SCFA stimulates the proliferation of epithelium cells in the intestine *(37)* and reduces luminal pH *(6, 25, 38, 44)*.

We recently reported that both true absorption and apparent intestinal calcium absorption were significantly stimulated by FOS consumption in rats. No significant differences, however, were noted in endogenous net calcium excretion into feces between the rats fed FOS and the control animals. Fractional calcium absorption and its balance in the FOS-fed rats were significantly higher than in the control group, since there was no significant difference in calcium intake between the two groups. More than 95% of the absorbed calcium was retained in the body, and less than 5% of that was excreted into urine in both groups. Calcium balance in rats fed FOS was correlated significantly with true calcium absorption ($r^2 = 0.936, p < 0.01$), as in the control group ($r^2 = 0.994, p < 0.01$). Thus calcium retention in bone mainly depended on absorbed calcium *(22)* (Fig. 1).

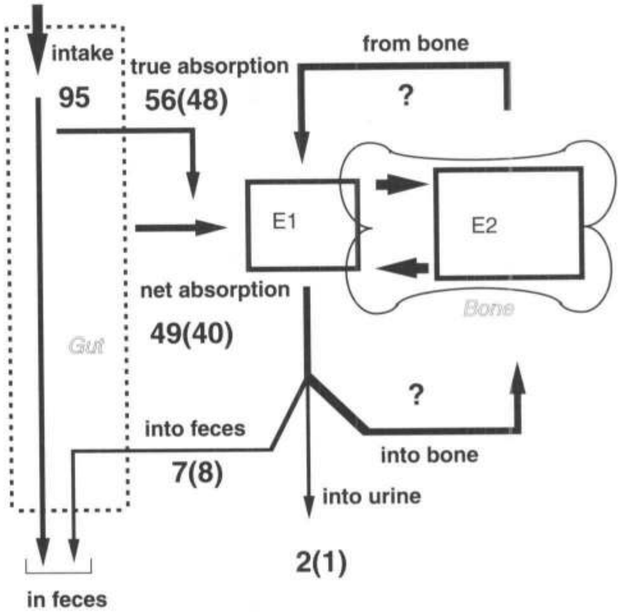

Fig. 1. Schematic illustration of a two-compartment open model of calcium metabolism in rats.

The data are described as mg/day in rats fed with fructooligosaccharides or without (in parentheses). In this model, the ^{45}Ca elimination curve is expressed by the following equation. $[^{45}Ca]pl = Ae^{-\alpha t} + Be^{-\beta t}$. $[^{45}Ca]pl$ is the plasma-specific activity (cpm/mg Ca), and t (hrs) is the time after ^{45}Ca injection. The four parameters (A, B, and α, β) are estimated by using the nonlinear least-squares method and the specific radioactivities of 6 plasma samples obtained at 2, 4, 6, 25, 49, and 73 hrs after ^{45}Ca injection. The six plasma specific radioactivities are calculated by dividing the radioactivity in each of the samples by the cold calcium concentration in the plasma sample obtained at the end of experiment.

FOS AND BONE

Several studies have been carried out to establish the role of minerals in bone. Bone stores 99% of the body's calcium, and calcium salts are responsible for the hardness of bone *(10)*. Meanwhile, it has been established that magnesium is a critical ion in mammals, not only as a cofactor for many enzymes of the energy extraction system and protein synthesis pathways, but also for bone formation. Thus it is suggested that these minerals may play an important role in bone structure or in the hardness of bone *(36)*.

The enhancement of apparent calcium and magnesium absorptions and their ratios, resulting from fructooligosaccharides (FOS)-feeding, have been reported in growing rats *(23–25)*. These findings would lead us to expect an increase in bone mass with FOS supplementation. Previous studies have relied on the mineral contents in ashed bone or on measurements of bone mineral density to investigate the effects of FOS on bone *(27, 29, 30)*. However, these methods cannot be used to examine bone structure or to measure mineral contents in a local area when rats have been fed a diet containing FOS.

We reported whether the enhanced mineral absorption resulting from FOS-feeding affects femoral bone structure and mineral contents in intact rats. Constant amounts of calcium (95 mg/d) and magnesium (8 mg/d) were fed to the rats. Mineral concentrations in diaphyses, which were considered to have been formed after dietary treatment *(21)*, were enhanced by FOS feeding *(41)*. Similar changes were also found in other regions that were close to the surface of trabecular bone. These increases are small but statistically significant. In fact, the weight percentages of Ca and Mg were enhanced, as calculated from a small area (7.5 × 10 μm) of the cortical or trabecular bone. Calcium in bone is usually characterized as hydroxyapatite $[(Ca_{10}(PO_4)_6(OH)_2)]$ *(10)*. Magnesium has been shown to bind to the surface of hydroxyapatite crystals and to retard its nucleation and growth *in vitro (1)*. In fact, the bones of rats fed excess magnesium have smaller mineral crystals than control rats. In contrast, the hydroxyapatite crystals in magnesium-deficient rats are significantly increased in size *(2, 4)*. Thus the enhanced weight percent of calcium and magnesium might be associated with the size of hydroxyapatite crystal. On the other hand, Fountos et al. *(7)* have suggested that *in vivo* measurements of the calcium/phosphorus ratio of bone may be useful for assessing skeletal aging or some pa-

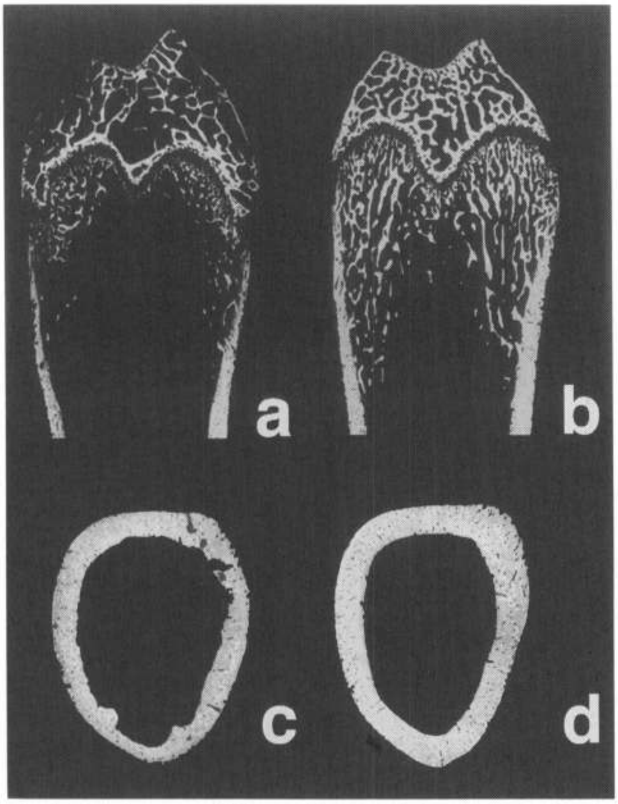

Fig. 2. Back-scattered electron images of the sagittal surface in the distal metaphysis (a and b) and diaphysis (c and d). The right femur was obtained from gastrectomized (GX) rats fed with (b and d) or without (a and c) fructooligosaccharides.

this phenomenon by using the tetracycline labeling method and computed microtomography, by which gastrectomy leads to an increase in bone resorption. Thus this gastrectomy-induced osteopenia may be due to an increase in bone resorption rather than to a decrease in bone formation. This gastrectomy-induced bone loss is completely prevented by FOS consumption in rats *(19)* (Fig. 2). Therefore, our results suggest that the decrease in bone mass is quite likely to reflect increased bone resorption in the gastrectomized rats, and FOS-feeding might inhibit this phenomenon.

Peak bone mass in humans is achieved after sexual maturity and is maintained for two decades. Thereafter the mass of nealy all bones declines until death. Thus we would expect that high calcium and magnesium deposition into bone during the period of growth would increase the peak bone mass and prevent bone disorders that result from aging. There have been several reports on studies of rats to evaluate the beneficial effects of FOS on bone, which suggest that it may be a promising prebiotic treatment. If similar effects are found in humans, dietary supplementation with FOS could contribute to the treatment of osteopenia. Further human studies are required to evaluate the effects on bone metabolism.

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