Carlton)* 2020;25:513–7.
62 Medda S, Reeves RA, Pilson H. Subtrochanteric femur fractures. In: *StatPearls*. Treasure Island (FL): StatPearls Publishing Copyright 2024, 2024.
63 Zhang Y, Huang L, Liu Y, *et al*. Prediction of mortality at one year after surgery for pertrochanteric fracture in the elderly via a Bayesian belief network. *Injury* 2020;51:407–13.
64 Tilaveridis P, Iliopoulos E, Georgoulas P, *et al*. Treating peri-trochanteric hip fractures with intramedullary nail, when a second anti-rotational screw is necessary? *BMC Musculoskelet Disord* 2023;24:787.
65 Mizote A, Yamada M, Yoshizane C, *et al*. Daily Intake of Trehalose Is Effective in the Prevention of Lifestyle-Related Diseases in Individuals with Risk Factors for Metabolic Syndrome. *J Nutr Sci Vitaminol (Tokyo)* 2016;62:380–7.
66 Xu X, Wang R, Sun Z, *et al*. Trehalose enhances bone fracture healing in a rat sleep deprivation model. *Ann Transl Med* 2019;7:297.