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ORIGINAL ARTICLE

Fructooligosaccharide consumption improves the decreased dentin formation and mandibular defects following gastrectomy in rats

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OBJECTIVE: We examined the effects of fructooligosaccharides (FOS) consumption on gastrectomy-evoked osteopenia and disorders of dentin formation in rats.

MATERIALS AND METHODS: Male Sprague–Dawley rats ($n = 28$, 35-day old) were equally divided into two groups; sham-operated and gastrectomized, and sham-operation or total gastrectomy was performed. Four weeks after each surgery, the rats were divided into two sub-groups ($n = 7$ each); with or without 7.5% FOS-feeding for 6 weeks. Backscattered electron images of the mandibular sections were taken to calculate trabecular bone area, cortical bone area and total scan area. Thereafter, the dentin formation rate in maxilla were calculated using a fluorescent microscope.

RESULTS: Trabecular bone area and cortical bone area in GX rats were markedly decreased. FOS-feeding significantly counteracted this reduction, but not to the level seen in sham-operated rats. Total scan area in gastrectomized groups was significantly decreased. The dentin formation rate was not statistically different among the groups, except the gastrectomized group.

CONCLUSION: These results suggest that FOS consumption partially restored osteopenia and almost completely restored the reduction in dentin formation following gastrectomy in rats.

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Keywords: mandibula; incisor; dentin; gastrectomy; fructooligosaccharides; rat

Introduction

It has been shown that plant-based substances, such as grape seed proanthocyanidin extract, isoflavone and fructooligosaccharide (FOS), have beneficial effects on bone mass of rat mandibles and long bone in rats and mice, respectively (Morohashi *et al*, 2000; Ohta *et al*, 2002; Kamitani *et al*, 2004). The beneficial effects of FOS, which are low-molecular-weight indigestible carbohydrates (Oku *et al*, 1984; Tokunaga *et al*, 1986), on mineral absorption and bone have been well examined (Ohta *et al*, 1993; Morohashi *et al*, 1998). FOS increase bone mineral density, mineral concentration and volume in growing intact rats (Takahara *et al*, 2000). In addition, there is a significant relationship between the calcium concentration in bone and the apparent calcium absorption in FOS-fed rats (Morohashi *et al*, 1998). Thus, it has been considered that the enhanced calcium absorption following FOS consumption may be useful for mineralization.

Significant bone loss has been observed experimentally and clinically after total gastrectomy (Inoue *et al*, 1992; Mühlbauer *et al*, 1998; Ohta *et al*, 1998; Morohashi *et al*, 2000). It has been reported that gastrectomy-evoked osteopenia occurs in several bones, such as the tibia, femur, calvaria and vertebra. Recently, we demonstrated that total gastrectomy also induced mandibular bone loss, and decreased dentin formation in maxillary incisor (Morohashi *et al*, 2002).

Fructooligosaccharides consumption completely prevented gastrectomy-evoked osteopenia in rats (Ohta *et al*, 1998; Morohashi *et al*, 2000). Therefore, FOS affects bone structure in gastrectomized rats. However, it is not yet clear whether the defective bone and decreased dentin formation following gastrectomy are improved by FOS consumption. Thus, we used this experimental model to examine the effect of FOS consumption on the bone structure and dentin formation in gastrectomized rats.

Materials and methods

Twenty-eight 35-day-old male Sprague–Dawley rats were equally divided into two groups; sham-operated and gastrectomized. The initial body weight in each group was 129.0 ± 4.2 and 130.5 ± 4.5 g, respectively.

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Total gastrectomy, Roux-en-Y reconstruction, was performed essentially as described previously (Ohta *et al*, 1998). Briefly, the stomach and duodenum were preserved, and the pyloric sides of the duodenum and jejunum were then ligated. The esophagus was anastomosed to the jejunum, end-to-side. The duodenal segment bearing the bilio-pancreatic opening was grafted to a point 10 cm distal from the esophageal anastomosis to avoid reflux of digestive juice into the esophagus. The abdominal cavity of sham-operated rats was opened for approximately 50 min, i.e. the same length of time needed for the gastrectomy procedure. After each surgery, the rats were deprived of food for 24 h, and then allowed free access to pasteurized cow's milk for 2 days. Thereafter, the rats were fed a synthetic diet (18 g a day) containing 0.5% calcium for 25 days in individual metabolic cages. Four weeks after each surgery, the rats were divided into two sub-groups ($n = 7$); with or without 7.5% FOS-feeding (SH, SH + FOS, GX and GX + FOS) for 6 weeks. FOS consisted of 34% 1-ketose, 53% nystose and 10% 1F-b-fructofuranosyl nystose (Meioligo-P®; Meiji Seika Kaisha, Ltd, Tokyo, Japan). The base diets were prepared according to the modified AIN-93G formulation (Reeves *et al*, 1993). Dietary components were purchased from Oriental Yeast Co. (Tokyo, Japan) and Wako Pure Chemical Industries, Ltd (Tokyo, Japan). Vitamin B12 (0.5 mg kg^{-1}) was injected intramuscularly every other week in all of the rats. Body weight was recorded every week. This study was approved by Showa University.

To calculate dentin formation, tetracycline (10 mg kg^{-1}) and calcein (10 mg kg^{-1}) were administered by intraperitoneal injection, for use as time markers. Tetracycline was injected 1 day before killing, and calcein was injected 2 weeks before the injection of tetracycline. Ten weeks after surgery, rats were killed with an overdose of halothane, then the right mandibula and maxilla were removed and fixed with 4% paraformaldehyde and 2% glutaraldehyde in 0.05 M phosphate buffer (pH 7.4). The specimens were dipped in solution containing 70% methanol and 1% Villanueva bone-stain powder (Maruto Instrument Co., Ltd, Tokyo, Japan). The bones were dehydrated with a graded series of ethanol, defatted with acetone, and then embedded in polyester resin (Rigolac; Nissin EM Co., Ltd, Tokyo, Japan).

Undecalcified sections of the maxilla and mandibula were prepared and examined. The maxilla was sagittally sectioned along the center of the incisor and the mandibula was cut parallel to the long axis of the mesial root of the first molar (100 μm thick in each). Backscattered electron images of the mandibular sections were taken with a scanning electron microscope (Hitachi S-2500CX, Tokyo, Japan) after coating with carbon. Trabecular bone area, cortical bone area and total scan area were calculated using an image processor (Pro 2, Graftek, Mirmande, France). The sections were then polished to a thickness of < 10 μm and the dentin formation rate in maxilla and the enamel, dentin and dental pulp areas in mandibula were calculated using a fluorescent microscope (Axiophoto, Carl Zeiss, Germany) and the analysis software mentioned above.

Statistical analyses were performed using the SPSS statistical software package (SPSS version 6.0; SPSS, Chicago, IL, USA). One-way ANOVA with Tukey's significant difference test was used to evaluate differences in each variable among groups ($P < 0.01$). Values are expressed as the mean and standard deviation.

Results

None of the rats died throughout the experiment. Four weeks after surgery, the gain in body weight was significantly reduced in gastrectomized groups, compared with that in sham-operated rats. The body weight gain and reduction in food consumption in the gastrectomized group were lower than those in sham-operated rats throughout the experiment. Food consumption in the gastrectomized group was < 14 g a day (13.6 $\pm$ 0.4 on week 4 and 12.9 $\pm$ 0.7 on week 6). Supplementation with FOS partially counteracted these reductions caused by gastrectomy. Food consumption in the GX + FOS rats partially recovered as a consequence of FOS-feeding (13.3 $\pm$ 0.7 on week 4 and 16.8 $\pm$ 1.2 on week 6). Sham-operated rats consumed 18 g of each diet. Meanwhile, the body weight in GX + FOS rats was approximately 60 g greater than that in gastrectomized rats at the end of the experiment. A slight but statistically significant reduction was observed in GX + FOS rats compared with sham-operated rats (Table 1).

In the morphometric analysis of mandibular cross-sections, the trabecular and cortical bone areas in gastrectomized rats were markedly decreased: these values were less than half and a quarter, respectively, of those in sham-operated rats. On the contrary, while FOS-feeding significantly improved this reduction, the level was still lower than that in sham-operated. The values in the GX + FOS group were between those in the sham-operated and gastrectomized group. Total scan area was also significantly decreased in gastrectomized groups by approximately 20%. FOS-feeding did not appear to affect total scan area (Figure 1 and Table 2).

In cross-sections of the mandibular incisor, the dentin and enamel areas were significantly reduced in gastrectomized rats. Meanwhile, the dental pulp area was

Table 1 Gain in the body weight (g) at 4 and 10 weeks after the sham-operation or gastrectomy ($n = 7$)

	SH	*SH + FOS*	*GX*	*GX + FOS*
4 weeks	160 $\pm$ 3.9	158 $\pm$ 5.2	108 $\pm$ 9.5[a]	113 $\pm$ 7.6[a]
10 weeks	306 $\pm$ 10	298 $\pm$ 12	175 $\pm$ 17[a]	241 $\pm$ 12[a,b]

All values are expressed as mean $\pm$ s.d. in rats fed with or without fructooligosaccharide (FOS) from 4 weeks after operations.
There was no significant difference between SH and SH + FOS rats.
[a]Significant difference from sham-operated (SH) rat; $P < 0.01$.
[b]Significant difference between GX and GX + FOS rats; $P < 0.01$.

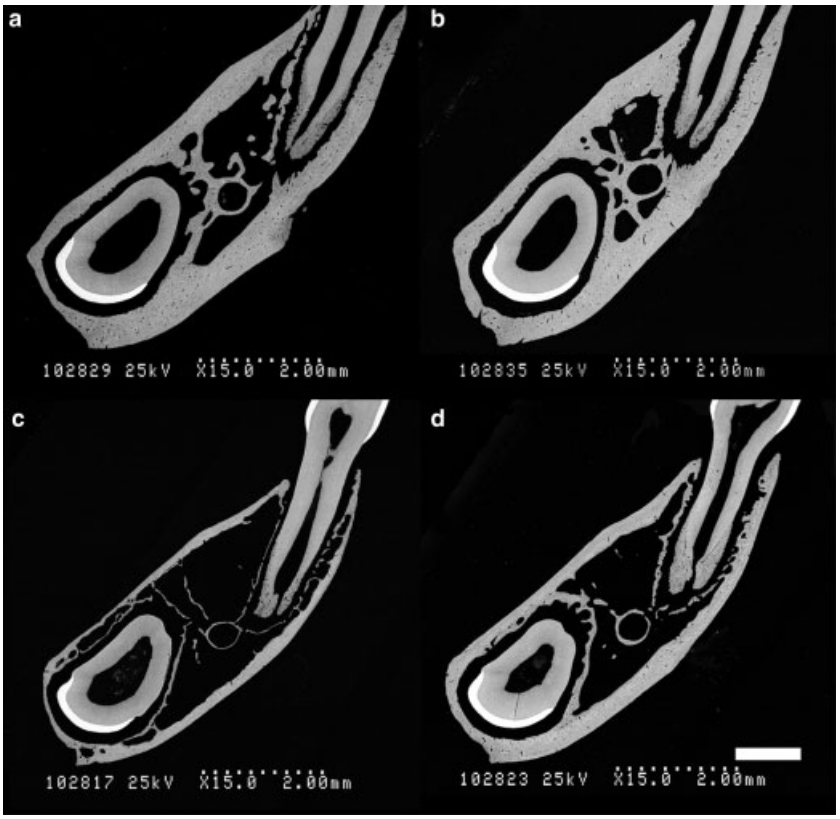

Figure 1 Backscattered electron images of the mandibular sections with a scanning electron microscope, 10 weeks after surgery. The mandibula was obtained from sham-operated rat (**a**), sham-operated rat fed with fructooligosaccharides (**b**), gastrectomized rat (**c**), gastrectomized rat fed with fructooligosaccharides (**d**). Bar = 1 mm

Table 2 Bone morphometry in cross-sections of mandibula (mm^2, $n = 7$)

	SH	*SH + FOS*	*GX*	*GX + FOS*
TBA	1.34 ± 0.16	1.31 ± 0.19	0.31 ± 0.05[a]	0.52 ± 0.12[a,b]
CBA	5.35 ± 0.21	5.53 ± 0.40	2.04 ± 0.19[a]	3.34 ± 0.70[a,b]
TA	13.61 ± 0.73	13.07 ± 0.55	11.17 ± 1.04[a]	11.75 ± 0.85[a]

The values of trabecular bone area (TBA), cortical bone area (CBA) and total scan area (TA) were calculated from backscattered electron images of mandibular cross-sections. All values are expressed as mean ± s.d. in rats fed with or without fructooligosaccharide (FOS). There was no significant difference between SH and SH + FOS rats.
[a]Significantly different from sham-operated (SH) rats; $P < 0.01$.
[b]Significant difference between GX and GX + FOS rats; $P < 0.01$.

Table 3 Morphometry in cross-sections of mandibular incisor (mm^2, $n = 7$)

	SH	*SH + FOS*	*GX*	*GX + FOS*
Dentin	1.79 ± 0.17	1.65 ± 0.15	1.46 ± 0.11[a]	1.67 ± 0.21[b]
Enamel	0.28 ± 0.02	0.28 ± 0.02	0.24 ± 0.02[a]	0.26 ± 0.02
Pulp	0.66 ± 0.15	0.70 ± 0.14	0.56 ± 0.12	0.38 ± 0.15[a,b]
Total	2.73 ± 0.10	2.64 ± 0.16	2.25 ± 0.08[a]	2.31 ± 0.14[a]

The values for the mandibular incisor were calculated from cross-sections (Villanueva bone-stain). All values are expressed as mean ± s.d. in rats fed with or without fructooligosaccharides (FOS). There was no significant difference between SH and SH + FOS rats.
[a]Significantly different from sham-operated (SH) rats; $P < 0.01$.
[b]Significant difference between GX and GX + FOS rats; $P < 0.01$.

significantly reduced in GX + FOS rats. The total area (sum of dentin, enamel and dental pulp cavity) in gastrectomized groups was also reduced by gastrectomy. There were no significant differences in any of the variables between the SH and SH + FOS groups (Table 3).

There was no significant difference in the dentin formation rate, calculated from the incisor in sagittally sectioned maxilla, among the groups, except that the gastrectomized group showed an approximately 25% reduction relative to the sham-operated group. On the contrary, FOS consumption almost completely restored the reduction in the dentin formation rate following gastrectomy, however, there was still a 7% reduction relative to the sham-operated group (Figure 2).

Discussion

It is well known that gastrectomy is followed by early satiety and body weight loss in most patients (Sanchez-Cabezudo Diaz-Guerra and Larrad Jimenez, 2002). Body weight loss in gastrectomized rats has also been reported (Zittel *et al*, 2002). Meanwhile, indigestible carbohydrates such as FOS, in addition to their selective effects on microflora in the large intestine, influence many aspects of bowel function through fermentation (Cummings *et al*, 2001). Overall, FOS appear to enhance indices of gut health by positively altering gut microbial ecology (Swanson *et al*, 2002). Thus, the effects of FOS on body weight and food consumption might result from the improvement of bowel function in gastrectomized rats. It is conceivable that gastrectomized rats had, at least in part, a reduced bone area

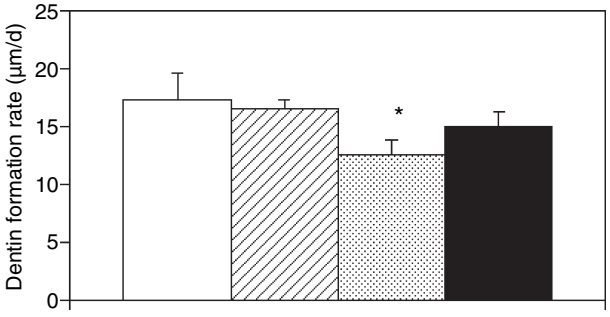

Figure 2 Dentin formation rate, calculated from the incisor in sagittally sectioned maxilla. The values were calculated from the distance between tetracycline and calcein labels during 2 weeks before killing. Sham-operated rats (open bar), sham-operated rats fed fructooligosaccharides (dashed bar), total gastrectomized rats (dotted bar) and total gastrectomized rats fed fructooligosaccharides (closed bar). There was no significant difference in the dentin formation rate among groups, except the GX group ($P < 0.01$). All values are expressed as mean + s.d.

because of a reduced body weight. Thus, the influence of FOS on bone may be more clearly shown by considering a weight-matched group. Unfortunately, such a group was not used in this experiment.

Marked cortical and cancellous bone loss was observed in GX rats in this study, as in our previous report (Morohashi *et al*, 2002). FOS consumption for 6 weeks partially improved gastrectomy-evoked mandibular bone loss (Table 2). A reduction in calcium absorption and femoral bone loss in gastrectomized rats have been reported previously (Ohta *et al*, 1998; Morohashi *et al*, 2000). Meanwhile, FOS-feeding enhances calcium retention resulting from stimulated calcium absorption (Morohashi *et al*, 1998). These stimulatory effects of FOS have been explained by the fermentation of FOS in the large intestine to produce short-chain fatty acids, which in turn reduce luminal pH (Ohta *et al*, 1994) and induce the proliferation of epithelial cells (Sakata, 1987). Considering previous reports, calcium absorbed in the large intestine by FOS-feeding is also likely to be retained in the mandibula.

Cortical bone area and trabecular bone area in the GX + FOS group increased compared with those in the gastrectomized group. However, these variables did not reach the levels in the SH group. In addition, the total scan area in the gastrectomized and GX + FOS groups was smaller than that in the sham-operated group (Table 2). Recently, age-related changes in craniofacial growth was examined in growing rats (Tanimoto *et al*, 2003). They demonstrated that the mandible shows substantial growth from 7 to 9 weeks of age. In this study, gastrectomy was performed at 5 weeks of age and FOS-feeding was started at 9 weeks of age. Thus, the final mandibular volume in GX + FOS rats may have been nearly achieved before FOS supplementation. In fact, FOS-feeding did not appear to have affected total scan area in GX + FOS rats at the end of the experiment. On the contrary, it has been reported that FOS consumption completely prevents femoral bone loss following gastrectomy when rats are fed FOS from 6 to 10 weeks of age (Morohashi *et al*, 2000). In general, there is an age-related decrease in bone formation (Kanekawa and Shimizu, 1998). In this study, FOS was supplied to rats after peak bone modeling and remodeling. Therefore, cortical and cancellous bone formation may have been somewhat improved.

A reduction in dentin formation following gastrectomy has been reported (Morohashi *et al*, 2002). In this study, similar results were obtained in gastrectomized rats and the reductions were improved by FOS consumption (Figure 2 and Table 3). A possible explanation for these findings is as follows. The parathyroid hormone (PTH) level in blood is significantly increased in gastrectomized rats (Morohashi *et al*, 2002). It is well known that the serum calcium concentration is inversely proportional to the PTH level in blood. While calcium absorption is reportedly reduced in gastrectomized rats, hypocalcemia is not observed (Ohta *et al*, 1998). Thus, it is unlikely that calcium malabsorption is the main cause of hyperparathyroidism. While a high-phosphorus diet induces nutritional hyperparathyroidism in rats (Schaafsma and Visser, 1980; Demeter *et al*, 1991), gastrectomy significantly enhances phosphorus absorption in the intestine: gastrectomized rats show approximately 60% higher phosphorous absorption than control rats. In addition, this enhancement is reduced by FOS consumption (Ohta *et al*, 1998). It has been reported that PTH inhibited odontogenesis in cultured molar (Sakakura *et al*, 1989). It has been suggested that PTH may inhibit the transformation of preodontoblasts into odontoblasts *in vitro* (Sakakura, 1987). It is likely that elevated PTH along with a high absorption of phosphorus might inhibit dentin formation, and FOS consumption might suppress this phenomenon.

An analysis of TRAP 5b and osteocalcin, biochemical serum markers of bone turnover, suggested that there is a shift in the balance to favor bone resorption rather than formation for 4 weeks after gastrectomy in rats (Surve *et al*, 2001a). In histomorphometry in an 8-week study, the number of osteoclasts in the distal femur was reduced while the osteoclast surface was increased in gastrectomized rat (Surve *et al*, 2001b). Those researchers also suggested that the reduction in the number of osteoclasts reflects the reduced trabecular bone volume while the increased osteoclast surface is consistent with the accumulation of osteoclasts on the remaining bone. Thus, it is likely that bone resorption predominates over bone formation in gastrectomy-evoked osteopenia. While gastrectomy in rats results in osteopenia and impaired dentin formation, many putative factors, such as changes in mineral metabolism, calcium-regulating hormones and bone turn-over following surgery, may act simultaneously. FOS consumption almost completely restored the reduced dentin formation and partially recovered mandibular bone disorders following gastrectomy. These beneficial results suggest that a nutritional trial, such as with FOS supplementation, may be important for understanding calcification in the gastrectomized rat. Indeed, calcium and magnesium, important minerals for calcification, in each region of the bone surface were increased in rats fed FOS. There

was a significant relationship between absorbed calcium and calcium contents in bone, and a similar relationship was found for magnesium (Takahara *et al*, 2000). Thus, the stimulatory effect of FOS might contribute to an improvement of bone disorders and the dentin formation rate in gastrectomized rat. However, the mechanism of this improvement in osteopenia following gastrectomy at the cellular level is still unclear. Further studies are required to evaluate the relationship between the absorption of minerals and the activities of bone cells. In addition, Tahiri *et al* (2003) suggested that FOS may influence calcium absorption in the late postmenopausal phase. In contrast, FOS had no effect on calcium absorption in young, healthy men (Heuvel *et al*, 1998). Meanwhile, it has been reported that the ingestion of moderate doses of FOS improves intestinal Mg absorption and status in postmenopausal women (Tahiri *et al*, 2001). Thus, whether or not FOS has a beneficial effect on mineral absorption and bone in humans is still controversial. Further studies are needed to examine the effects of FOS on mineral and bone metabolism in humans.

References

Cummings JH, Macfarlane GT, Englyst HN (2001). Prebiotic digestion and fermentation. *Am J Clin Nutr* **73:** 415S–420S.

Demeter JG, De Jong SA, Oslapas R (1991). High phosphate diet-induced primary hyperparathyroidism: an animal model. *Surgery* **110:** 1053–1060.

Heuvel EG, Schaafsma G, Muys T (1998). Nondigestible oligosaccharides do not interfere with calcium and nonheme-iron absorption in young, healthy men. *Am J Clin Nutr* **67:** 445–451.

Inoue K, Shiomi K, Higashide S (1992). Metabolic bone disease following gastrectomy: assessment by dual energy X-ray absorptiometry. *Br J Surg* **79:** 321–324.

Kamitani Y, Maki K, Tofani I (2004). Effects of grape seed proanthocyanidins extract on mandibles in developing rats. *Oral Dis* **10:** 27–31.

Kanekawa M, Shimizu N (1998). Age-related changes on bone regeneration in midpalatal suture during maxillary expansion in the rat. *Am J Orthod Dentofacial Orthop* **114:** 646–653.

Morohashi T, Sano T, Ohta A (1998). True calcium absorption in the intestine is enhanced by fructooligosaccharide-feeding in rats. *J Nutr* **128:** 1815–1818.

Morohashi T, Ohta A, Yamada S (2000). Dietary fructooligosaccharides prevent a reduction of cortical and trabecular bone following total gastrectomy in rats. *Jpn J Pharmacol* **82:** 54–58.

Morohashi T, Hirama Y, Takahara S (2002). Defects in mandibular bone area, enamel iron content, and dentine formation following gastrectomy in rats. *Arch Oral Biol* **47:** 499–504.

Mühlbauer RC, Schenk RK, Chen D (1998). Morphometric analysis of gastrectomy-evoked osteopenia. *Calcif Tissue Int* **62:** 323–326.

Ohta A, Osakabe N, Yamada K (1993). Effect of fructooligosaccharides and other saccharides on Ca, Mg and P absorption in rats. *J Jpn Soc Nutr Food Sci* **46:** 123–129.

Ohta A, Baba S, Takizawa T (1994). Effects of fructooligosaccharides on the absorption of magnesium in the magnesium-deficient rat model. *J Nutr Sci Vitaminol* **40:** 171–180.

Ohta A, Ohtsuki M, Hosono A (1998). Dietary fructooligosaccharides prevent osteopenia after gastrectomy in rats. *J Nutr* **128:** 106–110.

Ohta A, Uehara M, Sakai K (2002). A combination of dietary fructooligosaccharides and isoflavone conjugates increases femoral bone mineral density and equal production in ovariectomized mice. *J Nutr* **132:** 2048–2054.

Oku T, Tokunaga T, Hosoya N (1984). Nondigestibility of a new sweetener, "Neosugar" in the rat. *J Nutr* **114:** 1574–1581.

Reeves PG, Rossow KL, Lindlauf J (1993). Development and testing of the AIN-93 purified diets for rodents: results on growth, kidney calcification and bone mineralization in rats and mice. *J Nutr* **123:** 1923–1931.

Sakakura Y (1987). Effects of parathyroid hormone on odontogenesis of the mouse embryonic molar tooth in vitro. *Calcif Tissue Int* **40:** 49–54.

Sakakura Y, Fujiwara N, Sugawara M (1989). In vitro effects of calcitonin and/or parathyroid hormone on odontogenesis of mouse embryonic molars. *J Dent Res* **68:** 1279–1284.

Sakata T (1987). Stimulatory effect of short-chain fatty acids on epithelial cell proliferation in the rat intestine: a possible explanation for trophic effects of fermentable fibre, gut microbes and mucosal trophic factors. *Br J Nutr* **58:** 95–103.

Sanchez-Cabezudo Diaz-Guerra C, Larrad Jimenez A (2002). Analysis of weight loss with the biliopancreatic diversion of Larrad: absolute failures or relative successes? *Obes Surg* **12:** 249–252.

Schaafsma G, Visser R (1980). Nutritional interrelationships between calcium, phosphorus and lactose in rats. *J Nutr* **110:** 1101–1111.

Surve VV, Andersson N, Alatalo S (2001a). Does combined gastrectomy and ovariectomy induce greater osteopenia in young female rats than gastrectomy alone? *Calcif Tissue Int* **69:** 274–280.

Surve VV, Andersson N, Lehto-Axtelius D (2001b). Comparison of osteopenia after gastrectomy, ovariectomy and prednisolone treatment in the young female rat. *Acta Orthop Scand* **72:** 525–532.

Swanson KS, Grieshop CM, Flickinger EA (2002). Fructooligosaccharides and *Lactobacillus acidophilus* modify gut microbial populations, total tract nutrient digestibilities and fecal protein catabolite concentrations in healthy adult dogs. *J Nutr* **132:** 3721–3731.

Tahiri M, Tressol JC, Arnaud J (2001). Five-week intake of short-chain fructo-oligosaccharides increases intestinal absorption and status of magnesium in postmenopausal women. *J Bone Miner Res* **16:** 2152–2160.

Tahiri M, Tressol JC, Arnaud J (2003). Effect of short-chain fructooligosaccharides on intestinal calcium absorption and calcium status in postmenopausal women: a stable-isotope study. *Am J Clin Nutr* **77:** 449–457.

Takahara S, Morohashi T, Sano T (2000). Fructooligosaccharide consumption enhances femoral bone volume and mineral concentrations in rats. *J Nutr* **130:** 1792–1795.

Tanimoto K, Imada M, Ohno S (2003). Association between craniofacial growth and urinary bone metabolic markers (pyridinoline, deoxypyridinoline) in growing rats. *J Dent Res* **82:** 28–32.

Tokunaga T, Oku T, Hosoya N (1986). Influence of chronic intake of new sweetener (Neosugar) on growth and gastrointestinal function of the rat. *J Nutr Sci Vitaminol* **32:** 111–121.

Zittel TT, Glatzle J, Muller M (2002). Total gastrectomy severely alters the central regulation of food intake in rats. *Ann Surg* **236:** 166–176.