Enhanced absorption of calcium by chondroitin in the rat small intestine

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Abstract: To study the possibility of utilizing sea shells as a new calcium source, a trial calcium product-
UNICAL® which is the calcium citrate and calcium lactic added with chondroitin (IAM= increase absorption
materials), was prepared from sea shells and a test on its intestinal absorption of calcium in rats was conducted
using situ recirculation method. Intestinal absorption of the calcium compound from sea shells and facilitating
of intestinal calcium absorption by IAM were examined by the in situ recirculation method. The calcium
compound from sea shells clearly resulted in increased absorption rate, and IAM in facilitating intestinal
calcium absorption. Based on those findings, the calcium compound composed of the calcium from sea shells
and IAM was concluded as highly effective in facilitating intestinal calcium absorption.

Key words: Chondroitin, Intestine, Absorption, Calcium, Rat

Introductions

Many reports revealed that adequate calcium intake prevents skeletal disorders during childhood and
adolescence1-9. Calcium not only constitutes the main mineral element of the bone and the teeth. And it also
plays an important role in maintaining various functions of organisms, such as regulation of immune mechanism,
muscles contraction, nerve system and hormones. It is therefore thought to be important to assure a constant and
sufficient amount of calcium in an organism. Although calcium uptake in humans has generally attracted broad
attention, since its shortage is nowadays associated with hypertension, obesity, coronary heart diseases, endocrine
diseases and bone diseases, it has been pointed out that in general it has not reached a sufficient level1-9.

Recently, much attention has also been drawn to the quality of calcium. People in European countries and
North American countries take much of their calcium from milk. Their calcium intake is 1.5 to 3 times more than that
of Japanese. However, it has been reported that the incidence of fractures in European and North American
countries are about 3 times more than that of Japan and differences in bone-salt amount is within 10%6. These
finding could be attributed to difference in race and diet. The difference of the calcium source is also suggested to
the reason of the findings.

To study the possibility of utilizing sea shells as a new calcium source, a trial calcium product-
UNICAL® which is the calcium citrate and calcium lactic added with chondroitin (IAM= increase absorption materials), was
prepared from sea shells and a test on its intestinal absorption of calcium in rats was conducted using situ recirculation method.

Material and Methods

Animals

Twenty five eight-week old male Wistar rats (approximately
200g, Gnea Japan, Tokyo, Japan) were individually housed
in stainless-steel wire-mesh cages with an auto-flush
cleaning unit in a room maintained at 24 ± 1°C and 55 ±
5% relative humidity. Lighting condition was set at 12 hours
automatic-lighting cycle. During preliminary housing
period, rats were fed sterilized solid stock diet (CA-1,
Crea Japan, Tokyo, Japan) and supplied filtered tapped
water by an automated water supply unit ad libitum.

Test calcium

1. UNICAL® LDM which was calcium lactic prepared
from sea shells added with chondroitin, 2. UNICAL® CTM which was calcium citric prepared from sea shells added with chondroitin, 3. Milk which was calcium prepared from drinking milk, 4. CaCO₃ which was calcium carbonate and 5. CaL which was calcium lactic (Table 1).

**Preparation of intestine recirculation area**

All rats were fasted for 24 hours and an abdominal median incision was performed under urethane anesthesia (1.2g/kg). The choledoch proximal to the duodenum was tied to eliminate the effect of the bile, and the area of the intestine from the pyloric part to the part 6 cm below was assigned for recirculation. L-shaped tubes were set at the starting and end points of the recirculation and tied with suture. Sterilized saline was preheated to 37°C and poured into the L-shaped tube to clean the intestine. After cleaning, a light was placed close to the rats to prevent the loss of body temperature.

**Preparation of recirculation solution**

Artificial gastric juice and intestinal juice were prepared by following manner. For the gastric juice 2g of sodium chloride was added into 24ml diluted hydroxide solution and fill up to 1000ml with ion exchanged water (pH:about1.2). For the intestinal juice 118ml of 0.2N sodium hydroxide solution and ion exchanged water were added to 250ml of 0.2M monobasic potassium phosphate solution to make 1000ml of solution (pH: about 6.8). 2mg various test calcium was dissolved in 10ml artificial gastric juice. The detail of test calcium was described in Table 1. Thirty minutes later, this solution was added to 10ml artificial intestinal juice. The pH was adjusted to 6.5 and exactly 100ml was obtained for the recirculation solution.

**Recirculation**

100ml-recirculation solution was preheated to 37°C. The perista pump and the reservoir containing the recirculation solution with each test calcium supplement and intestine were set to from a loop-shaped circulation. The solution was circulated from the start point proximal to the pyloric to the lower part at a speed of 1ml/min. 1ml recirculation solution was collected from the loop immediately after the recirculation and at 10, 20, 30, 40, 50 and 60 minutes after. Ca concentration (decreased amount of Ca) was successively measured.

**Measurement of Ca concentration**

0.5ml-recirculation solution was mixed with lanthanum chloride to prevent inhibition caused by impure ingredients. Measurement was performed using Shimazu AA-640 atomic absorption spectrophotometer (Shimazu, Kyoto, Japan).

Blood from the rats was collected before and after the circulation procedure. The plasma Ca concentration was measured by OCPC method using the ABBOTT-VISION analyzer.

**Statistical analysis**

All Ca concentration tests were repeated for 5 times. The values were expressed as the mean ± SD for 5 animals. All the Ca concentration test data were analyzed statistically using Student’s t test and the significant level was set at 0.05.

![Graph](image-url)  
*Fig.1 Time course of Ca absorption in rat intestine*
Table 1  Test materials

<table>
<thead>
<tr>
<th>Material</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. UNCAL® LDM</td>
<td>Calcium lactic prepared from sea shells added with IAM (chondroitin)</td>
</tr>
<tr>
<td>2. UNCAL® CTM</td>
<td>Calcium citric prepared from sea shells added with IAM (chondroitin)</td>
</tr>
<tr>
<td>3. Milk</td>
<td>Calf milk</td>
</tr>
<tr>
<td>4. CaCO₃</td>
<td>Calcium carbonate</td>
</tr>
<tr>
<td>5. CaL</td>
<td>Calcium lactic</td>
</tr>
</tbody>
</table>

Table 2  Intestinal absorption of calcium in rat duodenum by recirculating method

<table>
<thead>
<tr>
<th>Material</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDM</td>
<td>4.12±0.47</td>
<td>6.02±0.35</td>
<td>6.94±0.16</td>
<td>7.08±0.39</td>
<td>7.34±0.14</td>
<td>7.99±0.38</td>
<td>6.58±1.36</td>
</tr>
<tr>
<td>CTM</td>
<td>3.84±0.25</td>
<td>5.39±0.37</td>
<td>6.31±0.36</td>
<td>6.06±0.27</td>
<td>6.81±0.29</td>
<td>7.29±0.35</td>
<td>5.95±1.22</td>
</tr>
<tr>
<td>Milk</td>
<td>2.34±0.12</td>
<td>3.44±0.59</td>
<td>4.38±0.46</td>
<td>5.05±2.21</td>
<td>4.84±2.42</td>
<td>6.35±3.70</td>
<td>4.40±1.38</td>
</tr>
<tr>
<td>CaCO₃</td>
<td>1.32±0.57</td>
<td>1.65±0.58</td>
<td>2.63±0.58</td>
<td>2.96±1.00</td>
<td>3.29±0.58</td>
<td>4.28±0.58</td>
<td>2.67±1.11</td>
</tr>
<tr>
<td>CaL</td>
<td>0.47±0.81</td>
<td>2.32±0.81</td>
<td>2.32±0.81</td>
<td>3.25±0.85</td>
<td>4.19±0.03</td>
<td>5.12±0.83</td>
<td>2.95±1.63</td>
</tr>
</tbody>
</table>

Each value (Mean±S.D.) represents percentage of total intestinal absorption of calcium (n=5).

Table 3  Plasma levels of calcium in rat before and after the recirculation

<table>
<thead>
<tr>
<th>Material</th>
<th>Before recirculating</th>
<th>After recirculating</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDM</td>
<td>9.20±0.08</td>
<td>9.17±0.05</td>
</tr>
<tr>
<td>CTM</td>
<td>9.23±0.05</td>
<td>9.27±0.05</td>
</tr>
<tr>
<td>Milk</td>
<td>9.07±0.05</td>
<td>9.00±0.14</td>
</tr>
<tr>
<td>CaCO₃</td>
<td>9.17±0.05</td>
<td>9.17±0.05</td>
</tr>
<tr>
<td>CaL</td>
<td>9.23±0.05</td>
<td>9.27±0.05</td>
</tr>
</tbody>
</table>

Each value (Mean±S.D.) represents plasma level of calcium (n=5).

Results

Five types of calcium were tested in the study. Chronological changes in rats’ intestinal absorption rate of these test materials (decreases of calcium in the recirculation solution) were showed in Table 2 and Fig.1. LDM and CTM showed significantly higher absorption rate than any other material. Also, LDM and CTM showed significantly higher absorption rate than the rest of the three materials especially at 20 minutes after the start of
the recirculation. A significant low level of Ca absorption rate was observed in CaCO3 in comparison with other materials at 50 and 60 minutes.

Comparing the mean absorption rate during the 60 minutes period (mean ± SD), all the materials of Ca from sea shell with chondroitin showed higher rates in the order of LDM: 6.89 ± 1.98% > CTM: 5.28 ± 2.15% > Milk: 3.86 ± 1.74% > CAL: 2.95 ± 1.49% > CaCO3: 2.69 ± 0.99%.

Plasma Ca concentration before and after the recirculation were showed in the Table 3. Plasma Ca concentration in the rat measured before and after the recirculation all showed normal values. No significant difference before and after the recirculation and the difference among rats were observed.

Discussion

Calcium supplements are becoming an important source of dietary calcium and a basic defense against osteoporosis. Recent studies have also focused on a cancer-preventive role for calcium2,9. Oral calcium supplement is very popular method of fortifying inadequate dietary calcium because its cheap cost, convenient intake way and minimal incidence of side effects though a controversy still exists as to its effect on preventing age-related decrease of bone mass8,10. Now there are more than a dozen commonly prescribed calcium supplements and hundreds of different formulations commercially available11-15. Calcium carbonate is the major constituent in sea shell and hydroxyapatite calcium, and is a preferred source of calcium supplements because of its high density of elemental calcium, about 40% by weight. Calcium citrate is another very popular formulation and there are now effervescent preparations available. At 21% by weight, the citrate preparations have a lower calcium content but are considered much more soluble than calcium carbonate, especially important in achlorhydric patients. The citrate ion may help to modulate the propensity for the development of renal stones13.

The calcium citrate combined with IAM made from sea shell, is sort of oral calcium supplements. The results suggested a positive effect on bone débilitation in animals; this process was recovered by UNICAL® diet.

Inadequate dietary calcium during the critical growth and building period may result in failure to reach peak bone mass, causing osteopenia, osteoporosis, decreased skeletal integrity, and increased risk of fracture in later life3,17. The importance of ample calcium intake in early life was made evident by recent clinical studies18,29 and experimental studies21.

Generally, calcium supplementation is recognized as accelerating bone formation with less inhibition of bone resorption19. The efficiency of various kinds of calcium in organisms can be evaluated by their nutrition availability determined by such factors as intestinal absorption efficiency, concentration in the blood and uptake to the bone. Intestinal absorption efficiency in particular is considered to be a crucial element in evaluated calcium sources. Intestinal calcium absorption comprises two processes; a saturable, vitamin-D dependent, transcellular movement that occurs largely in duodenum, and nonsaturable process that is paracellular and relatively invariant with age or vitamin-D status22-24. The present study investigated in the intestinal absorption rate of chondroitin-added calcium compounds prepared from sea shells and the effects of chondroitin in facilitating absorption. The rats were tested after a 24-hour fast and no other treatment was performed such as removal of the thyroid or supplying feed free of vitamin D and calcium. Ligation of the bile duct was performed, however, to eliminate the effects of bile in intestinal absorption. Since bile is involved in absorption of fat-solution vitamins (vitamin D), this operation could have caused decreased absorption of vitamin D. Therefore, it is possible to say that the present intestinal calcium absorption test was conducted under suppressed conditions, although the suppressing effects might not have been as strong as in rats with feed free of vitamin D. Furthermore, when a low calcium diet is provided to rats prior to tests, intestinal calcium absorption tends to be higher to compensate25,26. Therefore, without this pretreatment, it is possible to say that the present absorption test was performed under negative conditions. No significant difference, however, was found when the results of Cal and CaCO3 were compared with those of similar tests with pretreatment27, and thus it is safe to say that the effects of not having these pretreatments were minimal during the 60 min observation period.

The results of the calcium absorption test conducted under these conditions showed significantly increased intestinal calcium absorption in all the groups with calcium from sea shells. LDM, which contained calcium derived from sea shells, showed 1.6 times higher mean
absorption rate than CaL which is generally known to have the best absorption rate\(^6\). This higher absorption rate of UcL can be attributed to the presence of lactic acid. Gunshin et al\(^{14}\) found that lactose had the highest absorption rate of the several carbohydrates in their study of calcium uptake by brush border membrane vesicles. According to the study by Shiraki\(^9\), patients with lactose intolerance show a decreased amount of bone salt, possible because of calcium absorption decrease. Hashizume\(^9\) reported the effects of insulin on absorption and utilization of calcium, and Davie et al\(^{15}\) reported inhibition of calcium absorption resulting from decreased carbohydrate in low-energy diet food. These findings showed that glucose and its metabolism was clearly related to intestinal calcium absorption, and lactose in particular played an important role in facilitating calcium absorption. Judging from the results of the present study, however, it seems unlikely that lactic acid in facilitating the calcium absorption since LDM showed a higher mean absorption rate than CaL. Therefore, the higher absorption rate can be attributed rather to component of sea shells since CTM, a calcium from sea shells and from other sources, showed a lower absorption rate. It seemed more likely that certain substances contained in sea shells affected intestinal absorption, resulting in significantly higher calcium absorption in all the groups with calcium from sea shells than the contrast groups.

Sea shells showed a very high level of calcium utilization compared to other marine organisms and they are constantly under the influence of strong calcium ion in the process of fertilization, development and growth\(^{20}\). Kaneko et al\(^{11}\) studied sea urchins (Strongicentrotus pulcherrimus), their calcium absorption from sea water and its distribution in their bodies. Analyzing sea shells highly efficient and rapid calcium transport ability, they discussed the important role of a certain substance in their body fluid. It is plausible, but not proved at present, that this unknown substance facilitated the intestinal absorption of calcium from sea shells.

The plasma calcium concentration checked before and after the recirculation were all within the normal range\(^3\), showed no significant differences. It is likely that the plasma concentration was not affected because of the amount of calcium (2mg) in the recirculation solution and of the short period (60 min).

Concerning the effects of chondroitin (IAM), a mucopolysaccharide, on intestinal calcium absorption, the mean absorption rate of LDM was 2.2 times more than that of CaL, and CTM also showed a 2.0, 2.2 times higher than CaL, CaCO3.

Details of IAM effect on intestinal calcium absorption and its mechanism are not yet fully revealed. The only study in this field is done by Gunshin et al\(^{14}\) who confirmed the specificity of lactose in calcium intake. They further observed that the specificity of lactose disappeared after they treated brush border membrane vesicles with sialidase. Calcium intake in the presence of lactose markedly increased, but similar results were also obtained in the presence of manitol. These results indicated that sialic acid residue in chondroitin on the surface of brush border membrane vesicle was disengaged and facilitated calcium absorption, which can occur not only with lactose but also with other glucose. Thus, it was suggested that the facilitating effects of lactose in calcium absorption resulted not from its direct action to brush border membrane vesicles but from its interaction with chondroitin on the vesicle surface.

It is not possible to determine in the present study whether IAM was involved in this mechanism. It is quite plausible, however, that IAM was involved in transporting free calcium ion into the cells on the surface of brush border membrane vesicles. Further studies, such as in the area of membrane digestion, are needed to reveal details.

To present study at least clearly shows that IAM was involved in facilitating intestinal calcium absorption. It further suggests that this efficiency was due to the total effects of IAM facilitating calcium transport into the cells to the blood.

**Conclusion**

Intestinal absorption of the calcium compound from sea shells and facilitating of intestinal calcium absorption by IAM were examined by the in situ recirculation method. The calcium compound from sea shells clearly resulted in increased absorption rate, and IAM in facilitating intestinal calcium absorption. Based on these findings, the calcium compound composed of the calcium from sea shells and IAM was concluded as highly effective in facilitating intestinal calcium absorption.

**References**

1) Matkovic V, FontanaD, TominaC, Goel P, Chesaul CH \(\text{III}\) (1990)