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ORIGINAL CONTRIBUTION

Comparison of native or reformulated chicory fructans, or non-purified chicory, on rat cecal fermentation and mineral metabolism

Christian Demigné
Heidi Jacobs
Corinne Moundras
Marie-Jeanne Davicco
Marie-Noëlle Horcajada
Annick Bernalier
Véronique Coxam

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C. Demigné · C. Moundras
M.-J. Davicco · M.-N. Horcajada
V. Coxam
INRA, Centre Clermont-Ferrand–Theix
UMR1019, Unité Nutrition Humaine
St Genès Champanelle, 63122 France

H. Jacobs
COSUCRA-Groupe Warcoing
Warcoing, Belgium

A. Bernalier
INRA, Centre Clermont-Ferrand-Theix
Unité de Microbiologie
St Genès Champanelle, France

V. Coxam (✉ for the ASM group)
Unité Nutrition Humaine
UFR Médecine, UMR1019
Univ Clermont 1
Clermont-Ferrand, 63001 France
Tel.: +33-473/624-284
Fax: +33-473/624-638
E-Mail: coxam@clermont.inra.fr

Abstract Chicory inulin has been identified as an effective prebiotic to promote active fermentation and lactobacilli proliferation in the large intestine, and to enhance calcium (Ca) digestive absorption and deposition in bones. The aim of this study was to compare, in a growing rat model, the effects on digestive fermentations and mineral metabolism of diets containing 7.5% inulin, using either a purified native inulin ($_{NAT}$Inulin) or a reformulated inulin ($_{REF}$Inulin, based on a combination of short- and long chain fructans) or dehydrated chicory. All the inulin diets elicited a marked enlargement of the cecum and acidification of the cecal contents ($P < 0.01$) and these diets promoted succinic acid rich fermentation together with substantial amounts of short-chain fatty acids (SCFA), especially butyrate. After 1 month of adaptation, all the inulin diets strongly enhanced Ca absorption compared to controls ($P < 0.01$), but this effect was no more observed after 3 months of adaptation. Magnesium (Mg) absorption was stimulated by the inulin diets after 1 and 3 months experiment. Bone parameters were significantly affected by the chicory diet (enhanced distal bone mineral density and breaking load) whereas the purified inulin diets were less effective. In conclusion, with the present model, both $_{NAT}$Inulin and $_{REF}$Inulin exerted similar effects as to (1) cecal fermentation and profile of end-products of bacterial metabolism, (2) stimulation of Ca and Mg digestive absorption and (3) overall effects on bone parameters. The particular effects of the chicory crude fractions on digestive fermentation and bone parameters suggest possible synergisms between inulin-type fructans and other nutrients.

Key words inulin – chicory – microflora – calcium – bone – rat

Introduction

Consumption of Ca-rich foods such as milk or dairy products, or even Ca supplementation of food has been consistently recommended to prevent osteoporosis, and its consequences on fracture risk, especially in postmenopausal women [20]. Nevertheless, results from recent prospective cohort studies suggests that Ca intake is not systematically associated with hip fracture risk and no reduction of this risk could be identified with Ca supplementation [3]. In fact, it has

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been recognized that the nutritional prevention of osteopenia and osteoporosis should also pay attention to various other nutrients such as fermentable carbohydrates, proteins, potassium (K), Mg and various vitamins (C, K, B_{12} for example) [8]. Some of these nutrients stimulate the apparent absorption of Ca, others are more effective to reduce calciuria or to improve Ca fixation in the bone matrix [4]. In this view, it has been demonstrated that ingestion of non-digestible carbohydrates, especially fructo-oligosaccharides (FOS) or fructans in general, increases Ca absorption in rats [5, 6, 13, 25] or in humans [7, 25]. The effects of fructans are hightly dependent on modifications of the digestive microflora, namely an increase in *bifidobacteria* and some possible alterations of *clostridia* populations, which generally results in an acidification of the colonic lumen [15, 21]. These changes also lead to variations in the SCFA concentration, particularly that of butyrate and, in some cases, of propionate [13, 21, 26].

Fructans are reserve carbohydrates comprising fructose molecules linked or not to a terminal sucrose molecule [21], particularly abundant in plant species such as chicory, dahlia or Jerusalem artichoke. Inulin is a generic term to cover all $\beta(2,1)$ linear fructans and chicory inulin is a complex mixture of linear β fructans with a degree of polymerization (DP) ranging from 2 to 60. Through partial enzymatic hydrolysis, an oligofructose (OFS) fraction may be obtained with a DP of 2–8; (average DP: 4) whereas specific separation technologies yield long-chain inulin fractions (DP 10–60; average DP > 20) [30]. Specific products are obtained by blending a long-chain inulin and oligofructose fractions. The aim of this reformulation of inulin is to optimize the rate of fermentation by providing OFS (readily fermented in the proximal colon) on the one hand, and long-chain inulin (supposed to be less rapidly fermented, hence more available in the distal colon) on the other hand. This should prevent (1) excessively acidic fermentation in the proximal colon together with lactic acid accumulation and gas over-production, and (2) a shortage of substrates for the distal colonic microflora with untoward consequences (bacteriolysis, abnormal fermentation) [15]. Some investigations suggest that longer chain fructan fractions could be more effective than OFS for stimulating Ca absorption in vivo [10, 17] and *in vitro* prebiotic effects [34]. To determine whether fructans such as purified native inulin or reformulated inulin (through blending of OFS and long-chain inulin fractions), or non-purified chicory (inulin: 50–55 % of dry weight, also providing other fibers, minerals and various phytomicronutriments) would exert different effects on Ca status and/or bone characteristics, groups of rats were adapted to 7.5% inulin diets, differing in the origin of the inulin, compared to an inulin-free control diet. The study was carried out using diets designed to mimic some features of western human diets (acidogenic, moderate Ca, Mg and K content, high Na content), know to exert unfavourable effects on Ca status [12].

Materials and methods

Experimental design

Animals

Dietary factors liable to prevent osteopenia/osteoporosis have been frequently evaluated in aged animals (for example ovariectomized female rats) as a model for human postmenopausal osteoporosis [24, 25]. Nevertheless, it is now recognized that the earlier step of peak bone mass (PBM) constitution in young adults is critical in the proneness to osteopenia 20 or 30 years later and that PBM is also affected by lifestyle factors including diet and physical activity [31]. Therefore, young adult rats were used as the animal model. The male Wistar rats used in the experiment were purchased from Janvier (Le Genest St Isle, France) and housed individually. The animals were subjected to 12–12 h light–dark cycles, fed ad libitum and had free access to demineralized water. The study was conducted in accordance with the regional Ethics Committee (France).

Diet conditions

At the beginning of the study, the rats were 3 months old and the experiment continued for a 3 further months. During a 2-week adaptation period, the animals were maintained on a standard diet to record their daily food intake and body weight. Then, they were randomly assigned to one of four dietary groups ($n = 10$ animals per group): a control diet group and three inulin diet groups. The control diet was designed to mimic the 'western dieting' and its proneness to latent metabolic acidosis using: (1) a level of protein slightly in excess of maintenance needs (20% casein), (2) a relatively high Na/ K ratio, (3) a provision of major cations such as chloride (NaCl, KCl) or phosphate ($CaHPO_4$) and (4) omission of potentially alkalinizing anions such as citrate or carbonate. Two of the inulin diet groups included purified chicory inulin, either a reformulated inulin enriched in short-chain and long-chain fructans ('$_{REF}$Inulin' group) or a native inulin ('$_{NAT}$Inulin' group), while the third inulin group included dehydrated chicory

Table 1 Composition of the diets, daily food intake and body weight gain

Diet group	Control	$_{REF}$Inulin	$_{NAT}$Inulin	Chicory
Composition (g/kg)				
Casein	200	200	200	200
Sucrose	150	150	150	150
Corn oil	50	50	50	50
NaCl	15	15	15	15
KCl	4.8	4.8	4.8	4.8
$CaHPO_4$, $2(H_2O)$	21.3	21.3	21.3	21.3
MgO	0.8	0.8	0.8	0.8
$_{REF}$Inulin*	0	81.7	0	0
$_{NAT}$Inulin*	0	0	80.6	0
Dehydrated chicory[a]	0	0	0	141.5
Trace elements mix (AIN-93)[b]	10	10	10	10
Vitamin mix (AIN-93)[b]	10	10	10	10
Wheat starch (*qs* 1,000 g)	538	456	458	397
Sodium	5.90	5.90	5.90	6.60
Potassium	2.51	2.51	2.51	3.70
Calcium	4.95	4.95	4.95	5.96
Magnesium	0.47	0.47	0.47	0.68
Food intake (FI)[c] (g/d)				
FI after 1 month experiment	25.8 ± 1.7	24.4 ± 2.2	24.1 ± 1.4	28.1 ± 1.6
FI after 3 months experiment	22.0 ± 0.5	22.6 ± 1.5	21.9 ± 0.4	22.8 ± 1.0
Body weight (bw)) (g)				
bw after 1 month experiment	438 ± 4	431 ± 5	428 ± 5	450 ± 7
bw after 3 months experiment	631 ± 16	584 ± 12	600 ± 16	627 ± 17

[a] $_{REF}$Inulin: 91.8% inulin; $_{NAT}$Inulin: 93.0% inulin; Dehydrated chicory: 53.0% inulin
[b] AIN-93; Bethlehem, PA, USA
[c] No significant difference between the diet groups after 1 month or 3 months experiment, except for bw after 1 month experiment between '$_{Nat}$Inulin' and 'Chicory' diet groups. The differences between 1 month experiment and three months experiment were significant, except for food intake in the $_{REF}$Inulin and $_{NAT}$Inulin diet groups

('Chicory' group). The $_{REF}$Inulin batch contained 91.8% inulin, the $_{NAT}$Inulin batch 93.0% and the 'Chicory' batch 51.7%; the quantities added at the expense of starch were adjusted to obtain a final 7.5% inulin level in the diets (Table 1).

During the experiment, body weight was recorded twice weekly. After one month and three months exposure to the diets, the rats were transferred into Nalgene metabolic cages (UAR, Villemoisson, 93360 Epinay/Orge) with feeder chamber outside the cage, to minimize food spillage. The separator device allows fecal pellets to roll downside of a stainless steel funnel (also directing urine to a collector vessel), to be collected in tubes. After a 3d-adaptation period, food intake was controlled daily, and urine and feces were collected for three consecutive days. At the end of the experiment, rats were fasted for 12 h then anesthesized by a mixture of Imalgen 1000 (Merial, Lyon, France) 0.75 ml/kg bw and Vetranquil 1% (CEVA santé Animale, Libourne, France) 0.25 ml/kg bw. Blood and the cecum were collected then the animals were killed by an overdose of anesthesia. Femurs were cleaned from adjacent tissues and withdrawn. Left femur was harvested in saline solution (9 g NaCl/l) and frozen (−20°C) until mechanical testing. Right femur was placed in 80% alcohol until BMD was measured.

Physical measurements

Bone mineral density

BMD and BMC were assessed by dual-energy X-ray absorptiometry, using a Hologic QDR-4500 A X-ray bone densitometer (Hologic, Massy, France). Total femoral BMD, metaphyseal BMD and diaphyseal BMD were determined. For metaphyseal BMD and diaphyseal BMD measurements, scans were cut and analyzed as follows: the first cut of the femur was performed at the upper third, and the next cut was made at the lower third. Diaphyseal BMD, which is rich in cortical bone, corresponded to the density of the second third of the femur. Metaphyseal BMD, which mainly contains cancellous bone, was calculated as the mean of the femoral proximal metaphysis density and the femoral distal metaphysis density.

Femoral mechanical testing

Femoral length and mean diaphyseal diameter were measured with a precision caliper (Mitutoyo, Shropshire, UK). The femoral failure load was determined using a three-point bending test, with a Universal Testing Machine (Instron 4501, Canton, MA, USA). The two lower supports were separated by a distance

of 20 mm and an upper crosshead roller was applied in front of the middle of the bone until failure at a speed of 0.5 mm/min to guarantee that 85–90% of the bone flexure was due to bending [27].

Biochemical analysis

Cations and anions analysis

Urinary Ca, Mg and K were determined by atomic absorption spectrophotometry (Perkin Elmer 400, Norwalk, CT, USA). For atomisation, each sample was diluted appropriately with distilled water and 0.1% lanthanum chloride (Ca, Mg determination) or 0.1% cesium chloride (K determination). For analysis of anions, biological samples were diluted with milliQ water (urine 400-fold, cecal supernatants 1,000-fold). Cecal supernatants were obtained after 15 min centrifugation (8,000*g*) of around 300 mg of cecal contents. The anions (inorganic and organic, including SCFA and lactate) were analyzed using a DX320 Dionex (Sunnyvale, CA, USA) chromatograph on an AS-11 pellicular column with appropriate standards.

Osteoblastic activity

Plasma osteocalcin (OC) was measured by RIA, using rat 125I-labeled OC, a goat anti-rat OC antibody and a donkey anti-goat secondary antibody (Biochemical Technologies, Stoughton, MA, USA). The sensitivity was 0.01 ng/ml. The intra- and inter-assay precisions were 6.8 and 8.9%, respectively.

Bone resorption

The urinary deoxypyridinoline (DPD) excretion rate (nmol/24 h) was determined by competitive RIA, using a rat monoclonal anti-DPD antibody adsorbed to the inner surface of a polystyrene tube and 125I-labeled DPD (Pyrilinks-D RIA kit, Metra Biosystems, Mountain View, CA, USA). The sensitivity was 2 nmol/l. The intra- and inter-assay precisions were 4 and 6%, respectively. A remodeling index could therefore be calculated as the $OC/DPD_{(creatinine)}$ ratio.

Statistical methods

Results are expressed as means with their standard errors and inulin source effects were determined by one-way ANOVA. Significant differences between groups were identified by Tukey's tests and a trend test analysis was also performed for bone parameters (Instat/GraphPad Software, La Jolla, CA, USA).

Results

Food intake, body weight gain and cecal fermentation

As shown in Table 1, food intake was 25.8 g/d after 1 month of adaptation in controls, and there was no significant difference between the diet groups (indicating that the bitter sesquiterpene lactones of chicory did not alter food intake in the 'Chicory' diet group). There was a significant decline of food intake after three months exposure to the diets in rats adapted to the 'Control' or 'Chicory' diet ($P<$) but not in those adapted to purified inulin diets. The body weight of the rats ranged from 428 to 450 g after one month of adaptation (differing significantly only between the '$_{NAT}$Inulin' and 'Chicory' diet groups), and from 584 to 631 g after 3 months experiment. The initial daily weight gain (data not presented) was 8.08 ± 0.66 g/d in controls and it declined markedly after three months adaptation (1.43 ± 0.43 g/d in controls); no significant difference between diet groups being foound at 1 or 3 months experiment. There was a significant enlargement of the cecum in rats adapted to the inulin diets ($P < 0.01$), compared to controls, but the highest effect (cecum weight 9.80 g) was found in rats adapted to the 'Chicory' diet (Table 2). There was in parallel a significant rise in the cecal wall weight in rats adapted to the purified inulin diets (3.0–3.5 g vs. about 1 g in controls), less marked in those fed the 'Chicory' diet. The digestive content mass (total cecal weight-cecal wall weight difference), which governs the pool size of cecal compounds, was much greater in the 'Chicory' diet group than in the '$_{REF}$Inulin' and '$_{NAT}$Inulin' diet groups (7.23 vs. 3.81 and 4.74 g, respectively). The presence of gas in the cecum was noticed in the purified inulin diet group, unlike in the 'Control' and 'Chicory' diet groups. The cecal content pH was close to neutrality in control rats and definitely acidic in rats fed the inulin diets, with means ranging from 5.7 to 5.9 ($P < 0.001$ vs. 'Control', no significant difference between inulin diet groups).

Anionic end-products of bacterial metabolism and inorganic anions, expressed in terms of cecal pool (concentration × cecal content volume), displayed qualitative and quantitative differences between the 'Control' group (limited amounts of SCFA) and the inulin diet groups (larger amounts of SCFA, together with succinate and lactate) (Table 2). There was also a difference between the purified inulin diet groups (presence of lactate) and the 'Chicory' diet group (lactate undetectable). In 'Control' rats, SCFA were essentially acetate (73.4% of total SCFA) together with small amounts of propionate and butyrate. In rats fed the inulin diets, acetate was still the major SCFA but

Table 2 Physiological parameters and anions pools in the cecum

Diet group	Control	$_{REF}$Inulin	$_{NAT}$Inulin	Chicory
Cecal physiological parameters				
Cecum weight (g)	3.05 ± 0.16^{a}	6.77 ± 0.45^{b}	8.25 ± 0.41^{bc}	9.80 ± 0.49 ^{c}
Cecum wall weight (g)	1.15 ± 0.07^{a}	2.96 ± 0.27^{b}	3.51 ± 0.21^{b}	2.56 ± 0.26^{b}
Cecal content (g)	1.90 ± 0.60^{a}	3.81 ± 0.41^{b}	4.74 ± 0.31^{b}	7.23 ± 0.53^{b}
Cecal pH	6.68 ± 0.06^{b}	5.94 ± 0.15^{a}	5.66 ± 0.17^{a}	5.83 ± 0.18^{a}
Cecal anions pools (μmoles)				
Acetate (AC)	96.9 ± 7.0^{a}	156.1 ± 22.7^{b}	189.3 ± 12.3^{bc}	415.1 ± 46.3^{b}
Propionate (PR)	25.8 ± 1.7^{a}	42.2 ± 7.7^{ab}	49.8 ± 7.8^{b}	119.6 ± 12.4^{c}
Butyrate (BU)	9.2 ± 1.0^{a}	63.9 ± 15.6^{b}	72.2 ± 12.5^{b}	88.6 ± 11.9^{b}
AC/PR/BU molar ratio	73.4/19.5/7.1	59.5/16.1/24.4	60.8/16.0/23.2	66.6/19.2/14.2
Lactate	Traces	27.9 ± 11.3^{b}	41.7 ± 18.6^{b}	2.2 ± 0.1^{a}
Succinate	12.7 ± 3.8^{a}	108.6 ± 24.7^{b}	163.9 ± 28.2^{b}	259.5 ± 56.5^{b}
Chloride	34.8 ± 3.3^{a}	32.2 ± 3.6^{a}	28.0 ± 4.8^{a}	68.7 ± 8.8^{b}
Sulfate	8.3 ± 0.6^{a}	8.9 ± 1.7^{a}	9.4 ± 0.9^{a}	8.0 ± 0.8^{a}
Phosphate	1.6 ± 0.1^{a}	41.8 ± 8.2^{b}	54.9 ± 8.7^{b}	61.0 ± 14.4^{b}

Values are means ± SEM for 10 rats in each group. Values not sharing the same superscript are significantly different ($P \leq 0.05$)

large amounts of propionate and butyrate were also present, especially in the 'Chicory' diet group. Differences between this last group and the 'Control' group were highly significant ($P < 0.001$) whereas differences with the '$_{REF}$Inulin' and '$_{NAT}$Inulin' diet groups were not significant, except for acetate and butyrate. In terms of molar ratio, the butyrate ratio was particularly high in the '$_{REF}$Inulin' and '$_{NAT}$Inulin' diet groups (24.4 and 23.3%, respectively) compared to the 'Chicory' diet group (14.2%). Inorganic anions were also altered by dietary inulin, especially phosphate which was present in small amounts in 'Control' rats, but was considerably increased by the inulin diets (both concentration and pool values).

Mineral balance

Intake of the major cations (Na, K, Ca, Mg) was very similar in the 'Control', '$_{REF}$Inulin' and '$_{NAT}$Inulin' diet groups, whereas it was substantial higher in the 'Chicory' diet group. After 1 month of exposure to the experimental diets, lower values of the fecal excretion of Ca (−36 to −72%, $P < 0.01$) and Mg (−73 to −78%, $P < 0.001$) were observed in rats adapted to the purified inulin diets ($P < 0.01$) than in 'Control' rats (Table 3). The K excretion was slightly higher than in 'Control' rats ($P < 0.05$) in the purified inulin diet groups, and much higher in the 'Chicory' diet group ($P < 0.01$). Urine Ca excretion was not significantly altered, whereas Mg excretion was significantly greater than in 'Control' rats in all the inulin diet groups. The apparent absorption of Ca was +19 mg/d in 'Control' rats (14% of Ca intake), but markedly higher ($P < 0.01$) in rats adapted to the '$_{REF}$Inulin' and '$_{NAT}$Inulin' diet groups (+51 and +58 mg/d, 40–46% of Ca intake), without any difference between these two groups. In rats fed the 'Chicory' diet, the Ca balance was also higher than in 'Control' rats

Table 3 Parameters of the mineral metabolism after 1 month exposure to the experimental diets

Diet group	Control	$_{REF}$Inulin	$_{NAT}$Inulin	Chicory
Intake (mg/d)				
Calcium	138 ± 4^{a}	127 ± 5^{a}	127 ± 5^{a}	183 ± 7^{b}
Magnesium	10.0 ± 0.3^{a}	9.6 ± 0.4^{a}	9.3 ± 0.4^{a}	14.6 ± 0.7^{b}
Potassium	62 ± 2^{a}	60 ± 3^{a}	58 ± 2^{a}	89 ± 4^{b}
Fecal excretion (mg/d)				
Calcium	119 ± 8^{b}	76 ± 10^{a}	69 ± 8^{a}	133 ± 4^{b}
Magnesium	6.3 ± 0.6^{b}	1.7 ± 0.2^{a}	1.4 ± 0.3^{a}	6.5 ± 0^{b}
Potassium	1.9 ± 0.5^{a}	4.4 ± 0.8^{b}	5.0 ± 0.8^{b}	12.0 ± 1.7^{c}
Urine excretion (mg/d)				
Calcium	3.0 ± 0.6	4.1 ± 0.4	4.5 ± 0.4	3.3 ± 0.2
Magnesium	3.2 ± 0.3^{a}	6.3 ± 0.6^{b}	6.2 ± 0.4^{b}	5.8 ± 0.4^{b}
Potassium	44 ± 4^{a}	40 ± 3^{a}	39 ± 1^{a}	82 ± 4^{b}
Apparent digestive absorption (mg/d)				
Calcium	19 ± 9^{a}	51 ± 7^{b}	58 ± 12^{b}	50 ± 4^{b}
Magnesium	3.7 ± 0.6^{a}	8.0 ± 0.6^{b}	7.9 ± 0.5^{b}	8.0 ± 0.5^{b}
Potassium	60 ± 3^{a}	55 ± 4^{a}	51 ± 3^{a}	82 ± 4^{b}
Apparent retention (mg/d)				
Calcium	16 ± 5^{a}	47 ± 8^{b}	53 ± 12^{b}	47 ± 5^{b}
Magnesium	0.5 ± 0.3^{a}	1.6 ± 0.4^{a}	1.7 ± 0.3^{a}	3.3 ± 0.5^{b}
Potassium	7 ± 2^{a}	15 ± 3^{b}	14 ± 3^{b}	3 ± 2^{a}

Values are means ± SEM for 10 rats in each group. Values not sharing the same superscript are significantly different ($P \leq 0.05$)

(+50 mg/d) but, since this group had the highest supply of Ca, absorption represented only 27% of Ca intake. Since calciuria represents a minor percentage of dietary Ca intake (2–4%), retention mirrored the digestive absorption, with definitely positive values in rats adapted to the inulin diets. The apparent absorption of Mg (+3.7 mg/d in control rats) was almost doubled in rats fed the various inulin diets, up to around +8 mg/d. In contrast to Ca, Mg urine excretion represented a substantial percentage of the intake and, as a result, retention values were relatively low (0–2 mg/d), except with the 'Chicory' diet (+3.3 mg/d). The major part of dietary K was absorbed in the intestine and recovered in urine,

Table 4 Parameters of bone metabolism

Diet group:	Control	$_{REF}$Inulin	$_{NAT}$Inulin	Chicory	*P* for trend
Bone mineral content (g)	0.646 ± 0.056[a]	0.671 ± 0.057[a]	0.688 ± 0.084[a]	0.706 ± 0.047[b]	0.041
Diaphysal bone mineral density (g/cm^2)	0.264 ± 0.004[a]	0.269 ± 0.006[a]	0.274 ± 0.006[a]	0.273 ± 0.005[b]	0.026
Metaphysal bone mineral density (g/cm^2)	0.264 ± 0.004	0.266 ± 0.004	0.268 ± 0.005	0.273 ± 0.004	0.060
Femoral failure load (Newtons)	146.2 ± 3.7	152.4 ± 6.6	149.8 ± 5.4	164.2 ± 7.0	0.056
Plasma Osteocalcin (nmol/l)	2.48 ± 0.09	2.45 ± 0.10	2.57 ± 0.13	2.34 ± 0.12	>0.10
Urine DPD excretion rate (nmol/24 h)	15.1 ± 1.9	13.8 ± 2.2	12.8 ± 1.5	12.8 ± 1.2	>0.10
Remodeling index (OC/DPD ratio)	0.164	0.173	0.201	0.182	

Values are means ± SEM for 10 rats in each group. Values not sharing the same superscript are significantly different ($P \leq 0.05$)

but K retention was slightly higher in the purified inulin diet groups.

After 3 months exposure to the diets, there was practically no change in Mg and K intake/fecal excretion/urine excretion. On the other hand, some of the Ca balance parameters were altered (Fig. 1), essentially the level of Ca fecal excretion: the Ca balance was thus slightly negative in 'Control' rats (−11 mg/d), whereas it was close to 0 in the two groups of rats adapted to the purified inulin diets and negative in rats adapted to the 'Chicory' diet −24 mg/d).

Chicory contains some citrate and malate anions, together with various cations (K, Mg, Ca), but this was not sufficient to exert a noticeable alkalinizing effect since the urine pH in the 'Chicory' group did not differ from that in the other inulin diet groups, even if slight but detectable citraturia (3 mg/d, to compare to around 1 mg/d in the other groups, data not presented) and a relatively low calciuria and magnesiuria was observed in this diet group.

Bone status parameters

Total bone mineral content (BMC) and diaphyseal bone mineral density (dBMD) of femurs were significantly higher only in rats adapted to the 'Chicory' diet ($P < 0.05$), and there was a significant linear trend for both BMC and dBMD, as well in the inulin diet groups ($P = 0.041$ and 0.026, respectively) (Table 4). The femoral failure load was not significantly altered by the diet conditions. Plasma concentrations of osteocalcin (marker of osteoblast activity) and the urine excretion of DPD (corrected for creatinine, marker of osteoclasts activity) were not significantly modified, nor was the remodeling index (namely the OC/DPD ratio).

Discussion

It is generally accepted that fructans effects on Ca and Mg metabolism are largely dependent on their impact on microbial fermentation in the large intestine. All the inulin diets led to an acidification of the cecal content, in the 5.7–6.0 range, and an enlargement of the cecum in parallel to an hypertrophy of the cecal wall. The cecal content was particularly high with chicory, which could reflect the additional effect of some cell-wall fibers present in chicory (pectin 5% and cellulose + hemicellulose 11% of dry matter) besides inulin itself. Inulin is known to promote microbial proliferation in the large intestine [15, 21], leading to a noticeable accumulation of lactic acid in the cecum of rats adapted to the purified inulin diets, together with an enlargement of the SCFA pool, especially that of butyrate [9, 16]. This was actually observed in the present study, with the highest value for the butyrate pool being observed in rats fed the 'Chicory' diet. One of the most salient features of the cecal fermentations was the presence of large amounts of succinate in the cecum of rats fed the inulin diets and succinate was the main end-product of bacterial metabolism in terms of carbon units with these diets. Succinate is generally considered as an intermediate in the metabolic pathway yielding propionate [13, 15], but here there was poor correlation between succinate and propionate except, to a certain extent, in rats adapted to the 'Chicory' diet. Acetate and succinate are the major fermentation products when the substrate is abundant, whereas succinate is decarboxylated to produce propionate when carbon and energy sources are limiting [15]. Whether fructose metabolism is responsible for succinate is still uncertain, but it was observed that some *bifidobacteria* are able to grow as fast on oligofructose as on fructose, with succinic acid being the major metabolite produced by both strains [29]. SCFA are considered as readily absorbed compounds in the large intestine, in part due to the fact that they are relatively lipophilic when protonated [11], but little is known of the actual effectiveness of succinate absorption in the large intestine. Wolfram et al. [33] described a Na-dependent carrier for succinate in the large intestine but Umesaki et al. [28] reported that succinate (or lactate) transport across the colonic mucosa was much slower than that of SCFA. It must be noted that the various parameters of cecal digestion displayed practically no

difference between the '$_{REF}$Inulin' and the '$_{NAT}$Inulin' diet groups, suggesting that differences in the repartition of the various fructan chains have a limited impact on microbial fermentation in our model.

After 1 month of adaptation to the experimental diets, apparent Ca absorption was relatively low in 'Control' rats, whereas adaptation to '$_{REF}$Inulin' or '$_{NAT}$Inulin' diets was characterized by a high rate of Ca absorption (no significant differences between these two types of inulin). Chicory consumption was accompanied by a high rate of fecal Ca excretion and, despite a higher dietary Ca intake than in the other diet groups, the final digestive balance did not differ from that observed in the purified inulin groups. Components in chicory able to counteract the enhancing effects of inulin on Ca absorption in chicory could be invoked, since chicory contains some oxalate, sulfate and pyroglutamate [2] but it is also conceivable that a threshold level of absorbed Ca, which is associated to the needs for skeletal growth, was achieved in the 'Chicory' group. In contrast, the reduction of Ca and Mg excretion in rats adapted to the purified inulin diets, compared to control rats, probably reflects a stimulatory effect on large intestine absorption [5, 10, 17, 34]. In this connection, consistent with direct measurements of Ca absorption from the large intestine in rats [13, 22], most of the available data support the view that the large intestine is the site of inulin action on Ca absorption: a recent study in humans suggests that about 70% of the increase of Ca absorption occurring after an oral dose of inulin should be ascribed to colonic digestion [1]. The mechanisms involved are still a matter of discussion: acidification of colonic content and greater Ca solubilization, modification of colonic mucosal permeability, altered expression of some genes (calbindin, aquaporin-8, carbonic anhydrase 3...) or more specific effects of SCFA [14, 17–19]. Compared to humans, low-grade acidosis induces a moderate hypercalciuria in rats [23, 32], possibly due to the fact that calciuria represents a minute percentage of Ca intake in this species (2–4%), in contrast to Mg or K.

The relationship between Ca absorption and bone parameters is not straightforward, because it is governed by complex factors affecting osteoblastic/osteoclastic activity balance, as well as the rate of renal Ca elimination [25]. Due to the inherent variability of the measured parameters, changes in these markers upon inulin exposure reached significance only for a limited number of parameters. It is noteworthy that the changes in bone status parameters were more pronounced in rats adapted to the 'Chicory' diet: animals of this group had the highest BMC and diaphyseal BMD, in spite of a Ca retention similar to that observed in the purified inulin diet groups. Diaphyseal BMD reflects mainly cortical bone, while

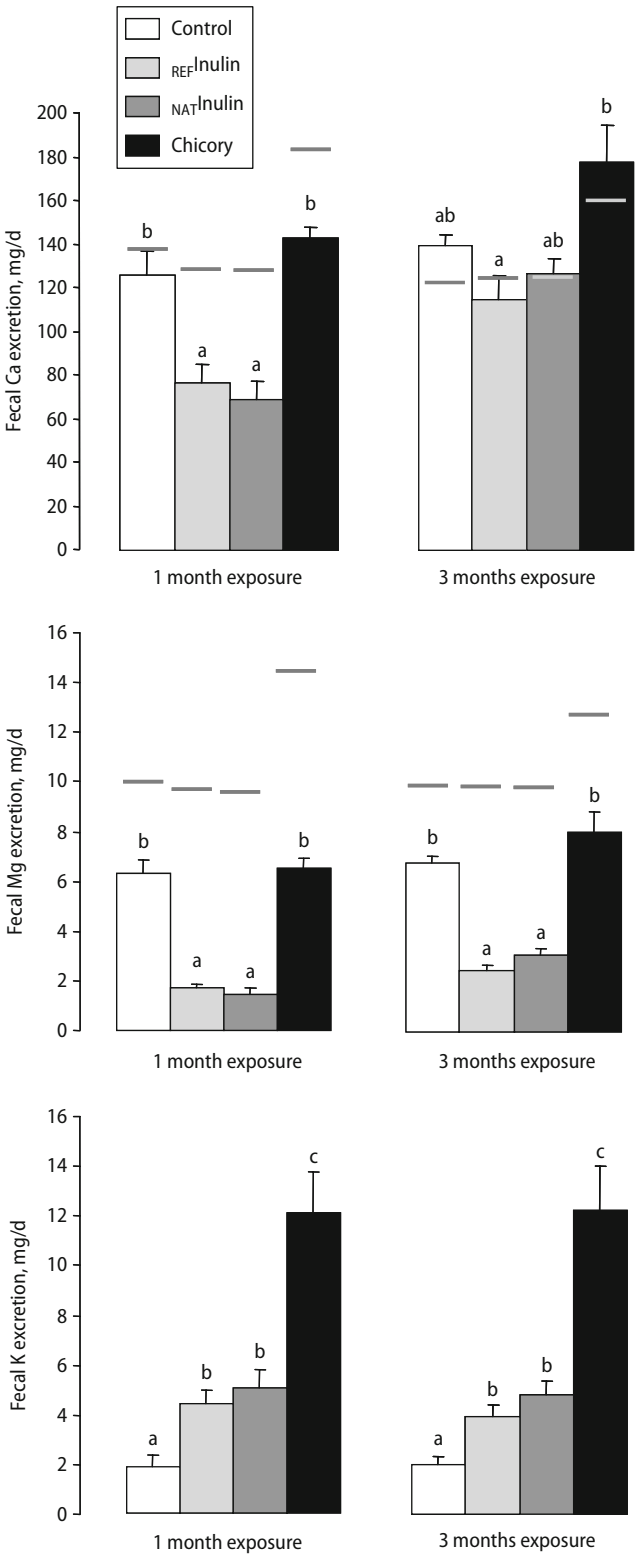

Fig. 1 Fecal excretion of calcium, magnesium or potassium in rats after 1 month or 3 months exposure to the experimental diets. Values are means ± SEM for 10 rats in each group and values not sharing the same superscript are significantly different ($P \leq 0.05$). *Grey bars* indicate daily intake (mg/d) for Ca and Mg, K intake being out of the scale for fecal K excretion

metaphyseal bone represents mainly trabecular bone. In contrast to this study, it was shown that OFS prevented the loss of mainly trabecular bone [24] and trabecular bone tissue is predominantly affected by menopause. This discrepancy could be explained by the fact that our study was carried out on growing rats, more representative of peak bone mass constitution. The major difference between the chicory diet and purified inulin diet groups was the presence of larger amounts of SCFA and succinate in the former group than in the latter. Whether absorption and metabolism of these compounds could also exert an effect independent of Ca absorption rate on bone metabolism is still uncertain.

In conclusion, with this rat model, differences in the chain length profile of inulin fractions did not alter (1) stimulation of cecal fermentation and the profile of end-products of bacterial metabolism, (2) stimulation of Ca and Mg digestive absorption and (3) the overall effects on bone parameters. Chicory crude fractions had the most marked effect on digestive fermentation but only a slight effect on bone metabolism. The slightly but significantly higher BMD and BMC compared to the other groups could be attributed to the higher feed consumption at the beginning of the experiment and/or to additive effects induced by other nutrients relevant for bone growth, like Mg, K or trace elements in the product.

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References

1. Abrams SA, Hawthorne KM, Aliu O, Hicks PD, Chen Z, Griffin IJ (2007) An inulin-type fructan enhances calcium absorption primarily via an effect on colonic absorption in humans. J Nutr 137:2208–2212
2. Barlianto H, Maier G (1995) Acids in chicory roots and malt. Zeitschrift Lebensmitteluntersuchung Forschung A 201:375–377
3. Bischoff-Ferrari HA, Dawson-Hughes B, Baron JA, Burckhardt P, Li R, Spiegelman D, Specker B, Orav JE, Wong JB, Staehelin HB, O'Reilly E, Kiel DP, Willett WC (2007) Calcium intake and hip fracture risk in men and women: a meta-analysis of prospective cohort studies and randomized controlled trials. Am J Clin Nutr 86:1780–1790
4. Cashman KD (2005) Diet, nutrition and bone health. J Nutr 137(suppl):2507S–2512S
5. Coudray C, Bellanger J, Castiglia-Delavaud C, Rémésy C, Vermorel M, Rayssiguier Y (1997) Effect of soluble or partly soluble dietary fibres supplementation on absorption and balance of calcium, magnesium, iron and zinc in healthy young men. Eur J Clin Nutr 51:375–380
6. Delzenne N, Aertssens J, Verplaetse H, Roccaro M, Roberfroid M (1995) Effect of fermentable fructo-oligosaccharides on mineral, nitrogen and energy digestive balance in the rat. Life Sci 57:1579–1587
7. Holloway L, Moynihan S, Abrams SA, Kent K, Hsu AR, Friedlander AL (2007) Effects of oligofructose-enriched inulin on intestinal absorption of calcium and magnesium and bone turnover markers in postmenopausal women. Br J Nutr 97:365–372
8. Kitchin B, Morgan SL (2007) Not just calcium and vitamin D: other nutritional considerations in osteoporosis. Curr Rheumatol Rep 9:85–92
9. Kolida S, Gibson GR (2007) Prebiotic capacity of inulin-type fructans. J Nutr 137:2503S–2506S
10. Kruger MC, Brown KE, Collett G, Layton L, Schollum LM (2003) The effect of fructooligosaccharides with various degrees of polymerization on calcium bioavailability in the growing rat. Exp Biol Med (Maywood) 228:683–638
11. Kunzelmann K, Mall M (2003) Electrolyte transport in the mammalian colon: mechanisms and implications for disease. Physiol Rev 82:245–289
12. Lanham-New SA (2008) The balance of bone health: tipping the scales in favor pf potassium-rich, bicarbonate-rich foods. J Nutr 138:172S–177S
13. Levrat MA, Rémésy C, Demigné C (1991) High propionic acid fermentations and mineral accumulation in the cecum of rats adapted to different levels of inulin. J Nutr 121:1730–1737
14. Lutz T, Scharrer E (1991) Effect of short-chain fatty acids on calcium absorption by the rat colon. Exp Physiol 76:615–618
15. McFarlane S, McFarlane G (2003) Regulation of short-chain fatty acid production. Proc Nutr Soc 62:67–72
16. Morrison DJ, Mackay WG, Edwards CA, Preston T, Dodson B, Weaver LT (2006) Butyrate production from oligofructose fermentation by the human faecal flora: what is the contribution of extracellular acetate and lactate? Br J Nutr 96:570–577
17. Nzeusseu A, Dienst D, Haufroid V, Depresseux G, Devogelaer JP, Manicourt DH (2006) Inulin and fructo-oligosaccharides differ in their ability to enhance the density of cancellous and cortical bone in the axial and peripheral skeleton of growing rats. Bone 38:394–399
18. Ohta A, Motohashi Y, Sakai K, Hirayama M, Adachi T, Sakuma K (1998) Dietary fructooligosaccharides increase calcium absorption and levels of mucosal calbindin-D9k in the large intestine of gastrectomized rats. Scand J Gastroenterol 33:1062–1068
19. Raschka L, Daniel H (2005) Mechanisms underlying the effects of inulin-type fructans on calcium absorption in the large intestine of rats. Bone 37:728–735
20. Rizzoli R, Boonen S, Brandi ML, Burlet N, Delmas P, Reginster JY (2008) The role of calcium and vitamin D in the management of osteoporosis. Bone 42:246–249
21. Roberfroid MB (2005) Introducing inulin-type fructans. Br J Nutr 93(Suppl 1):S13–S25
22. Rémésy C, Levrat MA, Gamet L, Demigné C (1993) Cecal fermentations in rats fed oligosaccharides (inulin) are modulated by dietary calcium level. Am J Physiol 264:G855-G862

23. Sabboh H, Horcajada MN, Coxam V, Tressol JC, Besson C, Rémésy C, Demigné C (2005) Effect of potassium salts in rats adapted to an acidogenic high-sulfur amino acid diet. Br J Nutr 94:192–197
24. Scholtz-Ahrens KE, Açil Y, Schrezenmeier J (2002) Effects of oligofructose or dietary calcium on repeated calcium and phosphorus balances, bone mineralization and trabecular structure in ovariectomized rats. Br J Nutr 88:365–377
25. Scholz-Ahrens KE, Ade P, Marten B, Weber P, Timm W, Açil Y, Gluer CC, Schrezenmeir J (2007) Prebiotics, probiotics, and synbiotics affect mineral absorption, bone mineral content, and bone structure. J Nutr 137:838S–846S
26. Tsukahara T, Iwasaki Y, Nakayama K, Ushida K (2003) Stimulation of butyrate production in the large intestine of weaning piglets by dietary fructooligosaccharides and its influence on the histological variables of the large intestinal mucosa. J Nutr Sci Vitaminol (Tokyo) 49:414–421
27. Turner CH, Burr DB (1993) Basic biomechanical measurements: a tutorial. Bone 14:595–608
28. Umesaki Y, Yajima T, Yokokura T, Mutai M (1979) Effect of organic acid absorption on bicarbonate transport in rat colon. Pflugers Arch 379:43–47
29. Van der Meulen R, Adriany T, Verbrugghe K, De Vuyst L (2006) Kinetic analysis of bifidobacterial metabolism reveals a minor role for succinic acid in the regeneration of NAD+ through its growth-associated production. Appl Environ Microbiol 72:5204–5210
30. Van Loo J (2004) The specificity of the interactions with intestinal bacterial fermentation by prebiotics determines their physiological efficacy. Nutr Res Rev 17:89–98
31. Weaver CM (2008) The role of nutrition in optimizing peak bone mass. Asia Pac J Clin Nutr 17(Suppl 1):135–137
32. Wiederkehr M, Krapf R (2001) Metabolic and endocrine effects of metabolic acidosis in humans. Swiss Med Wkly 131:127–132
33. Wolfram S, Badertscher M, Scharrer E (1994) Carrier-mediated transport is involved in mucosal succinate uptake by rat large intestine. Exp Physiol 79:215–226
34. van de Wiele T, Boon N, Possemiers S, Jacobs H, Verstraete W (2007) Inulin-type fructans of longer degree of polymerization exert more pronounced in vitro prebiotic effects. J Appl Microbiol 102:452–460