JBMR Plus, 2025, **9**, ziaf031
https://doi.org/10.1093/jbmrpl/ziaf031
Advance access publication: February 17, 2025
Research Article

Prevention of glucocorticoid-induced impairment of bone metabolism—a randomized, placebo-controlled, single centre proof-of-concept clinical trial

Giulia Gregori[1], Lisa Johansson[1,2], Lena Silberberg[1], Henrik Imberg[3,4], Per Magnusson[5], Marcus Lind[4,6,7], Mattias Lorentzon[1,8,*]

[1]Department of Internal Medicine and Clinical Nutrition, Sahlgrenska Osteoporosis Centre, Institute of Medicine, University of Gothenburg, SE-413 45 Gothenburg, Sweden
[2]Department of Orthopedics, Sahlgrenska University Hospital, SE-431 80 Mölndal, Sweden
[3]Statistiska Konsultgruppen, SE-414 63 Gothenburg, Sweden
[4]Department of Molecular and Clinical Medicine, University of Gothenburg, SE-413 45 Gothenburg, Sweden
[5]Department of Clinical Chemistry and Department of Biomedical and Clinical Sciences, Linköping University, SE-581 83 Linköping, Sweden
[6]Department of Medicine, Sahlgrenska University Hospital, SE-413 45 Gothenburg, Sweden
[7]Department of Medicine, NU-Hospital Group, SE-451 53 Uddevalla, Sweden
[8]Geriatric Medicine, Sahlgrenska University Hospital, SE-431 80 Mölndal, Sweden

*Corresponding author: Mattias Lorentzon, Geriatric Medicine, Institute of Medicine, Sahlgrenska Academy, Sahlgrenska University Hospital, Building K, 6th Floor, SE-431 80 Mölndal, Sweden (mattias.lorentzon@medic.gu.se)

Abstract

Oral glucocorticoid (GC) therapy rapidly and deleteriously affects bone metabolism and blood glucose regulation. The gut microbiota regulates bone metabolism and a prior study found that *Limosilactobacillus reuteri* ATCC PTA6475 (*L. reuteri*) reduced bone loss over 12 mo in older women. Mice treated either with broad-spectrum antibiotics or with *L. reuteri* did not experience GC-induced trabecular bone loss. This proof-of-concept, randomized, double-blind, placebo-controlled trial aimed to investigate if daily supplementation with *L. reuteri*, compared with placebo, could mitigate or prevent the negative effects of oral GC on bone turnover and blood glucose regulation in healthy young adults. Twenty-one men and 29 women, aged 18-45, were randomized to either placebo or *L. reuteri* (1×10^{10} CFU/d) treatment for 2 wk, followed by open-label oral prednisolone 25 mg daily for 7 d. Primary outcomes were changes in blood bone status indices (osteocalcin, C-terminal telopeptide cross-links of collagen type-I (CTX), and type-I procollagen intact N-terminal propeptide [PINP]) from baseline to 7 d after starting oral GC. Secondary endpoints included changes in blood glucose levels using continuous glucose monitoring during the same period (ClinicalTrials.gov NCT04767711). Blood samples were collected from participants in the morning after overnight fasting. Forty-six participants completed the 30-d study. The *L. reuteri* and placebo groups were well balanced in terms of baseline characteristics (age, BMI, sex, dietary intake, and physical activity). No significant differences were found between *L. reuteri* vs placebo for percent changes in CTX (−0.3 [95%CI −19.2-18.7], $p = .98$) or PINP (4.2 [−6.3-14.8], $p = .43$), or in osteocalcin levels (14.2 [−7.8-36.3], $p = .21$), although the group-to-group difference in osteocalcin was larger. There was no effect of treatment on mean blood glucose (−0.1 [−0.3-0.1] mmol/L, $p = .28$). In conclusion, we failed to detect a significant effect of *L. reuteri* supplementation on GC-related adverse effects on bone status indices in this proof-of-concept RCT. Larger studies are needed to identify any potential smaller effects.

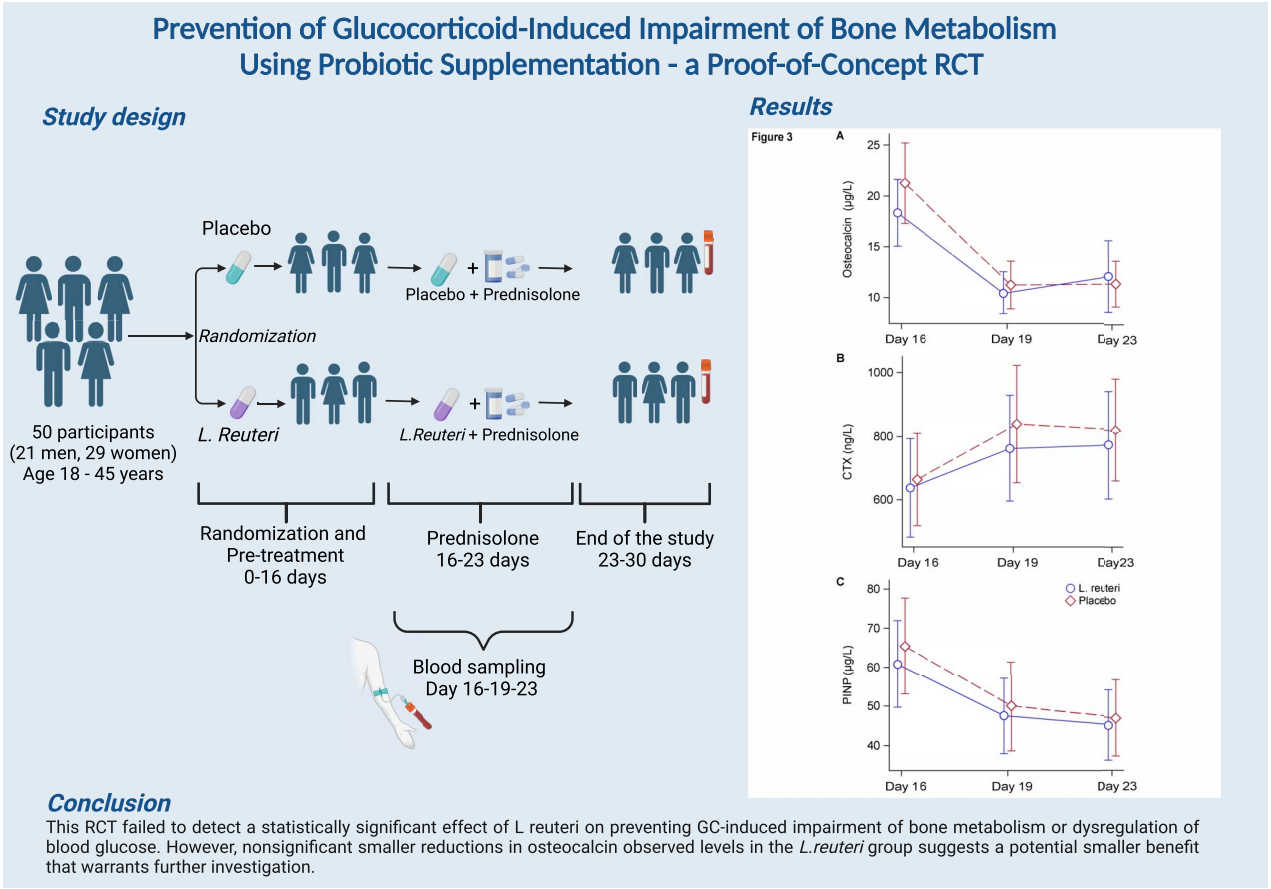
Keywords: oral glucocorticoid treatment, bone status indices, probiotic, gut microbiota, blood glucose

Lay Summary

Oral glucocorticoid (GC) therapy, commonly used for various medical conditions, can quickly and negatively affect bone health and blood sugar levels. The gut microbiota plays a significant role in bone metabolism. Previous research showed that a specific probiotic, *L. reuteri* ATCC PTA 6475 (*L. reuteri*), reduced bone loss in older women over a year. This study aimed to investigate if *L. reuteri* daily intake could prevent the negative effects of oral GC on bone metabolism and blood glucose levels in healthy young adults. In this trial, 50 participants were given placebo or *L. reuteri* for 2 wk, followed by a week of oral GC (prednisolone). Primary and secondary outcomes were changes in bone status indices and blood glucose levels before and after GC treatment. Results showed no significant differences between the *L. reuteri* and placebo groups in bone status indices or blood glucose levels. Thus, *L. reuteri* did not prevent the adverse effects of GC on bone turnover or blood glucose. Further studies are needed to explore any potential smaller effects of *L. reuteri* on these health aspects.

Received: November 25, 2024. **Revised:** February 5, 2025. **Accepted:** February 6, 2025

Graphical Abstract



Introduction

Glucocorticoid (GC) therapy is extensively used in clinical practice to treat inflammatory conditions such as rheumatoid arthritis, inflammatory bowel disease, bronchial asthma, allergies, ankylosing spondylitis, and some forms of cancers.[1,2] Despite its recognized adverse effects, GC treatment remains prevalent, with approximately over 1.2% of the US population on long-term GC therapy.[3] Oral GC administration induces rapid and profound effects on bone metabolism, characterized by increased osteoblast apoptosis and prolonged osteoclast survival, leading to enhanced bone resorption, resulting in bone loss, especially of the trabecular bone, and a subsequent increased fracture risk.[4] Oral GC use increases the risk of nonvertebral, vertebral and hip fracture, in a dose-dependent fashion,[5] and fracture may occur in 30%-50% of patients on chronic GC therapy.[6,7] Vertebral fractures related to GC therapy often remain asymptomatic. X-ray–based morphometric assessments reveal that over one-third of postmenopausal women undergoing chronic oral GC therapy (exceeding 6 mo) have experienced at least 1 vertebral fracture.[7] Additionally, prolonged oral GC use increases the risk of other severe complications such as myopathy with reduced muscle strength and function[8] and may also increase the risk of infections.[9] Furthermore, oral GC treatment increases the risk of steroid induced hyperglycemia in patients with preexisting diabetes and of steroid-induced diabetes in those without.[10] Within days of high-dose (60 mg) oral GC administration, glucose tolerance decreases and bone turnover shifts toward decreased bone formation and increased resorption.[11] Bone formation and bone resorption can be estimated by measuring serum bone status indices.[12,13] Osteocalcin and type I procollagen intact N-terminal propeptide (PINP) reflect bone formation by osteoblasts, and C-terminal telopeptide cross-links of collagen type I (CTX) reflects osteoclastic bone resorption.

Emerging evidence implicates the gut microbiota in the regulation of bone metabolism.[14,15] A placebo-controlled randomized clinical trial (RCT) has shown that *L. reuteri* 6475 (*L. reuteri)* reduced bone loss over 12 mo by half in older women,[16] a finding that could not be verified in another RCT investigating younger, early postmenopausal women.[17] Recently, an experimental study on mice revealed that gut microbiota modulation, either through broad-spectrum antibiotics or *L. reuteri* administration, diminished or abolished the GC-induced bone loss in the spine and femur.[18] Furthermore, GC was found to induce intestinal barrier breaches, an effect that could also be prevented with *L. reuteri* supplementation.[18] Thus, findings from both experimental studies in mice and a clinical trial in older women suggest that *L. reuteri* could be investigated as a means to protect against bone loss due to a variety of mechanisms.

This RCT was performed with the aim to determine if daily supplementation with *L. reuteri* compared with placebo was able to mitigate or prevent the negative effects of oral GC on bone turnover and on blood glucose regulation in healthy young adult men and women.

Materials and methods

Study design

This double-blind, randomized, placebo-controlled, single-center clinical trial was performed in the greater Gothenburg area (southwest Sweden) between May and December 2022. The study protocol including study procedures, inclusion and exclusion criteria, and predefined outcomes were registered at ClinicalTrials.gov prior to study start (number NCT04767711). The study was approved by the Swedish Ethical Review Authority (Dnr 2021-00786) and the Swedish Medical Products Agency (Eudra-CT number 2021-000275-36).

Participants

Participants between 18 and 45 yr old were asked to participate by letter ($n = 22\,706$). In total, 222 (1%) men and women who contacted the clinic underwent a phone screening process out of which 149 did not meet inclusion criteria and 10 declined to participate. Out of the 63 assessed for eligibility and invited to a first screening visit, 11 did not meet inclusion criteria and 2 withdrew consent, resulting in 50 healthy men ($n = 21$) and women ($n = 29$), who met all inclusion criteria, had no exclusion criteria, and consented to participate (Figure 1). Inclusion criteria were willingness to participate and to adhere to the protocol (study protocol in the Supplement), availability throughout the study period, healthy men and women 18-45 yr old, and having received a vaccination for Covid-19. Exclusion criteria were history of diabetes or glucose intolerance defined as an abnormal oral glucose tolerance test (OGTT), condition of obesity (BMI >30 kg/m^2), history of adrenal disease or impairment, previous (within the last 5 yr) or current use of antiresorptive therapy (systemic hormone therapy (estrogen), bisphosphonates, strontium ranelate or denosumab), participation in other clinical trials, current antibiotics treatment or within the last 2 mo prior to inclusion, current and within the past 2 mo use of probiotic supplement, untreated hyperthyroidism or hyperthyroidism within the last 5 yr, known untreated hyperparathyroidism, rheumatoid arthritis, diagnosed with disease causing secondary osteoporosis (chronic obstructive pulmonary disease, inflammatory bowel disease, celiac disease, or diabetes mellitus), or any systemic disease that could affect bone loss, as judged by the investigator, recently diagnosed malignancy (within the last 5 yr), systemic skeletal disease (eg, Paget's disease and osteogenesis imperfecta), oral corticosteroid use, history of peptic ulcer, diagnosed osteoporosis, current smoking or other use of nicotine containing products, pregnancy, history of any psychiatric disorder (psychosis, depression, anxiety disorder, or bipolar disorder).

Randomization and masking

Eligible participants were included and randomized (double-blinded) to either placebo or *L. reuteri* treatment for 2 wk and then in addition received open-label oral prednisolone 25 mg daily for 7 d. The study product consisted of capsules of freeze-dried *L. reuteri* (BioGaia AB) with the dose 5×10^9 CFU and 200 IU of cholecalciferol mixed with maltodextrin powder, taken twice daily, yielding a total daily dose of 1×10^{10} CFU/d, or placebo that contained 200 IU of cholecalciferol and maltodextrin powder only.

Randomization was carried out by the supplier of *L. reuteri* (BioGaiaAB) and was generated using a web-based randomization tool (http://www.randomization.com). The investigators had no access to the randomization code and remained blinded until study end and database lock.

Procedures

After randomization, study participants consumed either *L. reuteri* or placebo for 30 d (27 d minimum). Study subjects were asked to refrain from physical exercise, other than walking, from day 14 until day 23, and keep their normal diet during the study period. After the 14-d run-in period (11 d minimum), all participants started to take 25 mg of oral prednisolone every morning for 7 d. At the 14-d visit, a 24-h glucose monitoring for the following 10 d was performed. At the next visit on day 16, fasting morning blood samples and feces samples were collected, and after on the same visit, participants started their oral GC treatment. At the 19-, 23- and 30-d visits, blood and feces samples were collected in the morning prior to taking the GC dose (Figure 2).

Questionnaires

Standardized questionnaires were administered to collect information regarding lifestyle habits, medical and drug history, risk factors for osteoporosis and fracture, dietary intake (Food Frequency Questionnaire [FFQ]),[19] exercise and physical activity habits (IPAQ),[20] and gastrointestinal symptoms (GSRS).[21]

Blood biochemistry

Fasting blood samples were collected in the morning, serum and plasma extracted and stored at -80 °C until further analyses. Samples from the baseline visit (day 16) were collected prior to oral GC treatment start. Fasting blood glucose was analyzed using fresh blood at screening visit (with the addition of glycated hemoglobin A1c [HbA1c]) and at 16-, 19-, 23-, and 30-d at the Department of Clinical Chemistry, Sahlgrenska University Hospital. Analyses of osteocalcin, PINP and CTX were performed at the Department of Clinical Chemistry, Linköping University Hospital. Serum osteocalcin was measured with the N-Mid Osteocalcin ELISA (Immunodiagnostic Systems Holdings Ltd.), which detects intact osteocalcin (amino acids 1-49) and the N-terminal-Mid osteocalcin fragment (amino acids 1-43), with an assay performance of analytical range 0.5-100 μg/L, intra-assay CV of <4%, and inter-assay CV of <7%. Serum PINP was measured with the UniQ radioimmunoassay (Aidian Oy), with an assay performance of analytical range 5-250 μg/L, intra-assay CV of <5%, and inter-assay CV of <6%. CTX was assessed in EDTA plasma samples by the CrossLaps ELISA (Immunodiagnostic Systems Holdings Ltd.), with an assay performance of analytical range 20-3380 ng/L, intra-assay CV of <6%, and inter-assay CV of <10%.

Oral glucose tolerance test

Fasting morning blood glucose was measured at the screening visit, followed by ingestion of 24 cl of glucose syrup, containing 75 g of sugar. Blood glucose measurement was performed after 2 h. Inclusion in the study depended on having a normal fasting blood glucose (≤ 6 mmol/L) and OGTT (<7.8 mmol/L).

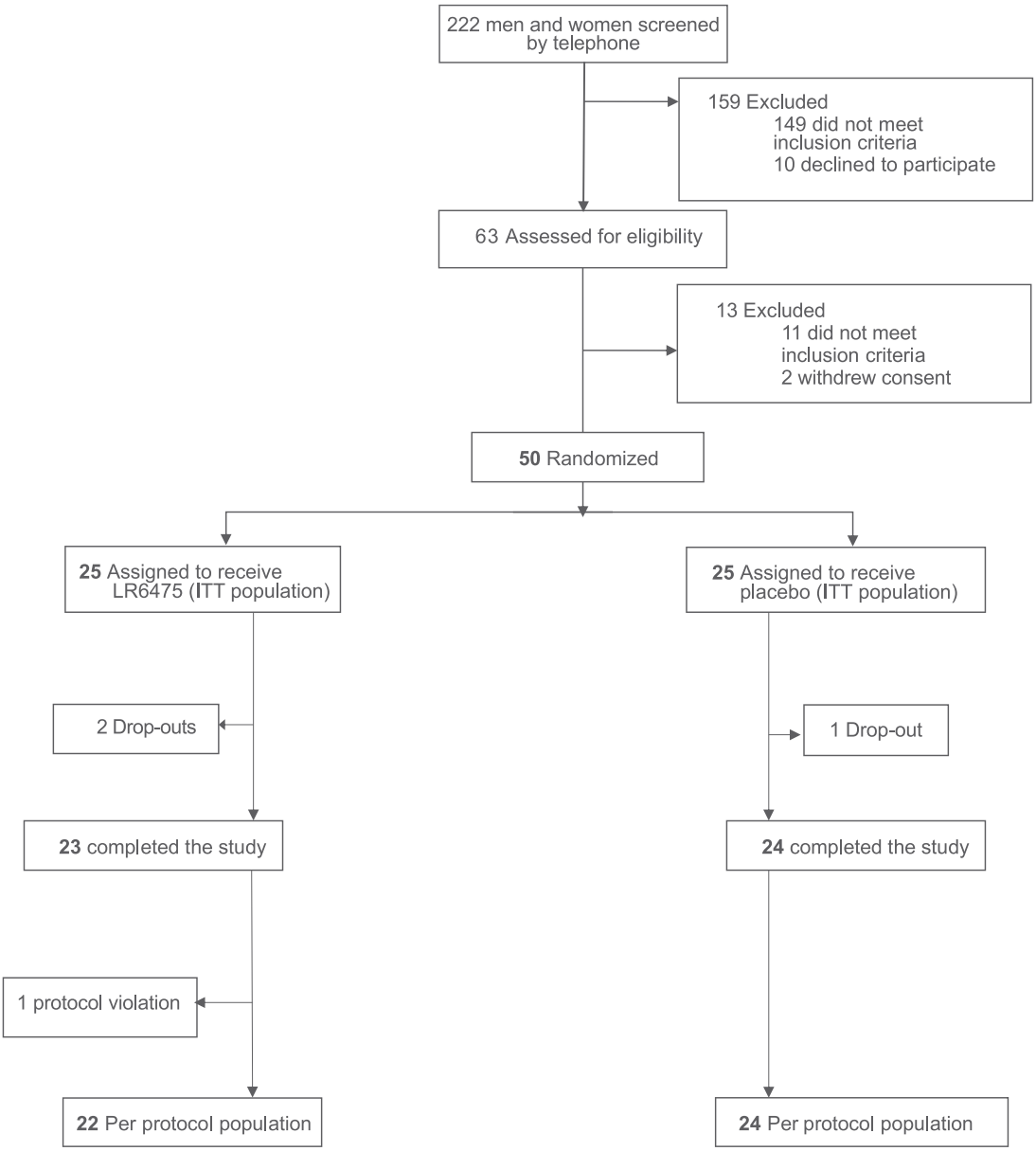


Figure 1. Flowchart of the study cohort, showing the number of persons screened, eligible, randomized, and analyzed, according to the CONSORT guidelines.

Continuous glucose monitoring

Continuous glucose monitoring (CGM) using FreeStyle Libre Pro iQ (Abbott Diabetes Care Ltd.) started at day 14, allowing a 48-h observation period prior to commencement of oral prednisolone dosing. CGM was used from day 14 until ending prednisolone treatment on day 23.

Primary and secondary outcomes

The primary outcome was the percent change in serum bone status indices osteocalcin, CTX, and PINP after 7 d of treatment with prednisolone, with *L. reuteri* compared with placebo. Secondary outcomes were changes in the following CGM endpoints after 7 d of treatment with prednisolone: mean glucose, SD of glucose values, time in range (TIR; 3.9-10 mmol/L [70-180 mg/dL]), time in tight range (TITR; 3.5-7.8 mmol/L [63-141 mg/dL]), time above range (TAR; >10 mmol/L [>180 mg/dL]), time above tight range (TATR; >7.8 mmol/L [>141 mg/dL]), and TAR level 2 (>13.9 mmol/L [>250 mg/dL]) with *L. reuteri* compared with placebo. Exploratory CGM endpoints included time in hypoglycemia (<3.0 mmol/L [<54 mg/dL]), time below range (<3.9 mmol/L [<70 mg/dL]), coefficient of variation (CV = SD/mean) of glucose values, and mean amplitude of glycemic excursions (MAGE). Fasting blood glucose was evaluated after 3, 7, and 14 d of treatment with prednisolone. Additional secondary endpoints listed in the study protocol included serum markers of intestinal permeability (endotoxin levels), serum and feces markers of intestinal inflammation (lipocalin-2 and calprotectin), and gut microbiota composition, were not analyzed or evaluated.

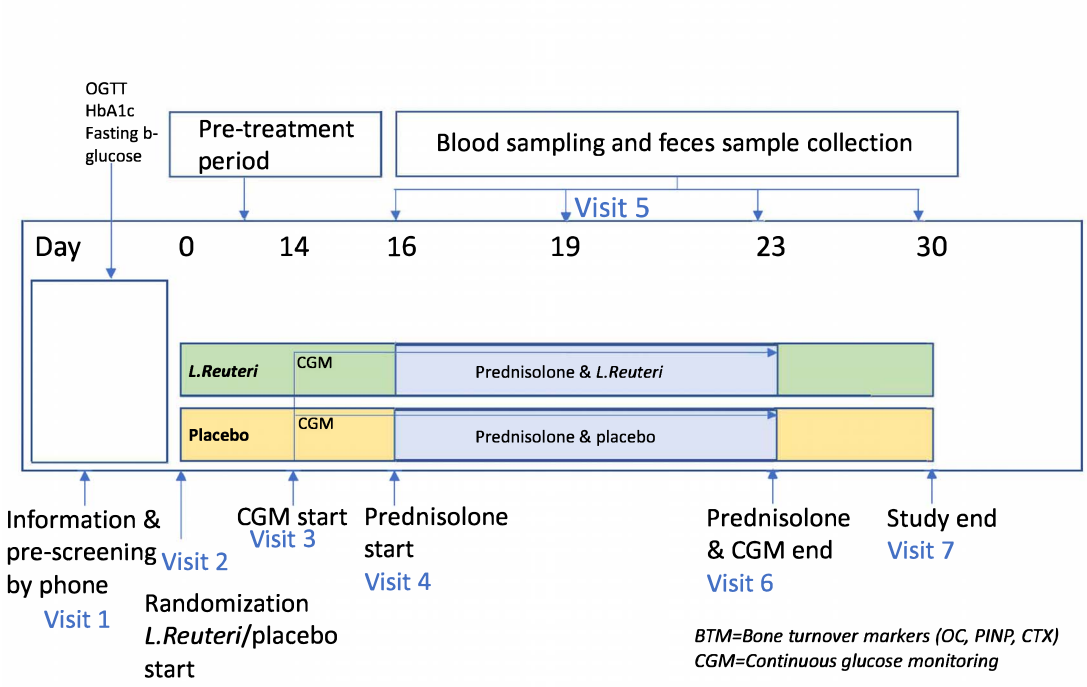


Figure 2. Timeline of the study procedures, including screening, randomization (day 0), pre-treatment period with *L. reuteri* or placebo (day 0-16), treatment prednisolone (day 16-23), and study end (day 30). Abbreviation: *L. reuteri*, *Limosilactobacillus reuteri* ATCC PTA 6475.

Statistical analyses

Descriptive data are presented using the mean and SD or median and IQR for numeric variables. Categorical variables are presented in numbers and percentages.

Statistical analyses were performed according to intention-to-treat (ITT), including all randomized subjects. Comparisons between groups were performed using analysis of covariance (ANCOVA), adjusting for baseline values. Robust standard errors (HC3 method) were employed for non-normally distributed variables for osteocalcin, CTX, PINP, and CGM endpoints TATR, TAR, and TAR level 2. Missing data were handled using multiple imputation by chained equations, using regression imputation with randomization group and measurements at preceding visits as auxiliary variables. Log-normally distributed data (osteocalcin, CTX, and PINP) were log-transformed prior to imputation. Times in glycemic ranges were imputed within the feasible range 0%-100%. This study was designed to detect group-to-group differences of at least 21% with >90% statistical power, assuming an SD of 17% and a drop-out rate of 8% with a minimum of 42 study participants completing the study.

Sensitivity analyses without imputation of missing data were performed using repeated measures ANOVA and ANCOVA. An unstructured covariance matrix for the correlation between repeated measures was used. Sensitivity analyses for modeling assumptions were performed using the Fisher-Pitman nonparametric permutation test for the mean difference. Corresponding CIs were calculated by test inversion. Sensitivity analyses evaluating the treatment effect in men and women and for participants with a high (higher than median) or low (lower than or equal to median) BMI were performed by similar methods as above.

All tests were 2-tailed and conducted at the 5% significance level with corresponding 95% CIs for the treatment effect. The overall type I error rate of the 3 primary endpoints were controlled using a sequential alpha-splitting test procedure. Further details may be found in the Statistical Analysis Plan in the Supplements. The prespecified primary efficacy analysis was the analysis of covariance on log-transformed data (osteocalcin, CTX, and PINP), with treatment effect as fold change between groups. However, for ease of interpretation and consistency with previous publications, results in the manuscript are presented as the mean difference in percentage changes between groups.

All analyses were performed using SAS/STAT Software, Version 9.4 of the SAS System for Windows (SAS Institute Inc.).

Results

A total of 50 healthy men and women with a mean age of 30 yr (SD 8 yr) were randomly assigned to receive placebo or *L. reuteri* (1×10^{10} CFU/d) treatment for 2 wk and then in addition 25 mg of open-label oral prednisolone daily for 7 d. The ITT analysis included 50 participants ($n = 25$ in the *L. reuteri* group and $n = 25$ in the placebo group). Four participants were excluded in the per protocol population (PP), of which 3 did not complete the study and 1 had a protocol violation ($n = 3$ in the *L. reuteri* group and $n = 1$ in the placebo group; Figure 1).

At randomization, the groups were overall well balanced in terms of anthropometrics, dietary intake, physical activity, gastrointestinal symptoms according to the GSRS, bone status indices (osteocalcin, CTX, PINP), HbA1c level, OGTT, and

Table 1. Demographics and baseline characteristics of the ITT population.

Characteristic	*L. reuteri* (*n* = 25)	Placebo (*n* = 25)
Age (yr)	30.4 (8.6)	29.4 (7.3)
Female sex	14 (56%)	15 (60%)
Height (cm)	176 (9.3)	175 (9.2)
Weight (kg)	73.8 (12.5)	71.1 (12.8)
Body mass index (kg/m^2)	23.7 (2.7)	23.1 (2.9)
Time from screening to randomization (d)	6.2 (5.5)	5.0 (4.1)
GSRS total score	1.25 (0.29)	1.37 (0.33)
Energy intake (kcal/d)	1530 (370)	1490 (370)
Protein intake (g/d)	63.7 (14.1)	59.1 (17.9)
Fat intake (g/d)	58.8 (16.7)	57.6 (18.7)
Carbohydrates intake (g/d)	168 (50.4)	160 (41.7)
Fiber intake (g/d)	17.7 (7.6)	19.6 (8.5)
Salt intake (g/d)	5.3 (1.3)	5.0 (1.6)
Calcium intake (mg/d)	919 (300)	867 (260)
Physical activity (METs/wk)	2066 (996-3760)	1775 (1345-3123)
Osteocalcin (μg/L)	18.3 (8.1)	21.3 (9.6)
CTX (ng/L)	639 (386)	665 (348)
PINP (μg/L)	60.8 (27.6)	65.4 (29.9)
HbA1c (mmol/mol)	31.3 (2.9)	31.5 (2.7)[a]
Oral glucose tolerance test, 2-h glucose value (mmol/L)	4.9 (1.2)	5.1 (1.1)[a]
Fasting blood glucose (mmol/L)	5.1 (0.4)	5.2 (0.4)[a]

Continuous variables are presented as mean (SD) or median (IQR). Categorical variables are presented as numbers and percentage. Abbreviations: GSRS, gastrointestinal symptom rating scale; HbA1c, glycated hemoglobin A1c; ITT, intention-to-treat; *L. reuteri*, *Limosilactobacillus reuteri* ATCC PTA 6475; MET, metabolic equivalent of task; PINP, type I procollagen intact N-terminal propeptid. [a] *n* = 24.

fasting blood glucose (Table 1). Demographics and baseline characteristics of the PP population are presented in Table S1 and Table S2 in the Supplement.

There were significant changes in osteocalcin, CTX, and PINP between baseline, day 3 and day 7 in both the *L. reuteri* and placebo groups (Table S3). There were no significant differences between *L. reuteri* and placebo in the ITT population with regards to primary or secondary outcomes. From baseline to day 7, osteocalcin decreased by 29.2 % in the *L. reuteri* group (p = .02) and by 45.7% (p < .001) in the placebo group. After 7 d of treatment with prednisolone, the adjusted mean difference between *L. reuteri* treatment and placebo in percentage change of bone status indices was 14.2 (95% CI −7.8 to 36.3, p = .21) for osteocalcin, −0.3 (−19.2 to 18.7, p = .98) for CTX, and 4.2 (−6.3 to 14.8, p = .43) for PINP (Table 2, Figure 3; descriptive data by visit in Table S2 in the supplement). Similarly, there was no difference in mean glucose levels measured by CGM, with a mean difference of −0.1 mmol/L (95% CI −0.3 to 0.1, p = .28). Other secondary outcomes, including TITR, TIR, TAR, TAR level 2, and SD of glucose values, also showed no effect of treatment with *L. reuteri* compared with placebo (Table 2).

In the PP population, similar results were found. For the primary endpoints, the difference in percent change of osteocalcin was 15.2 (95% CI −9.2 to 39.6, p = .22), for CTX 1.4 (−15.4 to 18.1, p = .87), and for PINP 4.4 (−6.7 to 15.4, p = .43). No significant differences between groups were seen for secondary endpoints (Table S4 and Table S5 in the supplement).

Sensitivity analyses using repeated measures modeling techniques (ANOVA and ANCOVA), nonparametric permutation tests, and analyses stratified by sex and BMI, revealed similar results, with no significant differences between treatment groups (Table S6 and Table S7 in the supplement).

During the study duration, both groups had an equal proportion of participants experiencing adverse events. No serious adverse events or discontinuation due to adverse events were reported in either group (Table 3).

Discussion

The present proof-of-concept RCT aimed to evaluate the efficacy of *L. reuteri* supplementation in mitigating or preventing GC-induced impairment of bone metabolism and dysregulation of blood glucose in healthy adults. Despite the rigorous study design and execution with a low drop-out rate and high compliance, the results did not demonstrate a statistically significant *L. reuteri* treatment effect on the primary and secondary outcomes after 7 d of prednisolone administration in the ITT and per-protocol-populations.

The primary outcomes, represented by the percentage change in bone status indices osteocalcin, CTX, and PINP, showed no significant differences between the *L. reuteri* and placebo groups. Specifically, the adjusted mean difference in percentage change was 14.2% for osteocalcin, −0.3% for CTX, and 4.2% for PINP. Although these results were not statistically significant, the nonsignificant but larger difference observed for osteocalcin levels suggests a potential but smaller positive effect of *L. reuteri* on osteocalcin regulation, a bone formation marker and an indicator of energy metabolism. In contrast to PINP and CTX, osteocalcin (undercarboxylated) has been found to upregulate insulin production in the pancreas and enhance insulin sensitivity in skeletal muscle and adipose tissue in mice,[22] although these effects are no fully elucidated in humans.[23] Although the increase in mean blood glucose was numerically greater in the placebo than the *L. reuteri* group, there was no significant difference.

The mechanisms of action for the *L. reuteri* effect on bone metabolism has not been fully elucidated. In a murine model, μCT demonstrated that *L. reuteri* treatment increased male trabecular bone parameters, including BMD, bone volume

Table 2. Primary, secondary, and exploratory efficacy analyses: the effect of *L. reuteri* on bone status indices and glucose values after 7 d of treatment with prednisolone on the ITT population.

Endpoint	*L. reuteri* (*n* = 25)	Placebo (*n* = 25)	Adjusted mean diff. (95% CI)	*p*-value
Primary endpoints, change from BL to 1 wk				
Osteocalcin (% change)	−29.2 (56.2)	−45.7 (12.3)	14.2 (−7.8, 36.3)	.21
CTX (% change)	28.8 (34.0)	28.3 (26.6)	−0.3 (−19.2, 18.7)	.98
PINP (% change)	−24.5 (22.2)	−28.8 (12.3)	4.2 (−6.3, 14.8)	.43
Secondary endpoints, change from BL to 1 wk				
Mean glucose (mmol/L)	0.6 (0.4)	0.8 (0.4)	−0.1 (−0.3, 0.1)	.28
SD of glucose values (mmol/L)	0.4 (0.2)	0.5 (0.2)	−0.1 (−0.2, 0.1)	.40
Time in tight range (% 3.5-7.8 mmol/L)	−8.1 (8.3)	−9.9 (9.0)	1.6 (−3.9, 7.1)	.57
Time above tight range (% >7.8 mmol/L)	10.0 (6.1)	11.6 (7.9)	−1.9 (−6.3, 2.4)	.38
Time in range (% 3.9-10 mmol/L)	1.9 (8.0)	0.4 (6.5)	1.2 (−0.8, 3.4)	.23
Time above range (% >10 mmol/L)	1.4 (1.7)	2.5 (4.2)	−0.6 (−2.0, 0.9)	.46
Time above range level 2 (% >13.9 mmol/L)	0.0 (0.1)	0.0 (0.1)	−0.0 (−0.0, 0.0)	.16
Exploratory endpoints, change from BL to 1 wk				
Time in hypoglycemia (% <3.0 mmol/L)	0.3 (0.9)	−0.4 (1.1)	0.0 (−0.1, 0.1)	.44
Time below range (% <3.9 mmol/L)	3.2 (8.1)	−2.7 (4.0)	−0.1 (−0.8, 0.7)	.88
CV of glucose values (%)	5.6 (3.6)	6.1 (4.2)	−0.7 (−2.9, 1.5)	.51
Mean amplitude of glycemic excursions (mmol/L)	1.0 (0.6)	1.2 (0.7)	−0.2 (−0.6, 0.2)	.25
Fasting blood glucose, BL to day 3 (mmol/L)	−0.2 (0.5)	−0.1 (0.3)	−0.0 (−0.2, 0.2)	.86
Fasting blood glucose, BL to 1 wk (mmol/L)	−0.3 (0.3)	−0.1 (0.4)	−0.1 (−0.3, 0.0)	.09
Fasting blood glucose, BL to study end (mmol/L)	−0.1 (0.3)	0.0 (0.4)	−0.1 (−0.3, 0.1)	.35

Descriptive data are presented as means and SDs. Statistical analyses were performed using analysis of covariance (ANCOVA), adjusting for baseline values. Robust standard errors (HC3 method) were employed for non-normally distributed variables osteocalcin, CTX, and P1NP, and CGM endpoints TATR, TAR, and TAR level 2. Abbreviations: BL, baseline; CGM, continuous glucose monitoring; CV, coefficient of variation; *L. reuteri*, *Limosilactobacillus reuteri* ATCC PTA 6475; PINP, type I procollagen intact N-terminal propeptide; TAR, time above range; TATR, time above tight range.

Table 3. Summary of adverse events on the safety population.

	L. reuteri (*n* = 25)	Placebo (*n* = 25)
Any AE, *n* (%)	9 (36)	9 (36)
Any SAE, *n* (%)	0 (0)	0 (0)
Any treatment-related AE, *n* (%)	2 (8)	3 (12)
Any treatment-related SAE, *n* (%)	0 (0)	0 (0)
Any AE leading to discontinuation[a], *n* (%)	0 (0)	0 (0)

Data are presented as numbers and percentages. Abbreviations: AE, adverse event (gastro-intestinal symptoms, dizziness during oral glucose tolerance test, common cold, headache, dry skin under both eyes, upper respiratory tract infection, threw up, flu-like symptoms); *L. reuteri*, *Limosilactobacillus reuteri*; ATCC PTA 6475; SAE, serious adverse event. [a]Discontinuation of study product.

fraction, trabecular number, and trabecular thickness in the distal femur metaphyseal region as well as in the lumbar vertebrae.[24] In contrast, in an ovariectomized (Ovx) mouse model mimicking menopause, *L. reuteri* treatment significantly protected Ovx mice from bone loss. This protection was accompanied by decreased osteoclast bone resorption markers and activators (tartrate-resistant acid phosphatase isoform 5b and RANKL) as well as osteoclastogenesis in *L. reuteri* treated mice. Consistent with this, *L. reuteri* suppressed Ovx-induced increases in bone marrow CD4+ T-lymphocytes (which promote osteoclastogenesis) and directly suppressed osteoclastogenesis in vitro.[14] In vitro studies indicated that this strain has strong tolerance to acid environments, as do many other *L. reuteri* strains and that it has the unusual ability to interfere with TNF-alpha mediated propagation of inflammatory responses in human macrophages.

Prior studies have shown that GC therapy increases osteoblast apoptosis and prolongs osteoclast survival, leading to enhanced bone resorption and significant bone loss, especially in the trabecular bone.[4,25,26] In the study by Schepper et al.[18] the involvement of the gut microbiota and intestinal barrier function in glucocorticoid-induced osteoporosis (GIO) was explored. They demonstrated that GC treatment not only induces osteoblast and osteocytes apoptosis but also causes significant changes in the gut microbiota composition and increases intestinal permeability. Their murine experiment showed that depletion of the microbiota through broad-spectrum antibiotics prevented GC-induced bone loss, indicating the critical role of the gut microbiota in GIO. Furthermore, treatment with *L. reuteri* was effective in preventing bone loss and maintaining intestinal barrier integrity. The study also identified that GC treatment increased the abundance of certain microbial taxa and caused intestinal barrier leaks, which were mitigated by *L. reuteri* and antibiotics. Moreover, bone-specific overexpression of Wnt10b was found to prevent GIO, suggesting that targeting gut microbiota and enhancing intestinal barrier function could serve as novel therapeutic strategies for GIO prevention. These findings highlight the gut-bone axis and propose the gut microbiota as a potential therapeutic target for managing GC-induced bone deterioration.[18]

As expected, oral GC treatment had great and rapid effects on the levels of all measured bone status indices.[11] Although the differences between groups were negligible for CTX and PINP, the magnitude of difference (14.2%) observed for osteocalcin is intriguing and warrants further study. Since the study

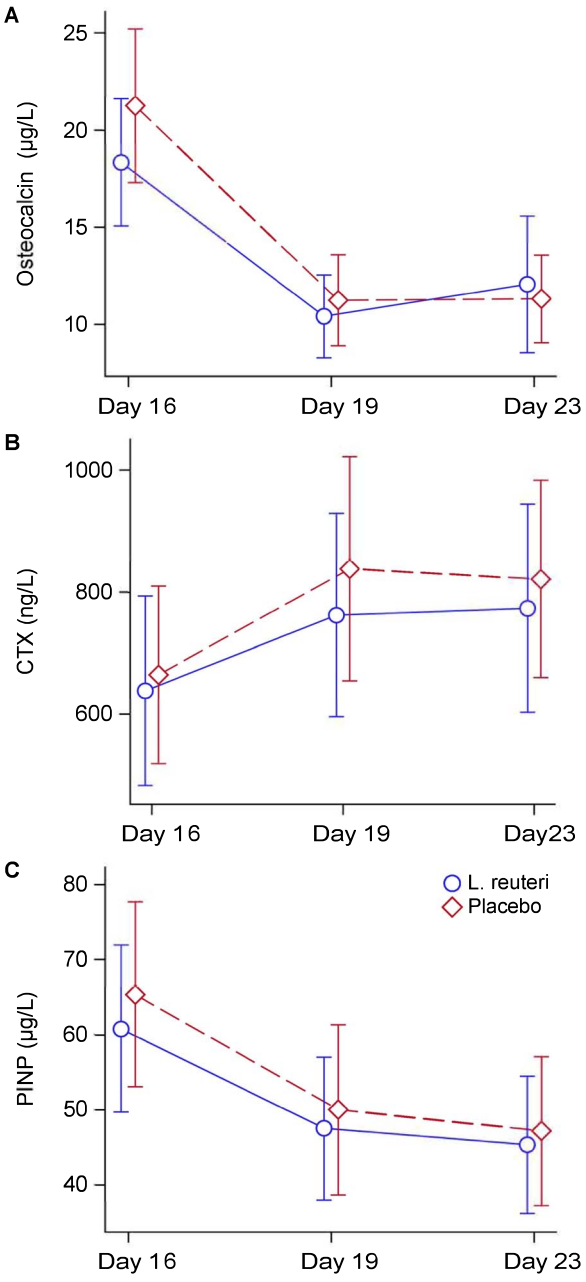


Figure 3. Changes in bone status indices osteocalcin, PINP, and CTX during the study period in persons treated with *L. reuteri* ($n = 25$) and placebo ($n = 25$) on the ITT population. The points and error bars represent means with 95% CIs. Bone status indices and CGM were analyzed prior to prednisolone treatment on day 16 (visit 4), and during prednisolone treatment on day 19 (visit 5) and day 23 (visit 6). Abbreviations: CGM, continuous glucose monitoring; ITT, intention-to-treat; *L. reuteri*, *Limosilactobacillus reuteri* ATCC PTA 6475; PINP, type I procollagen intact N-terminal propeptide.

had 80% power to detect a difference between groups of 21%, it cannot be concluded if the lack of significance for change in osteocalcin was due to a lack of treatment effect or due to insufficient statistical power in the study sample. The anticipated effect of a 21% group-to-group difference is in line with the previously demonstrated size of the effects of the weak antiresorptive raloxifene, which reduced osteocalcin by 20% after 52 wk treatment in postmenopausal women with osteopenia.[27]

Dysbiosis increases with age and has been associated with diseases like obesity, diabetes, and osteoporosis. Probiotic supplements may be superfluous in people with healthy gut microbiota and have the greatest effect in those with dysbiosis. In this study, participants were healthy young adults, with an expected relatively healthy gut microbiota. This could have limited the potential impact of *L. reuteri* supplementation. We recently demonstrated that supplementation with *L. reuteri* vs placebo had no effect on bone loss or bone turnover over 2 yr in an RCT of 239 early postmenopausal women. Interestingly, an interaction between BMI and treatment effect was observed, indicating a treatment effect in those with high BMI, suggesting that *L. reuteri* supplementation might be influenced by the baseline metabolic status, associated with dysbiosis, of the participants. Dysbiosis due to advanced age could also have contributed to the previous RCT demonstrating a reduction of bone loss in older postmenopausal women with *L. reuteri* supplementation compared with placebo.[17]

The study has several limitations that should be acknowledged. The relatively small sample size and short duration of GC administration may have contributed to the lack of significant findings, given that the group-to-group differences did not reach the anticipated 21% target. Additionally, the healthy young adult population may not fully represent those most at risk for GC-induced bone loss, such as older adults or individuals with chronic inflammatory conditions requiring long-term GC therapy. For ethical reasons, older or frail individuals, or those with prediabetes, could not be included due to the risk of adverse events such as fractures and hyperglycemia because of the relatively high oral GC dose given.

Although the groups were well-balanced, indicating successful randomization, several potential sources of bias, such as vitamin D levels and family history of osteoporosis, were not accounted for, which could have influenced the results. The study also has strengths. The utilized RCT design allows testing of causality, and the study was successful in terms of a low drop-out rate and high compliance to the intervention. Furthermore, extensive testing procedures were used, and state-of-the-art glucose measurements undertaken. This proof-of-concept randomized controlled trial (RCT) was the first to assess whether *L. reuteri* could counteract the effects of GCs on bone metabolism. The findings can inform future studies by providing an effect size to support power calculations for larger trials.

In conclusion, this RCT did not demonstrate a statistically significant effect of *L reuteri* on preventing GC-induced impairment of bone metabolism or dysregulation of blood glucose. However, the observed nonsignificant smaller reductions in osteocalcin levels in the *L. reuteri* group suggests a potential smaller benefit that warrants further investigation.

Acknowledgments

We acknowledge the expert assistance of Walaa Bader, Department of Clinical Chemistry, Linköping University Hospital, Sweden.

Author contributions

Giulia Gregori and Lisa Johansson contributed equally.

Giulia Gregori (Data curation, Investigation, Methodology, Project administration, Visualization, Writing—original draft), Lisa Johansson (Data curation, Investigation, Methodology, Supervision, Validation,

Visualization, Writing—review & editing), Lena Silberberg (Data curation, Investigation, Methodology, Project administration, Resources, Software, Writing—review & editing), Henrik Imberg (Formal analysis, Methodology, Software, Visualization, Writing—review & editing), Per Magnusson (Formal analysis, Investigation, Methodology, Writing—review & editing), Marcus Lind (Conceptualization, Investigation, Methodology, Writing—review & editing), and Mattias Lorentzon (Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing—review & editing).

Supplementary material

Supplementary material is available at *JBMR Plus* online.

Funding

The Swedish Research Council (VR), ALF/LUA grants from the Sahlgrenska University Hospital, and BioGaia AB.

Conflicts of interest

L.J. has received lecture fees from UCB Pharma, all outside the submitted work. M.L. has received lecture fees or consulting fees from Astellas, Amgen, UCB Pharma, Medison Pharma, Jansen-Cilag, Viatris, Medac, Gedeon Richter, and Parexel International, all outside the submitted work. All other authors have no conflicts of interest.

Data availability

Data cannot be made publicly available for ethical and legal reasons. Such information is subject to legal restrictions according to national legislation. Specifically, in Sweden confidentiality regarding personal information in studies is regulated in the Public Access to Information and Secrecy Act (SFS 2009:400). The data underlying the results of this study might be made available upon request, after an assessment of confidentiality. There is thus a possibility to apply to get access to certain public documents that an authority holds. In this case, the University of Gothenburg is the specific authority that is responsible for the integrity of the documents with research data. Questions regarding such issues can be directed to the head of the Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden. Contact information can be obtained from medicin@gu.se.

References

1. Reichardt SD, Amouret A, Muzzi C, et al. The role of glucocorticoids in inflammatory diseases. *Cells*. 2021;10(11): 2921. https://doi.org/10.3390/cells10112921
2. Bruscoli S, Febo M, Riccardi C, Migliorati G. Glucocorticoid therapy in inflammatory bowel disease: mechanisms and clinical practice. *Front Immunol*. 2021;12:691480. https://doi.org/10.3389/fimmu.2021.691480
3. Overman RA, Yeh JY, Deal CL. Prevalence of oral glucocorticoid usage in the United States: a general population perspective. *Arthritis Care Res (Hoboken)*. 2013;65(2):294–298. https://doi.org/10.1002/acr.21796
4. Buckley L, Humphrey MB. Glucocorticoid-induced osteoporosis. *N Engl J Med*. 2018;379(26):2547–2556. https://doi.org/10.1056/NEJMcp1800214
5. van Staa TP, Leufkens HG, Abenhaim L, Zhang B, Cooper C. Oral corticosteroids and fracture risk: relationship to daily and cumulative doses. *Rheumatology (Oxford)*. 2000;39(12):1383–1389. https://doi.org/10.1093/rheumatology/39.12.1383
6. Frenkel B, White W, Tuckermann J. Glucocorticoid-induced osteoporosis. In: Wang J-C, Harris C, editors. *Glucocorticoid Signaling: From Molecules to Mice to Man*. Springer New York; 2015. p. 179-215. https://doi.org/10.1007/978-1-4939-2895-8_8
7. Angeli A, Guglielmi G, Dovio A, et al. High prevalence of asymptomatic vertebral fractures in post-menopausal women receiving chronic glucocorticoid therapy: a cross-sectional outpatient study. *Bone*. 2006;39(2):253–259.
8. Pereira RM, Freire de Carvalho J. Glucocorticoid-induced myopathy. *Joint Bone Spine*. 2011;78(1):41–44. https://doi.org/10.1016/j.jbspin.2010.02.025
9. Dixon WG, Suissa S, Hudson M. The association between systemic glucocorticoid therapy and the risk of infection in patients with rheumatoid arthritis: systematic review and meta-analyses. *Arthritis Res Ther*. 2011;13(4). R139 https://doi.org/10.1186/ar3453
10. Roberts A, James J, Dhatariya K, the Joint British Diabetes Societies (JBDS) for Inpatient Care. Management of hyperglycaemia and steroid (glucocorticoid) therapy: a guideline from the Joint British Diabetes Societies (JBDS) for inpatient care group. *Diabet Med*. 2018;35(8):1011–1017. https://doi.org/10.1111/dme.13675
11. Kauh E, Mixson L, Malice MP, et al. Prednisone affects inflammation, glucose tolerance, and bone turnover within hours of treatment in healthy individuals. *Eur J Endocrinol*. 2012;166(3): 459–467. https://doi.org/10.1530/EJE-11-0751
12. Lombardi G, Jørgensen NR, Harvey NC, et al. Guidelines for the correct use of the nomenclature of biochemical indices of bone status: a position statement of the Joint IOF Working Group and IFCC Committee on Bone Metabolism. *Clin Chem Lab Med*. 2024;63(4):704–711. https://doi.org/10.1515/cclm-2024-1148
13. Lorentzon M, Branco J, Brandi ML, et al. Algorithm for the use of biochemical markers of bone turnover in the diagnosis, assessment and follow-up of treatment for osteoporosis. *Adv Ther*. 2019;36(10, 10):2811–2824. https://doi.org/10.1007/s12325-019-01063-9
14. Britton RA, Irwin R, Quach D, et al. Probiotic L. reuteri treatment prevents bone loss in a menopausal ovariectomized mouse model. *J Cell Physiol*. 2014;229(11):1822–1830. https://doi.org/10.1002/jcp.24636
15. Tyagi AM, Yu M, Darby TM, et al. The microbial metabolite butyrate stimulates bone formation via T regulatory cell-mediated regulation of WNT10B expression. *Immunity*. 2018;49(6): 1116–31 e7.
16. Nilsson AG, Sundh D, Backhed F, Lorentzon M. Lactobacillus reuteri reduces bone loss in older women with low bone mineral density: a randomized, placebo-controlled, double-blind, clinical trial. *J Intern Med*. 2018;284(3):307–317.
17. Gregori G, Pivodic A, Magnusson P, et al. *Limosilactobacillus reuteri* 6475 and prevention of early postmenopausal bone loss: a randomized clinical trial. *JAMA Netw Open*. 2024;7(6):e2415455.
18. Schepper JD, Collins F, Rios-Arce ND, et al. Involvement of the gut microbiota and barrier function in glucocorticoid-induced osteoporosis. *J Bone Miner Res*. 2020;35(4):801–820. https://doi.org/10.1002/jbmr.3947
19. Christensen SE, Möller E, Bonn SE, et al. Relative validity of micronutrient and fiber intake assessed with two new interactive meal- and web-based food frequency questionnaires. *J Med Internet Res*. 2014;16(2):e59.
20. Craig CL, Marshall AL, Sjöström M, et al. International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc*. 2003;35(8):1381–1395. https://doi.org/10.1249/01.MSS.0000078924.61453.FB
21. Svedlund J, Sjödin I, Dotevall G. GSRS?A clinical rating scale for gastrointestinal symptoms in patients with irritable bowel syndrome and peptic ulcer disease. *Dig Dis Sci*. 1988;33(2):129–134. https://doi.org/10.1007/BF01535722
22. Kirk B, Feehan J, Lombardi G, Duque G. Muscle, bone, and fat crosstalk: the biological role of myokines, osteokines, and adipokines. *Curr Osteoporos Rep*. 2020;18(4):388–400. https://doi.org/10.1007/s11914-020-00599-y
23. Booth SL, Centi A, Smith SR, Gundberg C. The role of osteocalcin in human glucose metabolism: marker or mediator.

Nat Rev Endocrinol. 2013;9(1):43–55. https://doi.org/10.1038/nrendo.2012.201
24. McCabe LR, Irwin R, Schaefer L, Britton RA. Probiotic use decreases intestinal inflammation and increases bone density in healthy male but not female mice. *J Cell Physiol*. 2013;228(8):1793–1798. https://doi.org/10.1002/jcp.24340
25. Bouvard B, Legrand E, Audran M, Chappard D. Glucocorticoid-induced osteoporosis: a review. Clinical reviews in bone and mineral. *Metabolism*. 2010;8(1):15–26.
26. Gado M, Baschant U, Hofbauer LC, Henneicke H. Bad to the bone: the effects of therapeutic glucocorticoids on osteoblasts and osteocytes. *Front Endocrinol (Lausanne)*. 2022;13:835720. https://doi.org/10.3389/fendo.2022.835720
27. Delmas PD, Davis SR, Hensen J, Adami S, van Os S, Nijland EA. Effects of tibolone and raloxifene on bone mineral density in osteopenic postmenopausal women. *Osteoporos Int*. 2008;19(8):1153–1160. https://doi.org/10.1007/s00198-007-0545-3

JBMR Plus, 2025, 9_ziaf031
https://doi.org/10.1093/jbmrpl/ziaf031
Research Article