

Prebiotic Food Intake may Improve Bone Resorption in Japanese Female Athletes

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Research article

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Abstract

Background: Several studies have reported that prebiotics are beneficial in improving bone health due to their balancing effect on the gut microbiota. As numerous Japanese female athletes have issues related to bone health, a better understanding of the bioactivity of prebiotics are essential. The present study aimed to attempt continuous intake of prebiotic food would balance the gut microbiota, resulting in improved bone metabolism turnover marker among the Japanese female athletes.

Methods: The participants included 29 female athletes aged 18–25 years. They were requested to consume their habitual foods or drinks with one pack of prebiotic food every day for 12 weeks. Dietary intake, energy expenditure, body composition, blood sample, and faecal gut microbiota was assessed during this intervention period.

Results: Body composition, total energy intake, and total energy expenditure of the participants revealed no significant changes during the intervention period. The occupation ratio of *Bifidobacterium spp.* was significantly increased at 3 and 4 weeks ($18.0\% \pm 8.3\%$ and $17.6\% \pm 8.5\%$, respectively) compared to that of pre-intervention ($11.7\% \pm 7.3\%$) ($p = 0.019$ and $p = 0.035$, respectively). Serum TRACP-5b level was significantly decreased at 12 weeks (363 ± 112 mU/dL) compared to that at baseline (430 ± 154 mU/dL) ($p = 0.018$).

Conclusions: These results suggest that continued intake of inulin and lactulose fortified prebiotic food might have beneficial effects on bone health and gut microbial environment among the female athletes. Further studies are warranted to identify the mechanism of the prebiotics-gut-bone axis.

Trial registration: Umin Clinical Trials Registry, UMIN000029589. Registered 17 October 2017.

Background

Inadequate energy intake is establishing immense attention as the main factor underpinning various unfavorable health issues in female athletes worldwide. The American College of Sports Medicine defined the concept of the Female Athlete Triad model in 1997 [1] and renewed it in 2007 [2]. This concept highlights the importance of adequate energy intake to assist the athletes' health, such as bone health, reproductive function, and sport performance. Poor bone health in female athletes occurs when athletes consume inadequate energy with or without disordered eating. For example, inadequate energy intake negatively affects bone metabolism turnover, leading to rapid increases in bone resorption and decreased bone formation [3, 4]. Similarly, it contributes to reduced bone mineral density and increased risk of stress fractures [5, 6]. Female athletes may be at an increased risk of bone disorders, including osteoporosis during or after retiring from their athletic careers [7]. Thus, adequate energy intake is essential for these athletes to prevent impaired bone health.

Nutritional intervention to improve bone health in female athletes addresses chronic energy deficiency and improves energy intake to meet the energy demands. A previous study reported that an increase in

habitual energy intake is a useful nutritional strategy for solving women's health problems [8]. However, many female athletes believe that lighter the body weight, better the performance [9]. This idea is considered to cause energy and nutrient deficiencies. In reality, it is difficult to increase energy intake, macro- and micro-nutrient requirements for maintaining their health and sports performance.

Prebiotics such as inulin and lactulose are selectively metabolized by microbes inside and on the body surface, and several studies have reported that both inulin and lactulose have prebiotic effects [10–12]. A prebiotic is defined as a substrate that is selectively utilized by host microorganisms, thus conferring health benefits [13]. These effects occur via prebiotic–microbial interactions in the large intestine. Moreover, a recent review summarized the gut microbiota involved in bone remodelling [14]. Furthermore, a previous study revealed that the combination of prebiotics may have synergistic effects on health by allowing prolonged fermentation throughout the large intestine [15]. The combined intake of prebiotics may provide nutritional benefits in female athletes. From these contexts, we have developed a new food with low energy including prebiotics such as inulin and lactulose. However, no studies have assessed whether the combination of prebiotics affects bone health among female athletes. Therefore, a better understanding of the combination effect of prebiotics among female athletes is essential.

The present study aimed to determine whether continuous intake of prebiotic food would balance the gut microbiota, resulting in an improved bone metabolism turnover marker among Japanese female athletes.

Method

Participants

Thirty female athletes aged 18–30 years were recruited in this study. One female athlete (long-distance running) was excluded due to personal reasons. In total, 29 of the 30 female athletes were intervened. The sports events were as follows: rhythmic gymnastics ($n = 15$), middle- and long-distance running ($n = 6$), soccer ($n = 6$), and figure skating ($n = 2$). Exclusion criteria were as follows: 1) regular use of medication that may influence metabolism or hormones; 2) injury; 3) smoking; 4) history of fractures in the last 6 months; 5) pregnancy; 6) milk allergy; and 7) use oral contraceptives and antibiotics. All athletes were adequately informed about the study by verbal and written descriptions, and written informed consent was obtained before commencing the study. All procedures were approved by Ethics Review Committee of Waseda University on Research with Human Subjects (approval number: 2017-096) following the Declaration of Helsinki. This study was registered in the University Hospital Medical Information Network Clinical Trials Registry (UMIN000029589).

Dietary intervention

All participants were requested to take one pack of prebiotic food every day for 12 weeks. The participants were instructed to mix it with their daily diet according to their preference. This milk flavored prebiotic food was produced by Morinaga Milk Industry Co., Ltd. (Tokyo, Japan) as per the request from the research team. One pack (25 g) provided 100 kcal of energy, 2.5 g of inulin, 1.0 g of lactulose, 100 mg

of calcium, and 0.5 µg of vitamin D (Table 1). Participants were instructed to record their status of food intake during the intervention period.

Table 1
Nutritional composition of the prebiotic food used in this study

	Amount per bag
Energy (kcal)	100
Protein (g)	3.5
Fat (g)	2.8
Carbohydrate (g)	15.0
Inulin (g)	2.5
Lactulose (g)	1.0
Calcium (mg)	100
Vitamin D (µg)	0.5
Dietary fiber (g)	1.0

Anthropometric and body composition measurements

Height was measured to 0.1 cm using a stadiometer (YG-200, Yagami, Aichi, Japan). Body weight was measured by the participants themselves to the nearest 0.05 kg using an electronic scale (UC-321, A&D, Tokyo, Japan) after urination and under morning fasting conditions during the intervention period. Body mass index (BMI) was calculated by dividing the body weight by height squared. Fat mass, fat-free mass (FFM), bone area, bone mineral content (BMC), bone mineral density (BMD), and z-score of the whole body were measured using dual-energy X-ray absorptiometry (DXA) (QDR-4500DXA Scanner, Hologic; Marlborough, MA) during the pre- and post-intervention periods. All scans were performed and analyzed using Hologic software by an orthopaedic surgeon who is one of the coauthors of this study.

Dietary intake

The total energy intake (TEI) and macro- and micronutrient intake were investigated using a 3-day consecutive weighed food record with a food scale at pre-, mid-, and post-intervention periods. Participants were instructed to record their daily consumption and to take photos of all foods and beverages, including their labels. After completing the dietary record forms, the participants were interviewed by a well-trained dietitian, and their nutritional status was analyzed using the nutritional analysis software (Wellness 21, Top Business System, Okayama, Japan). Energy, macronutrient, and micronutrient intakes were calculated based on the Japanese Standard Food Composition Table 2015 published by the Ministry of Education, Culture, Sports, Science and Technology.

Energy expenditure

Total energy expenditure (TEE) was measured for consecutive 7-days with an accelerometer (HJA-750C Active Style Pro, Omron Healthcare Co., Ltd., Kyoto, Japan) at pre-, mid-, and post-intervention periods. These monitors were constantly updated with the most recent values for the participant's height, weight, and age. All participants were requested to wear an accelerometer on their waist, except when bathing and swimming. Moreover, the participants were instructed to record the time period of attaching and detaching the device, getting into bed and waking up, and doing their daily chores.

Analysis of fecal gut microbiota

Fecal gut microbiota samples were collected pre-intervention, at 1, 2, 3, 4, and 8 weeks, and post-intervention. These samples were collected by the participants themselves in collection tubes with guanidine thiocyanate solution (Techno Suruga Laboratory Co., Ltd., Shizuoka, Japan). DNA was extracted from these samples, and the samples were analyzed by the terminal restriction fragment length polymorphism (T-RFLP) method. All fecal gut microbiota samples were analyzed using T-RFLP by Techno Suruga Laboratory Co., Ltd. (Shizuoka, Japan). The abundance of each terminal restriction fragment (T-RF) was calculated by first dividing them into 29 operational taxonomic units (OTUs) by the previous researches [16, 17]. Each OTU was quantified as a percentage of the total OTU area, expressed as a percentage of the area under the curve (%AUC). The reference database, human fecal gut microbiota T-RFLP profiling (<http://www.tecsrg-lab.jp/>), was used to putatively identify the bacteria in each classification unit and the corresponding OTU.

Blood samples

Blood samples at pre-intervention, at 4 and 8 weeks, and post-intervention were collected in the morning after overnight fasting for bone metabolism turnover marker, bone-specific alkaline phosphatase (BAP), tartrate-resistant acid phosphatase 5b (TRACP-5b), 25-hydroxyvitamin D₃ (25(OH)D₃), and estradiol (E₂). After clotting, the blood samples were separated via centrifugation at 3000 rpm for 15 min (Spectrafuge™ 6C, Labnet International Inc., USA), and the remaining serum was separated into aliquots and frozen at -80 °C until further analysis. TRACP-5b was analyzed using the enzyme immunoassay method. BAP was analyzed using an electro chemiluminescent immunoassay method. 25(OH)D₃ was analyzed using the liquid chromatography-tandem mass spectrometry method. E₂ was determined using a chemiluminescent immunoassay. All blood samples were analyzed by the LSI Medience Corporation (Tokyo, Japan).

Statistical analyses

Data are presented as means ± standard deviations (SD) or medians with interquartile range. The Kolmogorov–Smirnov test was used to check the non-normal data. In order to achieve homogeneity, data were log transformed. Statistical analyses were performed using statistical software (SPSS ver. 24.0, IBM Corporation, Armonk, NY, USA). Repeated-measures one-way analysis of variance (ANOVA) was used to assess the differences in nutritional status, exercise status, fecal gut microbiota analysis, and blood sample analysis. Bonferroni *post hoc* corrections for equal variance and the Games–Howell procedure for unequal variance were used to identify significantly different measurement points. Paired t-test or Mann–

Whitney test were used to compare between the physical characteristics data during the pre- and post-intervention period. Statistical significance was set at $p < 0.05$ in two-sided tests for all analyses.

Result

The intake rate of prebiotic food during intervention was $76\% \pm 21\%$. The comparison of body composition and bone parameter variables at pre- and post-intervention is presented in Table 2. No significant changes in any parameter, including body weight, were observed after the intervention. The energy status and macro- and micronutrient intakes of the participants during intervention are presented in Table 3. TEI and TEE at mid- and post-intervention were not significantly different compared with that at pre-intervention.

Table 2

Physical characteristics of the participants during the interventions

	Pre			Post			<i>p</i> -value
Age (y)	20	±	1	-	-	-	-
Height (cm)	160.2	±	5.3	-	-	-	-
Body weight (kg)	51.2	±	5.3	51.3	±	5.3	0.620
BMI (kg/m ²)	19.9	±	1.7	20	±	1.8	0.557
Fat mass (kg)	9.8 (7.0, 10.6)			9.3 (7.4, 10.9)			0.184
Fat-free mass (kg)	40.6	±	4.2	40.7	±	4.4	0.902
Bone area (cm ²)	1845	±	118	1842	±	125	0.620
BMC (g)	2109	±	189	2096	±	215	0.557
BMD (g/cm ²)	1.143	±	0.069	1.136	±	0.066	0.184
z-score	1.3	±	1.2	1.2	±	1.2	0.242

Data were expressed as mean ± SD or median (inter quartile range); BMI, body mass index; BMC, bone mineral contents; BMD, bone mineral density. *p* values < 0.05 represent significantly different mean.

Table 3

Energy status, macro nutrient, and micronutrient intakes of the participants

	Pre		Mid		Post		<i>p</i> -value
Energy status							
TEI (kcal)	1724	± 441	1672	± 524	1603	± 575	0.295
TEE (kcal) [†]	2999 (2819, 3118)		3014 (2846, 3221)		3076 (2877, 3242)		0.405
Macronutrients							
Protein (g)	63.7	± 24.1	65.8	± 28.5	63.8	± 30.4	0.774
Fat (g)	59.1	± 18.1	60.8	± 22.1	57.3	± 26.2	0.688
Carbohydrate (g)	228.4	± 62.0	209.2	± 66.8	203.4	± 67.1	0.069
Micronutrients							
Calcium (mg) [†]	418 (316, 713)		480 (290, 765)		364 (231, 694)		0.088
Iron (mg) [†]	5.4 (3.7, 10.3)		10.6 (5.9, 13.1)		5.2 (3.6, 10.4)		< 0.001
Vitamin D (µg) [†]	4.4 (2.4, 7.9)		3.3 (2.3, 7.6)		3.6 (1.7, 9.6)		0.998
Vitamin K (µg) [†]	146 (68, 326)		127 (58, 382)		171 (68, 314)		0.787
Dietary fiber (g)	11.5	± 6.6	12.7	± 6.0	10.7	± 6.3	0.086
Data were expressed as mean ± SD or median (inter quartile range).							
† Log transformed variables for non-normally distributed variables were used for analysis.							
Mid date revealed the nutritional composition of prebiotic food intake.							

Human gut microbiota composition mainly comprises members of approximately 10 bacterial flora groups (Fig. 1). As illustrated in Fig. 1, one of the fecal gut microbiota significantly changed during the intervention. The occupation ratio of *Bifidobacterium spp.* was significantly increased at 3 and 4 weeks (18.0% ± 8.3% and 17.6% ± 8.5%, respectively) compared to that during pre-intervention (11.7% ± 7.3%) ($p = 0.019$ and $p = 0.035$, respectively), and further an increasing trend was observed at 2 week and post-intervention (17.3% ± 7.0% and 17.1% ± 7.6%, respectively) compared to pre-intervention ($p = 0.057$ and $p = 0.073$, respectively). No statistically significant changes were observed in the composition of other fecal gut microbiota during intervention.

Figure 2a, 2b, and 2c depict the changes in bone turnover metabolism markers at pre-intervention, weekly, and post-intervention period. Serum TRACP-5b level decreased significantly at 8 weeks and post-intervention in this study. *Post hoc* testing revealed that TRACP-5b at post-intervention (363 ± 112 mU/dL) was lower than that at pre-intervention (430 ± 154 mU/dL) ($p = 0.018$), and TRACP-5b at 8 weeks revealed a decreasing trend compared to that with the pre-intervention ($p = 0.059$). No significant changes were observed in other bone turnover metabolism markers during the intervention. E₂ levels from pre- to post-intervention were 46 pg/ml (median; 33–87, interquartile range), 48 pg/mL (median; 33–95, interquartile

range), 55 pg/mL (median; 31–89, interquartile range), and 44 pg/mL (median; 25–92, interquartile range), respectively. Moreover, E_2 levels did not reveal any significant change during the intervention.

Discussion

The main finding of the present study revealed that consuming inulin and lactulose fortified prebiotic food for 12 weeks balanced the gut microbiota, resulting in suppressed bone resorption marker without body weight gain among the Japanese female athletes. This finding provides novel insights into the existing literature indicating that the combination of prebiotics may play a pivotal role in modifying gut microbiota and bone turnover metabolism markers among the Japanese female athletes.

Inulin is an indigestible, water-soluble dietary fiber, that is, a polysaccharide with a glucose molecule attached to the reducing end of the fructose chain [18], whereas lactulose is a synthetic disaccharide comprising fructose and galactose that cannot be digested or absorbed by humans [19]. Several studies have reported that short-term administration of inulin or lactulose increased *Bifidobacterium* spp. in the gut microbiota [10, 20, 21], which reduced bone resorption markers in postmenopausal women and healthy young men [22–24]; however, only limited studies have demonstrated that supplemented prebiotics change the gut microbiota and bone turnover metabolism markers in humans [24]. According to a consensus statement, prebiotics were recognized to particularly stimulate *Bifidobacterium* spp. (bifidogenesis) [13]. Short-chain fatty acids (SCFAs) are metabolites generated by gut microbial fermentation from prebiotics [25]. Indeed, a previous study reported the direct suppression of osteoclast synthesis [26]. After ingesting prebiotic food for 2 weeks, the occupation of *Bifidobacterium* spp. was higher than that before intervention and was maintained at a higher level until post-intervention. This is in accordance with the results of previous studies [10, 20, 21]. Thus, the combination of inulin and lactulose in the present study might contribute to the “prebiotic effect.”

Energy intake deficiency may exhibit uncoupled bone metabolism turnover, which decreases bone formation, increases bone resorption and/or a combination of the two, thereby consequently inducing unfavourable bone disorders. Subsequent reduction in the estrogen levels and associated reproductive dysfunction may also be indirectly influenced by bone health among the female athletes. Participants in this study were in an energy-deficient state, and the bone resorption marker during their pre-intervention was above the reference value [27]. These results were in accordance to those observed in previous studies [4, 28]. In particular, energy deficiency elevates bone resorption markers in athletes. If the balance between bone resorption and bone formation is maintained (coupling), bone mass and bone mineral contents remain balanced; however, when uncoupling in bone metabolism turnover occurs and bone resorption becomes more dominant, bone mineral density decreases. TRACP-5b is recognized as a bone resorption marker with low intra- and inter-day variations [29]. The finding of reduced bone resorption markers in this study may prove beneficial for preventing issues related to bone health among female athletes.

Adequate consumption of calcium and enough vitamin D status contributes to bone health [30, 31]. Daily consumption of milk fortified with calcium 1,200 mg/d and vitamin D 15 µg/d resulted in improved serum 25 (OH) D₃ and suppressed bone turnover marker in postmenopausal women [32]. Serum 25(OH)D₃ plays a crucial role in maintaining bone health in humans [33]. In this study, any significant increase in serum 25(OH)D₃ status did not observe. Taking these matters into account, the amount of calcium 100 mg/d and vitamin D 0.5 µg/d in the prebiotic food may have been insufficient to improve serum 25 (OH) D₃; however, the combination of prebiotics in this study may have significantly suppressed a bone resorption marker.

Since serum E₂ plays a crucial role in maintaining normal bone health in females, De Souza et al. reported that low E₂ levels were involved with suppressed bone formation and increased bone resorption [34]. Since E₂ did not show any significant changes during this intervention, it is inferred that E₂ did not affect bone turnover metabolism.

Body composition is related to athletic performance [35–37]. During intervention, no changes were observed in BW, FFM, and FM. Cialdella-Kam et al. found that a 6-month intervention provided an extra energy (+ 360 kcal/day) that could successfully reverse the menstrual status, resulting in weight gain (+ 1.6 kg) [38]. In contrast, our study did not induce substantial weight gain (+ 0.1 kg). While habitual EI increment and body weight gain are useful strategies for solving women's health problems [8, 39], such strategies may not be practically feasible for long-distance runners and rhythmic gymnasts. Increased body fat reduces athletic performance [40]. Among the competing female athletes that require daily weight control, the prebiotic food used in this study may provide "bone protection effect" without having a significant impact on body composition. Regarding energy, macronutrient, and micronutrient intake, participants did not change their dietary intake throughout the study. A previous study suggested that an increase in certain micronutrients (i.e., calcium and vitamin D) has a favorable effect on the bone health [41, 42]. Since daily meals include various nutrients, it is necessary to interpret the results from a comprehensive perspective. Hence, no differences were observed in the bone-related micronutrients in the participants, which indicates that TRACP-5b was significantly decreased during this study.

The advantage of this study is that it was conducted in multiple events where female athletes participated in intense training every day. Previous studies were conducted in postmenopausal women and adolescent girls. The rate of bone turnover varies with age; therefore, it was unclear whether prebiotic intake in female athletes would prove beneficial for bone health. Thus, the present findings can be used as a nutritional strategy in sports fields. In contrast, this study has certain limitations. First, our participants could not perform placebo trials. Because our participants in this study had a high-performance level, it was difficult to get female athletes to commit to long-term intervention. Second, we did not measure SCFAs in fecal samples or serum proinflammatory cytokines. Thus, our study could not explicitly explain the prebiotics-gut-bone axis. Future studies should clarify the impact of prebiotic food on gut microbiota and bone turnover metabolism in other athletes.

Conclusion

We conclude that continued intake of inulin and lactulose fortified prebiotic food over 12-weeks may balance the gut microbiota, thereby resulting in improved bone resorption markers without body weight gain among the Japanese female athletes. The findings of this study can be applied to prevent bone health related disorders among the female athletes.

Abbreviations

BMI: Body mass index; FFM: Fat-free mass; BMC: Bone mineral content; BMD: Bone mineral density; DXA: Dual-energy X-ray absorptiometry; TEI: Total energy intake; TEE: Total energy expenditure; T-RFLP: The terminal restriction fragment length polymorphism; BAP: Bone-specific alkaline phosphatase; TRACP-5b: Tartrate-resistant acid phosphatase 5b; 25(OH)D₃: 25-hydroxyvitamin D₃; E₂: Estradiol

Declarations

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Availability of data and materials

Not applicable

Authors' contributions

MT and TI conceived, and MT designed the study. TI, ET, and ST supervised the data collection. TI analysed the data. TI and MT drafted the first manuscript. All authors approved the final version of the manuscript.

Ethics approval and consent to participate

All procedures were approved by the University Ethics Review Committee on Research with Human Subjects following the Declaration of Helsinki (approval number: 2017-096). A written informed consent was obtained from all subjects prior to the study.

Consent for publication

All participants were informed that their data collected may be published and signed informed consent prior to the experiment.

Competing interests

All authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Figures

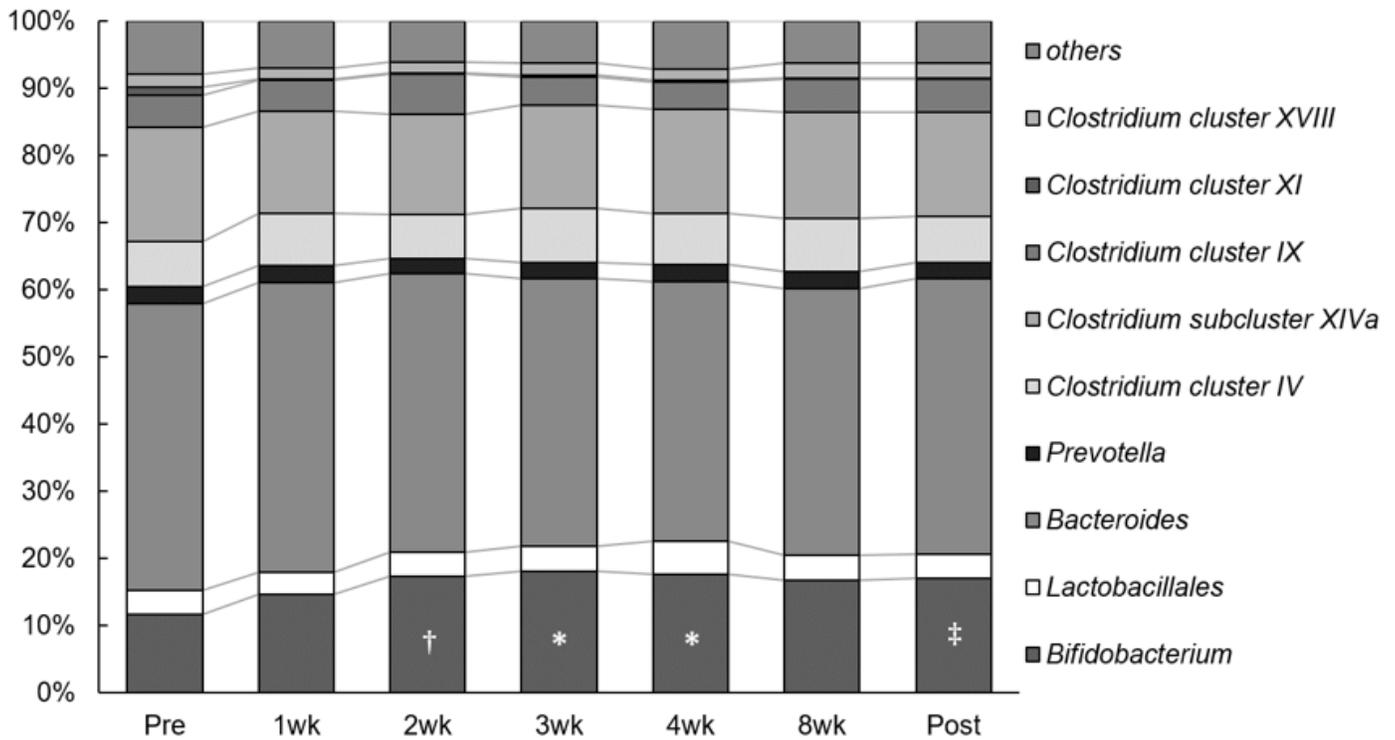


Figure 1

Change in gut microbiota diversity at 12 weeks * $p < 0.05$ versus pre-intervention at the same genus. †, ‡ $p < 0.10$ versus pre-intervention at the same genus. No significant differences were found in the gut microbiota without *Bifidobacterium* spp.

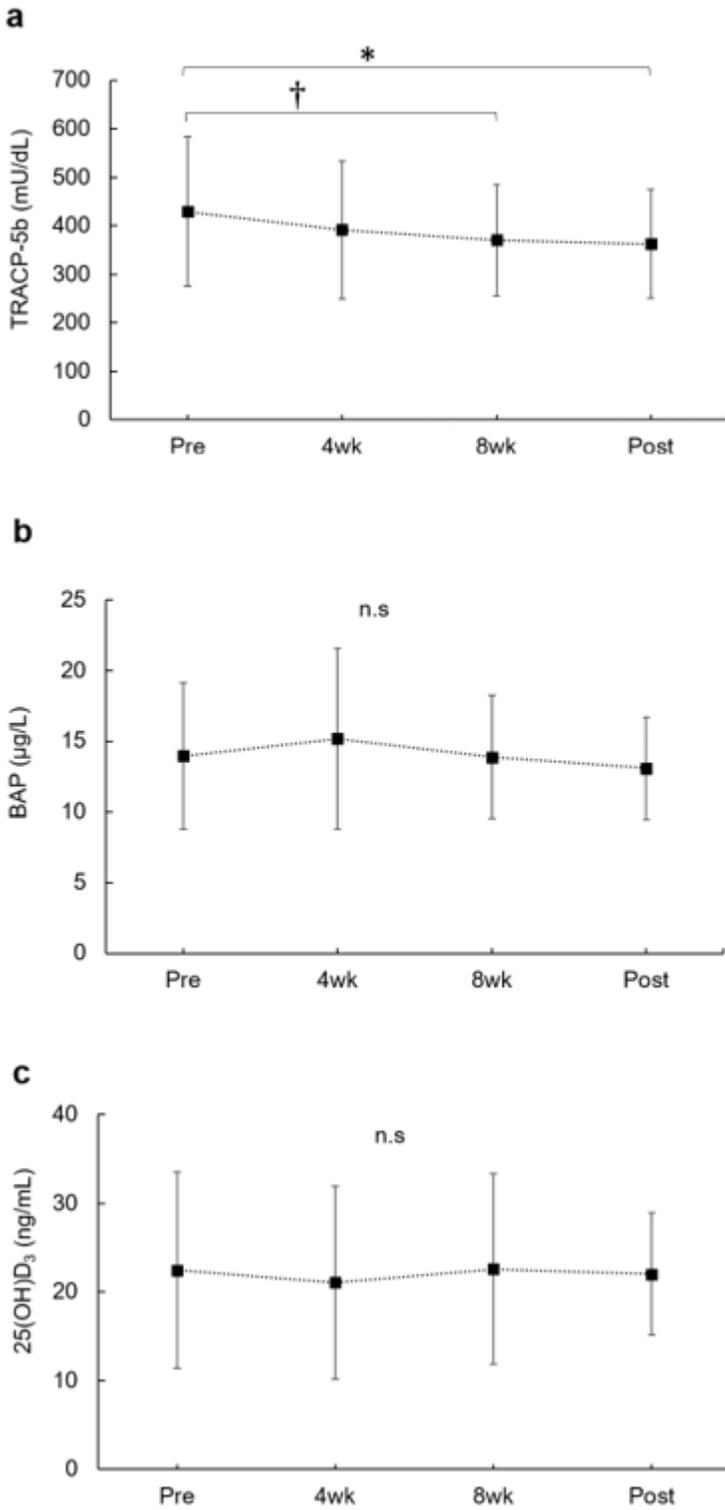


Figure 2

Change from pre-intervention for bone-related markers during 12 weeks of the intervention. (a) Change in TRACP-5b for 12 weeks. (b) Change in BAP for 12 weeks. (c) Change in 25(OH)D₃ for 12 weeks. * $p < 0.05$ was considered as statistically significant. † $p < 0.10$ was considered as trend toward a significant.