Can *Lactobacillus acidophilus* decrease the risk of postmenopausal osteoporosis in women?

Short Description

Background

The current recommendations for treating bone diseases are estrogen therapy and pharmacological agents. However, those treatments increase the risk of cancers and are not prescribed for long-term use. *Lactobacillus acidophilus* in ovariectomized mice could inhibit osteoclastogenic cells and promote anti-osteoclastogenic cells.

Aim of study

This study aims to evaluate the effect of *L. acidophilus* supplementation on calcium status and bone densitometry in postmenopausal women in a randomized, double-blind placebo-controlled study.

Study design

This study has three steps; namely, **1st step** is to prepare the diet supplementation of probiotics (*L. acidophilus* 1×10^9 CFU). **2nd step** is to perform the randomized, double-blind placebo-controlled clinical trial. **3rd step** is to analyze the effects of *L. acidophilus* in postmenopausal women.

Methods & Analysis

60 women of postmenopausal osteoporosis will be recruited by following inclusion and exclusion criteria. Participants will be randomized and divided into two groups to take a capsule containing probiotics and a placebo for 12 weeks. Before and after the intervention, several parameters will be conducted, such as the bone turnover and hormones parameter in blood using ELISA, the calcium mineralization in hair sample using AAS, the bone densitometry analysis in bone using DXA.

Research Design

Study settings

This study will be conducted within two (2) years for research activity and one (1) year for the publication period. There are three steps within this study; namely, **1st step** is to prepare the sample of *L. acidophilus* as diet supplementation. Probiotic capsules will contain *L. acidophilus* 1×10^9 CFU. **2nd step** is to perform the randomized, double-blind placebo-controlled clinical trial of *L. acidophilus* supplementation for twelve weeks (Figure 1). **3rd step** is to analyze the effects of *L. acidophilus* in postmenopausal women.

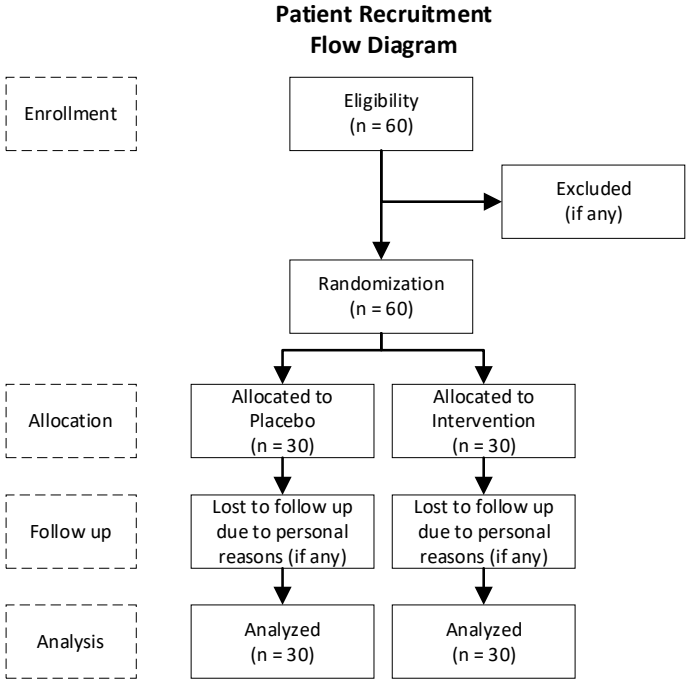

Figure 1. Flow diagram of patient recruitment

Eligibility criteria (inclusion, exclusion criteria)

The inclusion and exclusion criteria are provided in figure 2. Informed consent should be obtained from all patients, and the patients will be asked to notify the researchers if medical changes occurred during the intervention.

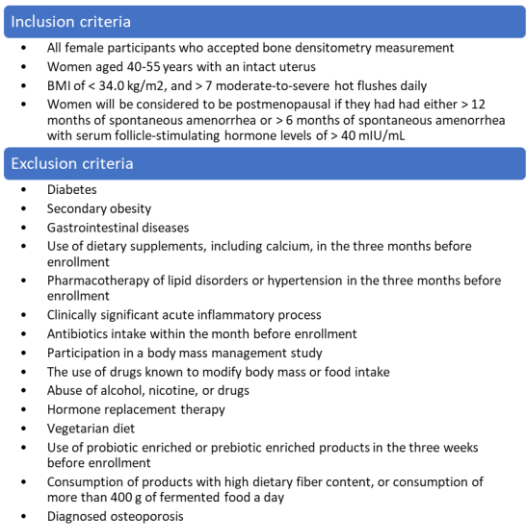

Figure 2. Inclusion and Exclusion criteria

Interventions or exposures

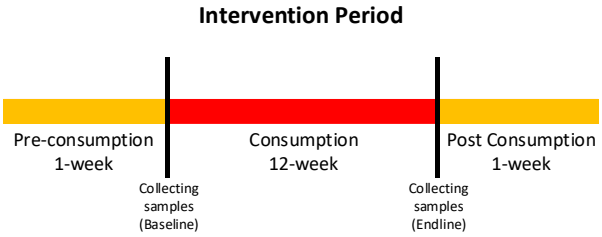

Figure 3. Diagram of the intervention period

The intervention period during this study can be seen in figure 3. The diet intervention in this experiment will be performed for 12 weeks, as Ahn et al. (2019) described. Two groups will be designated in this study. Group 1 is the placebo group that will receive a capsule that consists of the excipient. The excipient will be composed of maize starch and maltodextrins (Skrypnik et al., 2019). Group 2 is the probiotic group that will receive a diet capsule consisting of *Lactobacillus acidophilus* with a dose of $1x10^9$ CFU with the excipient described by Hosseini et al. (2019). In addition, it is essential to deliver self-administration of probiotic agents orally in the morning with fasting conditions to evaluate probiotic effectiveness. Subsequently, patients will be asked to keep the diet in refrigerated condition (2-7 °C).

Primary endpoints

This study will consider the change from baseline in the frequency and severity of hot flushes as primary endpoints.

Secondary endpoints

Meanwhile, the health-related quality of life, sleep, satisfaction with treatment, and cumulative amenorrhea and breast pain will be considered as secondary endpoints.

Sample size calculation

To calculate sample size, this study refers to the Soleimani et al. (2017) study. Using a formula suggested for clinical trials, having 25 participants in each group will be considered adequate

while considering a type I error (a) of 0.05 and type II error (b) of 0.20 (power ¼ 80%), with 1.61 as the SD and 1.30 as the mean distinction of CTX for markers of bone resorption and BSAP for marker of bone formation as the key variable. By assuming five dropouts in each group, the final sample size will be 30 participants in each group.

Methods assignment of interventions

Randomization will be performed using a computerized random number generator, which generated the random sequence based on simple randomization. All subjects will be identified by a trial identification number and assigned a treatment code. The researchers and participants will be blinded to the treatment code until the end of the study when data will be collected and evaluated. The groups will be decided after the completion of the data analysis.

Variable assessment

All information is containing 3 days dietary records, body composition measurement, and fasting blood samples will be collected at the beginning and end of the study. DIETA 6.0 and 5D computer program by Instytut Żywności i Żywienia, Poland will be used for analyzing 3 days averages of macronutrients and micronutrient intakes. A validated questionnaire obtains personal and demographic information. Anthropometric measurements will be measured using body composition analysis.

Biomarkers of bone turnover and hormones

This study will analyze the biomarkers of bone turnover and hormones as described by Villareal et al. (2016). Briefly, venous blood samples will be obtained after fasting overnight and analyzed in the laboratory. Enzyme-linked immunosorbent assay (ELISA) will be used to measure C-terminal telopeptide of type I collagen (CTX) and tartrate-resistant acid phosphatase isoform-5b (TRAP5b) as markers of bone resorption and bone-specific alkaline phosphatase (BSAP) as a marker of bone formation. Radioimmunoassay will be used to measure N-terminal propeptide of type I procollagen (PINP) as an additional marker of bone formation. ELISA is also used to measure high-molecular-weight adiponectin and insulin-like growth factor-1. Chemiluminescent immunoassays will be used to measure parathyroid hormone (PTH) and cortisol.

Hair sample

A hair strand of 1 cm of length is collected from the occipital region. The sample will be taken by a member of the study team shortly after the hair had been washed with a shampoo not containing functional components. Each woman is instructed on how to wash her hair. Moreover, it will be clearly explained to patients that reliable results could be obtained only by complying with this procedure. Hair spray or hair dye is forbidden during the study (Skrypnik et al., 2019).

Calcium analysis

The calcium contents of the hair and serum will be determined after digestion in 65% (w/w) spectra pure HNO3 using a Microwave Digestion System. Upon digestion and dilution with deionized water, the concentrations in the mineral solutions are measured using flame atomic absorption spectrometry (Skrypnik et al., 2019).

Bone densitometry analysis

This analysis will be conducted in the accredited laboratory in Poznan, Poland. Subsequently, the subjects are asked to undergo bone mineral densitometry using a DEXA scan. The scan will be performed on a Hologic DXA scan machine.

Products used in the research

1. A capsule consists of Maize starch and maltodextrins for the Placebo Group.
2. A capsule contains *Lactobacillus acidophilus* with a dose of $1x10^9$ CFU for the Probiotic Group.

Ascertainment Feasibility

The study will be conducted in the Department of Obesity and Metabolic Disorders Treatment and Clinical Dietetics, Karol Marcinkowski University of Medical Sciences (PUMS), Poznan, Poland. This place receives patients who meet the inclusion criteria described above.

Techniques and actual available equipment

The study will be registered to the Ethics Committee of Poznan Authority before the study started and followed by sending documents to the Nutricia Foundation Committee in September 2021. Practically, the activity will be held in the Hospital of PUMS under specialist doctor and expertise healthcare worker control. Meanwhile, sample measurements will be analysed at Poznan University of Life Sciences, and DXA measurement will also be held in the Department of Human Nutrition and Dietetics, Poznan University of Life Sciences, Poland.

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