Note

Accelerating Effect of Chitosan Intake on Urinary Calcium Excretion by Rats

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The effect of chitosan on calcium (\(^{44}\)Ca) metabolism was investigated in rats. The whole-body retention of \(^{44}\)Ca by rats fed on a 5% chitosan diet was significantly decreased when compared with that of rats fed on a cellulose diet, but showed no significant difference from that of rats fed on a fiber-free diet. Although there was no significant difference in the fecal excretion of \(^{44}\)Ca between the chitosan group and the cellulose or fiber-free group, the urinary excretion of \(^{44}\)Ca was significantly increased in the chitosan group when compared with the cellulose group. These results suggest that dietary chitosan would affect the calcium metabolism in animals.

Key words: chitosan; calcium; rat

It has been reported that chitosan has such biological actions as suppressing serum cholesterol elevation\(^{11}\) and inhibiting lipid absorption.\(^{32}\) It is also known that chitosan, an amino group derived from glucosamine which is a constituent of saccharide, binds jointly with heavy metal ions.\(^{31}\) Although it is clear that chitosan is free of acute toxicity,\(^{41}\) the biological effects of its continuous intake remain quite obscure. It is important to clarify the effect of chitosan on the absorption and metabolism of nutrients to better understand chitosan’s usefulness and safety. However, its relation with various minerals in vivo remains largely unclear. We carried out experiments, mainly involving balance studies, with radioactive calcium (\(^{44}\)Ca) in order to investigate the effect of chitosan on Ca metabolism.

Eight-week-old, male Wistar rats (body weight of 250–300 g; Japan SLC Co., Hamamatsu, Japan) were individually housed in metabolic cages by which separate collection of feces and urine could be achieved. A diet containing chitosan (Chitosan F, \(3 \times 10^4\) average molecular weight, \(89\%\) degree of deacetylation; Kimitsu Chemical Industries Co., Tokyo, Japan) and another diet containing cellulose (Oriental Yeast Co., Tokyo, Japan), both at the level of \(5\%\), were prepared with AIN-76\(^{31}\) as a base. Another fiber-free diet was prepared with corn starch used as a substitute for dietary fiber. The animals were divided into three diet-based groups each consisting 10 rats, and were given the respective diets ad libitum for 40 days. They were also given free access to tap water.

Radioactive Ca (\(^{44}\)Ca, \(^{44}\)CaCl\(_2\), \(7.4\) MBq/mg of Ca specific activity, Amersham Life Science, UK) was purchased through Japan Isotope Association. \(^{44}\)Ca was diluted with sufficient physiological saline to make its specific activity 15 kBq/ml. After 40 days on the experimental diet, half the animals in each group were each given orally a single dose of \(^{44}\)Ca equivalent to 7.5 kBq with a gastric cannula. In order to assess the effect of Ca incorporated in the body, the remaining animals were intravenously given \(^{44}\)Ca via the caudal vein at a dose identical to the oral dose.

After administering the \(^{44}\)Ca, each rat was immediately placed in a polystyrene container for whole-body measurement by a small animal counter (Armac model 446, Packard Instrument Co., U.S.A.) to determine the exact dose of \(^{44}\)Ca received by each animal, and to observe chronological changes in the whole-body retention of \(^{44}\)Ca. Feces and urine were collected daily and their radioactivity measured for 14 days.

Significant differences in the data were studied by the following techniques: homogeneity of variance was first studied by Bartlett’s method \((p<0.05)\); Duncan’s multiple-range test \((p<0.05)\) was performed if the variance was homogeneous; and Scheffe’s test \((p<0.05)\) was conducted if there was no homogeneity.

Figure 1 shows the effect of chitosan on the whole-body \(^{44}\)Ca retention. In comparison with the cellulose group, the whole-body retention of \(^{44}\)Ca given orally was significantly decreased in the chitosan group on day 2 after administering \(^{44}\)Ca and thereafter, but no significant difference was observed between the chitosan group and the fiber-free group.

It is well known that strontium and calcium absorbed by the

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Fig. 1. Whole-body Retention of \(^{44}\)Ca after Single Oral and Intravenous Administrations.
Each value is the mean for 5 rats. Values with different letters are significantly different \((p<0.05)\).
body are mostly distributed in the bones. In our experiment, \(^{47}\text{Ca}\) was retained at a high rate, presumably due to migration to the bones, in all the groups given \(^{47}\text{Ca}\) intravenously. Throughout the test period, however, the whole-body \(^{47}\text{Ca}\) retention in the chitosan group remained significantly lower than that of the cellulose group. In comparison with the fiber-free group, on the other hand, the whole-body \(^{47}\text{Ca}\) retention in the chitosan group tended to be lower.

Figure 2 shows \(^{47}\text{Ca}\) excretion into the feces. No significant differences were noted in either the daily excretion or cumulative excretion between the chitosan group and the cellulose or fiber-free group, regardless of whether \(^{47}\text{Ca}\) was administered orally or intravenously.

Figure 3 shows \(^{47}\text{Ca}\) excretion in the urine. Cumulatively, the urinary excretion of \(^{47}\text{Ca}\) tended to be greater in the chitosan group given \(^{47}\text{Ca}\) orally or intravenously, and was significantly greater in comparison with the cellulose group. Those animals given \(^{47}\text{Ca}\) intravenously resulted in the excretion being significantly higher in the chitosan group than in the cellulose or fiber-free group. There are several reports concerning the behavior of chitosan in relation to strontium. Strontium is known to behave similarly in vivo to Ca. Nishimura et al. have reported that the oral administration of chitosan was effective for removing radioactive strontium, a fission product, from the gastrointestinal tract, and that continuous intake of chitosan inhibited the reabsorption of radioactive strontium and accelerated its excretion to significantly decrease its whole-body retention, thus being useful for removing radioactive substances. They also found that a 5% chitosan diet significantly decreased the absorption of \(^{85}\text{Sr}\) by the body as compared with the effect of the fiber-free diet. On the other hand, a large number of reports have pointed out that a long-term massive intake of food containing abundant dietary fiber inhibited the intestinal absorption of Ca. Oka et al. have examined Ca transport from the intestine in rats fed on two diets respectively containing cellulose and glucomannan at 20% for seven weeks. They found that there was no difference in Ca transport between the cellulose group and the fiber-free control group, but that Ca transport in the glucomannan group was significantly decreased in comparison with that of the control group. We fed the diet containing chitosan at 5% to rats for 40 days and examined the behavior of Ca after orally or intravenously administering \(^{47}\text{Ca}\). We found that the whole-body \(^{47}\text{Ca}\) retention by the chitosan group was less than that by the cellulose group, and that there was no difference between the chitosan and fiber-free groups in the whole-body retention of orally administered \(^{47}\text{Ca}\).

Orally administered \(^{47}\text{Ca}\) is incorporated into various tissues of the body via intestinal absorption. Immediately following its administration, there was no intergroup difference in the whole-body retention of \(^{47}\text{Ca}\) as shown in Fig. 1. Neither was there any intergroup difference in \(^{47}\text{Ca}\) excretion in the feces. However, its urinary excretion tended to be greater in the chitosan group than in the fiber-free or cellulose group. These results indicate that orally administered chitosan acted to increase the excretion of Ca rather than to inhibit its absorption. Similar behavior was found from the results obtained by the intravenous administration of \(^{47}\text{Ca}\); data for whole-body retention indicate that \(^{47}\text{Ca}\) was promptly taken up in the bones. Similarly to oral administration, the whole-body retention by the chitosan group was significantly lower than that by the cellulose group. This is presumably because \(^{47}\text{Ca}\) in the bones was decreased.

In comparison with the fiber-free diet, the chitosan diet significantly promoted strontium excretion, although no
significant difference was noted between these groups in the excretion of $^{47}$Ca (orally administered). This suggests that chitosan tended to decreased bone salt by promoting the excretion of Ca in the body, although to a lesser extent than with the excretion of strontium. That a difference in whole-body $^{47}$Ca retention was shown between the chitosan and cellulose groups, but not between the chitosan and fiber-free groups, in this study can be explained by the fact that cellulose had no substantial effect on Ca abosorption, while chitosan presumably lost its property as fiber when extensively degraded by bacterial enzymes in the digestive tract.

The urinary excretion of intravenously administered $^{47}$Ca was significantly higher by the chitosan-fed animals, which suggests that Ca metabolism was affected by the chitosan products formed in the gastrointestinal tract. Absorbed chitosan products may provoke Ca transport from the bone. Further studies on the effects of glucosamine and chitosan oligosaccharides on Ca metabolism are warranted.

The results obtained by this study indicate that chitosan increased urinary Ca excretion and thus affected Ca metabolism in vivo. Chitosan at a relatively high dose of 5% in the diet may have adverse effects such as decreasing bone salt with long-term administration, although the degree of such an effect was not as substantial as the effect on strontium, a non-nutrient. This may be attributable to the active transport mechanism for Ca that functions in response to vital needs.

In any case, a long-term intake of chitosan may greatly affect Ca metabolism in vivo, suggesting that calcium intake should be increased when chitosan is used as dietary fiber.

References