The beneficial effect of the sap of Acer mono in an animal with low-calcium diet-induced osteoporosis-like symptoms

Geun-Shik Lee1, Hyuk-Soo Byun1, Man-Hee Kim1, Bo-Mi Lee1, Sang-Hwan Ko1, Eui-Man Jung1, Ki-Seob Gwak2, In-Gyu Choi2, Ha-Young Kang3, Hyun-Jin Jo3, Hak-Ju Lee3 and Eui-Bae Jeung1*

1Laboratory of Veterinary Biochemistry and Molecular Biology, College of Veterinary Medicine, Chungbuk National University, Cheongju, Chungbuk 361-763, Republic of Korea
2Department of Forest Sciences, College of Agriculture & Life Sciences, Seoul National University, Seoul 151-921, Republic of Korea
3Division of Wood Chemistry and Microbiology, Department of Forest Products, Korea Forest Research Institute, Seoul 130-712, Republic of Korea

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The sap of Acer mono has been called ‘bone-benefit-water’ in Korea because of its mineral and sugar content. In particular, the calcium concentration of the sap of A. mono is 37.5 times higher than commercial spring water. In the current study, we examined whether A. mono sap could improve or prevent osteoporosis-like symptoms in a mouse model. Male mice (3 weeks old) were fed a low-calcium diet supplemented with 25, 50 or 100 % A. mono sap, commercial spring water or a high calcium-containing solution as a beverage for 7 weeks. There were no differences in weekly weight gain and food intake among all the groups. Mice that were given a low-calcium diet supplemented with commercial spring water developed osteoporosis-like symptoms. To assess the effect of sap on osteoporosis-like symptoms, we examined serum calcium concentration, and femur density and length, and carried out a histological examination. Serum calcium levels were significantly lower in mice that received a low-calcium diet supplemented with commercial spring water (the negative control group), and in the 25 % sap group compared to mice fed a normal diet, but were normal in the 50 and 100 % sap and high-calcium solution groups. Femur density and length were significantly reduced in the negative control and 25 % sap groups. These results indicate that a 50 % sap solution can mitigate osteoporosis-like symptoms induced by a low-calcium diet. We also examined the regulation of expression of calcium-processing genes in the duodenum and kidney. Duodenal TRPV6 and renal calbindin-D9k were up-regulated dose-dependently by sap, and the levels of these factors were higher than those attained in the spring water-treated control. The results demonstrate that the sap of A. mono ameliorates the low bone density induced by a low-calcium diet, most likely by increasing calcium ion absorption.

Acer mono: Calcium-processing genes: Osteoporosis: Sap

Sap is the fluid transported in the xylem cells or phloem sieve tube elements of a plant. Sap consists primarily of water, but also contains mineral elements, sugars, hormones and other nutrients. Other liquid components found in plants or that are exuded by plants, such as latex, resins and mucilage, are sometimes incorrectly referred to as sap(1). Acer mono is a rounded, deciduous tree with five to seven bright green, almost heart-shaped, lobular leaves that turn yellow in autumn. Yellow flowers appear on erect umbels with these leaves, but may also appear without them. The sap of A. mono contains sugar, and can be ingested directly as a drink, or concentrated into a syrup by boiling(2) and then used as a sweetener. In Korea, the sap of A. mono has been called ‘bone-benefit-water’ because of its abundant calcium and magnesium ion content. The medicinal uses of A. mono include the leaves as an irritant and the bark as an astringent(3), however, the pharmacological effect of the sap on bone health has not been examined.

The most common abnormality of bone is osteoporosis, which is a decrease in the density of the bone mass that causes thinning and weakening of the bone. There are two main categories of osteoporosis: type I and type II. Type I osteoporosis occurs only in post-menopausal women, and is due to estrogen deficiency. Type II osteoporosis occurs in both men and women (about two times more frequently in women), and is due to ageing and calcium deficiency. In animal models, a low-calcium diet leads to the development of radiologic rickets and histologic features of osteomalacia(4). These symptoms were defined as osteoporosis-like symptoms in the present study.

To clarify the potential therapeutic nature of ‘bone-benefit-water’, an animal model of low-calcium diet-induced
osteoporosis-like symptoms has been generated. Using this model, the effect of sap on the active calcium transport system has also been examined. Calcium ions are actively absorbed from the duodenum and reabsorbed in kidney. In the calcium active transport system, several proteins mediate calcium influx, transfer through the cytosol and extrusion into the bloodstream. In the active calcium-transporting organs, these steps are carried out by calcium entry channel proteins of the outer cell membrane, cytosolic buffering or transfer proteins, and excretory pump proteins\(^{(5-7)}\). Two highly selective calcium channels located in the apical cell membrane are the major portals of calcium ion entry, transient receptor potential vanilloid 6 (TRPV6) and 5 (TRPV5). Calbindin-D9k (CaBP-9k) is an intracellular calcium ion-binding protein that is thought to participate in shuttling calcium ions from the apical to the basolateral membrane, where calcium ions are extruded. However, the proteins involved in intracellular calcium transport vary somewhat depending on the tissue. In the epithelial cells of the duodenum, the transport pathway involves calcium entry primarily through specialized TRPV6 channels, and is highly dependent on intracellular free-calcium concentration. Maintenance of the ‘open’ state of TRPV6 requires the constant buffering action of intracellular calcium-binding proteins.

In mammalian intestinal cells, CaBP-9k is the major intracellular calcium-binding protein. The final step of extrusion of calcium into the blood requires ATP. In the kidney, trans-cellular calcium ion transport is carried out by cells lining the distal part of the nephron. Calcium ion entry is facilitated by TRPV5 in the apical membrane. Calcium then binds CaBP-9k and diffuses through the cytosol to the basolateral membrane, where it is extruded, directly or via CaBP-9k, by excretory pump proteins\(^{(6)}\).

In the current study, we examined the effect of the sap of A. mono on osteoporosis-like symptoms. Mice were fed a low-calcium diet to induce osteoporosis-like symptoms during their growth stage (from 3 to 10 weeks of age). Solutions of increasing percentages of sap, commercial spring water or a high-calcium-content solution were supplied to the mice, and after 7 weeks, bone mineral density and at the centre point of the tibia.

**Materials and methods**

**Mineral concentration of the sap of Acer mono**

The mineral contents of A. mono sap and commercial spring water (Samsan, Jeju Special Self-governing Province Development Corp., Jeju-si, Jeju-do, Korea) were analysed using an inductively coupled plasma emission spectrometer (ICP-1000 IV; Shimadzu, Tokyo, Japan). All samples (10 ml) were digested with the addition of 20 ml nitric acid to remove all organic matter, reduced in volume with heating, filtered through no. 42 filter paper (Whatman, Maidstone, UK), and then diluted to a final volume of 50 ml before being processed by an inductively coupled plasma emission spectrometer.

**Animals and treatments**

Male ICR mice (3 weeks old) were obtained from KOATECH (Pyeongtaek-si, Gyeonggi-do, Korea). All animals were housed in polycarbonate cages and acclimated to an environmentally controlled room (23 ± 2°C, relative humidity 50 ± 10%, frequent ventilation and 12 h light/dark cycle) prior to use. Thirty mice were divided into six groups (five in each group). Osteoporosis-like symptoms were induced in five groups by feeding with a low-calcium diet (AIN-76A purified rodent diet with 1 % phosphorus and 0.02 % calcium, DYET 113294; Dyets Inc., Bethlehem, PA, USA) for 7 weeks. One group was fed a normal diet (AIN-76A purified rodent diet with 0.8 % phosphorus and 1.1 % calcium, DYET #113295; Dyet Inc.; normal group) for the same period of time.

To assess the preventative effect of A. mono sap on osteoporosis-like symptoms, a daily total of 30 ml commercial spring water (negative control group), or a solution of 25, 50 or 100 % sap, or a high-calcium-content rescue solution (7.5 mM-CaCl\(_2\) and 3.0 mM-NaH\(_2\)PO\(_4\) based on the Krebs–Ringer buffer, rescue group) were restrictively supplied to the mice from 3 to 10 weeks of age. Body weights were measured weekly for the duration of the experiment. The Ethics Committee of the Chungbuk National University approved all experimental procedures and use of animals.

**Bone mineral density and histomorphometry**

Mice were killed at 10 weeks of age by CO\(_2\) inhalation. The hind legs were removed and fixed in a 10 % neutralized formalin solution for 3 d. The legs were then washed with water, and the skin and muscle were eliminated. The femur was dried overnight and bone mineral density was analysed using a Direct View CR 500 (Eastman Kodak Co., Rochester, NY, USA). The tibia was decalcified using Plank-Rychlo’s solution, and the bone was embedded in paraffin, sectioned and deparaffinized in xylene. Sections were hydrated in a series of descending ethanol concentrations, then stained with haematoxylin and subjected to histomorphometric analyses using light microscopy. Osteoid thickness was measured at the centre point of the tibia.

**Real-time PCR using TaqMan™ Probe**

Total RNA was prepared from the duodenum and kidney using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA). First strand complementary DNA (cDNA) was prepared by subjecting total RNA (1 μg) to reverse transcription using mMLV RT (Invitrogen Life Technologies Inc.) and random primers (9 mer; TaKaRa Bio. Inc., Otsu, Shiga, Japan)\(^{(8,9)}\). Real-time PCR was performed in a 20 μl reaction volume containing 10 μl TaqMan Universal PCR Master Mix (Applied Biosystems, Foster, CA, USA), 1 μl 20 × Assays-on-Demand™ Gene Expression Assay Mix (Applied Biosystems; TRPV6, Mm00499069_m1, NM_022413-2; TRPV5, Mm01166209_m1, NM_001007572-2; CaBP-9k, Mm00486654_m1, NM_009789-2; TRPV1, Mm00446968_m1, NM_013556-2) and 3 μl cDNA. Amplification was carried out using a 7300 Real-Time PCR System (Applied Biosystems) and the following cycle parameters: initial denaturation at 50°C for 2 min; 90°C for 10 min; forty cycles of denaturation at 95°C for 15 s; annealing and extension at 60°C...
for 1 min. Expression levels were determined using RQ software (Applied Biosystems). The expression of TRPV6, TRPV5 and CaBP-9k was normalized to that of HPRT1.

Biochemical and hormonal analysis
Blood was collected from each mouse into serum separator tubes and subjected to centrifugation at 3000 rpm for 10 min. Serum calcium levels were determined using ASAN Ca-Lq Reagents (Asanpharm, Kyeonggido, Korea). Serum PTH levels were measured using a Mouse Intact PTH ELISA Kit (ALPCO Diagnostics, Salem, NH, USA), according to manufacturer’s instructions.

Data analysis
Data were analysed by non-parametric one-way ANOVA using the Kruskal–Wallis test, followed by Dunnett’s test for multiple comparisons to the negative control group. Data were ranked according to these tests. Statistical analysis was performed using SPSS for Windows (SPSS Inc., Chicago, IL, USA). *P*<0.05 was considered statistically significant.

Results

The mineral content of *Acer mono* sap
The concentration of sixteen mineral ions was measured in the sap of *A. mono* and commercial spring water. The potassium, calcium and magnesium levels of sap were 16, 37 and 3.9 times higher than commercial spring water, respectively, while the level of sodium in sap was 0.68 times lower (Table 1). A small amount of silicon was detected in both solutions. Manganese was detected only in the sap, and iron was detected only in commercial spring water. No other minerals were detected in either liquid.

**Table 1.** Mineral content (mg/l) of the sap of *Acer mono* and commercial spring water* (Mean values with their standard errors)

<table>
<thead>
<tr>
<th>Commercial spring water</th>
<th>Mean</th>
<th>SEM</th>
<th>Sap of <em>A. mono</em></th>
<th>Mean</th>
<th>SEM</th>
<th>Rescue solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>7.34</td>
<td>0.05</td>
<td>117.96</td>
<td>2.86</td>
<td></td>
<td>300.6</td>
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<tr>
<td>Ca</td>
<td>4.15</td>
<td>0.27</td>
<td>155.32</td>
<td>3.94</td>
<td></td>
<td>58.97</td>
</tr>
<tr>
<td>Na</td>
<td>27.86</td>
<td>0.97</td>
<td>16.90</td>
<td>0.49</td>
<td></td>
<td>92.92</td>
</tr>
<tr>
<td>Mg</td>
<td>3.68</td>
<td>0.12</td>
<td>14.39</td>
<td>0.44</td>
<td></td>
<td></td>
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<tr>
<td>P</td>
<td>ND</td>
<td></td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Si</td>
<td>0.08</td>
<td>0.00</td>
<td>0.07</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>ND</td>
<td></td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mn</td>
<td>ND</td>
<td></td>
<td>0.35</td>
<td>0.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fe</td>
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<td>0.08</td>
<td>0.10</td>
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<tr>
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<td>ND</td>
<td></td>
<td>ND</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>ND</td>
<td></td>
<td>ND</td>
<td></td>
<td></td>
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<tr>
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<td>ND</td>
<td></td>
<td>ND</td>
<td></td>
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<td></td>
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<tr>
<td>Mo</td>
<td>ND</td>
<td></td>
<td>ND</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Cd</td>
<td>ND</td>
<td></td>
<td>ND</td>
<td></td>
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<tr>
<td>Pb</td>
<td>ND</td>
<td></td>
<td>ND</td>
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</tr>
<tr>
<td>Cr</td>
<td>ND</td>
<td></td>
<td>ND</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ND, not detected.

* For details of procedures, see Materials and methods.

**The effect of *Acer mono* sap on osteoporosis-like symptoms**
To elucidate the beneficial effect of sap on osteoporosis-like symptoms, mice from the weaning (3 weeks old) to mature (10 weeks old) stage were fed low-calcium diets supplemented with increasing percentage solutions (0, 25, 50 and 100 %) of sap, or a rescue solution containing a high concentration of calcium ions, and femur mineral densities were analysed using computer radiography (Fig. 1). The femurs of the normal group were bright and clear in radiographic images, while the corresponding images of the negative control group, which received commercial spring water, were dark and indistinct, which indicated that the mice had developed osteoporosis-like symptoms (Fig. 1 (A)). The rescue group (which received high calcium-containing water) exhibited improved femur brightness in radiographs. To determine the effect of sap on osteoporosis-like symptoms more precisely, the density of the distal (D) and proximal (P) femur heads was analysed using Direct View CR 500 software (Fig. 1 (B)). The densities of the distal and proximal femur heads of the normal group were significantly higher than all of the low-calcium diet groups. However, the bone densities of the 50 and 100 % sap solution groups and the rescue group were significantly higher than the 25 % sap solution and negative control group. Femur length was also analysed (Fig. 2 (C)), and we found that mice treated with commercial spring water alone, or a 25 % sap solution, had significantly shorter femurs than normal mice. The femur lengths of the groups that received 50 % or higher sap solutions were not significantly different from the normal group. The effect of sap on low-calcium diet-induced osteoporosis-like symptoms was further analysed by bone histomorphometry. The tibia was sectioned and stained with haematoxylin, and osteoid thickness was analysed (Fig. 2 (A)). The thickness of the negative group was significantly less than the normal group, and thickness gradually increased with increasing percentage sap solutions (Fig. 2 (B)). The tibia thicknesses of all of the low-calcium diet groups were significantly less than the normal group. Supplementation with 50 % or greater sap solutions or rescue solution improved tibia thickness compared to the negative control group. The present results suggested that 50 % or higher percentage sap solutions improve low-calcium-induced osteoporosis-like symptoms.

**The effect of *Acer mono* sap on calcium-processing gene expression**
Based on the previous experiment, administration of a 50 % sap solution appeared to mitigate low-calcium diet-induced osteoporosis-like symptoms in mice. To begin to uncover the mechanism of the beneficial effect of sap on osteoporosis-like symptoms, we examined the expression of three duodenal and renal calcium-absorbing genes using real-time PCR. The expression of duodenal CaBP-9k was similar in mice that received sap, commercial spring water or a normal diet (Fig. 3 (A)). Mice that received the high calcium-containing rescue solution had significantly reduced expression levels of duodenal CaBP-9k compared with negative and normal dieted groups. The expression of duodenal TRPV6 was increased in the sap solution groups, but was decreased in the rescue group when compared with negative and normal dieted mice. In the kidney, the CaBP-9k expression levels of the
sap solution and control mice were higher than those of the mice in the negative and rescue groups, and CaBP-9k expression was down-regulated in the negative and rescue groups compared to the normal group (Fig. 3 (B)). The expression of renal TRPV6 was not altered in any of the low-calcium diet groups. However, renal TRPV5 expression was decreased in the negative, sap solution and rescue groups compared to the normal group. These distinct patterns of regulation of duodenal and renal calcium-processing gene expression suggested that the mechanism that improves osteoporosis-like symptoms in the sap solution group involves not only augmentation of calcium levels, but also enhanced activity of the calcium active transport system.

**Fig. 1.** The effect of *Acer mono* sap on osteoporosis-like symptoms assessed using bone radiography. Femurs were obtained from mice and analysed as described in the Materials and methods. (A), Representative femurs of each of the indicated treatment groups. (B, C) Bone density at the distal (B) and proximal (C) heads of all femurs (five per group). Values are means of duplicate measurements of all samples with their standard errors depicted by vertical bars. Data are expressed as a percentage of the density of a normal femur. D, distal head of femur; P, proximal head of femur; Negative, low-calcium diet plus commercial spring water; Normal, normal diet plus commercial spring water; Rescue, low-calcium diets plus high-calcium solution. Mean values were significantly different from those of the normal group: *P*<0·05. Mean values were significantly different from those of the negative control group: †*P*<0·05.

**Discussion**

Maple syrup is a sweetener made from the sap of maple trees. It is often eaten with pancakes, waffles or French toast, and is used as an ingredient in baking and the preparation of desserts. *A. mono* is a kind of maple tree found in eastern Asia, and its sap can be ingested directly, without any processing. The sap of *A. mono* consists primarily of water, and contains minerals and sugars, similar to the sap of maple (1). In the current study, we examined the pharmacological effect of the sap of *A. mono*. This sap is believed to be beneficial to bone health because of its high mineral content. We first examined the mineral content of sap, and compared it to commercial spring water. We showed that potassium, calcium and magnesium were present at high levels in sap. The level of calcium ions was 37 times higher, and magnesium was 3·9 times higher in the sap of *A. mono* than in commercial spring water. We hypothesized
that it was the high calcium content of sap that prevented or improved the osteoporosis-like symptoms.

It is well known that calcium is essential to attain maximum bone mass\(^\text{[10,11]}\). Reduced calcium intake results in osteoporosis in man and in many other animal species\(^\text{[12]}\). Clinical and experimental nutritional secondary hyperparathyroidism rapidly develops in horses, dogs and cats fed diets high in phosphate and low in calcium\(^\text{[12]}\). Young rats and mice fed diets low in calcium develop osteoporosis-like symptoms, and ageing mice fed a high-calcium diet exhibit accelerated bone resorption with increased levels of dietary phosphate\(^\text{[12]}\). Similarly, ageing rats fed a diet with adequate calcium content developed progressively more severe osteoporosis as dietary phosphate was elevated\(^\text{[12]}\). Adult dogs fed diets high in phosphate but adequate levels of calcium developed rapid bone loss resembling osteoporosis, whereas young dogs developed severe nutritional secondary hyperparathyroidism characterized by fibrous osteodystrophy\(^\text{[12]}\). In the current study, we examined whether the high calcium content of the sap of \textit{A. mono} would affect bone density when given as a supplement to a low-calcium diet. Although we demonstrated that administration of solutions of increasing percentages of sap increase bone density and osteoid thickness in a mouse model of induced osteoporosis-like symptoms, the sap supplement was less efficient in improving or preventing the symptoms when compared with a high-calcium solution or a normal diet. The present results suggested that in addition to supplying calcium ions, there are other factors involved in the beneficial effect of sap on osteoporosis-like symptoms. The other factor might be potassium which was present at 17-fold higher concentration in sap than in commercial spring water. Potassium influences calcium homeostasis, particularly the urinary conservation and excretion of calcium\(^\text{[13]}\). Low-potassium diets increase urinary calcium losses while high-potassium diets reduce it\(^\text{[13]}\). High-potassium intake ameliorates bone resorption in high-salt diets and is associated with a higher baseline bone density.

\[\text{Fig. 2. The effect of } \textit{Acer mono} \text{ sap on osteoporosis-like symptoms assessed using bone histomorphometry. Tibias were removed from mice and processed as described in the Materials and methods. (A), Representative tibias of the indicated treatment groups. (B) Osteoid thickness taken at the middle of the bone for all femurs (five per group).} \]

```
(A) Negative 25% 50% 100% Rescue Normal

200 μm

200 μm

200 μm

200 μm

(B) *

(C) †

Tibia thickness (μm)

Femur length (mm)

25% 50% 100% Rescue Normal

25% 50% 100% Rescue Normal

(Figures for tibia thickness and femur length are not shown here.)

Mean values were significantly different from those of the normal group: * \(P < 0.05\). Mean values were significantly different from those of the negative control group: † \(P < 0.05\).
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\[\text{https://doi.org/10.1017/S0007114508959195}\]
mineral density and lower levels of bone loss\(^{14,15}\). Taken together, the present results suggest that the sap of *A. mono* improves low-calcium diet-induced osteoporosis-like symptoms via augmentation of mineral levels.

To clarify the mechanism of the sap’s effect on osteoporosis-like symptoms, we examined the expression of calcium active transport genes. Based on the results of previous studies\(^{9,16-18}\), we examined the expression of TRPV5, TRPV6 and CaBP-9k. TRPV5 and TRPV6 are calcium channels found in the apical membranes of intestinal and renal epithelial cells, and have been proposed as the mediators of calcium uptake during trans-cellular calcium transport\(^{19}\). They are highly related proteins expressed mainly in cells involved in calcium absorption or re-absorption in the duodenum and kidney\(^{20}\). TRPV6 is distributed in the duodenum, jejunum, ileum and kidney, and in exocrine tissues such as the pancreas, prostate, mammalian gland and sweat gland\(^{19,21,22}\). Hormonal vitamin D-induced expression of TRPV5 occurs in rabbit renal epithelial cells, and TRPV5 has been identified in human, rat and mouse kidney. Dietary calcium also regulates TRPV6 mRNA expression of duodenoctyes and renal distal convoluted tubules\(^{23}\). In the current study, we demonstrated that duodenal TRPV6 mRNA expression is induced by sap solutions, independent of the dose of sap. Renal TRPV6 was not influenced by any of the dietary beverage conditions. TRPV5 mRNA was induced by sap and the high-calcium rescue solution, but the level of expression was not significantly higher than normal levels. CaBP-9k is a cytosolic protein with a high affinity for calcium ions\(^{23,24}\). In mammals, a number of tissues and organs, including the intestine, uterus, placenta, kidney and bone, express CaBP-9k\(^{16-18,25-31}\). Intestinal CaBP-9k is involved in intestinal calcium absorption and is regulated at the transcriptional and post-transcriptional levels by 1,25-dihydroxyvitamin D3 (1,25-(OH)\(_2\)D\(_3\)), the hormonal form of vitamin D, in rodents\(^{32-34}\). It has also been shown that the expression of duodenal CaBP-9k may be linked to 1,25-dihydroxycholecalciferol in man\(^{17}\). Renal CaBP-9k is expressed at distal convoluted tubules, which are believed to facilitate calcium re-absorption\(^{35}\). In the current study, we showed that duodenal CaBP-9k mRNA levels were not altered by sap.

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**Fig. 3.** The effect of *Acer mono* sap on the expression of calcium-processing genes. Duodenal (A; transient receptor potential vanilloid 6 (TRPV6), calbindin-D9k (CaBP-9k)) and renal (B; TRPV6, transient receptor potential vanilloid 5 (TRPV5), CaBP-9k) mRNA were prepared from mice of the indicated treatment groups (five per group) as described in the Materials and methods. Relative gene expression of CaBP-9k, TRPV5 and TRPV6 was examined using real-time PCR. Values are means of duplicate measurements of all samples with their standard errors depicted by vertical bars. Data are expressed as a percentage of the expression of HPRT1, an internal control gene. Negative, low-calcium diet plus commercial spring water; Normal, normal diet plus commercial spring water; Rescue, low-calcium diets plus high-calcium solution. Mean values were significantly different from those of the normal group: *P* < 0·05. Mean values were significantly different from those of the negative control group: †P < 0·05.
Sap of *A. mono* improves osteoporosis-like symptoms in mice

Table 2. Serum calcium and parathyroid hormone (PTH) analysis†‡ (Mean values with their standard errors)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Serum calcium levels (mg/l) Mean SEM</th>
<th>Serum PTH (pg/ml) Mean SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>70.1* 10.1</td>
<td>41.78* 9.97</td>
</tr>
<tr>
<td>Sap of <em>Acer mono</em></td>
<td>25 % 72.3* 8.2</td>
<td>35.07* 4.34</td>
</tr>
<tr>
<td></td>
<td>50 % 86.6 5.1</td>
<td>52.70* 17.04</td>
</tr>
<tr>
<td></td>
<td>100 % 90.2 13.2</td>
<td>46.98* 9.00</td>
</tr>
<tr>
<td>Rescue</td>
<td>86.6 6.8</td>
<td>5.41† 1.30</td>
</tr>
<tr>
<td></td>
<td>Normal 91.4 4.5</td>
<td>14.20† 5.63</td>
</tr>
</tbody>
</table>

Mean values were significantly different from those of the normal group: *P* < 0.05. Mean values were significantly different from those of the negative control group: ††P < 0.05.

† For details of procedures, see Materials and methods.

However, sap solution increased renal CaBP-9k mRNA expression. The high calcium-containing rescue solution significantly decreased duodenal CaBP-9k and TRPV6 transcription. In addition, it has previously been shown that high calcium intake attenuates CaBP-9k and TRPV6 gene expression in mice. Thus, while sap appeared to regulate duodenal TRPV6 and renal CaBP-9k expression, this effect was different from that of the rescue solution. The present result indicated that the underlying mechanism of regulation of these genes by sap may not involve calcium ions. If the high calcium content of sap was involved in the regulation of CaBP-9k and TRPV6, one would also expect the effect of sap on the expression patterns of these genes to be similar to previous studies.

Generally, a calcium-deficient diet decreases serum calcium concentration and accelerates PTH secretion. In the current study, we demonstrated that the serum calcium levels of mice that received a 50 % or higher sap solution were similar to mice that were fed a normal diet. The present results suggest that the amount of calcium in a 50 % sap solution is sufficient to maintain normal calcium homeostasis. PTH plays a major role in regulating calcium homeostasis. PTH regulates the conversion of 25-hydroxyvitamin D to the active metabolite 1,25-(OH)2D3. PTH also activates dihydropyrimidine-sensitive channels that mediate calcium entry. It has also been shown that microtubule-dependent exocytosis stimulated by PTH is required for the activation of calcium channels and calcium influx. 1,25-(OH)2D3 inhibits PTH secretion and parathyroid cell proliferation through the action of the vitamin D receptor. It has also been shown that PTH secretion and bone mineralization correlate with calcium levels, rather than the activation state of the 1,25-(OH)2D3 receptor signalling pathway. In the current study, serum PTH was up-regulated by a low-calcium diet compared to the normal group; however, the mice in the rescue group showed significantly lower serum PTH levels. The present results suggest that sap improves osteoporosis-like symptoms via a different mechanism than the high calcium-containing solution, similar to the regulation of the calcium-processing genes.

In summary, the positive effect of sap on osteoporosis-like symptoms was evaluated by measuring serum calcium concentrations, femur density and length, and by a histological examination. Serum calcium levels were significantly lower in mice that received a low-calcium diet supplemented with commercial spring water, and in the 25 % sap solution group compared to the normal group, but not in the 50 and 100 % sap solution groups, or the rescue group. Femur density and length were significantly reduced in the negative and 25 % sap solution groups. The present results imply that a 50 % or higher sap solution could ameliorate osteoporosis-like symptoms induced by a low-calcium diet. The results of the histological analysis correlated with the bone density results. In addition, we examined the expression of calcium-processing genes in the duodenum and kidney, and showed that duodenal TRPV6 and renal CaBP-9k were up-regulated by sap in a dose-independent manner. We propose that the sap of *A. mono* may improve low-calcium diet-induced osteoporosis-like symptoms by augmenting mineral levels in the body.

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References


