

THE INFLUENCE OF MULTI-STRAIN PROBIOTIC SUPPLEMENTATION ON CALCIUM AND MAGNESIUM STATUS IN WOMEN WITH NON-MORBID OBESITY

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ABSTRACT

Background. Obesity is a significant global health issue, associated with many metabolic disorders. Magnesium (Mg) and calcium (Ca) play critical roles in body metabolism, and deficiencies in these minerals can increase complications associated with obesity. This study aimed to investigate the effects of twelve weeks of multi-strain probiotic supplementation on Ca and Mg metabolism in obese women.

Material and methods. The study is a multicentre, randomised, double-blind, placebo-controlled trial conducted at the University of Medical Sciences in Poznań and the University of Life Sciences in Poznań. Ninety obese women, aged 45–70, with a BMI > 30 kg/m², participated in the study. Participants were randomly assigned to either a high-dose (HD) or low-dose (LD) probiotic or placebo group. The probiotic groups received the Ecologic Barrier multi-strain probiotic combination.

Results. Hair Ca concentration was significantly lower in the LD group after the intervention ($p < 0.05$), while no significant changes were observed in serum calcium or magnesium levels. Additionally, osteocalcin levels were significantly decreased in the HD group compared to baseline ($p < 0.05$), indicating a potential effect of probiotics on bone metabolism. No significant differences were observed in oestradiol (E2), parathyroid hormone (PTH), or Procollagen Type 1 N-Terminal Propeptide (PINP) levels between the baseline and post-intervention.

Conclusion. The probiotic supplementation may influence calcium metabolism and bone turnover, as reflected in changes in hair calcium and osteocalcin levels. Further research is needed to explore the underlying mechanisms and their long-term clinical relevance.

Keywords: obesity, calcium, magnesium, probiotic

INTRODUCTION

Excess body mass is one of the top five risk factors globally for disability-adjusted life years and related deaths. The largest growth in obesity since 1975 has been in adult women. Currently, the percentage of women with obesity ranges from 0.2% in Vietnam to 65.3% in Samoa (Jaacks, 2019). Two billion people (30% of the world's population) suffer from obesity,

and the disease is a contributing factor in three million deaths annually (Hassabou and Farag, 2020). The per-person medical costs for individuals with obesity were over six times higher than those for individuals who are overweight, with total costs estimated at nearly \$114 billion. Between 2001 and 2016, medical costs associated with obesity more than doubled,

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reaching \$260.6 billion in 2016 worldwide (Cawley et al., 2021).

Magnesium is a vital nutrient involved in energy metabolism, protein synthesis, and enzymatic reactions such as those catalysed by phosphofructokinase and hexokinase (Toprak et al., 2017). In obesity, magnesium metabolism is often disrupted, with lower serum levels linked to increased urinary loss and inadequate dietary intake. This deficiency contributes to insulin resistance, inflammation, and obesity-related complications (Askari et al., 2021). Importantly, around half of Western populations fail to meet magnesium intake requirements, which may exacerbate obesity risk (Costello et al., 2016). Magnesium deficiency is linked to higher obesity prevalence and metabolic dysfunction. A 2021 meta-analysis found that patients with obesity consistently have lower serum magnesium compared to normal-weight controls. Supplementation trials suggest that magnesium improves waist circumference, BMI (body mass index), and insulin sensitivity in overweight and obese adults (Askari et al., 2021).

The vital mineral Ca regulates energy distribution in fat tissue and how adipocyte lipid metabolism is regulated (Lu et al., 2021). Mature adipocytes undergo apoptosis when their intracellular Ca^{+2} levels are consistently elevated (Sergeev, 2009). There is still debate, although several epidemiological studies have connected adult obesity to a Ca intake shortage (Larsen et al., 2014). One potential mechanism by which calcium reduces body fat is through enhancing thermogenesis and increasing energy expenditure. Calcium has been shown to play a role in regulating energy balance (Srivastava and Veech, 2019). From a nutritional perspective, both Ca and Mg are essential elements which deficiencies may worsen obesity-related outcomes, making them relevant targets for dietary or probiotic interventions.

The human microbiota comprises nearly 10,000 species and subspecies of microorganisms, with the gut microbiome alone containing over nine million genes. The primary microorganisms in the gut microbiota include *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*, *Fusobacteria* and *Verrucomicrobia*. Notably, *Firmicutes* and *Bacteroidetes* dominate, making up around 90% of the gut microbiota (León Aguilera et al., 2022). It has been indicated

that phylum-level changes in gut microbiota composition, a decrease in bacterial diversity, and alterations of functional genes and metabolic activities are associated with obesity (Bervoets et al., 2013; Pedersen et al., 2013). Thus, dietary intervention or modulation of the gut microbiota has the potential to prevent or treat obesity and obesity-related metabolic diseases (Zhang et al., 2018).

Probiotics are only advised as adjuvant therapy for the decrease of cardiovascular disease biomarkers; nevertheless, most studies indicate that they have many health advantages (Do et al., 2018). In one study, they improved BMI and weight by 3–5%, but they did not have a statistically significant effect on HbA1c, cholesterol, triglycerides, insulin resistance (HOMA-IR), or liver function (Koutnikova et al., 2019). However, studies on probiotics are contradictory. Another study showed that probiotics led to improvements in weight, fasting glucose, insulin levels, and inflammation markers, but did not significantly change cholesterol or insulin resistance levels (FAO/WHO Working Group, 2002). The influence of multi-strain probiotic supply on metabolic parameters is still a topic of a wide range of scientific studies (Skrypnik et al., 2018). However, multi-strain probiotics are not well investigated, although it is supposed that they may exert synergistic effects through broader microbial interactions and metabolite production compared to single strains.

Probiotics may influence calcium and magnesium status primarily by improving mineral bioavailability. Specific strains enhance mineral solubility in the gut, reduce luminal pH, upregulate mineral transporters, and generate short-chain fatty acids that facilitate absorption (Taylor et al., 2020). Probiotics may also improve intestinal barrier integrity and reduce gut inflammation, which supports more efficient nutrient uptake. Taken together, multi-strain probiotics, through combined and complementary mechanisms, have the potential not only to enhance calcium and magnesium utilization but also to contribute to obesity management by modulating metabolic pathways.

Animal studies support this interaction. Magnesium deficiency alters gut microbiota composition, with time- and dose-dependent effects on *Bifidobacterium* and *Lactobacillus* populations (García-Legorreta et al., 2020; Pyndt Jørgensen et al., 2015). These findings

suggest a bidirectional relationship, where magnesium influences microbiome diversity and, conversely, probiotics may enhance magnesium utilization. However, more human studies are needed in order to confirm these clinical implications.

The influence of probiotics, especially multi-strain probiotics, on Ca and Mg metabolism in obesity remains uninvestigated. For baseline characteristics, the null hypothesis assumed no significant differences between the placebo, low-dose, and high-dose probiotic groups, while the alternative assumed at least one difference. In relation to the study aim, the main null hypothesis stated that probiotic supplementation would not affect calcium or magnesium status or bone turnover markers compared with the placebo, whereas the alternative proposed that supplementation would significantly modify one or more of these outcomes.

The novelty of this research lies in its exploration of the barely studied impact of multi-strain probiotic supplementation on calcium and magnesium metabolism in obese women, whereas earlier work has focused on outcomes such as body weight, lipid profile, or glucose regulation. An innovative aspect of this trial is its dose-comparative design, where both low and high probiotic doses were compared with a placebo, allowing assessment of possible dose-dependent effects (Kaczmarczyk et al., 2022; McCabe and Parameswaran, 2018). Moreover, the inclusion of both serum and hair mineral analyses provides a comprehensive perspective, capturing both short- and longer-term changes in mineral status. The findings, particularly the decrease in hair calcium with low-dose probiotics and reduced osteocalcin levels with high-dose probiotics, suggest that probiotics may modulate calcium homeostasis, bone turnover, and fat distribution (Suliburska et al., 2021). By linking gut microbiota modulation with mineral and bone health, this study offers a novel contribution to the understanding of probiotic supplementation in obesity management.

MATERIALS AND METHODS

The current study was designed as a multicentre, randomised, prospective, double-blind, comparative, placebo-controlled trial conducted at two different locations: the Department of Treatment of Obesity, Metabolic Disorders and Clinical Dietetics, Poznań

University of Medical Sciences, and the Institute of Human Nutrition and Dietetics, Poznań University of Life Sciences. The study protocol was approved by the Poznań University of Medical Sciences Ethics Committee (approval no. 871/15 with amendments 439/17 and 93/19; approval year: 2015) and registered at ClinicalTrials.gov (NCT03100162; <https://clinicaltrials.gov/ct2/show/NCT03100162>). All procedures were conducted in accordance with the Declaration of Helsinki and followed CONSORT guidelines, including preparation of a CONSORT flow diagram (Fig. 1).

The primary outcome of the study was the change in serum calcium (Ca) and magnesium (Mg) from baseline to 12 weeks. Secondary outcomes included hair Ca and Mg, parathyroid hormone (PTH), oestradiol (E2), bone turnover markers (PINP, osteocalcin), anthropometric measures, and adverse events. All outcomes were measured at baseline and at week 12. The study was conducted between 27 Feb 2016 and 02 Feb 2018, with participant recruitment, intervention, and follow-up occurring within this period.

Participants

Two hundred and thirty-two women with obesity were screened at the outpatient department of Poznań University of Medical Sciences. Patients were enrolled provided they met each of the subsequent inclusion criteria: written informed consent, female sex, age range 45–70 years, body mass index (BMI) > 30 kg/m², abdominal obesity (waist circumference > 80 cm), body fat percentage > 33%, as determined by electrical bioimpedance, and stable body mass for one month before enrolment (with a permitted deviation of ±1 kg). The following conditions were considered exclusions: diabetes, secondary obesity, gastrointestinal disorders, use of dietary supplements in the three months before enrolment, pharmacotherapy for lipid disorders or hypertension in the three months before enrolment, clinically significant acute inflammatory process, use of antibiotics within the month before enrolment, participation in a body mass management study, use of medications known to alter body mass or food intake, abuse of alcohol, nicotine, or drugs, hormone replacement therapy, vegetarian diet, use of probiotic enriched or prebiotic enriched products in the three weeks before enrolment, consumption of high dietary fibre products, or more than 400 g of fermented food per day were the

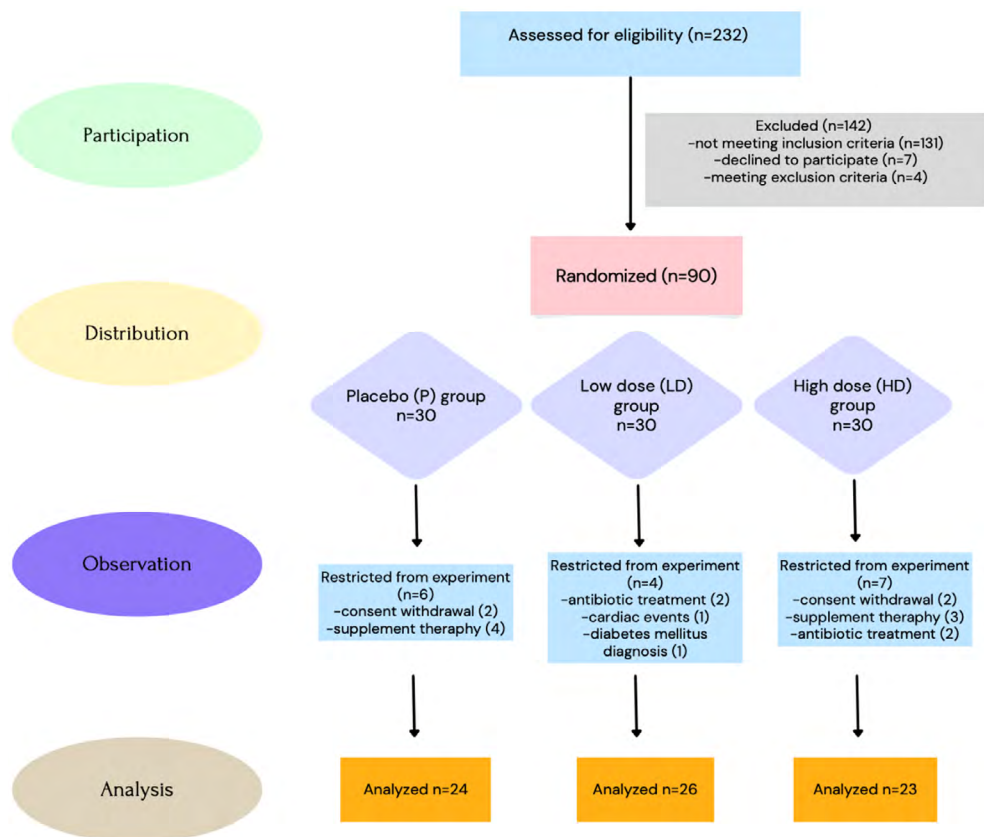


Fig. 1. Flow diagram
Source: prepared by authors.

exclusion criteria. Participants who satisfied any of the exclusion criteria were not admitted into the research study. The patient was also required to discontinue the experiment immediately if any of these exclusion criteria arose during the study. A total of 142 patients were excluded from the study enrolment. Ninety individuals, in total, fulfilled all the inclusion criteria and met none of the exclusion criteria, submitted written informed consent, were randomly assigned, and were enrolled in the study by research staff. Sample size was calculated to detect a between-group difference, with $\alpha = 0.05$ and 80% power, requiring 30 participants per group. In a 1:1:1 allocation ratio, the 90 patients who were enrolled were randomly allocated to one of two probiotic groups (HD – high dose, $n = 30$; LD – low dosage, $n = 30$) and a placebo group (P, $n = 30$). Both the volunteers and the researchers were blinded to the patient distribution. The research staff provided each

participant with a unique code for identification. Using permuted blocks of size four, Winclove (Winclove Probiotics, Amsterdam, Netherlands) computer-generated the randomisation formula. An independent statistician, not involved in enrolling participants, generated the randomisation sequence. Study staff then enrolled participants and allocated them to their respective intervention groups based on these codes. Participants, investigators delivering the interventions, and laboratory personnel performing outcome assessments were all kept blinded to group assignments. In the event of a medical emergency necessitating unblinding, a predefined protocol allowed access to the specific participant's allocation while preserving the blinding of the rest of the study. The study staff working with the patients were unable to change the randomisation. Power loss due to dropouts, which reduced the number of participants from 90 to 73 (24 in the placebo

group, 26 in the low-dose group, and 23 in the high-dose group), was addressed using an intention-to-treat (ITT) analysis, in which all randomized participants were included in the analysis according to their original group assignment. This approach ensured that the study retained sufficient statistical power to detect the pre-specified effect size despite participant attrition. The flowchart of the study in Fig. 1 shows the number of patients lost to follow-up and the reasons.

Probiotic

The Ecologic Barrier multi-strain probiotic combination (Winlove Probiotics, Amsterdam, Netherlands) was given to the probiotic groups in the form of sachets containing freeze-dried powder. The probiotic combination was administered daily to Group LD at a dose of 2.5×10^9 colony-forming units (CFU). A daily dosage of 1×10^{10} CFU of the probiotic combination was administered to Group HD. Equal amounts of the following bacterial strains were present in the probiotic mixture: *Bifidobacterium bifidum* W23, *B. lactis* W51, *B. lactis* W52, *Lactobacillus acidophilus* W37, *L. brevis* W63, *L. casei* W56, *L. salivarius* W24, *Lactococcus lactis* W19, and *Lc. lactis* W58. Maltodextrins and maize starch made up the excipient. The excipient alone, packaged in the same sachets, was given to the placebo group. The taste, smell, and appearance of the placebo and probiotic combination were identical. Before consuming, the participants were told to dissolve the contents of the sachet in a glass of room-temperature water. The twelve-week experimental intervention was conducted. Additionally, the patients were instructed not to modify their regular physical exercise.

Rationale for strain selection and dosing

Strain selection

The chosen strains were selected based on their documented effects on metabolic regulation, gut barrier function, and modulation of systemic inflammation in adults with obesity. For instance, *Bifidobacterium bifidum* has been associated with the modulation of gut microbiota composition and improvement in metabolic parameters (Kim et al., 2022). Similarly, *Lactobacillus acidophilus* has demonstrated potential in reducing body weight, fat mass, and inflammation, while enhancing intestinal barrier integrity (Kang et al., 2022).

Dosing

The selected doses of 2.5×10^9 CFU (low dose) and 1×10^{10} CFU (high dose) were based on previous clinical trials indicating that these ranges are both safe and effective for modulating gut microbiota and improving metabolic outcomes in obese populations (Kaczmarczyk et al., 2022). These dosages are consistent with those used in other studies investigating the effects of probiotics on obesity and related metabolic disorders (Li et al., 2022).

Anthropometrics

Anthropometrics was measured at baseline and the end of the intervention. Following an overnight fast and rest, the patients were measured for anthropometry in a metabolic laboratory while wearing loose clothing and no shoes. Electronic scales were used to measure body mass, with a precision of 0.1 kg. The height was measured with a precision of 0.5 cm. Mass divided by height squared (kg/m^2) was used to compute BMI. The circumference of the waist was measured between the lower ribs and the iliac crest, to the nearest 0.5 cm, using non-stretchable tape. Electrical bioimpedance (Bioscan 920-2, Maltron International, Essex, UK) was used to analyse body composition; percentile fat mass (FAT%) and percentile fat-free mass (FFM%) were recorded. An examination of the body's composition was conducted to verify that the percentage of body fat exceeded 33%. Fig. 2 shows the methodology of this paper.

Biochemical analysis

Commercial ELISA (enzyme-linked immunosorbent assay) kits were utilised (for E2, PTH, PINP, and OC, Abcam, Cambridge, UK; and Ca and Mg in serum, determined with AAS, FineTest, Wuhan Fine Biological Technology, Hubei, China). The instrument used was an absorption spectrophotometer (LEDetect96, Labexim, Lengau, Austria). The method used to test biochemical parameters was verified for accuracy and precision. Human serum was used as a control to ensure reproducibility (HUM ASY CONTROL 2, Sero, Billingstad, Norway). For E2, PTH, PINP, and OC, the intra-assay and inter-assay precision ($\text{CV} (\%) = \text{SD} / \text{mean} \times 100$) were less than 8% and less than 10%, respectively.

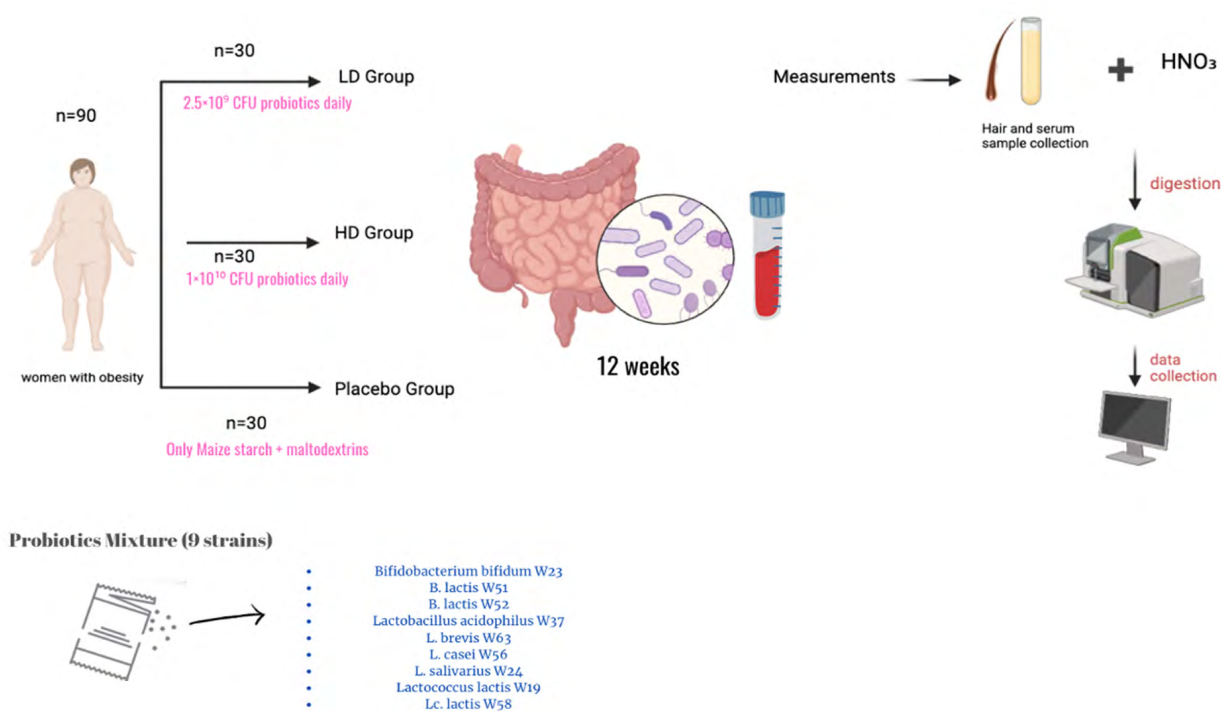


Fig. 2. Study design
Source: prepared by authors.

Hair sample collection

Hair was chosen as a biomarker for calcium and magnesium status because it reflects long-term mineral accumulation, can be collected non-invasively, and remains relatively stable compared to blood or urine. A 1-cm-long hair strand was collected from the occipital area. Each sample was stored in a separate, labelled paper bag. A member of the study team collected the sample shortly after the hair had been washed with a shampoo containing no active ingredients. Participants were given instructions on how to wash their hair properly. It was emphasized that following these guidelines was essential to obtain accurate results. The use of hairspray or dye was strictly prohibited throughout the study. Hair that had been permed or dyed was excluded from analysis. Hair samples were cleansed with acetone and deionised water, then dried at $\leq 60^{\circ}\text{C}$ before their mass was recorded. Additionally, hair samples were taken both before and after the study was finished.

Blood sample collection

At baseline and at the end of the study, blood samples were also drawn from a forearm vein and placed in serum-separating tubes following an overnight fast. To conduct further biochemical and mineral analysis, serum samples were kept at -80°C .

Mineral analyses

Approximately 0.10–0.20 g of hair was digested with 5 mL of 65% HNO_3 in a Mars 5 microwave system (CEM, Matthews, NC, USA), using a programme that ramped to 180°C within 15 minutes and was maintained for 20 minutes. The cooled digests were quantitatively transferred to 50 mL volumetric flasks, diluted with ultrapure water, and adjusted to 2% HNO_3 for measurement. Quality control included blanks and certified reference materials prepared under identical conditions. After digestion and dilution with deionised water, the concentrations of Ca and Mg in the mineral solutions were measured using flame atomic

absorption spectrometry (AAS-3, Carl Zeiss, Jena, Germany). The mineral contents of the hair and serum were determined at wavelengths of 422.7 nm for Ca and 285.2 nm for Mg. The accuracy of the method was verified using certified reference materials (HUMASY CONTROL 2, Sero, Billingstad, Norway, and Human Hair NCS DC73347a, LGC, Teddington, UK) and was 95–98% for Ca and 99–103% for Mg.

Statistical analyses

The patients' randomisation codes remained blinded until the statistical analysis was conducted. Data are presented as means \pm standard deviations (SDs) and medians with quartiles (Q1–Q3). All statistical analyses were performed using Statistica 10.0 software (StatSoft, Krakow, Poland). The Shapiro–Wilk test was used to assess the normality of the data distribution. The Wilcoxon rank-sum test was performed to determine the statistical significance of variables at baseline and after the intervention. Group comparisons were made using the Kruskal–Wallis test, and correlations were analysed using Spearman's rank analysis.

A minimum sample size of 30 subjects per group was initially calculated to provide 80% power to detect a statistically significant between-group effect at an alpha level of 0.05. Although 90 participants were randomized, 17 participants dropped out during the study (Placebo $n = 6$, LD $n = 4$, HD $n = 7$), resulting in 73 completers (Placebo $n = 24$, LD $n = 26$, HD $n = 23$). To account for potential power loss due to these dropouts, an intention-to-treat (ITT) analysis was performed, in which all randomized participants were included according to their original group assignment. Sensitivity analyses were also conducted to assess the robustness of the findings.

The primary analyses focused on the pre-specified outcomes of serum Ca and Mg. Secondary outcomes including hair Ca and Mg, PTH, oestradiol E2, bone turnover markers (PINP, osteocalcin), anthropometric measures, and adverse events were considered exploratory or post hoc analyses derived from previously published data. This distinction ensures transparency in interpretation and minimizes the risk of data dredging. Statistical significance was set at $p < 0.05$. There were no significant changes to the analysis methods after the trial began.

RESULTS

Primary outcome: serum and hair calcium/magnesium levels

Before intervention, serum and hair Ca and Mg concentrations did not differ significantly between groups. After intervention, no significant changes were observed in serum Ca or Mg across groups (all $p > 0.05$). Hair Ca levels decreased significantly in the LD group compared with before intervention, whereas no changes were detected in the HD and placebo groups. Hair Mg remained stable in all groups (all $p > 0.05$). Between-group comparisons showed no significant differences in post-intervention serum or hair Ca/Mg levels. These data are summarized in Table 3.

Secondary outcomes: anthropometric and body composition

Anthropometric measurements (BMI, weight) did not differ between groups before or after intervention (all $p > 0.05$). Waist circumference decreased significantly after intervention in all groups. In body composition, subcutaneous adipose tissue area was significantly reduced in the HD group, with no changes in other fat or lean mass indices (all $p > 0.05$). Complete anthropometric and body composition data are presented in Tables 1 and 2.

Secondary outcomes: bone metabolism markers

No significant pre–post differences were found in PTH, PINP, or E2 levels within or between groups (all $p > 0.05$). Osteocalcin decreased significantly in the HD group compared with before intervention, but not in LD or placebo groups. Between-group comparisons revealed no significant differences in post-intervention bone markers. Data are shown in Table 4.

DISCUSSION

Key findings

This study evaluated the impact of multi-strain probiotic supplementation on Ca and Mg balance in obese perimenopausal women. Two main outcomes emerged: A significant decline in hair calcium was observed in the LD, 2 g/day probiotic group, with a similar but non-significant downward tendency in the HD

Table 1. Anthropometric measurements result before and after intervention for all groups

Parameters	Group	Before		After		p-value*
		mean ±SD	median [Q1; Q3]	mean ±SD	median [Q1; Q3]	
Weight, kg	P	91.11 ±11.27	87.45 [84.30; 96.85]	91.43 ±11.91	87.75 [83.53; 97.73]	NS
	LD	87.49 ±10.32	86.0 [81.0; 91.60]	87.40 ±11.84	87.60 [81.20; 91.35]	NS
	HD	88.15 ±8.39	89.65 [80.07; 94.56]	87.99 ±9.26	88.40 [78.60; 92.25]	NS
	p-value#		NS		NS	
BMI, kg/m ²	P	34.71 ±2.85	34.85 [32.22; 36.93]	34.86 ±3.04	35.15 [32.87; 36.73]	NS
	LD	34.24 ±3.65	34.70 [31.4; 38]	34.12 ±4.12	34.11 [30.70; 36.90]	NS
	HD	33.23 ±3.56	32.50 [30.65; 35.7]	33.06 ±3.82	32 [30.60; 34.70]	NS
	p-value#		NS		NS	
Waist circumferences, cm	P	110.77 ±7.52	111.0 [105.25; 115.75]	107.33 ±7.12	107.50 [102.75; 112.75]	< 0.0017
	LD	106.47 ±8.89	107.0 [102.0; 114.0]	101.18 ±10.02	104.0 [96.0; 110.0]	< 0.0016
	HD	106.66 ±7.89	106.50 [101.50; 109.75]	103.97 ±5.69	104.0 [100.0; 106.0]	p = 0.038
	p-value#		NS		NS	

Data are presented as median [Q1; Q3] and mean ±standard deviation; NS – not significant; #Kruskal–Wallis ANOVA test; *Wilcoxon rank–sum test, significantly different ($p < 0.05$, After: result of consuming multi-strain probiotic 12 week).

Table 2. Body composition analysis by using Bioelectrical impedance analysis before and after intervention for all groups

Parameter	Group	Before		After		p-value*
		mean ± SD	median [Q1; Q3]	mean ± SD	median [Q1; Q3]	
1	2	3	4	5	6	7
Fat, %	P	51.35 ±5.11	53.36 [48.1; 54.9]	50.28 ±5.94	52.23 [47.76; 53.35]	NS
	LD	49.42 ±5.14	50.32 [46.13; 54.43]	49.74 ±5.15	51.12 [45.6; 54.22]	NS
	HD	47.6 ±6.3	47.8 [43.8; 53.5]	46.6 ±7.8	46.32 [42.8; 52.2]	NS
	p-value#		NS		NS	
Fat, kg	P	46.88 ±8.31	45.54 [39.82; 51.66]	45.96 ±7.86	45.33 [39.07; 50.20]	NS
	LD	43.51 ±8.62	43.59 [37.69; 47.93]	43.77 ±9.39	43.82 [39.93; 48.34]	NS
	HD	42.31 ±8.27	41.18 [36.29; 49.16]	41.28 ±9.74	41.15 [35.60; 48.79]	NS
	p-value#		NS		NS	
FFM, %	P	48.66 ±5.10	46.64 [45.08; 51.91]	49.66 ±5.99	47.77 [46.65; 52.24]	NS
	LD	50.6 ±5.14	49.7 [45.57; 53.87]	50.26 ±5.15	48.89 [45.4; 54.4]	NS
	HD	52.41 ±6.26	52.16 [46.55; 56.18]	53.47 ±7.65	53.68 [47.85; 57.18]	NS
	p-value#		NS		NS	

Table 2 – cont.

	1	2	3	4	5	6	7
FFM, kg	P		44.84 ±7.03	44.10 [39.36; 48.66]	46.55 ±9.74	44.61 [40.11; 47.45]	NS
	LD		43.98 ±4.37	42.96 [41.81; 47.01]	42.91 ±4.22	41.89 [40.46; 44.52]	NS
	HD		46.02 ±5.0	45.90 [43.80; 49.90]	46.70 ±6.40	46.90 [43.40; 49.60]	NS
	<i>p</i> -value#		NS		NS		
TBW, %	P		37.06 ±4.88	34.82 [34.36; 39.78]	37.80 ±5.57	36.38 [34.70; 39.18]	NS
	LD		38.12 ±3.68	38.48 [34.43; 40]	37.51 ±3.22	36.37 [35.1; 39.71]	NS
	HD		39.30 ±4.90	37.70 [35.20; 41.80]	40.44 ±6.85	39.89 [37.25; 42.07]	NS
	<i>p</i> -value#		NS		NS		
TBW, ltr	P		33.71 ±5.65	33.07 [29.55; 35.26]	34.71 ±7.82	33.87 [30.44; 34.58]	NS
	LD		33.17 ±3.44	33.10 [30.57; 35.34]	32.48 ±3.60	31.90 [30.04; 33.97]	NS
	HD		34.68 ±4.30	34.85 [32.88; 37.72]	35.54 ±6.09	35.40 [32.79; 36.55]	NS
	<i>p</i> -value#		NS		NS		
FFMH, %	P		76.06 ±2.58	75.47 [74.26; 76.91]	75.98 ±2.74	75.68 [74.42; 76.27]	NS
	LD		75.45 ±2.57	75.17 [74.25; 76.39]	74.74 ±3.03	75.48 [73.36; 77.34]	NS
	HD		75.04 ±2.61	75.24 [73.35; 76.19]	75.51 ±3.16	75.53 [74.04; 76.40]	NS
	<i>p</i> -value#		NS		NS		
Visceral	P		221.44 ±66.96	193.50 [173.25; 267.50]	213.80 ±55.84	202.0 [185.0; 250.0]	NS
	LD		193.31 ±61	212 [146; 234]	186.42 ±63.59	180.50 [148.25; 227.25]	NS
	HD		197.27 ±46.44	191.50 [170.25; 231.25]	196.28 ±50.52	187 [163; 250]	NS
	<i>p</i> -value#		NS		NS		
Subcutaneous	P		295.33 ±67.38	297.0 [264.0; 342.50]	257.70 ±74.84	279.0 [238.0; 298.0]	NS
	LD		252.15 ±75.65	246.0 [215.0; 296.0]	234.94 ±55.90	235.50 [197.25; 283.25]	NS
	HD		282.66 ±63.35	272.50 [258.25; 319.75]	243.35 ±64.63	236.0 [190.0; 298.0]	0.035
	<i>p</i> -value#		NS		NS		

Data are presented as median [Q1; Q3] and mean ±standard deviation; NS – not significant; #Kruskal–Wallis ANOVA test; *Wilcoxon rank–sum test; FFM, % – Fat-Free Mass percentage; FFM, kg – fat-free mass in kilograms; TBW, % – Total Body Water percentage; TBW, ltr – Total Body Water in litres; FFMH, % – Fat-Free Mass Hydration percentage; Values for visceral and subcutaneous adipose tissue are expressed as cross-sectional area (cm²) at the L4–L5 vertebral level).

group. A reduction in serum OC levels occurred in the HD group, suggesting suppressed bone turnover and remodelling activity. No meaningful changes were detected for other biochemical markers, including PTH, PINP, and E2, and probiotic supplementation did not consistently alter magnesium status.

Comparison with previous studies

Our results partially correspond with previous evidence from both animal and human studies on probiotics and mineral regulation. Earlier research has documented enhanced calcium absorption and bone-related benefits from probiotics (McCabe and Parameswaran, 2018;

Table 3. Ca and Mg concentration of serum and hair samples before and after intervention for all groups

Mineral	Group	Serum, ug/mL						Hair, ug/g						
		before			after			before			after			
		mean ±SD	median [Q1; Q3]	mean ±SD	median [Q1; Q3]	mean ±SD	median [Q1; Q3]	mean ±SD	median [Q1; Q3]	mean ±SD	median [Q1; Q3]	mean ±SD	median [Q1; Q3]	<i>p</i> -value*
Mg	P	16.13 ±2.49	16.57 [14.57; 17.92]	16.86 ±2.48	16.92 [15.73; 18.86]	NS	54.64 ±15.25	55.76 [42.83; 65]	60.55 ±18.82	60.34 [53.47; 69.12]	NS			
	LD	16.43 ±2.38	17.46 [14.78; 18.28]	16.85 ±2.70	16.72 [15.42; 18.58]	NS	60.73 ±20.95	53.24 [44.53; 80.80]	68.26 ±41.88	55.34 [46.76; 77.93]	NS			
	HD	16.21 ±2.22	16.42 [14.33; 18.01]	16.94 ±3.07	16.05 [15.08; 18.40]	NS	68.24 ±30.35	67.81 [41.11; 91.06]	66.42 ±25.57	59.75 [44.85; 84.72]	NS			
	<i>p</i> -value#	NS	NS	NS	NS	NS	NS	NS	NS	NS				
Ca	P	108.13 ±11.63	109.77 [88.83; 112.68]	110.26 ±20.20	115.39 [103.68; 122.66]	NS	891.67 ±298.13	828.79 [643.90; 1090.49]	906.68 ±191.56	915.74 [784; 1042.21]	NS			
	LD	108.13 ±7.74	109.36 [104.22; 112.72]	104.98 ±15.55	107.42 [106.12; 116.12]	NS	1073.25 ±445.72	944.1 [817.38; 1208.37]	956.34 ±294.38	914.84 [823.06; 1043.19]	0.049			
	HD	105.66 ±14.46	111.72 [102.54; 114.36]	114.15 ±16.67	117.92 [107.39; 121.22]	NS	969.74 ±321.25	967.85 [676.64; 1121.23]	962.35 ±283.58	844.11 [752.07; 1201.28]	NS			
	<i>p</i> -value#	NS	NS	NS	NS	NS	NS	NS	NS	NS				

Data are presented as median [Q1; Q3] and mean ±standard deviation; NS – not significant; #Kruskal-Wallis ANOVA test; *Wilcoxon rank-sum test.

Table 4. Serum concentration of biochemical parameters before and after intervention for all groups

Parameter	Group	Before		After		<i>p</i> -value*
		mean ±SD	median [Q1; Q3]	mean ± SD	median [Q1; Q3]	
E2 ng/ml	P	24.58 ±6.29	25.59 [22.29; 29.72]	25.56 ±4.32	26.37 [23.25; 28.72]	NS
	LD	24.93 ±4.40	25.63 [21.62; 28.43]	24.79 ±5.09	25.37 [22.99; 28.27]	NS
	HD	24.79 ±3.44	25.58 [23.53; 26.81]	25.36 ±4.49	26.24 [22.88; 28.13]	NS
	<i>p</i> -value#	NS		NS		
PTH ng/ml	P	93.01 ±50.87	74.47 [63.78; 98.97]	107.02 ±71.8	79.85 [63.81; 115.66]	NS
	LD	100.13 ±67.02	75.47 [61.93; 98.70]	94.80 ±58.68	73.31 [61.01; 83.01]	NS
	HD	76.98 ±61.83	65.70 [55.35; 75.54]	78.95 ±34.68	65.08 [56.12; 89.93]	NS
	<i>p</i> -value#	NS		NS		
PINP ng/ml	P	26.68 ±31.68	9.38 [5.07; 41.70]	29.10 ±68.39	11.31 [1.49; 15.65]	NS
	LD	26.21 ±31.76	11.13 [6.67; 29.93]	34.09 ±64.99	13.30 [9.56; 19.20]	NS
	HD	38.17 ±58.55	16.91 [8.99; 35.04]	33.73 ±54.10	11.91 [8.15; 33.39]	NS
	<i>p</i> -value#	NS		NS		
OC ng/ml	P	6.54 ±2.86	5.69 [5.10; 6.64]	6.54 ±2.86	5.47 [5.23; 5.99]	NS
	LD	6.18 ±2.58	5.39 [4.81; 5.82]	6.05 ±2.22	5.31 [4.73; 5.95]	NS
	HD	6.18 ±2.48	5.32 [4.89; 6.11]	5.58 ±1.40	5.06 [4.69; 5.95]	0.024
	<i>p</i> -value#	NS		NS		

Data are presented as median [Q1; Q3] and mean ±standard deviation; NS – not significant; #Kruskal-Wallis ANOVA test; *Wilcoxon rank-sum test; E2 – oestradiol, a form of oestrogen; PTH – parathyroid hormone; PINP – procollagen type I N-terminal propeptide, a marker of bone formation; OC – osteocalcin.

Raveschot et al., 2020). *Lactobacillus* and *Bifidobacterium* strains have been shown to improve calcium solubility and uptake, often leading to higher bone mineral density (Ghanemi and Mac-Way, 2023; Narva et al., 2004b). Conversely, studies such as Rodrigues et al. (2012) demonstrated that while *Bifidobacterium longum* with yacon flour did not significantly change bone structure, it did increase mineral deposition.

In clinical settings, fermented milk containing probiotics has been linked to reduced PTH and elevated serum calcium, pointing to complex influences on bone turnover (Narva et al., 2004a). Against this background, our observed decreases in hair calcium and osteocalcin levels may reflect dose-dependent and context-specific effects of probiotics, especially under conditions such as obesity and menopause.

Mechanistic explanations

Several mechanisms may help explain our findings. Probiotics can influence calcium metabolism by modulating the gut microbiota, lowering intestinal pH, and enhancing the activity of calcium transporters such as TRPV6 and calbindin-D9k, thereby improving calcium absorption and favouring its retention in circulation or bone rather than peripheral tissues like hair (Korkmaz et al., 2013). They may also facilitate the redistribution of calcium from peripheral stores to skeletal tissue, as suggested by animal studies showing increased bone calcium following probiotic supplementation (Ghanem et al., 2004). Another possible pathway involves the calcium–magnesium relationship: since magnesium regulates intracellular calcium levels and probiotics can improve Mg absorption

(Varvara and Vodnar, 2024), even subtle shifts in magnesium may influence calcium handling, despite the absence of significant changes in our study. Finally, the decline in osteocalcin observed in the HD group points toward reduced osteoblast activity and lower bone remodelling, potentially reflecting dose-dependent effects of probiotics in combination with the metabolic and hormonal alterations associated with obesity and menopause. The integrated interactions of these mechanisms and their influence on mineral metabolism in obesity are summarized in Fig. 3.

Obesity paradox and menopausal context

The interpretation of our findings should be considered in the context of the unique physiology of obese perimenopausal women. Obesity is frequently associated with greater bone mineral density because of mechanical loading and elevated levels of oestrogen and insulin; however, it also induces chronic low-grade inflammation, characterized by increased cytokines such as TNF- α , IL-6, and CRP, which stimulate osteoclast activity and disrupt mineral homeostasis (Mendonça et al., 2022). At the same time, the menopausal decline

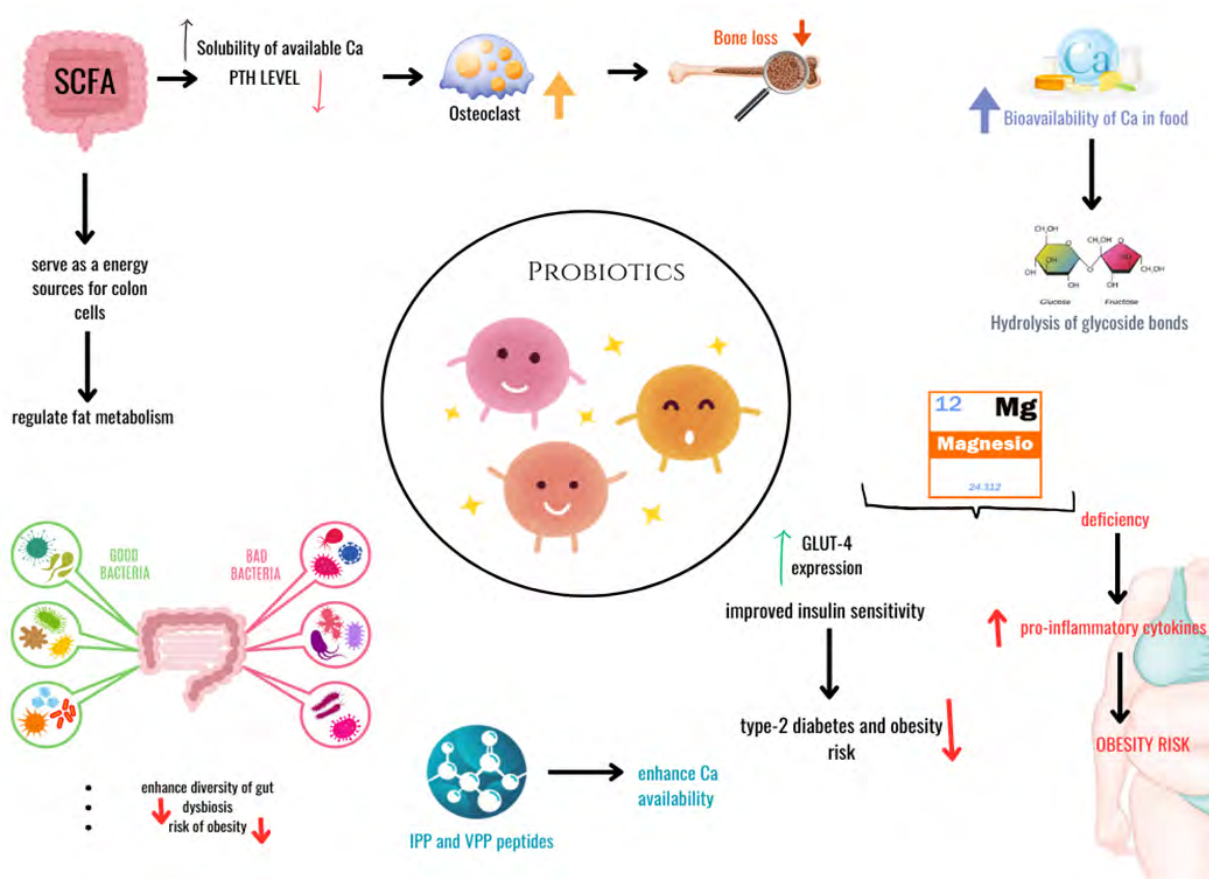


Fig. 3. Effects of probiotics on obesity and calcium and magnesium metabolism. Proposed mechanisms through which probiotics support mineral metabolism and metabolic health. Probiotics help maintain gut microbiota balance, generate short-chain fatty acids (SCFAs), modulate lipid metabolism, and increase calcium solubility and absorption. They also promote hydrolysis of glycosidic bonds and interact with bioactive peptides (IPP and VPP) to enhance calcium availability. Magnesium contributes to insulin sensitivity by regulating GLUT-4 expression, whereas its deficiency elevates inflammatory cytokines and obesity risk. Collectively, probiotics may reduce bone loss, improve metabolic regulation, and lower susceptibility to obesity and type 2 diabetes

Source: prepared by authors.

in oestrogen compromises calcium and magnesium absorption and reduces bone turnover, thereby increasing skeletal fragility (Anandacoomarasamy et al., 2008). Probiotics could potentially counteract some of these processes by lowering systemic inflammation and enhancing mineral uptake, but in our study, this remains speculative since the data did not provide direct confirmation.

Summary

In conclusion, our trial demonstrated two main outcomes of probiotic supplementation: a reduction in hair calcium (notably in the LD group) and lower serum osteocalcin (in the HD group). These results, which diverge from some previous reports of probiotic-induced improvements in calcium metabolism, may reflect dose-dependent actions along with obesity- and menopause-related physiological influences. Larger studies, including direct assessments of bone mineral density and controlled animal models, are required to clarify the clinical relevance and underlying mechanisms of these mineral shifts.

Clinical implications

Although there were no significant changes in BMI or total body fat percentage, the significant reduction in subcutaneous adipose tissue (SAT) in the high-dose group indicates a potential role for probiotics in modulating fat distribution. Additionally, the decrease in hair calcium concentration in the low-dose group suggests a possible interaction between probiotic supplementation and calcium homeostasis. The reduction in osteocalcin levels in the high-dose group may also reflect an effect of probiotics on bone metabolism. These results highlight the potential of probiotics as a complementary approach in obesity management, particularly in improving fat distribution and regulating calcium metabolism. Further research is required to deeply analyse these effects and explore potential long-term benefits.

Study strong points

This study provides valuable insights into the effects of probiotic supplementation on calcium and magnesium metabolism in females with obesity. Additionally, the study examines both anthropometric and biochemical parameters, allowing for a comprehensive evaluation of metabolic changes. Moreover, we investigated the

effect of a multi-strain probiotic, which is a scientific approach that is still not common in studies on probiotic supplementation, and allows for the investigation of the synergy effect of probiotics and their broad spectrum of clinical effects. Furthermore, the design of our study enabled us to compare two different doses of probiotics, providing insight into the dose dependence of the supplementation effect. Overall, these results underscore the promising health benefits of probiotic supplementation.

Study limitations

The primary limitation of this study was the relatively small sample size, primarily due to the stringent inclusion and exclusion criteria. These criteria helped ensure a high level of homogeneity within the study group, minimising factors that could compromise the quality of the results. Nevertheless, potential limitations should be acknowledged, including the possibility that external contamination (e.g., shampoos, dyes, or environmental exposure) may occur and that inter-individual differences in hair growth rates may influence mineral incorporation. Variability may therefore be introduced, and caution should be exercised when results are interpreted. Our study does not thoroughly investigate magnesium metabolism, and existing changes may have been very subtle, difficult to detect, and may have affected aspects of Ca-Mg homeostasis not assessed in our study. Despite the smaller sample size and relatively short duration, the study yielded several significant findings, highlighting potential avenues for future research.

Future studies

Future studies should aim to clarify whether probiotics influence the hormonal regulators of bone and mineral metabolism, such as osteocalcin, parathyroid hormone, vitamin D metabolism, oestrogen levels, and the Receptor Activator of Nuclear Factor κ B Ligand/Osteoprotegerin (RANKL/OPG) signalling pathways. Additionally, the mechanisms by which probiotics influence magnesium and calcium levels, as well as their interactions with hormonal pathways, require further elucidation through molecular and cellular-level research. Investigating the dose-response relationship is critical for optimising dosing regimens for specific clinical outcomes. Furthermore, there is a need

for larger, multicentre, randomised controlled trials to validate these findings across diverse populations, accounting for factors such as age, gender, dietary habits, and baseline health conditions. Future research should also explore the potential synergies between probiotics and other therapeutic interventions, such as dietary modifications or pharmacological treatments, to enhance clinical outcomes in managing obesity and metabolic and mineral disorders.

CONCLUSION

In this study, twelve weeks of multi-strain probiotic supplementation in women with obesity resulted in modest but measurable dose-dependent effects, specifically, a reduction in hair calcium levels at the lower dose and a decrease in circulating osteocalcin concentrations at the higher dose. While these divergent outcomes suggest potential regulatory roles of probiotics in mineral metabolism and bone-related biomarkers, their interpretation is limited by the small sample size, the post hoc design of the analysis, and the absence of consistent findings across other parameters. At present, there is insufficient evidence to claim clinically or metabolically meaningful effects, and these results should be regarded primarily as hypothesis-generating. Future research should focus on elucidating the mechanisms through which probiotics may influence calcium homeostasis, bone turnover, and mineral metabolism, while also testing different dose and duration regimens in larger and more heterogeneous populations to determine clinical relevance.

CONFLICT OF INTEREST

Winclove Probiotics kindly supplied both the active and the placebo products but did not have any additional role in the study design, data collection, and analysis, decision to publish, or preparation of the manuscript. The authors declare that they have no conflict of interest.

DECLARATIONS

Data statement

All data supporting this study has been included in this manuscript.

Ethical Approval

Not applicable.

Competing Interests

The authors declare that they have no conflicts of interest.

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