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ORIGINAL PAPER

BIFIDOBACTERIUM LACTIS BS01 AND *LACTOBACILLUS ACIDOPHILUS* LA02 SUPPLEMENTATION MAY CHANGE THE MINERAL BALANCE IN HEALTHY YOUNG WOMEN*

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Abstract

Gut microbiota is involved in the metabolism of calcium, iron, magnesium, selenium, copper, zinc, and silver. Previous research indicates that the mineral status of an organism is associated mostly with the mineral content in the diet, but there is also evidence for probiotic bacteria having an effect on facilitating mineral absorption. However, the effect of probiotic supplementation on the gut microbiota composition is not clear, with some studies pointing towards no effect, or towards individual and strain-specific efficacy. Hair samples were previously established as a biomarker of dietary mineral intake over the previous 6-8 weeks. The aim of this research was to test the hypothesis that prophylactic consumption of *Bifidobacterium lactis* BS01 and *Lactobacillus acidophilus* LA02 can impact the mineral status in healthy females. The study utilizes a randomized, single-blind, placebo-control design. 53 healthy females between 19 and 33 years old were enrolled, and 38 completed the trial. A 6-week supplementation with *Bifidobacterium lactis* BS01 and *Lactobacillus acidophilus* LA02 or placebo was conducted. Participants were asked not to change any of their diet and lifestyle habits during the study. The contents of Ca, Mg, Zn, Cu, and Fe in hair samples were determined by flame atomic absorption spectrometry. The outcome of this study provides evidence that *Bifidobacterium lactis* BS01 and *Lactobacillus acidophilus* LA02 strains might be useful for improving the mineral status in healthy people (without dietary intervention), increasing Ca, Mg, Fe, and decreasing Cu concentration in hair, changing the mineral balance. The results support the hypothesis that probiotics might possibly have antidepressant properties.

Keywords: hair, minerals, probiotics, supplementation, women.

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INTRODUCTION

The gastrointestinal tract is a natural habitat for many microbial species (Hu, Wang et al. 2016), including up to 1000 species of bacteria (Qin et al. 2010). The gut microbiota can be considered to be a bacterial organ located inside the host's organism. It complements human biology, allowing us to take advantage of bacteria's genetic and metabolic attributes, thus playing an important role in our functioning (Bäckhed et al. 2005). Gut bacteria prevent pathogenic colonization by competing for shared nutrients and niches, and also influence the host's immune responses (Kamada et al. 2013). Alterations in gut microbiota and associated increased gut permeability (which triggers an immune response) are now widely accepted as relevant to the etiology, course, and treatment of many neuropsychiatric disorders (Leclercq et al. 2014). The development of the HPA axis (hypothalamic-pituitary-adrenal axis) response is dependent on early gut colonization (Sudo et al. 2004), and some studies suggest that probiotics might have antidepressant properties (Liang et al. 2015).

Gut microbiota plays an important role in digestion and metabolism (Bäckhed et al. 2005). It has been linked to the development of obesity, cardiovascular disease, and metabolic diseases, such as type 2 diabetes (Claesson et al. 2012, Tremaroli, Bäckhed 2012). Studies on both animal models and humans show that microbiota can affect the energy yield from food and regulate fat storage (Skrypnik, Suliburska 2018). There is also evidence for probiotic bacteria having an effect on facilitating mineral absorption (Scholz-Ahrens et al. 2007) and a similar effect for prebiotics (which selectively promote the growth of bacteria species in the gut) (Scholz-Ahrens, Schrezenmeir 2002). A review of studies by Skrypnik and Suliburska (2018) indicates that gut microbiota is involved in the metabolism of calcium, iron, magnesium, selenium, copper, zinc, and silver.

The mineral status of an organism is associated with age but depends mostly on the mineral content in the diet (Wójciak et al. 2004). An Iranian study on 238 females reported normal iron status for only 49.8% of the subjects (Shams et al. 2010). Data from Poland indicate that dietary iron intake in young adult females (aged 19-30) provides only slightly above 50% of the recommended daily allowance (Wójciak et al. 2004).

A 2020 study by Willemse et al. (2020) reported that 42% of pregnant women subjected to the study had a calcium intake below the estimated average requirement of 800 mg/day. Despite the frequent use of food supplements containing calcium in the studied population (64.8% women at 8 weeks of gestation), such products do not provide sufficient amounts of calcium to make up for a deficient diet, median calcium content of 120.0 (60.0-200.0 mg/day). Due to the reported prevalence of mineral-deficient diets in females across the globe (Suliburska 2011), the ability of probiotics to facilitate mineral absorption might be essential in the improvement of the mineral status in women. The functioning of an organism is related not only to the content of minerals but also to the balance between them. Suliburska et al. (2011) argue that the proportions between zinc and copper are especially important because of the antagonistic interaction between them at the absorption and transport stages.

Probiotic products are the fastest-growing group of dietary supplements worldwide (Champagne et al. 2018). They have become increasingly popular pharmacy and grocery items and are widely used in different areas of medicine and nutrition (Adams 2015). Despite the claimed health benefits of such products, in most countries, food supplements are considered food. Therefore, they are not tested for efficacy or even safety before entering the market (Czajeczny et al. 2020*a*). The effects of probiotic supplementation on human health are still debated, with some data indicating that probiotic supplementation might not be a reliable way to change the gut microbiota composition (Zmora et al. 2018). Benton et al. ((2007) argue that probiotics might be effective only in clinical populations where there is room to grow. In our previous study, probiotic supplementation did not impact healthy females' anthropometric measures (Czajeczny et al. 2020*a*).

The aim of this research was to test the hypothesis that prophylactic consumption of probiotic bacteria can impact the mineral status in healthy females.

MATERIAL AND METHODS

Participants

53 female participants were recruited via internet ads and posters on the university campus. During an initial interview, a written informed consent and general health information were obtained from the participants. Participants with: 1) gastroenteric, 2) endocrine, 3) neurological, or 4) psychiatric disorders, 5) antibiotic treatment up to 3 months prior to the supplementation, and 6) current probiotic supplementation were excluded from the study. 38 participants (20 in the supplementation and 18 in the placebo group) completed the study. The most common reason for leaving the study was an antibiotic treatment during the supplementation period. Other reasons included withdrawal of consent, and in one case – the onset of a psychiatric disorder. The characteristics of the research group are presented in Table 1.

Study protocol

The study protocol was previously described in another publication (Czajeczny et al. 2020*a*) The study protocol was approved by the Poznan University of Medical Sciences Bioethics Committee (No. 1070/16, 05.01.2017).

Table 1

Research group details

Variable	Placebo	Supplementation
Age (years) mean ± SD Range Median	23.06±3.11 20–29 23	23.47±4.19 19–31 24
BMI (kg m^{-2}) mean ± SD Range Median	22.01±2.42 18.65–28.07 21.42	23.67±5.56 18.65–29.93 22.38

Hair samples were collected twice - at baseline and after treatment. They were taken by one researcher in an effort to minimize discrepancies between collecting techniques. Participants were provided one probiotic supplement or placebo capsule daily for 6 weeks, as recommended by the supplement's manufacturer. A second meeting was scheduled at the convenience of the participants but no later than 7 days after taking the last capsule. Participants were asked not to change any of their diet and lifestyle habits during the study. They were instructed to immediately contact a researcher in case any side effects of supplementation occurred. No participants reported any side effects of either supplement or placebo (Czajeczny et al. 2020*a*).

Supplementation

Bifidobacterium lactis BS01 (2 10^9 CFU) and *Lactobacillus acidophilus* LA02 (2 10^9 CFU) bacteria in the form of a probiotic supplement were used in this study. Placebo was prepared using empty capsules (similar in size and colour to supplement capsules) and maltodextrin as a filler (Steenbergen et al. 2015). Both products had the same taste, colour, and smell, and were provided to participants in ziplock bags. Participants were blinded to whether they received probiotic or placebo capsules.

Assessment of metal concentrations in hair

Previous publications by other authors described the preparation of hair samples and the determination of minerals in the hair, as well as reference values for hair elements (Chojnacka et al. 2010, Suliburska 2011). Hair samples, taken from six different points of the occipital scalp (about 0.5 g, 1.0 cm from the skin), were washed three times in unionized detergent, deionized water, acetone, and deionized water again, then dried to a dry mass (at 105°C). Wet mineralization in a nitric acid environment (65%, supra pure, Merck) using the Ethos Easy microwave mineralizer (Milestone Srl) was conducted. After proper dilutions, the contents of Ca, Mg, Zn, Cu, and Fe in the hair samples were determined by flame atomic absorption spectrometry using an iCE3500 spectrophotometer (ThermoFisherScientific). The accuracy of the method was verified with certified reference material (NCS DC 73347 human hair). Standards recovery was 99.6-102.9%. For details of the analysis see

Wojciak (2019). The reference values established on the basis of previous research by the authors (Wojciak et al. 2004, 2010, 2019) and other authors' research (Suliburska et al. 2015), were as follows: Ca – 600-1000, Mg – 40-60, Zn – 160-200, Cu – 10-20, Fe – 10-20 μg g^{-1} d.m.

Statistical analysis

The normality of distributions was tested with the Shapiro-Wilk test. The hair mineral concentration distribution was shown to deviate from normality. The Wilcoxon test was used to determine the differences between means before and after supplementation. The $\chi 2$ test was used to compare data distributions against normative / reference values. The data were presented using basic descriptive statistics: arithmetic mean, standard deviation, range, and median. All analyses were performed using the Statistica ver. 13.3 statistical program at a significance level of 0.05.

RESULTS AND DISCUSSION

The results in this study are presented in Tables 2 and 3. Table 2 contains data on hair mineral concentrations in placebo and supplementation groups before and after probiotic treatment. In the placebo group, changes in mean Ca, Mg, Zn, Cu, and Fe concentrations in hair samples were not significant. In the supplementation group, mean concentrations of Ca, Mg, and Fe significantly increased after the probiotic treatment. The mean concentration of Cu significantly decreased after the probiotic treatment, and the change in the mean concentration of Zn after the probiotic treatment was not significant. All of the differences between groups in terms of mean concentrations of minerals in hair samples at baseline (before treatment) were not significant, indicating successful randomization.

Table 3 presents the percentage distribution of participants according to hair mineral reference values (RVs). No significant changes (Ca, Mg, Zn, Cu, Fe) in distributions according to RVs were observed in the placebo group.

In the supplementation group, significant changes of distributions according to RVs were observed for Ca, Mg and Fe. The percentage of participants <RVs for Ca changed from 31% to 6%. A general shift towards >RVs was observed. The percentage of participants <RVs for Mg changed from 13% to 6%. A general shift towards >RVs was for Mg was also observed. The distribution of Fe RVs changed towards >RVs (from 25% >RVs pre-treatment to 63% >RVs post-treatment). The distribution of Cu RVs shifted towards <RVs, but this change was not statistically significant.

In both the supplementation and placebo groups, no participants were below RVs for Fe. The presented study investigated the effects of prophylactic *Bifidobacterium lactis* BS01 and *Lactobacillus acidophilus* LA02 supple-

Table 2

Hair mineral concentrations in the placebo and the supplementation group (μg g^{-1} d.m.)

Minerals		Placebo		Supplementation	
		before	after	before	after
Ca	mean±SD range median	975.8±308.8 475.8–1623.87 985.9	1003.4±285.0 (480.9–1524.8) 986.5	1077.6±578.7 (369.4–2286.1) 986.9	1424.3±505.5 (402.8–2560.8) 1375.4
Statistic *p*		0.1402		0.0005	
Mg	mean±SD range median	60.22±27.79 23.85–117.79 55.43	57.37±25.86 22.81–129.95 56.03	52.75±11.58 25.04–72.58 54.25	59.64±13.59 26.38–79.32 60.39
Statistic *p*		0.3137		0.0008	
Zn	mean±SD range median	231.8±73.9 143.3–415.3 210.7	221.8±68.3 138.5–402.8 207.2	243.5±94.2 143.6–556.6 222.5	245.8±53.3 166.8–383.1 242.0
Statistic *p*		0.1280		0.9204	
Cu	mean±SD range median	21.55±9.66 4.93–36.27 21.44	22.85±10.84 5.14–39.84 19.04	22.55±18.53 3.46–63.90 12.58	18.27±15.97 1.64–56.93 11.98
Statistic *p*		0.1084		0.0149	
Fe	mean±SD range median	22.82±7.20 13.07–37.58 19.71	23.03±7.90 13.50–36.56 20.64	17.95±3.71 12.88–26.50 18.10	20.82±4.32 14.25–30.99 20.71
Statistic *p*		0.8313		0.0002	

mentation on the mineral status in healthy, young females. The main aim of the study was to assess whether prophylactic consumption of probiotic supplementation can impact the mineral concentrations in hair.

The outcome of this study provides evidence that *Bifidobacterium lactis* BS01 and *Lactobacillus acidophilus* LA02 supplementation affects the mineral status in healthy females, increasing the calcium, magnesium, iron while decreasing the copper concentrations in hair. The probiotic treatment also affected the distribution of participants according to RVs. Calcium, magnesium, and iron distributions were shifted towards RV and >RV after the probiotic treatment. No significant changes were observed in the placebo group in both mean mineral concentrations in hair and percentage distribution according to RVs.

The diets of females around the world are low in calcium, magnesium, and iron (Suliburska 2011). Despite the commonly reported iron deficiency among adolescent and adult females (Alzaheb Al-Amer 2017, AlSheikh 2018), no participants in this study were <RVs for iron concentration in hair. In the authors' previous study (Suliburska 2011), 4% of females aged between 19 and 30 (age group similar to this study) were <RVs for iron. A significant increase in iron concentrations in hair after the probiotic treatment suggests

Table 3

Percentage distribution of subjects according to reference values (RVs) for hair minerals (%)

Specification		Placebo		Supplementation	
		before	after	before	after
Ca	<RVs	6	6	31	6
	RVs	47	47	19	6
	>RVs	47	47	50	80
Statistic (χ^2, *p*)		(0.00, 1.0000)		(34.12, 0.0000)	
Mg	<RVs	18	18	13	6
	RVs	41	47	56	44
	>RVs	41	35	31	50
Statistic (χ^2, *p*)		(0.88, 0.6431)		(8.48, 0.0144)	
Zn	<RVs	12	12	6	0
	RVs	29	24	25	25
	>RVs	59	65	69	75
Statistic (χ^2, *p*)		(0.67, 0.7136)		(3.84, 0.1465)	
Cu	<RVs	12	12	31	44
	RVs	29	41	31	25
	>RVs	59	47	38	31
Statistic (χ^2, *p*)		(3.42, 0.1813)		(3.61, 0.1648)	
Fe	<RVs	0	0	0	0
	RVs	53	47	75	38
	>RVs	47	53	25	63
Statistic (χ^2, *p*)		(0.72, 0.3961)		(29.30, 0.0000)	

that it may be useful for improving the iron status in iron-deficient individuals even without iron supplementation or dietary interventions (PEDLAR et al. 2018). Iron deficiency has been associated with depressive symptoms (HIDESE et al. 2018). Improvement of the iron status might be one of the mechanisms of hypothesized effect of a probiotic on mood. Other mechanisms involve changes in immune response and hypothalamic-pituitary-adrenal HPA axis activity. These mechanisms were further discussed in our previous article (CZAJECZNY et al. 2020*b*).

Magnesium deficits have also been associated with the development of depression in both human and animal studies (WANG et al. 2018). A 2017 study (BAMBLING et al. 2017) reported decreased depressive symptoms in patients with poor previous SSRI treatment outcomes after an 8-week treatment with a combination of *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, *Streptoccocus thermophiles* (total CFU of 2 $10^{10),}$ and magnesium orotate (1600 mg) divided into two daily doses. The study did not compare the effects of probiotics and magnesium supplementation separately; however, another study (Tarleton et al. 2017) reported attenuated depressive symptoms after just 2 weeks of magnesium chloride (248 mg of elemental magnesium per day) supplementation, and the results of this study suggest that probiotic consumption alone might improve magnesium absorption, potentially affecting depressive symptoms associated with magnesium deficit. This outcome further supports the hypothesis that probiotics might have antidepressant properties (Marotta et al. 2019) and might improve mineral absorption when combined with mineral supplements, improving their efficacy.

In this study, 31% of the supplementation group participants were <RVs for calcium before the probiotic treatment. This percentage dropped to 6% after the treatment, and the mean calcium concentration in hair improved significantly in the supplementation group. Calcium bioavailability depends not only on the source but also the age, transit time, the amount of calcium ingested, intestinal content, and type of diet. Higher content of phytate, oxalate, and dietary fibre in the diet, along with higher pH (alkaline condition), can interfere with calcium absorption (Dubey, Patel 2018). Probiotics produce short-chain fatty acids, which increase the solubility of available calcium (Dubey, Patel 2018). A study on mice with *Lactobacillus plantarum* NTU 102 fermented soy milk showed that through the production of enzyme phytase, probiotic treatment could increase the release of the depressed calcium and increase calcium availability at the site of absorption (Chiang, Pan 2011).

The observed decrease of the mean copper concentrations in hair after the probiotic treatment is in line with the previously reported inverse correlation of copper with calcium, magnesium, iron, and zinc levels in hair. This can be explained by the interactions between minerals at the stage of transport and absorption (Suliburska 2011).

In this study, no effect of probiotic treatment on zinc levels in hair was observed, but a recent in-vitro study reported a 2% increase in zinc bioaccessibility and a 4% increase in iron bioaccessibility after fermenting *Uapaca kirkiana (*indigenous fruit tree found in the miombo ecological zone in sub-Saharan Africa) fruit pulp with *Lactobacillus rhamnosus* yoba strain (Chawafambira et al. 2020). The authors argue that promoting probiotic foods might help improve nutrition in poor populations in sub-Saharan Africa.

CONCLUSIONS

The outcome of this study provides evidence that *Bifidobacterium lactis* BS01 and *Lactobacillus acidophilus* LA02 strains might be useful for improving the mineral status in healthy people. The reported increase in mineral concentrations in hair might be an effect of improved mineral absorption and transportation. It is important to note, however, that more is not always better and the optimal functioning of an organism is related not only to the content of minerals but also to the balance between them (SULIBURSKA 2011).

The multitude of bacterial strains used in probiotic products and combinations of probiotics with prebiotics, mineral supplements etc., make it difficult to draw general conclusions on the effects of probiotics on the mineral status and other health aspects. A meta-analysis by MCFARLAND et al. (2018) found strong evidence that the efficacy of probiotics is both strain-specific and disease-specific. Strains used in this study were beneficial for mineral status but not for anthropometric measures (CZAJECZNY et al. 2020*a*).

Authors' contribution

Dominik Czajeczny: funding acquisition, conceptualization, methodology, investigation, formal analysis, writing – original draft preparation, writing – reviewing and editing,

Karolina Kabzińska-Milewska: conceptualization, investigation, writing – reviewing and editing,

Rafał Wojciech Wójciak: conceptualization, methodology, formal analysis, supervision.

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