

Dietary long-chain inulin reduces abdominal fat but has no effect on bone density in growing female rats

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New strategies to improve Ca absorption and bone health are needed to address the current state of osteoporosis prevention and management. Inulin-type fructans have shown great promise as a dietary intervention strategy, but have not yet been tested in a young female model. Our objective was to investigate the effect of long chain (LC) inulin on bone mineralization and density in growing, female rats, as well as the quality of growth. Weanling Sprague–Dawley rats were assigned to inulin or cellulose treatments for either 4 or 8 weeks. Growth was measured weekly and quality of growth assessed using fat pad weights and dual-energy X-ray absorptiometry (DXA). Whole body (WB) and selected regions were analysed for bone mineral density (BMD) and body composition by DXA. Serum markers of bone turnover were assessed by enzyme-linked immunosorbent assays. Ca and P concentrations were determined in excised femurs by inductively coupled plasma spectrometry. Feeding inulin resulted in 4% higher femoral weight (adjusted for body weight) and 6% less feed intake. Inulin did not affect WB or regional BMD, but was associated with a 28% lower parametrial fat pad mass, 21% less WB fat mass and 5% less WB mass. In summary, LC-inulin lowered body fat mass, without consequence to bone density in growing female rats.

Inulin: Bone density: Adiposity: Rats

Inulin-type fructans are thought to promote bone health through positive actions on mineral retention leading to increased peak bone mass achievement and bone mass conservation during ageing. Experimental studies in animal models have reported beneficial effects on bone mineral content (BMC), bone mineral density (BMD) and gastrointestinal absorption of Ca and other minerals⁽¹⁾. There is strong evidence of enhanced BMC and BMD in growing male rodents fed inulin-type fructans^(2–6), as well as improved bone health in adult, ovariectomized rats^(7–10). Although results of short-term human trials have been mixed, a recent study in adolescent females has shown an increase in Ca absorption as well as BMC and BMD after feeding inulin-type fructans for 8 weeks and 1 year^(11,12). Effects of inulin on bone mineral retention have not, to the best of our knowledge, been reported in a growing female rodent model. This is an important model to develop, as the attainment of peak bone mass is especially critical in young, growing females in order to protect against osteoporosis.

Precise mechanisms for this effect have not been defined, although it is widely believed that the fermentation of inulin-type fructans in the large intestine results in SCFA production, leading to a decline in intestinal pH⁽¹⁾. Mineral solubility is thought to increase as pH drops in the large intestine, indirectly increasing mineral absorption through the paracellular pathway⁽¹⁾. Thus, enhanced mineral bioavailability could

explain the increased bone mass accretion and conservation reported with inulin feeding. Alternatively, a fructan-induced trophic effect may also explain increased paracellular mineral absorption in the colon⁽¹³⁾. Feeding inulin-type fructans to rats has been shown to promote ileal growth as well as mRNA levels of glucagon-like peptide-1 in the ileum and caecum⁽¹³⁾. Finally, there is preliminary evidence of increased caecal absorptive surface area, increased SCFA concentration and larger intestinal pools of soluble and ionized Ca (to promote the concentration gradient) as potential mechanisms to explain the effect of inulin on mineral absorption⁽¹⁴⁾.

In addition to effects on bone, inulin may positively impact growth quality with regard to body composition. Recent studies have reported decreased feed intake, weight gain and fat pad mass in male rats fed various inulin-type fructans^(15,16). However, data on the response of whole body (WB) fat and lean mass to inulin is limited. Body weight affects bone mass; however, the relative contributions of lean and fat mass to bone mass accretion have not been fully clarified⁽¹⁷⁾. Lean mass is positively associated with increased bone mass in the appendicular and axial skeletons^(17–20). Several studies have reported a negative association between fat mass and bone mass^(17,21,22). Thus, a positive shift in body composition from fat to lean mass may promote bone mass accretion.

Abbreviations: BMC, bone mineral content; BMD, bone mineral density; DP, degree of polymerisation; DXA, dual-energy X-ray absorptiometry; LC-inulin, long-chain inulin; OLF, oligofructose; WB, whole body.

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Inulin-type fructans are considered non-digestible carbohydrates as the β -configuration of the anomeric C₂ in the fructose monomer is resistant to enzymatic hydrolysis⁽²³⁾. Both inulin and oligofructose (OLF) are by-products of chicory root, which can be distinguished by their degree of polymerization (DP). Inulin is a linear $\beta(2 \rightarrow 1)$ fructan with a DP of 2 to 60 (DP_{avg} 12) and OLF is produced by the partial enzymatic hydrolysis of inulin and has a DP of 2 to 8 (DP_{avg} 4)⁽²³⁾. Long-chain inulin (LC-inulin; DP_{avg} 25) and OLF-enriched inulin can also be produced⁽²³⁾. Beneficial effects on bone mineralization have been demonstrated with inulin, OLF and OLF-enriched inulin, but studies directly comparing these different types of fibre indicate that fructans with the highest DP produce positive effects of greater magnitude with regard to bone density^(4–6). Therefore, the objective of the present study was to investigate the effect of dietary LC-inulin on bone mineralization and density, as well as growth quality, in growing, female rats.

Materials and methods

Animals and diets

Forty-eight weanling (3-week old) female Sprague–Dawley rats weighing approximately 55–65 g were obtained from Charles River Laboratories (St Constant, Quebec, Canada). Animals were housed individually in stainless steel hanging cages in a temperature- (21–23°C) and humidity- (55%) controlled room with a light:dark cycle of 14:10 h. Following a 1 week acclimatization period to a nutritionally complete diet, rats were randomly assigned to treatment groups. Twenty rats were fed a diet containing 10% cellulose (CL diet) and twenty rats were fed a diet containing 5% LC-inulin (IN diet) (Beneo HP, Orafit Group, Tienen, Belgium; IN; DP_{avg} > 23 and 100% inulin) and 5% cellulose. Ten rats were killed from each group at the end of 4 weeks (CL-4 and IN-4) and after 8 weeks (CL-8 and IN-8). Weanling rats were chosen so as to assess a critical period in the development of peak bone mass. In addition, by the end of the dietary treatment, rats had likely not yet reached sexual maturity, thus limiting hormonal effects in the study.

Experimental diets were based on the AIN-93G formulation⁽²⁴⁾ and provided *ad libitum*. (With the exception of fibre (cellulose or inulin) the diets contained the following: 54% carbohydrate (31.3% maize starch, 13.2% maltodextrin, 10% sucrose); 21% egg white; 3.5% Zn-free AIN-93G-MX mineral mix; 1% Zn premix (5.775 g ZnCO₃/kg dextrose); 1% AIN-93-VX vitamin mix; 1% biotin (200 mg biotin/kg dextrose); 0.54% potassium phosphate; 0.25% choline; 0.0014% *tert*-butylhydroquinone; 7% soyabean oil.) Ca concentration was kept constant across diets at 5 mg/kg. Deionized water was also provided *ad libitum* in plastic bottles with stainless steel sipper tubes. Feed intake was determined daily and body weights were measured weekly. The protocol for animal care procedures received ethics approval from the University of Manitoba Protocol Management and Review Committee.

Tissue collection

All animals were killed by CO₂ asphyxiation and exsanguination in keeping with the guidelines of the Canadian Council on

Animal Care. Body weights were determined *post mortem* and trunk blood was collected and stored on ice until centrifuged to obtain serum (2400 rpm at 4°C, 10 min). Parametrial fat pads were excised, rinsed briefly with PBS and blotted dry before weighing and being frozen in liquid nitrogen. Blood samples were stored at –80°C until analysis.

Dual-energy X-ray absorptiometry scans

The WB, spine, femur and tibia of rat carcasses were analysed for bone area, BMC, BMD, lean body mass, fat body mass and total mass *in situ* by dual-energy X-ray absorptiometry (DXA, 4500A; Hologic Inc., Bedford, MA, USA; small animal software high resolution option). Animals were placed dorsally, in an anterior–posterior position. DXA has shown high-quality precision and accuracy in measuring BMC and BMD in small animals *in situ*⁽²⁵⁾. The precision error (CV%) for triplicate scans of bone area, BMC and BMD was 2.0, 1.4 and <0.1, respectively, for the WB, 5.9, 7.4 and 6.9, respectively, for the spine, 11.4, 7.8 and 4.8, respectively, for the femur and 8.9, 9.2 and 4.6, respectively, for the tibia.

Lean, fat and total body mass analysis was done on carcasses following removal of trunk blood, spleen, stomach, small and large intestine (including caecum) and parametrial fat pads. Lean fat mass analysis with DXA varies with the extent of evisceration but generally has a CV of <10% from anaesthetized rats to completely eviscerated rats, based on studies in our laboratory (unpublished data). The precision error (CV%) for triplicate scans of fat mass, lean mass, total body mass and % fat was 1.7, 0.4, 0.2 and 2.3, respectively.

Bone morphometry

Following *in situ* DXA analysis, right and left femurs were excised and thoroughly cleaned of soft tissue. Morphometric measures were taken with digital callipers as previously described⁽²⁶⁾ and recorded to the nearest 0.01 mm. All measures were reproduced in triplicate by the same trained examiner and included length, diaphysis width, femoral neck width and femoral head width.

Mineral analysis

Wet and dry weights of right femurs were determined prior to acid digestion for mineral analysis. Femurs were wet digested with trace-element grade nitric acid (4 ml per sample), as previously described⁽²⁷⁾ in disposable DigiPrep tubes (SCP Science, Baie d'Urfé, Quebec, Canada) over 6–7 h at 85°C in a DigiPrep HP heater (24 well; SCP Science). Acid digests were diluted appropriately with double deionized water prior to analysis of Ca and P by inductively coupled plasma optical emission spectrometry analysis (Varian Liberty 200, Varian, Canada). Detection limits were 0.1 mg/ml for Ca and 0.5 mg/ml for P.

Biochemical assays

Osteoblast and osteoclast activity were measured by enzyme-linked immunosorbent assays specific for rat osteocalcin (Rat-Mid Osteocalcin; Osteometer BioTech, Herlev Hovengrade, Denmark) and bone-related plasma degradation products

of C-terminal peptides of type I collagen in rats, respectively (CTX-I; Osteometer Biotech). Osteocalcin is considered a marker of osteoblast activity (bone formation), whereas CTX-I is a marker of osteoclast activity (bone resorption). The assay was performed according to the manufacturer's instructions. Samples were analysed in duplicate and agreement was $\cong 82\%$.

Statistical methods

Data were analysed for main effects and interactions of inulin feeding over time by two-way ANOVA with a level of significance of 0.05, using SAS software version 9.1 (SAS Institute, Cary, NC, USA). Data were checked for normality and homogeneity of variance and transformed when necessary. Transformed data are indicated where appropriate; however, only non-transformed means are reported. In the case that data transformation could not correct non-normal data, significant main effects were verified by a non-parametric test (Kruskal–Wallis analysis of ranks). Significant differences were determined among treatment groups with Duncan's multiple range test. All data are reported as means with their standard errors of the mean.

Results

Growth and feed intake

Initial body weights at the start of the study ranged from 96 (SEM 4) to 112 (SEM 5) g and were not different among treatment groups. End-point body weight was not different between IN and CL treatments at 4 or 8 weeks (Table 1). When calculated as weight gain there was 10 g difference in diet groups but this effect did not reach significance either (CL 146 (SEM 7) g; IN 136 (SEM 6) g; $P=0.10$). There was a trend toward a lower weight gain at weeks 4 and 5 ($P=0.09$) for IN-fed rats. Feed intake was 6% lower in IN-fed rats when calculated as average intake per d (Table 1) and 6% lower as total feed intake (CL 706 (SEM 57) g; IN 663 (SEM 52) g; $P=0.02$). When analysed on a weekly basis, feed intake was lower in IN-fed rats at weeks 3, 4 and 5 ($P=0.03$, $P<0.01$ and $P<0.01$), with a trend toward a lower intake at week 6 ($P=0.09$).

Bone mass

From baseline to end-point at 8 weeks, bone area, BMC and BMD of WB, femur, tibia and spine increased over time (Table 2). However, there were no changes in any parameter due to IN. BMC was also corrected for body weight (data not shown) but results were not different among groups.

Femur morphometry and mineralization

Morphometric measures of the excised femur also tended to be higher with time from baseline to 4 or 8 weeks and there were no effects due to IN (Table 3). Femoral dry weight, length and length corrected for body length were higher at week 8 than week 4. In contrast, femoral neck width was 4% less at week 8 than week 4. Femoral dry weight corrected for body

Table 1. Effects of inulin on body weight, feed intake, body length and tail length*§ (Values are means with their standard errors)

Measurement	CL-4 (n 10)		CL-8 (n 10)		IN-4 (n 10)		IN-8 (n 10)		Diet effect (n 20)			Time effect (n 20)			
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	P	Mean	SEM	P	
Body weight (g)	221	5	274	7	211	2	268	8	CL	247	7	0.23	216	3	< 0.01
Feed intake (g/d)	17.1	0.4	17.3	0.5	16.4	0.4	16.2	0.4	IN	240	8	0.02	271‡	5	0.75
Body length (mm)	203	2	217	1	201	1	214	1	CL	17.4	0.3	0.12	16.7	0.3	< 0.01
Tail length (mm)	185	2	205	2	187	2	204	2	IN	16.3†	0.2	0.94	16.9	0.4	< 0.01
									IN	21.0	0.2		21.5‡	0.1	< 0.01
									CL	19.5	0.3		18.6	0.2	< 0.01
									IN	19.5	0.3		20.4‡	0.1	< 0.01

CL, cellulose diet; IN, inulin diet.

*No interaction between diet and time was found.

†Indicates a significant effect of diet.

‡Indicates a significant effect of time.

§For details of animals and procedures, see Materials and methods.

Table 2. Effect of inulin on *in situ* bone area (BA), bone mineral content (BMC) and bone mineral density (BMD) in the whole body, femur, tibia and spine*¶
(Values are means with their standard errors)

Measurement	CL-4 (n 10)		CL-8 (n 10)		IN-4 (n 10)		IN-8 (n 10)		Diet effect (n 20)			Time effect (n 20)				
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	P	Mean	SEM	P		
Whole body																
BA (cm ²)	49.99	1.07	57.58	0.86	48.63	0.55	57.29	1.62	CL	53.8	1.1	0.46	Wk 4	49.3	0.6	<0.01
									IN	53.0	1.3		Wk 8	57.4‡	0.9	
BMC§ (g)	6.04	0.16	8.15	0.18	5.94	0.09	8.16	0.25	CL	7.1	0.3	0.80	Wk 4	6.0	0.1	<0.01
									IN	7.1	0.3		Wk 8	8.2‡	0.2	
BMD (g/cm ²)	0.121	0.002	0.142	0.003	0.122	0.002	0.142	0.002	CL	0.131	0.003	0.59	Wk 4	0.122	0.001	<0.01
									IN	0.132	0.003		Wk 8	0.142‡	0.002	
Femur																
BA (cm ²)	1.13	0.04	1.31	0.03	1.09	0.03	1.30	0.04	CL	1.22	0.03	0.49	Wk 4	1.11	0.02	<0.01
									IN	1.19	0.03		Wk 8	1.30‡	0.02	
BMC (mg)	327	10	444	13	312	11	430	15	CL	385	0.02	0.26	Wk 4	319	0.01	<0.01
									IN	371	0.02		Wk 8	497‡	0.01	
BMD (mg/cm ²)	292	11	341	12	287	9	332	7	CL	317	10	0.53	Wk 4	290	7	<0.01
									IN	310	7		Wk 8	336‡	7	
Spine																
BA§ (cm ²)	1.63	0.05	1.61	0.16	1.63	0.03	1.77	0.04	CL	1.62	0.08	0.38	Wk 4	1.63	0.03	0.53
									IN	1.70	0.03		Wk 8	1.69	0.08	
BMC (mg)	308	14	384	41	305	8	423	14	CL	346	23	0.47	Wk 4	307	8	<0.01
									IN	364	16		Wk 8	404	22	
BMD (mg/cm ²)	188	4	234	8	188	3	240	6	CL	211	7	0.61	Wk 4	188	3	<0.01
									IN	214	7		Wk 8	237	5	

CL, cellulose diet; IN, inulin diet; Wk, week.

* No interaction between diet and time was found.

† Indicates a significant effect of diet.

‡ Indicates a significant effect of time.

§ Data were not normal but had homogeneity of variance; effects were verified by Kruskal–Wallis analysis of ranks for main effect of time and found to be significant (χ^2 , $P < 0.01$ whole body BMC; χ^2 , $P = 0.02$ Spine BA).

|| Data were log transformed to achieve normality.

¶ For details of animals and procedures, see Materials and methods.

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Table 3. Effect of inulin on femur morphometry and mineralization*||
(Values are means with their standard errors)

Measurement	CL-4 (n 10)		CL-8 (n 10)		IN-4 (n 10)		IN-8 (n 10)		Diet effect (n 20)			Time effect (n 20)				
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	P	Mean	SEM	P		
Femur																
Dry weight§ (mg)	297	5	418	12	302	7	418	13	CL	358	15	0.80	Wk 4	300	5	<0.01
									IN	360	15		Wk 8	418‡	9	
mg weight:g body weight	1.35	0.03	1.53	0.02	1.43	0.03	1.56	0.03	CL	1.44	0.03	0.03	Wk 4	1.39	0.02	<0.01
									IN	1.49†	0.02		Wk 8	1.55‡	0.02	
Length§ (mm)	29.8	0.2	32.9	0.2	29.5	0.2	32.3	0.3	CL	31.3	0.4	0.08	Wk 4	29.6	0.1	<0.01
									IN	30.9	0.4		Wk 8	32.6‡	0.2	
mm length:mm body length	1.47	0.01	1.52	0.01	1.47	0.01	1.51	0.01	CL	1.49	0.01	0.86	Wk 4	1.47	0.01	<0.01
									IN	1.49	0.01		Wk 8	1.52‡	0.01	
Head width (mm)	3.98	0.04	4.13	0.04	4.00	0.05	4.03	0.04	CL	4.05	0.03	0.35	Wk 4	3.99	0.03	<0.01
									IN	4.02	0.03		Wk 8	4.08‡	0.03	
Neck width (mm)	2.84	0.04	2.75	0.05	2.92	0.05	2.74	0.05	CL	2.80	0.03	0.44	Wk 4	2.88	0.03	<0.01
									IN	2.83	0.04		Wk 8	2.75‡	0.04	
Diaphysis width (mm)	3.88	0.04	3.88	0.08	3.85	0.03	3.94	0.05	CL	3.90	0.03	0.74	Wk 4	3.87	0.03	0.42
									IN	3.90	0.03		Wk 8	3.91	0.05	
Ca (mmol/g dry weight)	5.85	0.06	5.93	0.06	5.84	0.08	6.04	0.04	CL	5.89	0.04	0.37	Wk 4	5.84	0.05	0.03
									IN	5.94	0.05		Wk 8	5.98‡	0.04	
P (mmol/g dry weight)	3.69	0.05	3.66	0.03	3.55	0.06	3.69	0.03	CL	3.68	0.03	0.22	Wk 4	3.62	0.04	0.21
									IN	3.62	0.03		Wk 8	3.68	0.02	

Long-chain inulin and bone density

CL, cellulose diet; IN, inulin diet; Wk, week.

* No interaction between diet and time were found.

† Indicates a significant effect of diet.

‡ Indicates a significant effect of time.

§ Data were not normal but had homogeneity of variance; effects were verified by Kruskal–Wallis analysis of ranks for main effect of time and found to be significant (χ^2 , $P < 0.01$ femur weight; χ^2 , $P < 0.01$ femur length).

|| For details of animals and procedures, see Materials and methods.

weight increased over time from baseline and was 4% higher in IN rats.

Femoral Ca and P concentrations were higher with time from baseline (Table 3). There were no effects due to IN feeding.

Serum markers of bone metabolism

Serum osteocalcin concentrations were lower with time from baseline (data not shown). There was a trend toward higher osteocalcin levels in IN rats (CL 323 (SEM 27) nmol/l; IN 374 (SEM 29) nmol/l; $P=0.02$); however, data were not normal and did not have homogeneity of variance. As log transformation was not able to correct these issues, mean values of main effects were analysed by Kruskal–Wallis analysis of ranks and found to be not significantly different ($P=0.15$). Serum CTX-I concentrations decreased from baseline over time, but there were no effects associated with IN feeding (CL 31.1 (SEM 2.8) nmol/l; IN 35.2 (SEM 3.6); $P=0.16$ log transformed).

Fat and lean body mass

Parametrial fat pad weight decreased over time and was 28% lower in IN rats (Table 4). When corrected for body weight, the difference between IN and CL groups was 26% (Fig. 1 (a)). When analysed by DXA, IN feeding had similar effects on WB fat mass, resulting in 21% lower fat mass (Fig. 1 (b)) and 17% lower WB fat mass as a percentage of total body mass (Fig. 1 (c)). Furthermore, fat pad weights and fat mass measured by DXA were highly correlated (R^2 0.73). WB lean mass (\pm BMC) and WB total body mass increased over time but only WB total body mass decreased with IN treatment (5%, Table 4).

Discussion

Previous studies have examined the effects of inulin and OLF on bone density in growing male and adult ovariectomized female rodent models but this is the first study to examine the impact of LC-inulin on bone density in a growing female model. This study shows that LC-inulin did not enhance bone density in either the axial or appendicular skeletons over approximately one and two bone modelling–remodelling stages (4 and 8 weeks) in growing female rats. However, important and novel findings of the present study include the considerable reductions (21–28%) in parametrial fat pad mass and WB fat mass in IN-fed females (Fig. 1 (a, b)). This result is in partial agreement with studies in male Wistar rats fed OLF and synergy 1 inulin (a 1:1 mixture of OLF and LC-inulin) for 3 weeks, which reported a 30% reduction in epididymal fat pad mass⁽²⁸⁾, suggesting that inulin feeding may reduce abdominal fat gain. However, fat pad mass of male rats fed only LC-inulin in the Cani *et al.* (2004) study did not differ from the control after 3 weeks, which does not agree with the present findings in females. This disparity may reflect the differential response in males and females to inulin of varying DP or may be related to the length of the study (3 weeks *v.* 8 weeks in the present study). Similarly, preliminary studies in our own laboratory have found no change in WB fat mass or lean mass (assessed by DXA) or epididymal

Table 4. Effect of inulin on abdominal fat and body composition (as determined by dual-energy X-ray absorptiometry analysis)*†
(Values are means with their standard errors)

Measurement	CL-4 (n 10)		CL-8 (n 10)		IN-4 (n 10)		IN-8 (n 10)		Diet effect (n 20)			Time effect (n 20)		
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	P	Mean	SEM	P
Parametrial fat pad weight (g)	1.4	0.1	3.7	0.4	1.1	0.1	2.5	0.4	CL	2.5	0.3	1.2	0.1	<0.01
WB lean mass + BMC§ (g)	174	4	213	6	168	2	209	5	IN	1.8†	0.3	3.1‡	0.3	<0.01
WB lean mass – BMC§ (g)	168	4	205	6	162	2	201	5	CL	193	6	171	2	<0.01
WB mass (lean + BMC + fat) (g)	197	6	248	7	186	2	237	7	IN	188	5	211‡	4	<0.01
									CL	186	6	165	2	<0.01
									IN	181	5	203‡	4	<0.01
									CL	223	7	192	3	<0.01
									IN	212‡	7	243‡	5	<0.01

CL, cellulose diet; IN, inulin diet; WB, whole body; BMC, bone mineral content; Wk, week.

*No interaction between diet and time was found.

†Indicates a significant effect of diet.

‡Indicates a significant effect of time.

§Data were not normal but had homogeneity of variance, effects were verified by Kruskal–Wallis analysis of ranks for main effect of time and found to be significant (χ^2 , $P<0.01$ WB lean mass \pm BMC).

||Data were log transformed to achieve normality.

¶For details of animals and procedures, see Materials and methods.

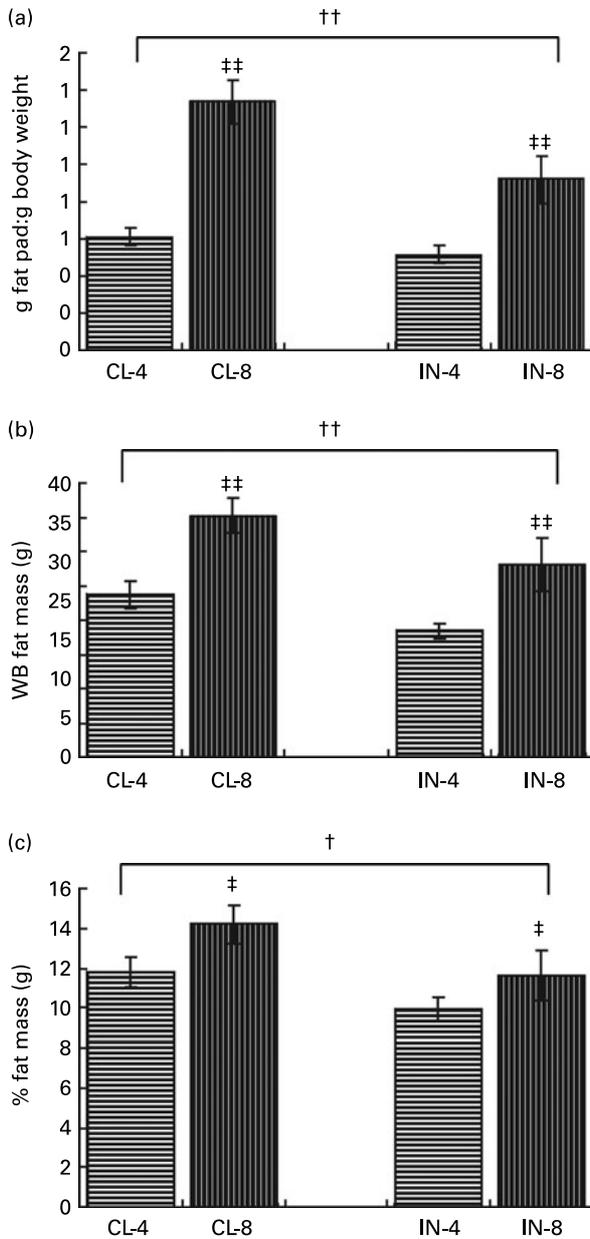


Fig. 1. Effects of inulin on parametrial fat pad mass adjusted for (a) body weight, (b) whole body (WB) fat mass analysed by dual-energy X-ray absorptiometry (DXA) and (c) total fat mass as % total body mass analysed by DXA in growing female rats at 4 and 8 weeks. Values are means with their standard errors of the means for ten rats. Statistical differences among means are indicated by †($P < 0.05$), ††($P < 0.01$; diet effect) and ‡($P < 0.05$), ‡‡($P < 0.01$; time effect). Data were log transformed to achieve normality (a), (b). CL, cellulose diet; IN, inulin diet. For details of animals and procedures, see Materials and methods.

fat pad mass in male growing Sprague–Dawley rats fed 10% LC-inulin for 6 weeks (unpublished data).

Based on the considerable reductions in visceral fat pad weight, WB fat mass and % fat mass (Fig. 1), there appears to be a significant reduction in adiposity associated with inulin feeding. This shift may reflect changes in both subcutaneous and visceral fat mass, although visceral fat is likely to be more amenable to inulin treatment. The reduction in

adiposity can be at least partially explained by the decrease in feed intake (Table 1) and WB mass (Table 4), as well as the trend toward less weight gain. It is also interesting that the changes in feed intake (and weight gain) were driven by differences during weeks 3–6, which trailed off during weeks 7 and 8. This is likely due to the decrease in growth rate due to ageing, making any differences due to diet more evident during the rapid growth phase. However, there may also be adaptation to the fibre present in the diet and long-term follow-up studies should address this concern.

There are very few studies reporting the effects of inulin on body composition, as assessed by DXA. A recent study in growing male Wistar rats fed a control diet, 5% inulin, or 5% OLF diet for 3 months reported significant improvements in bone density but no change in mean fat mass or lean mass when assessed by DXA or body weight⁽⁶⁾. However, specific fat pad weights were not reported by the authors and thus it is difficult to draw comparisons with the present study.

Nonetheless, the reduction in abdominal adiposity reported here has potential implications for the prevention and management of obesity. In fact, there is considerable evidence that inulin-type fructans protect against hepatic steatosis⁽²⁹⁾, fasting and postprandial triacylglycerolaemia⁽²³⁾, increased energy intake and gain in body weight and fat mass in rats^(15,16). The mechanism(s) of these effects are thought to work through the modification of gut-associated peptides (involved in the regulation of appetite and body weight) by fermentation products of inulin-type fructans⁽¹⁵⁾ and may explain the reduction in feed intake seen in the present study.

In the present study, there was a modest effect of LC-inulin on bone, as femoral weight was higher in IN-fed rats, when corrected for body weight (Table 3). A previous study has reported no change in bone size or length in male rats fed inulin⁽⁶⁾. However, these animals were 4 to 5 months of age at the study termination. Thus, it is possible that inulin may positively impact upon bone formation during earlier, more rapid growth stages although this change may only be a transient effect as it was not reflected by increases in bone area when measured by DXA.

The lack of change in the bone resorption marker CTX-I does not agree with previous studies in growing, male rats^(5,6), but is not surprising in light of the lack of response in bone density in the present study. These disparities may be related to several factors including sex, age, duration of feeding and DP of inulin. For example, 5.0–5.5% inulin, OLF and mixed-inulin diets have produced significant increases in BMD and decreases in bone resorption rates in growing male rats from 4 weeks⁽⁵⁾ to 22 weeks⁽⁴⁾ of feeding. However, similar results have not been reported and/or studied either with LC-inulin or in growing females. Thus, it is possible that LC-inulin, inulin or OLF could improve BMD in growing females over a longer feeding period. Alternatively, inulin or a mixed DP inulin source may be more beneficial than LC-inulin in females. In fact, a recent study has shown enhanced femoral BMD, Ca absorption and femoral Ca concentration and reduced bone turnover in a 9 month old, ovariectomized female rat model fed 5.5% diets of mixed inulin and OLF or enriched inulin for only 3 weeks⁽¹⁰⁾. Therefore, the role of inulin-type fructans in achieving peak bone mass in growing female models requires further investigation.

An increase in bone Ca concentration in response to inulin-type fructans has been reported^(3,10) and is thought to be mediated through a dose-dependent effect on intestinal Ca absorption⁽³⁰⁾. A strong correlation has also been reported between intestinal Ca absorption and bone Ca concentration⁽³⁾. However, inulin has not been shown to affect P absorption, and bone concentrations of this mineral in response to inulin-type fructans have not been widely reported. The impact of LC-inulin on bone mineralization in this study was negligible with regard to femoral Ca and P concentrations (Table 3).

The finding that femoral neck width was 4% lower at week 8 than week 4 is surprising. The narrowing of the femoral neck width is a concern as this is a site of heightened fracture risk⁽³¹⁾. However, femoral neck is thought to be under strong genetic control⁽³²⁾. Thus, this narrowing may be the result of species-specific bone re-modelling for growth and elongation of the femur and not necessarily associated with mineralization and strength.

In summary, the present study demonstrates the effectiveness of LC-inulin in reducing regional and total fat mass in a growing female rat model. This reduction in fat mass has implications for the management of obesity. There were no significant effects of LC-inulin on bone density after 8 weeks in this model. Further studies should investigate the effect of inulin-feeding on bone density in growing females over longer periods of 6 to 12 months. It will also be of interest to compare the effectiveness of LC-inulin with inulin and OLF in both growing female and post-menopausal models. Finally, further work should be carried out on the capacity of inulin to modulate body composition in females in healthy and disease states. Healthy bone development may be compromised in obesity and is therefore a special concern for obese children and teenagers. Inulin may be an effective treatment to promote bone mineralization as well as weight management.

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References

- Weaver CM (2002) Inulin, oligofructose and bone health: experimental approaches and mechanisms. *Br J Nutr* **93**, Suppl. 1, S99–S103.
- Chonan O & Watanuki M (1996) The effect of 6'-galactooligosaccharides on bone mineralization of rats adapted to different levels of dietary calcium. *Int J Vit Nutr Res* **66**, 244–249.
- Takahara S, Morohashi T, Sano T, *et al.* (2000) Fructooligosaccharide consumption enhances femoral bone volume and mineral concentrations in rats. *J Nutr* **130**, 1792–1795.
- Roberfroid M, Cumps J & Devogelaer J (2002) Dietary chicory inulin increases whole-body bone mineral density in growing male rats. *J Nutr* **132**, 3599–3602.
- Kruger MC, Brown KE, Collett G, *et al.* (2003) The effect of fructooligosaccharides with various degrees of polymerization on calcium bioavailability in the growing rat. *Exp Biol Med* **228**, 683–688.
- Nzeusseu A, Dienst D, Haufroid V, *et al.* (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**, 394–395.
- Taguchi A, Ohta A, Abe M, *et al.* (1994) The influence of fructooligosaccharides on the bone of model rats with ovariectomized osteoporosis. *Sci Rep Meiji Seika Kaisha* **33**, 37–44.
- Chonan O, Matsumoto K & Watanuki M (1995) Effect of galactooligosaccharides on calcium absorption and preventing bone loss in ovariectomized rats. *Biosci Biotech Biochem* **59**, 236–239.
- Scholz-Ahrens KE, Acil Y & Schrezenmeir J (2002) Effect 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.
- Zafar TA, Weaver CM, Jones K, *et al.* (2004) Inulin effects on bioavailability of soy isoflavones and their calcium absorption enhancing ability. *J Agric Food Chem* **52**, 2827–2831.
- Abrams SA, Griffin IJ, Hawthorne KM, *et al.* (2005) A combination of prebiotic short- and long-chain inulin-type fructans enhances calcium absorption and bone mineralization in young adolescents. *Am J Clin Nutr* **82**, 471–476.
- Abrams SA, Griffin IJ, Hawthorne KM, *et al.* (2005) Relationships among vitamin D levels, parathyroid hormone, and calcium absorption in young adolescents. *J Clin Endocrinol Metab* **90**, 5576–5581.
- Topping DL & Clifton PM (2001) Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. *Physiol Rev* **81**, 1031–1064.
- 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.
- Cani PD, Neyrinck AM, Maton N, *et al.* (2005) Oligofructose promotes satiety in rats fed a high-fat diet: involvement of glucagon-like peptide-1. *Obes Res* **13**, 1000–1007.
- Delzenne NM, Cani PD, Daubioul C, *et al.* (2005) Impact of inulin and oligofructose on gastrointestinal peptides. *Br J Nutr* **93**, Suppl. 1, S157–S161.
- Janicka A, Wren TA, Sanchez MM, *et al.* (2006) Fat mass is not beneficial to bone in adolescents and young adults. *J Clin Endocrinol Metab* **92**, 143–147.
- Wang MC, Bachrach LK, Van Loan M, *et al.* (2005) The relative contributions of lean tissue mass and fat mass to bone density in young women. *Bone* **37**, 474–481.
- Crabtree NJ, Kibridge MS, Fordham JN, *et al.* (2004) The relationship between lean body mass and bone mineral content in paediatric health and disease. *Bone* **35**, 965–972.
- Petit M, Beck T, Shults J, *et al.* (2005) Proximal femur bone geometry is appropriately adapted to lean mass in overweight children and adolescents. *Bone* **36**, 568–576.
- Weiler HA, Janzen L, Green K, *et al.* (2000) Percent body fat and bone mass in healthy Canadian females 10 to 19 years of age. *Bone* **31**, 547–555.
- Young D, Hopper J, Macinnis R, *et al.* (2001) Changes in body composition as determinants of longitudinal changes in bone mineral measures in 8 to 26-year-old female twins. *Osteoporos Int* **12**, 506–515.

23. Roberfroid M (2005) Introducing inulin-type fructans. *Br J Nutr* **93**, Suppl. 1, S13–S25.
24. Reeves P, Nielsen F & Fahey GJ (1993) AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *J Nutr* **123**, 1939–1951.
25. Lochmüller EM, Vung J, Weusten A, *et al.* (2001) Precision of high-resolution dual energy x-ray absorptiometry measurements of bone mineral status and body composition in small animal models. *Eur Cells Mater* **1**, 43–51.
26. Reichling T & German R (2000) Bones, muscles, and visceral organs of protein-malnourished rats (*rattus norvegicus*) grow more slowly but for longer durations to reach normal final size. *J Nutr* **130**, 2326–2332.
27. Clegg M, Keen C, Lönnerdal B, *et al.* (1981) Influence of ashing technique on the analysis of trace elements in animal tissues. *Biol Trace Elem Res* **3**, 107–115.
28. Cani PD, Dewever C & Delzenne NM (2004) Inulin-type fructans modulate gastrointestinal peptides involved in appetite regulation (glucagon-like peptide-1 and ghrelin) in rats. *Br J Nutr* **92**, 521–526.
29. Delzenne NM, Daubioul C, Neyrinck A, *et al.* (2002) Inulin and oligofructose modulate lipid metabolism in animals: review of biochemical events and future prospects. *Br J Nutr* **87**, Suppl. 2, S255–S259.
30. Coxam V (2005) Inulin-type fructans and bone health: experimental approaches and mechanisms. *Br J Nutr* **93**, Suppl. 1, S111–S123.
31. Bloebaum RD, Lundeen GA, Shea JE, *et al.* (2004) Age-related mineralization heterogeneity changes in trabecular bone of the proximal femur. *Anat Rec A Discov Mol Cell Evol Biol* **281**, 1296–1302.
32. Alam I, Sun Q, Liu L, *et al.* (2006) Identification of a quantitative trait locus on rat chromosome 4 that is strongly linked to femoral neck structure and strength. *Bone* **39**, 93–99.