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Effects of probiotics supplementation on the hormone and body mass index in perimenopausal and postmenopausal women using the standardized diet. A 5-week double-blind, placebo-controlled, and randomized clinical study

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Abstract. – OBJECTIVE: The results of pioneering studies indicate that probiotics can alleviate menopausal symptoms (including cardiometabolic dysfunctions) and improve the quality of life of perimenopausal/postmenopausal women. However, the results of randomized control trials are scarce to evaluate whether the administration of probiotics could affect the balance of sex hormones during the menopause period.

PATIENTS AND METHODS: In this randomized, double-blind, and placebo-controlled study, 48 perimenopausal and postmenopausal women received multispecies probiotic Sanprobi Barrier in a dose of 2.5×10^9 (CFU) for five weeks. Dietary guidelines were introduced in both groups simultaneously (~1800 kcal/per day, whole grain, no-wheat meals). The study aimed to assess the variations in follicle-stimulating hormone (FSH), estradiol (E2), cortisol (as the hypothalamic-pituitary-ovarian axis hormone), and the body mass during the intervention.

RESULTS: At the endpoint, FSH level has increased significantly concerning the baseline after the probiotic intake (31.91 *vs.* 42.00 mIU/ml; $p < 0.009$). Also, in the placebo group, a strong trend to elevate FSH was observed (22.31 *vs.* 41.99 mIU/ml; $p = 0.055$). Body mass has crucially decreased in reference to the baseline in both groups (PRO: 27.90 *vs.* 26.30 kg/m^2, $p<0.001$; PBO: 25.90 to 24.60 kg/m^2, $p<0.001$).

CONCLUSIONS: Probiotics affect FSH levels in perimenopausal women while simultaneously representing a non-invasive strategy to impact hormonal homeostasis. They could potentially have an impact on cardiometabolic health.

Key Words:
Perimenopausal women, Probiotic therapy, Hormonal homeostasis, Body mass index.

Abbreviations

Body Mass Index (BMI); follicle stimulating hormone (FSH); hypothalamic-pituitary-adrenal (HPA) axis; adrenocorticotropic hormone (ACTH); corticotropin-releasing hormone (CRH); cardiovascular disease (CVD); metabolic syndrome (MetS); autism spectrum disorders (ASD); short chain fatty acid (SCFA); Peptide YY (PYY).

Introduction

Women's cardiometabolic risk significantly increases after shifting into menopause[1], with cardiovascular disease (CVD) being generally the leading cause of mortality in postmenopausal women[2]. A recent meta-analysis showed that postmenopausal women experienced about 1.5-4 times higher cardiometabolic risk. Moreover, the 3.5 times higher risk of developing metabolic syn-

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drome (MetS) in comparison to premenopausal women was also noted[3]. Cardiometabolic malfunctions are most often diagnosed in MetS patients. They are not only associated with the increasing age, but also at least partially, remain related to the decreased levels of ovarian hormones reservoir during the menopausal transition and beyond[1]. Correspondingly, higher odds of MetS possibility are present in patients with a larger reduction in estrogen in comparison to androgens, along with increased LDL-cholesterol (LDL-C) levels and reduced HDL-cholesterol (HDL-C) concentration during the postmenopausal period[4-6].

As a result, effective and safe methods of prevention of metabolic disorders are sought in aging women. Lifestyle interventions, including taking probiotics, are the subject of particular interest. It's caused by the fact that they have been proven to be effective in counteracting the cardiometabolic risk factors in healthy persons[7]. A study by Szulińska et al[9] demonstrated the favorable effect of the administration of probiotic combination consisting of *Bifidobacterium bifidum* W23, *Bifidobacterium lactis* W51, *Bifidobacterium lactis* W52, *Lactobacillus acidophilus* W37, *Lactobacillus brevis* W63, *Lactobacillus casei* W56, *Lactobacillus salivarius* W24, *Lactococcus lactis* W19, and *Lactococcus lactis* W58 on different metabolic outcomes, including glucose metabolism, lipid profile, waist circumference, visceral fat, serum uric acid level, and LPS concentration in obese postmenopausal women. Additionally, it has also been revealed that this bacterial consortium had caused improvement in both functional and biochemical markers of vascular dysfunction. In addition to this, it has reduced homocysteine concentration, oxidative stress, and inflammation[8]. It was hypothesized that the abovementioned formula might improve the epithelial barrier integrity. It would then serve as an inhibitor of the pro-inflammatory cytokines' synthesis. In addition to this, it would function as an effective tool in decreasing the endotoxin load[9-11]. However, the mechanism of this bacterial formula has not been fully explained. Chahwan et al[12] and Steenbergen et al[13] have shown the influence of this probiotic formula on the gut-brain axis, which suggests its impact on the endocrine system[14]. On the other hand, some studies have confirmed the expected cardioprotective effects of estrogens[15,16] and the others failed to prove such causation. Indeed, studies reporting that endogenous estrogens may be associated with higher CVD risk[17,18] and type 2 diabetes[19,20] existence. Furthermore, recent studies have gradually reported associations between follicle-stimulating hormone (FSH) and cardiometabolic risk factors in postmenopausal women. The research presented that lower FSH was associated with prediabetes and diabetes[21] and a higher prevalence of MetS[22,23]. As a consequence, the low level of FSH might potentially serve as a risk factor or a biomarker for CVD in postmenopausal women[24]. In a quite recent study, Jung et al[25] reported that serum FSH levels had correlated negatively with cardiometabolic risk factors of postmenopausal period in Korean women, suggesting that a low FSH can be a predictor for cardiovascular disease.

Having used the knowledge about the relationship between the gut microbiota and endocrine system, we decided to take the following steps. We have analyzed the hormones of the hypothalamic-pituitary-ovarian and hypothalamic-pituitary-adrenal axes in perimenopausal and postmenopausal women who were supplemented with multistrain probiotic. We have done it in order to check the hypothesis suggesting that the effect of such a treatment is associated with hormonal changes.

Patients and Methods

The study was designed as a 5-week, double-center (Department of Gynecology, Endocrinology and Gynecological Oncology, Pomeranian Medical University in Szczecin, Poland and Department of Human Nutrition and Metabolomics, Pomeranian Medical University in Szczecin, Poland), randomized, double-blind, placebo-controlled clinical trial. Ethical approval was obtained from the Bioethical Committee at Pomeranian Medical University in Szczecin (KB-0012/40/17) and written informed consents were obtained from all participants prior to inclusion.

The inclusion criteria were: (1) women aged 45-65 years.

The exclusion criteria were: (1) use of probiotics, proton pump inhibitors (PPIs) or any dietary supplements in the 6 months preceding the study; (2) intake of antibiotics within 6 months before the study; (3) clinically significant acute inflammatory process, (4) chronic intestinal inflammation and other gastrointestinal diseases; (5) nicotine, alcohol, or drug abuse; (6) hormone replacement therapy; (7) any chronic diseases in the medical history.

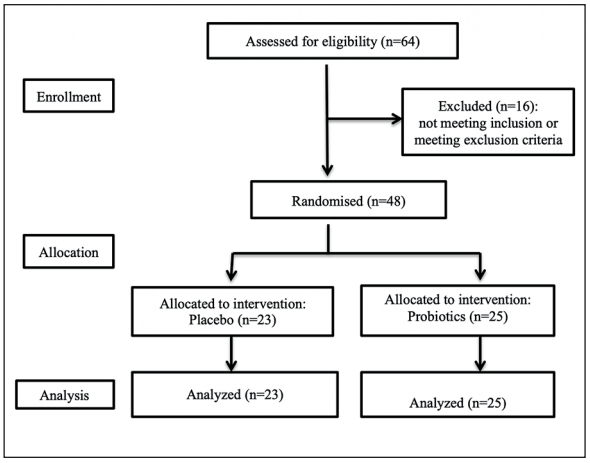

Figure 1. The flowchart of the recruitment, randomization and allocation in the study.

On the basis of the above criteria, there were 16 women who did not enter the study due to the presence of exclusionary criteria. Finally, 48 perimenopausal and postmenopausal women were eligible and provided with the informed consent. They were randomly assigned to the placebo or the probiotic group. Participants of the study and the investigators were blinded to the allocation until the completion of the study. A total of 48 participants (placebo group, n = 23; probiotic group, n = 25) were able to complete the 5-week intervention. The flowchart of the study is shown in Figure 1.

Intervention

The probiotic group received capsules containing powder of the probiotic mixture Sanprobi® Barrier (Sanprobi Sp. z o.o. Sp. k., Szczecin, Poland). Single capsule contained probiotic bacteria, namely: *Bifidobacterium bifidum W23, Bifidobacterium lactis W51, Bifidobacterium lactis W52, Lactobacillus acidophilus W37, Lactobacillus brevis W63, Lactobacillus casei W56, Lactobacillus salivarius W24, Lactococcus lactis W19, and Lactococcus lactis W58.* Products were stored at room temperature, in which declared bacteria content is warranted. The placebo group was given capsules containing excipients, i.e., maize starch and maltodextrins, and a small amount of vegetable protein. The placebo was indistinguishable in appearance, color, smell, and taste compared to capsules containing probiotic formulation. All participants were asked to administer 1 capsule orally three times a day (probiotic dose: 2.5×10^9 CFU/day) for the duration of 5 weeks with the following scheme: one during breakfast, one at dinner time, and one before going to sleep. Compliance was monitored at each visit. Participants were asked to return empty blisters at each clinical visit.

Randomization and Blinding

A simple randomization table was generated using the website tool, namely randomizer.org, by a designated investigator. All participants and investigators (including nutritionists, medical doctors, and laboratory technicians) were kept blinded until the end of the data analysis.

Dietary Intervention

A certificated nutritionist has prepared the weekly nutrition plan (five meals per day, identical for all participants). The caloric value did not exceed the amount of 1800 kcal per day. Before the beginning of the study, the dietician discussed the content of the menu with every patient separately. The nutritionist recommended to ingest the whole grain, low wheat products (rice, quinoa, buckwheat, oat, rye) having low and medium glycemic index. The promoted protein sources comprised poultry, fish (oily fish three times a week), eggs (four to five times a week). The amount of recommended fruits and vegetables included three portions of vegetables and two portions of fruits. The diet consisted of the recommended sources of fats (mainly vegetable fats, with a predominance of rapeseed oil and olive oil or a small amount of butter). The amount of fluids intake was calculated to be 35 mL/kg of the actual body weight (Figure 2).

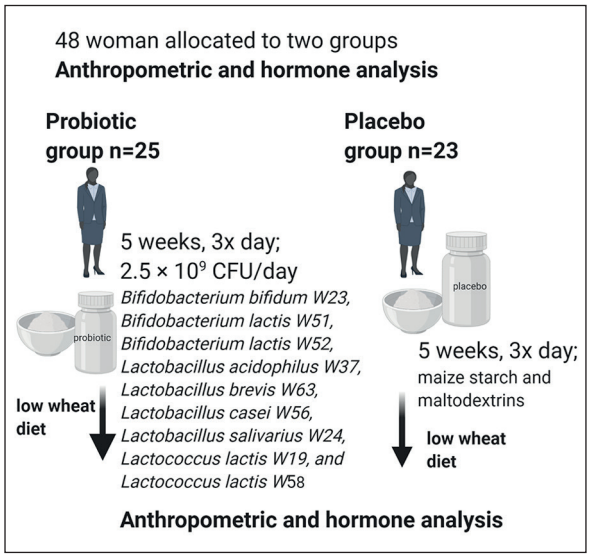

Figure 2. Dietary and probiotic intervention.

Study Parameters

The weight and height were measured by a medical stadiometer (Tanita SC-240MA, Tokyo, Japan), and BMI was calculated according to the standard calculation, i.e., BMI = body weight (kg)/(height)^2 (m)). The serum samples were obtained on days 3-5 (early follicular phase) of the menstrual cycle and in postmenopausal patients on any day for follicle-stimulating hormone (FSH), estradiol (E_2), and cortisol. All samples for hormonal tests were taken at the same hours to avoid errors resulting from the circadian rhythm of some hormones release, such as cortisol. These hormones were measured by the electrochemiluminescent immunoassay (ECLIA) using Cobas 8000 analyzer (Roche Diagnostics, Indianapolis, IN, USA), following the manufacturer's protocols: FSH in mIU/ml, estradiol in pg./ml, and cortisol in µg/dL. The parameters were measured before the study and after the completed 5-week intervention. The laboratory personnel was blinded to the outcomes.

Statistical Analysis

Statistics were conducted using MedCalc 19.2.6 software (Ostend, Belgium). The distribution of the continuous variables was evaluated by the Shapiro-Wilk normality test. Consequently, the nonparametric statistical analyses were conducted: Mann-Whitney or Wilcoxon, both performed in accordance with the appropriation usage. The significance level was adopted at $p<0.05$. In order to control the type I errors, the false discovery rate (FDR) approach has been used. The calculations were performed using a p. adjust function of the stats package in R (https://cran.r-project.org).

Results

The study parameters were assessed in 48 participants, aged 45.0-61.0 years (mean± SD 53± 4.8 years), among them 23 women who were randomized to a placebo group, and 25 to a probiotic group. None of the study participants dropped out during the trial. The compliance was more than 90% in all participants. The following adverse events were observed. Two persons reported abdominal bloating, which disappeared after two weeks of intervention. After the study had become unblinded, it was discovered that they came from the probiotic arm. The baseline characteristics of the groups are presented in Table I. Prior to the study, there were no significant differences in parameters between groups regarding the hormonal parameters of our interest.

After five weeks of intervention, we detected a significant growth of FSH level ($p<0.05$) in the probiotic group only. A significant decrease in BMI occurred before and after 5 weeks of study was found in both probiotic and placebo supplemented groups ($p<0.001$) (Table II).

Eventually, we decided to analyze endpoint parameters which only regard the intervention allocation. Due to the fact that the distribution of continuous variables was different from the normality, we refrained from using the ANCOVA test. Taking into account the parameters assessed at the beginning of the study as co-variants. However, as we insisted on keeping the baseline parameters in mind, we calculated the difference in the parameters marked at the end with respect to the start of the study in each group (delta). However, as presented in Table III, the differences between the endpoint and the baseline parameters (delta) were minor, as related to the allocation.

The following adverse events have taken place. Two persons have reported abdominal bloating, which disappeared after the passage of two weeks after the intervention took place. There were no participants that were dropped out throughout the whole study process. After the study got unblinded, it was found out that the abdominal bloating came from the probiotic arm.

Table I. Baseline characteristics of the study participants at baseline.

Variable	Probiotics group (n = 25)		Placebo group (n = 23)		
	Median	IQR (25-75 P)	Median	IQR (25-75 P)	FDR
Estradiol (pg./ml)	36.03	5.00-58.79	40.16	5.77-98.79	0.689
FSH (mIU/ml)	31.91	11.66-80.25	22.31	8.79-69.55	0.689
Cortisol (ug/dl)	12.61	9.30-20.10	13.61	10.56-18.54	0.812
BMI (kg/m^2)	27.90	23.83-31.10	25.90	23.88-29.13	0.689

[a]Mann-Whitney test, IQR - interquartile range; FDR- false discovery rate; BMI- Body Mass Index.

Table II. The comparison of parameters in both study and placebo groups before and 5 weeks after intervention.

Variable	Probiotics group (n = 25)						Placebo group (n = 23)					
	Baseline	IQR	After 5 weeks	IQR	p^b value	FDR	Baseline	IQR	After 5 weeks	IQR	p^b value	FDR
Estradiol (pg/ml)	36.03	5.00-58.79	24.80	5.00-91.93	0.548	0.657	40.16	5.77-98.79	32.00	9.41-92.65	0.418	0.501
FSH (mIU/ml)	31.91	11.69-80.25	42.00	12.34-92.68	0.009	0.018	22.31	8.79-69.55	41.99	8.09-79.39	0.055	0.11
Cortisol (ug/dl)	12.61	9.30-20.10	13.42	11.14-16.50	0.247	0.370	13.61	10.56-18.54	14.57	10.06-19.42	0.843	0.11
BMI (kg/m^2)	27.90	23.82-31.10	26.30	22.97-29.67	< 0.001	0.006	25.90	23.87-29.12	24.60	22.80-27.97	< 0.001	0.003

BMI- Body Mass Index, IQR - interquartile range, FDR- false discovery rate.

Table III. The difference in the endpoint and the baseline parameters (delta) in relation to the allocation.

Variable	Probiotics group (n = 25)		Placebo group (n = 23)		p^a	FDR	Cohen[d]
	Median	IQR (25-75 P)	Median	IQR (25-75 P)			
Estradiol_Δ	0.00	-9.61 to 1.89	0.00	-5.08 to 20.54	0.240	0.522	0.328
FSH_Δ	5.00	0.69 to 10.87	2.77	-0.61 to 10.20	0.451	0.522	0.158
Cortisol_Δ	-1.12	-4.49 to 2.84	-1.31	-3.32 to 2.85	0.439	0.522	0.2
BMI_Δ	-1.10	-1.60 to 0.20	-1.20	-1.70 to -0.20	0.522	0.522	1

BMI- Body Mass Index, IQR - interquartile range, FDR- false discovery rate.

Discussion

Finding innovative and safe therapies that can improve the quality of life and maintain proper body mass after menopause is a key challenge in modern society. Indeed, the problem has been steadily growing (with the growing population of ageing women), but there are only a couple of studies, including probiotics application in perimenopausal and postmenopausal women, conducted so far. Moreover, the mechanism of action of probiotics is poorly understood and requires further mechanistic studies.

For the first time, we discerned an increase in FSH concentration in the group of women receiving probiotics as compared to the group taking placebo. It might explain the mechanism of this probiotic's action. During the intake, it leads to the improvement of various parameters of the cardiometabolic risk in obese women after menopause[9]. The literature referring to the analyzed hormones during probiotic treatment is currently in short supply. Lim et al[26] showed a remarkable improvement in physical and sexual quality of life after 12-week administration *of Lactobacillus acidophilus* YT1, but without significant changes in blood FSH and estradiol levels or endometrial thickness. However, a recent trial by Zhang et al[27], delivered results looking similar to ours. Women diagnosed with polycystic ovary syndrome who were dosing 10.6 log CFU of Bifidobacterium lactis V9 once per day for 10 weeks experienced the following effect of CFU intake. They witnessed a decrease of the LH/follicle-stimulating hormone ratio. However, it only occurred in 9 out of 14 participants. The authors were able to show that such intervention increased the abundance of short chain fatty acid (SCFA) producing bacteria, which affected the secretion of gut-brain mediators, including ghrelin and Peptide YY (PYY), which further leads to the variations in sex hormones. This mechanism could play a role in our study as its participants used a standardized, normal fiber diet (in an average of 25 g/day) and this amount serves a substrate for the SCFA production.

We did not observe probiotic treatment actually affecting BMI, although a depletion of BMI has taken place in both investigated groups. Clinical trials showing a direct relationship between the probiotic therapy and the body weight in perimenopausal and postmenopausal women are ambiguous. In a 2018 a meta-analysis, comprising 15 primary studies, it was demonstrated that the administration of probiotics resulted in a significant reduction in body weight (BMI (-0.27 [-0.45, -0.08] kg /m^{-2}, I^2 = 57%), without any effect on fat mass (-0.42 [-1.08, 0.23] kg, I^2 = 84%)[28]. At the same time, an earlier meta-analysis by Park and Bae did not find either an association or a causation between the BMI decrease and the probiotic intake[29]. The most recent synthesis made by Jafar-Abadi et al[30], showed that neither probiotic (ingested as a supplement) nor probiotic foods reduced BMI. Szulińska et al[9] observed that the administration of the same multistrain product as used in our study had a favorable effect on waist and fat but did not affect body weight and BMI in obese postmenopausal women. Therefore, our results suggest that mainly healthy, norm caloric diet in perimenopausal and postmenopausal patients had an exceptional impact on the loss of weight but did not influence the hormonal balance.

Our study did not confirm the hypothesis that multistrain probiotic supplementation can affect the hypothalamic-pituitary-adrenal (HPA) axis in perimenopausal and postmenopausal women. We found a lack of impact of the probiotic intake on the cortisol level. Yet, it is known that the gut microbiota exerts control over the HPA axis[31], and the tested combination of probiotic strains positively influences the brain gut axis[12,13]. On the basis of animals' studies there is evidence that a role of gut microbiota composition is crucial for the regulation of the stress hormone-cor-

ticosterone, measured by cortisol in humans. The raised levels of circulating corticosterone in rodents were reduced by the administration of probiotics[32]. These correlations were found to be irrelevant in healthy humans but have been described as significant in patients with autism spectrum disorders (ASD)[33,34]. Accordingly, the probiotic supplementation was shown to decrease urinary cortisol excretion in humans[35,36]. Studies have revealed that the estrogens might have a preventive effect on the HPA axis hyperactivity. The estrogen's action through the adrenocorticotropic hormone (ACTH) and the corticotropin-releasing hormone (CRH), and the impact on the glucocorticoid receptor lead to the following consequences. The cortisol level gets regulated and reduced[37]. Nevertheless, in our work, the estradiol level has not been affected by the probiotic administration.

Strengths and Limitations

The strengths of the study are the design (randomized, placebo-controlled, double-blind intervention), a standardized diet, the same sex, and the similar age and hormonal status of the participants. The major limitation of this study is the relatively small number of examined individuals selected on the basis of rigorous inclusion and exclusion criteria. The observed differences in probiotic activity between the groups were not proven in the analysis of the intervention effect, which remarkably reduces the importance of this observation. Another limitation is a lack of microbial and metabolomic (mostly SCFA content) analysis of faeces, which could present the influence of probiotic bacteria on the gut microbiota composition and metabolic function. It should be the subject of further investigation of other parameters in the study group and would probably explain the positive effects of metabolic activity of probiotics. Additionally, we did not include dietary measures. Future studies with a longer duration of the intervention and larger sample sizes are required to confirm the validity of our findings.

Conclusions

The results demonstrated that multistrain probiotic administration significantly increases FSH levels in perimenopausal and postmenopausal women. This may be a mechanism of the favorable metabolic influence of probiotics in these groups of women.

Due to the limitations of the study, the findings should be interpreted with caution. Larger studies in perimenopausal and postmenopausal women are needed, along with an extended research in women at reproductive age, to confirm the probiotics influence on hormonal status.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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