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Dietary Chicory Inulin Increases Whole-Body Bone Mineral Density in Growing Male Rats[1]

Marcel B. Roberfroid,[2] J. Cumps* and J. P. Devogelaer[†]

Université Catholique de Louvain, B–1348 Louvain-la-Neuve, Belgium; *School of Pharmacy, Biostatistics, Université Catholique de Louvain, B–1200 Brussels, Belgium; [†]Department of Rheumatology, Saint Luc University Hospital, Université Catholique de Louvain, B–1200 Brussels, Belgium

ABSTRACT   Chicory inulin is a natural linear fructan that is not digested in the upper part of the gastrointestinal tract but is fermented in the cecocolon. It enhances calcium absorption in rats and improves femur and tibia mineral contents in gastrectomized or ovariectomized rats. We studied the effect of inulin (0, 5 and 10 g/100 g diet) on whole-body bone mineral content (WBBMC), whole-body bone area (WBBA) and whole-body bone mineral density (WBBMD) in live, growing male rats fed diets containing 0.2, 0.5 or 1 g Ca/100 g. Three experiments, each corresponding to one of the different dietary Ca concentrations, were performed using male Wistar rats ($n = 108$; 4 wk old). WBBMC was measured by dual-energy X-ray absorptiometry every 4 wk up to wk 22. Inulin increased WBBMC ($P < 0.05$) and WBBMD ($P < 0.001$) significantly but not WBBA at all ages and all dietary calcium concentrations. This is the first report to demonstrate that chicory inulin not only increases calcium absorption but also increases mineral parameters in whole-body bones.   J. Nutr. 132: 3599–3602, 2002.

KEY WORDS: • fructan • inulin • calcium • bone mineral density • rats

Calcium is the main component of bone tissue, giving it the structural integrity that is essential to support growth. Classically, to improve calcium balance and bone mineral density, an increase in dietary calcium intake, especially in growing children, has been recommended (1,2). However, for women in early menopause, this approach has been debated (3).

More recently, it has been shown that some dietary carbohydrates such as lactose or oligosaccharides improve calcium absorption (4). Among them, inulin-type fructans may play an important role (5). These are linear oligomers and polymers of fructose linked by $\beta(2–1)$ osidic bonds. They occur naturally in several edible plants and especially in chicory roots, from which they are extracted as inulin [average degree of polymerization ($DP_{av} = 10$)[3]] which can be partly hydrolyzed enzymatically to produce oligofructose ($DP_{av} = 4.5$) (5).

Both in experimental models and in humans, recent studies have consistently shown that adding inulin-type fructans to the diet increased calcium absorption by 10–25%, depending on the type of fructan, the DP of the fructan, diet supplementation dose and population (6–10). However, showing that Ca absorption is improved does not prove that Ca utilization in bone is increased.

A few studies have indicated that fructans improve femur and tibia mineral contents and densities in ovariectomized female rats and in gastrectomized male rats (11,12). The aim of this study was to investigate whether dietary supplementation with chicory inulin increased whole-body bone mineral parameters in live, healthy growing rats to test for the persistence of the increased accretion throughout the growth period.

MATERIALS AND METHODS

Study design.   Three experiments were performed each using 3 groups of 12 male Wistar rats (Iffa Credo, L'Arbresle, France) fed diets containing 0, 5 or 10 g inulin/100 g diet. In the 3 experiments, diets contained 0.2 g/100 g calcium (60% calcium-deficient diet), 0.5 g/100 g calcium (recommended intake for rats) or 1 g/100 g calcium (calcium-enriched diet) and were fed for up to 22 wk. Rats were housed individually in single stainless steel wire cages in a room at 25°C with an alternating 12-h light:dark cycle. They were 4–5 wk old at the start of the experiments.

Diets.   The purified diet AO4 (Unité d'Alimentation Rationnelle, France) was used as the basal diet. Except for Ca (0.2, 0.5 or 1.0 g/100 g) and inulin (10, 5 or 0 g/100 g), the diets had the following composition (in g/100 g), which varied slightly with the content of inulin: protein 15, 16, 16.8; lipids (saturated 12%, monounsaturated 30%, polyunsaturated 58%) 2.9, 3.0, 3.2; carbohydrates 53, 56, 59; mineral mix 6 g/100 g and vitamin mix[4] 1.3 g/100 g in all diets. The energy content of the diet was 3.1–3.3 kcal/g (13.0–13.7 kJ/g). In all diets, the Ca concentration (0.2, 0.5 or 1.0 g/100 g) was higher than the requirement for maximal body weight gain (13); compared with the requirement for maximal mineralization, however, only the diets containing 0.5 and 1 g/100 g Ca were adequate. All diets had a P concentration higher than the requirements both for maximal body growth and maximal mineralization. The Ca/P ratio in the diet containing 1 g/100 g Ca was higher than the recommendation (1.7 vs. 1.1 and 1.4 for body growth and mineralization, respectively). The

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[2] To whom correspondence should be addressed.
E-mail: roberfroid@pmnt.ucl.ac.be.

[3] Abbreviations used: DP, degree of polymerization, i.e., the number of (fructose and glucose) monomers in inulin; DXA, dual X-ray energy absorptiometry; WBBA, whole-body bone area; WBBMC, whole-body bone mineral content; WBBMD, whole-body bone mineral density.
[4] Mineral mix composition (mg/kg): P, 5,900; Na, 1900; K, 6,700; Mg, 2,000; Mn, 90; Fe, 240; Cu, 30; Zn, 85; Co, 1.5; I, 0.3. Vitamin mix composition (mg/kg except as noted): thiamine, 7; riboflavine, 6.5; niacin, 81.5; pyridoxine, 2.6; cyanocobalamin, 0.02; α-tocopherol, 30; menadione, 2.5; folic acid, 0.5; biotin, 0.04; choline, 1600; equivalent retinol, 2250 μg; cholecalciferol 375 μg.

diet containing 0.5 g/100 g Ca had a ratio close to the recommendation for maximal body growth but lower than the recommendation for maximal mineralization (0.8 vs. 1.1 and 1.4, respectively) and the diet containing 0.2 g/100 g Ca had a ratio (i.e., 0.3) lower than the defined requirements. RAFTILINE HP (ORAFTI, Tienen, Belgium) was used as the source of chicory inulin. It is a mixture of α-D-glucopyranosyl-(β-D-fructofuranosyl)$_{n-1}$-D-fructofuranosides with n ranging from 9 to 64 with an average of 24. Diets contained 0, 5 or 10 g inulin/100 g.

Measurement of whole-body bone mineral density. Whole-body bone mineral content (WBBMC; g), and whole-body bone area (WBBA; cm^2) were measured by dual X-ray absorptiometry (DXA) on QDR-1000W (Hologic, Bedford, MA) equipment with rat whole-body software. The precision of measuring WBBMC and whole-body bone mineral density (WBBMD; mg/cm^2) was determined using 33 Wistar rats measured 4 times in the anteroposterior incidence after careful repositioning. The CV ($\pm$ SEM) was 1.74 $\pm$ 0.15 (14). The method is extremely accurate as demonstrated by a correlation coefficient of 0.99 between total body Ca as measured by DXA and whole-body Ca content as measured by atomic absorption spectrometry of bone ash (15,16).

In the experiments reported here, WBBMC measurements were performed at the start of the experiments (4-wk-old rats) and at wk 10, 14, 18 and 22. The scanning time for whole-body analysis was ~20 min/rat, which is in agreement with previously published recommendations for optimization of the technique (17). Before measurement, rats were anesthetized with 0.1mL sodium pentobarbital/100 g body (Nembutal, Sanofi, Libourne, France) to immobilize them. From the WBBMC and WBBA, WBBMD was calculated. The protocol of the experiments met all ethical requirements for animal experiments according to Belgian and EU regulations.

Statistics. Data analyses were conducted using SAS 8.02 from the SAS Institute (Cary, NC) and followed the guideline recommended by the European Agency for the Evaluation of Medicinal Products (18). The experiments were designed to perform multiple comparisons to identify and quantify whether ages, calcium and/or inulin concentrations affected the bone mineral parameters (WBBMC, WBBA, WBBMD) without a priori knowledge of where the differences would be. Statistics were based on a repeated-measures ANOVA. The procedure included 4 consecutive steps: *1*) A priori tests (Kolmogrov-Smirnov and Shapiro-Wilkinson tests) to verify the normality of distribution of the differences between each individual value and the mean of its group. *2*) A "Levene" test to check for the homogeneity of variances in all the groups. When the variances were significantly heterogenous, a Geisser-Greenhouse correction was applied automatically. *3*) A parametric test using an extension of the General Linear Model (GLM, PROC MIXED SAS 8.02) to identify and quantify, based on F-tests, the contribution of age, calcium concentration and inulin intake (plus mixed effects) on the 3 bone mineral parameters (WBBMC, WBBA, WBBMD). *4*) A contrast analysis followed by a posteriori multiple comparison (Tukey's test) to identify which ages and which calcium and inulin concentrations were different in their effects on the 3 parameters.

Steps 1 and 2 were applied according the guidelines of the U.S. Environment Protection Agency (19). Effects and differences between treatments were considered significant at $P < 0.05$. In Table 1 and Figure 1, results are expressed as means $\pm$ SEM, $n = 10$–12.

TABLE 1

Whole-body bone mineral content (WBBMC), whole-body bone area (WBBA) and whole-body bone mineral density (WBBMD) of growing male Wistar rats (from wk 10 to 22 of age) fed diets containing 0.2, 0.5 or 1.0 g/100 g Ca and 0, 5 or 10 g/100 g inulin[1,2]

	Inulin, g/100 g	Week			
		10	14	18	22
Ca 0.2 g/100 g					
WBBMC, g	0	5.4 ± 0.5	7.1 ± 0.7	8.6 ± 0.9	9.2 ± 1.0
	5	5.8 ± 0.3	7.6 ± 0.3	9.0 ± 0.5	9.6 ± 0.5
	10	5.8 ± 0.2	7.4 ± 0.3	8.9 ± 0.4	9.5 ± 0.4
WBBA, cm2	0	48.1 ± 3.6	56.8 ± 4.2	64.7 ± 6.6	66.4 ± 6.3
	5	50.6 ± 2.2	58.5 ± 2.4	65.1 ± 3.9	66.1 ± 3.6
	10	48.1 ± 2.5	57.4 ± 2.8	63.4 ± 2.7	63.5 ± 4.1
WBBMD, mg/cm2	0	109 ± 3	125 ± 3	133 ± 5	139 ± 4
	5	115 ± 2	129 ± 4	138 ± 5	145 ± 4
	10	115 ± 3	127 ± 3	138 ± 2	147 ± 7
Ca 0.5 g/100 g					
WBBMC, g	0	6.0 ± 0.4	7.2 ± 0.4	8.0 ± 0.5	8.9 ± 0.8
	5	5.9 ± 0.3	7.4 ± 0.5	8.3 ± 0.6	9.3 ± 0.8
	10	5.7 ± 0.2	6.9 ± 0.2	8.0 ± 0.2	8.7 ± 0.2
WBBA, cm2	0	51.9 ± 2.6	57.1 ± 2.7	60.8 ± 3.5	65.0 ± 5.1
	5	49.6 ± 4.4	56.8 ± 4.1	61.0 ± 3.7	64.8 ± 5.4
	10	49.0 ± 1.1	54.9 ± 2.1	58.6 ± 1.2	61.8 ± 2.4
WBBMD, mg/cm2	0	114 ± 5	126 ± 4	133 ± 4	137 ± 5
	5	117 ± 4	126 ± 5	133 ± 7	140 ± 6
	10	116 ± 3	125 ± 4	136 ± 3	142 ± 3
Ca 1.0 g/100 g					
WBBMC, g	0	6.1 ± 0.6	7.5 ± 0.8	8.7 ± 1.0	9.4 ± 1.1
	5	6.4 ± 0.5	7.9 ± 0.6	9.3 ± 0.8	10.1 ± 0.9
	10	5.8 ± 0.5	7.4 ± 0.3	8.8 ± 0.8	9.5 ± 0.8
WBBA, cm2	0	50.4 ± 4.6	56.7 ± 5.2	61.7 ± 6.0	64.4 ± 6.0
	5	51.1 ± 2.0	58.2 ± 4.0	62.7 ± 4.1	66.0 ± 4.6
	10	47.9 ± 4.0	54.5 ± 2.4	66.0 ± 4.9	63.1 ± 4.7
WBBMD, mg/cm2	0	0.121 ± 0.003	0.132 ± 0.004	0.140 ± 0.006	146 ± 7
	5	0.124 ± 0.003	0.136 ± 0.003	0.146 ± 0.003	150 ± 4
	10	0.121 ± 0.003	0.136 ± 0.004	0.147 ± 0.005	151 ± 5

[1] Values are means $\pm$ SEM, $n = 10$–12.
[2] Results of the statistical analyses are given in Table 2.

RESULTS AND DISCUSSION

Body-weight gain. Except for rats fed the 0.2% Ca diet, the body weight gain of those fed diets containing 10% inulin was lower (-5 to -7%) ($P < 0.001$) than that of the control rats and of the rats fed the 5% inulin diet (data not shown).

Bone mineral composition. At the start of the experiments rats had WBBMC, WBBA and WBBMD that did not differ i.e., 2.11 ± 0.05 g, 26.6 ± 0.7 cm^2 and 84 ± 1 mg/cm^2, respectively. These values for whole-body bone parameters are in agreement with previously (albeit rarely) published values (15). Indeed, the published values of WBBMC and WBBMD for rats weighing 100 and 265 g, are 1.9 and 5 g, and 70 and 110 mg/cm^2, respectively.

Table 1 reports the bone mineral parameters in the different experimental groups as a function of the age of the rats. Table 2 gives the results of the statistical analysis and indicates which treatment or combination of treatments significantly affected WBBMC, WBBA and/or WBBMD. From wk 10 to wk 22, in all 3 Ca groups fed all levels of inulin, the effects of age, Ca and inulin concentrations were significant on WBBMD ($P < 0.001$) and on WBBMC ($P < 0.001$ or $P = 0.02$). WBBA, which is also a measure of bone size, was not affected by Ca concentration, but it was influenced by the duration of treatment, which had a positive effect ($P < 0.001$), and also by the highest, but not the lowest, dose of inulin. Indeed, in rats fed the diet containing 10 g inulin/100 g, WBBA was lower ($P < 0.05$) than in rats fed the control or the diet containing 5 g inulin/100 g. This is consistent with the effect of the highest dose of inulin on body weight. Rats with lower body weights are also likely to have smaller bones; even though the bones

TABLE 2

Results of the statistical analyses performed on the data reported in Table 1 to identify whether the age of the rats (wk 10–22), the Ca (0.2, 05, 1.0 g/100 g) and the inulin concentration (0, 5, 10 g/100 g) in the diet or any combination of these (mixed effects) significantly influenced whole-body bone mineral content (WBBMC), whole-body bone area (WBBA) and whole-body bone mineral density (WBBMD)[1]

	WBBMC	WBBA	WBBMD
Age			
Global effect	$P < 0.001$	$P < 0.001$	$P < 0.001$
Wk 14 vs. wk 10	$P < 0.001$	$P < 0.001$	$P < 0.001$
Wk 18 vs. wk 14	$P < 0.001$	$P < 0.001$	$P < 0.001$
Wk 22 vs. wk 18	$P < 0.001$	$P < 0.001$	$P < 0.001$
Dietary Ca			
Global effect	$P = 0.01$	NS[2]	$P < 0.001$
0.5 vs. 0.2 *g/100 g*	$P = 0.045$	NS	NS
1.0 vs. 0.5 *g/100 g*	$P < 0.001$	NS	$P < 0.001$
Dietary inulin			
Global effect	$P = 0.02$	NS	$P < 0.001$
5 vs. 0 *g/100 g*	$P = 0.012$	NS	$P < 0.001$
10 vs. 5 *g/100 g*	NS	$P = 0.02$	NS
Mixed effects			
Age × Dietary Ca	$P < 0.001$	$P < 0.001$	$P < 0.001$
Age × Dietary inulin	NS	NS	$P = 0.006$
Ca × Dietary inulin	NS	NS	NS
Age × Dietary Ca × Dietary inulin	NS	NS	NS

[1] See Materials and Methods for more details on the statistical procedures.
[2] NS, $P > 0.05$.
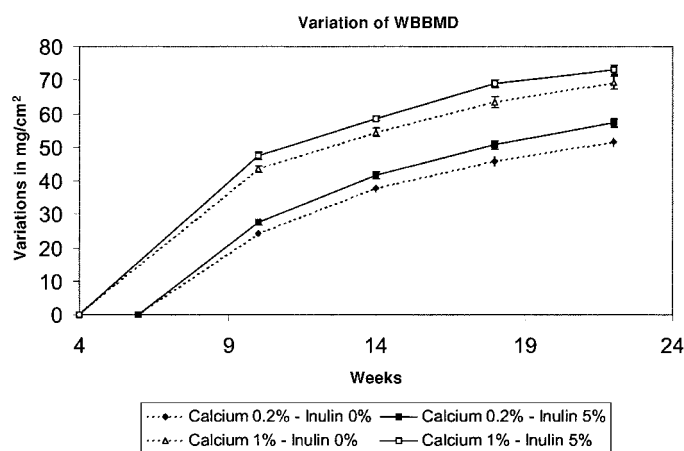
FIGURE 1 Increase in whole-body bone mineral density (WBBMD; mg/cm$^{2)}$) as a function of age (up to wk 22) of male Wistar rats fed a diet containing 0 and 5 g/100 g inulin and 0.2 or 1.0 g/100 g Ca. Values are means $\pm$ SEM, $n = 10$–12. Compared with the reference diet (Ca, 0.2 g/100 g, inulin, 0 g/100 g), increasing Ca concentration (1 g/100 g) and/or inulin concentrations (5 g/100 g) increased ($P < 0.001$) WBBMD at all ages (up to wk 22).

are smaller, they have the same mineral content and the same mineral density. In addition, both the Ca ($P < 0.001$) and inulin ($P < 0.05$) contents of the diet influenced the effect of age on WBBMD. Moreover, the Ca, but not the inulin concentrations, modified the effect of age on WBBMC and WBBA.

The effects of inulin on WBBMC and WBBMD were maximum when its concentration in the diet increased from 0 to 5 g/100 g ($P < 0.001$), but these parameters did not increase further when the concentration increased from 5 to 10 g/100 g ($P = 0.11$). Although increasing Ca intake from 0.2 to 0.5 g/100 g had no significant effect ($P = 0.093$), increasing it from 0.5 to 1.0 g/100 g had a significant effect ($P < 0.001$). The greatest effect of Ca intake was noted in a comparison of rats fed 0.2 g/100 g vs. 1.0 g/100 g ($P < 0.001$). Under optimal conditions (i.e., Ca, 1 g/100 g and inulin, 5 g/100 g), the increment of WBBMD as a function of age (from wk 10 to wk 22) was more marked ($P < 0.001$) than in rats fed the control diet (i.e., Ca, 0.2 g/100 g and inulin, 0 g/100 g) (**Fig. 1**).

Very few data have been published on whole-body bone parameters in rats measured by DXA (16). Data on whole-body bone parameters cannot be compared with data on specific bones (e.g., femur, tibia) because the former concern an average value that can mask differences between rapidly and slowly growing bones.

To our knowledge, this is the first experiment in which whole-body bone mineral parameters have been measured in live, growing male rats to study the influence of inulin-type fructans, and chicory inulin in particular, on Ca utilization. Adding inulin to the diet increased WBBMC and WBBMD at all ages (up to wk 22) and all dietary calcium concentrations.

These results confirm an increase in bone accretion as a consequence of adding inulin to the diet. They extend previous observations on specific bones isolated from ovariectomized female rats or gastrectomized male rats killed at particular time points [for a review, see (20)] to whole-body measurements in live, healthy growing rats. They demonstrate the persistence of the increased accretion throughout the period of growth. The increased Ca accretion in whole-body bones might be due to the previously reported increased intestinal Ca absorption, but a decrease in bone turnover cannot

be excluded, especially because Ca absorption was not measured in the experiments reported here.

Several hypotheses have been suggested to explain how inulin enhances Ca absorption: *1)* an increased calcium solubility in the colon due to pH reduction as a consequence of inulin fermentation; *2)* osmotic effects increasing fluid transfer in the colonic lumen and, as a consequence, an increased permeability between intracellular enterocyte junctions that facilitates diffusion (5); *3)* calcium/hydrogen exchange in the distal part of the colon activated by absorption of short-chain carboxylic acids (21); *4)* an increase in the cecocolonic concentration of the calbindin D9k protein known to be involved in the absorption of calcium (22).

The experimental data on the effect of chicory inulin on whole-body bone mineral content and density in growing male rats could be of paramount importance because they extend the scope of the work that was done recently in humans (8–10). In these studies, significantly increased calcium absorption occurred due to ingestion of inulin, oligofructose or a specific mixture of both. The long-term consequence of such an increase during growth remains to be evaluated. The data reported here support the hypothesis that increased Ca absorption due to the ingestion of inulin-type fructans might influence peak bone mass in a positive manner, especially in adolescents.

ACKNOWLEDGMENT

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