NFS Journal 3 (2016) 41–50

Contents lists available at ScienceDirect

NFS Journal

journal homepage: http://www.journals.elsevier.com/nfs-journal/

Original article

Effects of probiotics, prebiotics, and synbiotics on mineral metabolism in ovariectomized rats — impact of bacterial mass, intestinal absorptive area and reduction of bone turn-over

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ARTICLE INFO

Article history:
Received 14 December 2015
Received in revised form 9 March 2016
Accepted 14 March 2016
Available online 23 March 2016

Keywords:
L. acidophilus NCC90
Oligofructose + acacia gum
bone mineral
Bacteroides
Osteoporosis

ABSTRACT

Background: Defined prebiotics were shown to improve calcium balance and diminish bone loss. However, the effect of their combination with probiotics on gut ecology and bone metabolism has not yet been studied. We investigated whether the combination of a probiotic with a defined microbial strain results in improved bone mineralization, and whether this effect is associated with changes in gut ecology.
Methods: Eighty ovariectomized adult rats were allocated to five groups: group 1, sham-operated (SHAM); group 2–5, ovariectomized (OVX). Semipurified diets containing 0.7% calcium and 0.5% phosphorus were fed for 16 weeks, group 1 and group 2 got no supplements, group 3 (PRO) was supplemented with a potential probiotic (*Lactobacillus acidophilus NCC90*), group 4 (PRE) with prebiotics (oligofructose + acacia gum) and group 5 (SYN) with synbiotics (probiotics + prebiotics).
Results: Ovariectomy increased body weight and reduced bone weight, content of calcium, phosphorus and ash of bone, bone alkaline phosphatase (BAP), and bone structure. This was indicated by lower trabecular bone area, trabecular perimeter, and connectivity but higher epiphyseal breadth. Ovariectomy elevated the jejunal pH. The probiotic alone did not significantly affect bone mineralization and gut ecology. Rats on prebiotics had significantly higher amounts of cecal contents and lower pH in cecal and colonic contents. Their calcium balance tended to be increased ($p < 0.1$). Synbiotics reduced pH in different intestinal segments, significantly in cecum. They stimulated most the colonic absorption surface as indicated by colon weight. Only feeding synbiotics significantly prevented OVX-induced loss of calcium content in lumbar vertebrae (mg) with final values (mean ± SD) of 44.44 ± 2.94 (SHAM), 41.20 ± 4.59 (OVX), 41.63 ± 3.78 (PRO), 43.42 ± 3.07 (PRE), and 44.68 ± 2.28 (SYN). This effect was associated with higher counts of bifidobacteria in the short-term and *Bacteroides* in the long-term, and with a tendency for lower BAP with 128.7 ± 28.5 U/L vs. 155.3 ± 28.1 U/L in OVX ($p < 0.1$).
Conclusion: SYN exerted a synergistic effect on bone mineralization, presumably due to changes in gut microbiota and ecology associated with large bowel digesta weight (most likely reflecting microbial mass) and with large bowel weight (reflecting absorptive area), while bone turnover tended to be reduced as indicated by BAP.

Abbreviations: ANOVA, analysis of variance; Ar.MS, total area of mineralized structure; BAP, bone specific alkaline phosphatase; BMC, bone mineral content; BPm, trabecular bone perimeter; CFU, colony-forming unit; C.Th, cortical bone thickness; EpB, epiphyseal breadth; FOS, fructooligosaccharides; GOS, galactooligosaccharides; OVX, ovariectomized; PRE, prebiotics; PRO, probiotics; S.Ar, trabecular skeleton area; SCFA, short-chain fatty acid; SD, standard deviation; SHAM, sham-operated; SYN, synbiotics; T.Ar, bone tissue area; Tb.Ar, trabecular bone area; Tb.BrP, trabecular branch points; Tb.D, trabecular density; Tb.N, trabecular number; Tb.Pm, trabecular perimeter; Tb.Th, mean trabecular thickness; TBPf, trabecular bone pattern factor; TNF, tumor necrosis factor.

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http://dx.doi.org/10.1016/j.nfs.2016.03.001

1. Introduction

Osteoporosis is a multifactorial bone disease with increasing prevalence and importance, also because of ascending life expectancy. In the United States of America prevalence of osteoporosis is 10.3% and prevalence of low bone mass is 43.9% in adults aged 50 years and older [1]. For prevention and treatment of osteoporosis nutrition is one factor to be considered. Older adults (> 50 years) are recommended a diet that supplies adequate amounts of calcium (1000 mg/day for men and 1200 mg/day for women) [2]. Enhancers of bioavailability in the diet are a further approach to increase calcium absorption and bone mineral content (BMC), and approaches for improving bone health by bioactive foods and ingredients are discussed [3].

Prebiotics selectively stimulate the growth and/or activity of specific bacteria, mainly bifidobacteria and lactobacilli and by this deploy a microbiota-mediated health effect [4–8]. They include inulin-type fructans, fructooligosaccharides (FOS), galactooligosaccharides (GOS), as well as lactulose, sugar alcohols, resistant starch and complex polysaccharides such as acacia gum. Several non-digestible substrates, predominantly carbohydrates, are fermented in the large intestine. Among others, prebiotics increased the absorption of minerals and trace elements that have an impact on the mineralization of bone, increased BMC and bone trabecular structure, or reduced estrogen-deficiency-induced bone loss in the rat [for review see Refs. 7,9–14]. Inulin-type fructans also increased calcium absorption and bone mineralization in young healthy humans [15–17]. Probiotics are viable, defined microorganisms, in many cases bifidobacteria and lactobacilli, which alter the composition and/or activity of the microbiota of the host, provided that they have been ingested in sufficient number and survived the gastrointestinal transit. Thereby probiotics exert beneficial effects on the host's health and well-being [5,6]. Although beneficial effects of probiotics have been reviewed with respect to various diseases [6,18,19,20], there are only few studies on effects of probiotics on bone mineralization or osteoporosis [21]. A synbiotic is a combination of a specific prebiotic with a defined probiotic which exerts a beneficial effect beyond their individual effect, presumably by improving the gastro-intestinal survival and activity of beneficial microbes [5,20]. The investigated bacteria mainly belonged to the genus bifidobacteria or lactobacilli. Greater bone density in germ-free mice compared to conventional animals [22] suggests also a role of gut microbiota in bone mineralization. Accordingly, specific bacterial strains may display a probiotic potential with respect to bone preservation [12], but so far little is known about probiotics or synbiotics and their bone mineral preserving effect.

We have shown before that the beneficial effect of oligofructose on bone mineral content and trabecular structure depended on its amount in the diet, on the amount of calcium in the background diet, on the duration of intervention and on the investigated skeletal site [23]. We had observed stimulating effects of oligofructose on calcium absorption after 8 and 16 weeks but not after 4 weeks, and on calcium content and trabecular area in bone. The effects were significant if the dietary calcium was high (1%), but was less pronounced if the background diet was 0.5%. We now chose a dietary calcium content of 0.7% and phosphorus content of 0.5%. Thus the mineral contents were higher than the recommended amount for adult rats for maintenance of 0.5% for calcium and 0.3% for phosphorus, but their Ca:P ratio remained in the same range. In the present study we tested a potential probiotic strain (*Lactobacillus acidophilus NCC90*) and a potential prebiotic substance (oligofructose + acacia gum) for their short-term and long-term effect on bone mineralization of femora and lumbar vertebrae. We compared these supplements with the intake of the potential synbiotic (*L. acidophilus NCC90* plus oligofructose + acacia gum). The choice of the lactobacilli was based on a series of in vitro experiments showing enhancement of calcium absorption using human intestinal cell lines in which the *L. acidophilus* was the most promising bacterium among the ones tested (data not shown). Acacia gum was added, since it had been shown to be bifidogenic [24,25], to induce lactobacilli [25], and to be better tolerated than oligofructose alone [24,26]. To describe possible underlying mechanisms, we assessed parameters of gut ecology and performed microbiological analyses of feces.

2. Materials and methods

2.1. Study design, experimental groups, animals and diets

This experiment was approved by the German Institutional Animal Experiment Committee (Ministerium für Energiewende, Landwirtschaft, Umwelt und ländliche Räume des Landes Schleswig-Holstein). The study was performed with eighty virgin female Fisher-344 rats. Weanling rats were purchased from Harlan/Winkelmann, (Borchen, Germany). Until the age of 19 weeks, rats were fed ad libitum with a commercial standard rat diet. Two weeks before starting the intervention (study week −2) feed was switched from standard diet to a semipurified diet that was used as a control diet in studies with adult rats in our laboratory [23] providing all nutrients in sufficient amounts. Thereafter, at the age of 21 weeks (study week 0) rats were divided into five groups of 16 animals (Table 1). They were matched by body weight, and were sham-operated (SHAM) or ovariectomized (OVX, PRO, PRE and SYN).

Ovariectomy was done under anesthesia with intraperitoneal injection of xylazinhydrochloride/ketaminhydrochloride (Rompun®/Ketavet®). The intervention period of 16 weeks started by feeding 8.5 g per day of the semipurified diet (Table 2) to SHAM and OVX, or the semipurified diet supplemented with a probiotic (PRO), or with 2.5% of a prebiotic at the expense of corn starch (PRE) or with both as a synbiotic (SYN). The potential probiotic (*L. acidophilus NCC90*) was added to an acidified milk, both provided by Nestlé Research Center, Lausanne, Switzerland. The probiotic was supplied as fresh frozen bacteria culture as sets of vials stored in liquid nitrogen. Each vial contained sufficient bacteria for the preparation of one batch of probiotic feeding for 1 week. For this the acidified milk powder was reconstituted with demineralized water. One vial of thawed probiotic was freshly added to the milk slurry every week to get a concentration of $1–5 \times 10^8$ cfu per 100 g. One gram of the probiotic acidified milk was given each day, which was equivalent to $1–5 \times 10^6$ cfu per rat and day. The probiotic "yoghurt" was consumed by the groups PRO and SYN on top of their feed.

The prebiotic was a 50% mixture of Raftilose P95®, (Orafti, Belgium) and acacia gum (Fibergum®, CNI, France). The prebiotic mixture and the probiotic were provided by Nestlé Research Center, Lausanne, Switzerland. The form of calcium used in the diets was tri-calcium dicitrate tetrahydrate ($C_{12}H_{10}Ca_3O_{14}\cdot 4H_20$). To guarantee strict pair feeding the reservoirs were filled twice weekly with 8.5 g/day. In earlier studies this amount represented a restricted feed intake for about 30% and an ad libitum intake for 70% of the animals [23]. Again in this experiment, all rats had consumed all their feed after 3 or 4 days. There were no back-weights of feed. Rats had free access to the feed reservoir,

Table 1
Experimental groups.

		Dietary components				Matching	
		Ca	P	PRE	PRO	Body wt. (g)	
Group	OP	(g/kg diet)				Week 0	Global p
SHAM (Control 1)	Sham	7	5	0	No	166.7 ± 7.2	ns
OVX (Control 2)	OVX	7	5	0	No	169.6 ± 7.6	
PRO	OVX	7	5	0	Yes	169.1 ± 8.0	
PRE	OVX	7	5	25	No	169.9 ± 7.6	
SYN	OVX	7	5	25	Yes	170.7 ± 6.8	

Means ± SD, n = 15–16, p-value from ANOVA. SHAM = Sham-operation; OVX = ovariectomy;
PRO = OVX + Probiotics (1–5*10^6 cfu of *L. acidophilus NCC90/g*) mixed with yoghurt;
PRE = OVX + Prebiotics (fructooligosaccharide, Raftilose P95® + acacia gum (50:50);
SYN = OVX + Synbiotics (PRO + PRE).