Cola Beverage Consumption Induces Bone Mineralization Reduction in Ovariectomized Rats

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Received for publication October 7, 1999; accepted March 20, 2000 (99/187).

Background. A significant association of cola beverage consumption and increased risk of bone fractures has been recently reported. The present study was carried out to examine the relationship of cola soft drink intake and bone mineral density in ovariectomized rats.

Methods. Study 1. Four groups of 10 female Sprague-Dawley rats were studied. Animals from groups II, III, and IV were bilaterally ovariectomized. Animals from groups I and II received tap water for drinking, while animals from groups III and IV each drank a different commercial brand of cola soft drink. After 2 months on these diets, the following were measured: solid diet and liquid consumption; bone mineral density; calcium in bone ashes; femoral cortex width; calcium; phosphate; albumin; creatinine; alkaline phosphatase; 25-OH hydroxyvitamin D, and PTH. Study 2. Two groups of seven ovariectomized rats were compared. Group A animals received the same management as the group III animals from study 1 (cola soft drink and rat chow ad libitum), while rats from group B received tap water for drinking and pair-feeding. After 2 months plasmatic ionized calcium, phosphate, creatinine, albumin, calcium in femoral ashes, and femoral cortex width were measured.

Results. Study 1. Rats consuming cola beverages (groups III and IV) had a threefold higher liquid intake than rats consuming water (groups I and II). Daily solid food intake of rats consuming cola soft drinks was one-half that of rats consuming water. Rats consuming soft drinks developed hypocalcemia and their femoral mineral density measured by DEXA was significantly lower than control animals as follows: group I, 0.20 ± 0.02; group II, 0.18 ± 0.01; group III, 0.16 ± 0.01, and group IV, 0.16 ± 0.01 g/cm². Study 2. To rule out the possibility that these calcium and bone mineral disorders were caused by decreased solid food intake, a pair-fed group was studied. Despite a lower body weight, pair-fed animals consuming tap water did not develop bone mineral reduction or hypocalcemia.

Conclusions. These data suggest that heavy intake of cola soft drinks has the potential of reducing femoral mineral density. © 2000 IMSS. Published by Elsevier Science Inc.

Key Words: Cola, Carbonated beverages, Phosphate loads, Bone density, Osteoporosis, Hypocalcemia.

Introduction

The United States and Mexico are the countries that rank first and second, respectively, regarding cola soft drink consumption (1–3). Per capita consumption of cola soft drinks has shown an increasing trend since the 1960s in both countries, surpassing all other kinds of beverages such as water,
milk, beer, and coffee. Thus, considering the huge numbers of individuals exposed, health risks associated with cola beverage consumption may have important public health consequences.

Postmenopausal osteoporosis is the most frequent metabolic bone disease. Management of the medical complications of osteoporosis, such as bone fractures, imposes a heavy burden on health care systems. Currently, postmenopausal osteoporosis constitutes an important public health problem, a picture likely to worsen in the following years as the age of the population increases (4). The ovariectomized rat is a widely used model for studying osteoporosis (5–7). Estrogens are known to inhibit bone resorption, enhance osteoblast proliferation, and increase mRNA for the type I collagen α1 chain (8). Estrogen deficiency is also associated with a striking increase of cytokines responsible for increased bone resorption (9).

Recently, evidence has been provided that heavy cola soft drink consumption is associated with hypocalcemia both in clinical (2,3,10) and experimental settings (11). In another study, consumption of carbonated beverages on a short-term basis was reported not to adversely affect serum or urinary calcium metabolism markers (12).

Three published papers reported a significant association of cola beverage consumption and increased risk of bone fractures (13–15). Another study did not disclose any significant association between cola soft drink consumption and reduction of bone mineral density (16).

Mineral metabolism disorders associated with cola beverage consumption have been explained mainly by their phosphoric acid content (2,3,10,11), but other characteristics of cola soft drinks can also affect mineral metabolism. Caffeine, an important ingredient in this kind of drink, has been reported to increase fracture risk and reduce bone mineral density (7,17). Acid loads may also adversely affect calcium and bone metabolism (18–20). Thus, the objective of the present study was to examine the relationship of cola soft drink intake and bone mineral density in ovariectomized rats.

Materials and Methods

The protocol was approved by the Hospital’s Research and Ethics Committee (May 6, 1996, #015/96). All experimental procedures are in compliance with currently accepted guiding principles for care and use of animals.

Study I. Four groups of female Sprague-Dawley rats were studied. Each group consisted of 10 animals aged 9 months each at the beginning of the study, weighing from 280–320 g. The surgical intervention day was considered as the beginning of the study. Group I consisted of sham-operated animals; groups II, III, and IV consisted of bilaterally ovariectomized animals. Animals received general anesthesia with intraperitoneal (i.p.) ketamine (50 mg/kg) and dehydrobenzperidol (2 mg/kg). The ovaries were excised through a lumbo-dorsal incision. The vascular pedicle was identified and sectioned. Histological identification of ovarian tissue was carried out in all surgical specimens. All animals were fed with commercial rat chow (nutricubos) (Ralston Purina, St. Louis, MO, USA) ad libitum. Animals of groups I and II received tap water ad libitum for drinking. Animals from groups III and IV had no access to water. Group III animals drank the commercial brand of the most frequently consumed cola soft drink in Mexico, ad libitum. Group IV animals who received the commercial brand of cola soft drink to drink ranked second in the country, ad libitum. After 2 months of follow-up on the previously described diets, all animals were placed individually in metabolic cages for 3 days to measure their solid food and liquid consumption. Within 1 week after the previously mentioned procedure, all rats underwent dual energy X-ray absorptiometry (DEXA) using a Hologic QDR 1500 apparatus (Hologic, Inc., Waltham, MA, USA).

During the study, the animals were anesthetized with i.p. ketamine and dehydrobenzperidol at the same doses described previously to keep the animals immobilized. Animals were placed lying ventrally, then a whole-body scan, in a 182 × 162 mm area, and bone densitometry of lumbar spine, femur, and pelvis were carried out. The images obtained were analyzed with the software package Rat Whole Body V-5.67 (Hologic, Inc.). After the DEXA study, the animals were sacrificed by exsanguination through an abdominal aortic puncture. The blood sample was kept in a heparinized syringe for laboratory determinations.

Carcasses were dissected to obtain the femurs. Muscular masses were eliminated and bone was kept in 70% ethanol until assayed. Calcium was measured in bone ashes as follows: before the assay, bones were placed in dry recipients and kept at 37°C for 2 days; subsequently, dry weight was determined and registered. Bone was crushed in a mortar and placed in a porcelain melting pot, heated with a burner for 10 min in order to eliminate gases, and calcined at 550 ± 10°C for 6 h in a furnace. The ashes thus obtained were diluted in 1 mL of 65% nitric acid and 4 mL of deionized water. Calcium was measured with an ion-selective electrode with a BG Electrolyte potentiometer (IL Diagnostics, Lexington, MA, USA). A femur sample was processed for histology to measure cortex width. Bone tissue was fixed in 10% formaldehyde, decalcified, and embedded in paraffin. At the metaphysis level, longitudinal and transversal sections 6-μm in width were obtained and stained with hematoxylin-eosin and Masson’s trichrome. Cortex width was measured both in longitudinal and transversal sections using a graded lattice ocular lens in a light microscope by one of the members of our research group (LCM). Each sample was measured twice, blindly. Cortex width was considered the arithmetic mean of the measurements in one longitudinal and one transversal section. Total calcium, albumin, cre-
Liquid intake (mL/day) 35

Final weight (g) 336

Initial weight (g) 305

Ionized calcium (mmol/L) 1.41

Creatinine (mg/dL) 0.65

Albumin (g/dL) 1.71

25-hydroxyvitamin D (ng/dL) 41.07

Table 1. Body weight, liquid and solid food consumption of animals in the different groups of study 1

<table>
<thead>
<tr>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Sham)</td>
<td>(Ovariectomized control)</td>
<td>(Soft drink A)</td>
<td>(Soft drink B)</td>
</tr>
<tr>
<td>------------------</td>
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<td>---------------------</td>
</tr>
<tr>
<td>Initial weight (g) 305 ± 13</td>
<td>303 ± 15</td>
<td>306 ± 20</td>
<td>303 ± 16</td>
</tr>
<tr>
<td>Final weight (g) 336 ± 22</td>
<td>338 ± 26</td>
<td>344 ± 15</td>
<td>346 ± 19</td>
</tr>
<tr>
<td>Liquid intake (mL/day) 35 ± 7</td>
<td>37 ± 6</td>
<td>141 ± 5</td>
<td>144 ± 5</td>
</tr>
<tr>
<td>Solid food intake (g/day) 19.2</td>
<td>18.0 ± 1.8</td>
<td>11.8 ± 2.9</td>
<td>10.1 ± 3.3</td>
</tr>
</tbody>
</table>

Values are means ± SD. *p < 0.002 vs. groups I and II; †p < 0.001 vs. groups I and II.

Study 2. Fourteen 9-month-old female Sprague-Dawley rats weighing 280–320 g were ovariectomized as described previously and paired by weight. Group A consisted of the first animals of each pair (n = 7) and group B, the second animals of each pair (n = 7). All animals were placed in individual metabolic cages. Animals of group A were managed in identical form as group III animals from study 1, receiving a cola beverage ad libitum and rat chow ad libitum. Daily solid food consumption of each rat was measured and registered. Rats of group B were pair-fed: the amount of rat chow consumed by the group A mate the day before was offered to group B rats each morning, with tap water to drink ad libitum. After 2 months on this regime, the animals were sacrificed by exsanguination as described for the study 1 animals, and their femurs obtained. Plasma-ionized calcium, phosphate, creatinine, albumin, and calcium in femoral ashes were measured as previously described.

Statistics. Statistical analysis was carried out with SPSS for Windows 8.0 (Chicago, IL, USA) software. Results of the study 1 were analyzed with parametric one-way ANOVA. When significant differences were detected, the different group(s) was (were) found with a post-hoc test (Gabriel’s least significant difference). Results of study 2 were analyzed with the one-tailed Student t test for independent samples. Intra-observer concordance was assessed with κ concordance coefficient and Pearson’s r correlation coefficient. Differences were considered as significant when p < 0.05.

Table 2. Ionized calcium, phosphate, alkaline phosphatase, PTH, 25-hydroxy vitamin-D, albumin, and creatinine of the animals in the different groups from study 1

<table>
<thead>
<tr>
<th>Group I (Sham)</th>
<th>Group II (Ovariectomized control)</th>
<th>Group III (Soft drink A)</th>
<th>Group IV (Soft drink B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ionized calcium (mmol/L)</td>
<td>1.41 ± 0.05</td>
<td>1.40 ± 0.02</td>
<td>1.37 ± 0.05</td>
</tr>
<tr>
<td>Phosphate (mg/dL)</td>
<td>4.90 ± 0.99</td>
<td>4.86 ± 0.82</td>
<td>5.78 ± 0.83</td>
</tr>
<tr>
<td>Alkaline phosphatase (mg/dL)</td>
<td>115 ± 27</td>
<td>117 ± 32</td>
<td>81 ± 19</td>
</tr>
<tr>
<td>PTH (pg/μL)</td>
<td>30 ± 9</td>
<td>45 ± 17</td>
<td>63 ± 23</td>
</tr>
<tr>
<td>25-hydroxyvitamin D (ng/dL)</td>
<td>41.07 ± 22.7</td>
<td>26.58 ± 7.0</td>
<td>15.16 ± 9.6</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>1.71 ± 0.21</td>
<td>1.63 ± 0.55</td>
<td>1.31 ± 0.27</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.65 ± 0.08</td>
<td>0.64 ± 0.06</td>
<td>0.54 ± 0.05</td>
</tr>
</tbody>
</table>

Values are mean ± SD. *p < 0.001 vs. groups I and II; †p < 0.02 vs. group I; ‡p < 0.05 vs. groups I and II.
values are shown in Table 3. Variation coefficient of the DEXA measurements was 2%. BMD in femur was significantly lower in animals consuming soft drinks (groups III and IV) than in ovariec-tomized animals consuming water (group II), and BMD in group II animals was significantly lower than in non-ovariec-tomized animals consuming water (group I). BMD in pelvis was significantly lower in the three groups of ovariec-tomized rats (groups II, III, and IV) than in non-ovariec-tomized animals (group I). There were no significant differences between ovariec-tomized animals consuming cola soft drinks (groups III and IV), and ovariec-tomized animals consuming water (group II) with regard to pelvic BMD. Lumbar spine BMD showed no differences among the four groups. Calcium in femoral ashes was signifi-cantly lower in animals consuming soft drinks (groups III and IV) than in ovariec-tomized animals consuming water (group II), and in animals of group II than in non-ovariec-tomized animals consuming water (group I). Intra-observer concordance for histological measurement of fem-oral cortical width between the two blinded measurements for each slide was excellent (κ concordance coefficient = 0.85, Pearson’s r correlation coefficient = 0.92, p < 0.001). Again, femoral cortical width was significantly lower in ani-mals receiving cola beverages than in ovariec-tomized ani-mals receiving water (group II) and in group II than in non-ovariec-tomized animals receiving water.

Study 2. Initial body weight was not different between ani-mals in group A and group B. Final body weight was signif-icantly lower in group B than in group A. However, plasma-ionized calcium levels were significantly lower in group A than in group B. Calcium values in femoral ashes and femo-ral cortical width were also significantly lower in group A than in group B (Table 4). Plasmatic phosphate, creatine, and albumin levels were not different between the two groups (data not shown).

Discussion

The results of this study suggest that cola beverage con-sumption has the potential to reduce femoral density. The fact that three different methods such as DEXA, measure-ments of calcium in femoral ashes, and histological mea-surement of femoral cortical width consistently showed decreased bone density in the femurs in groups of rats receiving cola soft drinks compared with animals receiving water to drink further supports this conclusion. We could not demonstrate a soft drink intake-related bone density re-duction in pelvis and lumbar spine, but the trend shown in

Figure 1. Alkaline phosphatase. Animals consuming cola beverages (groups III and IV) show a significant decrease of alkaline phosphatase levels.

Figure 2. 25 Hydroxyvitamin D. Animals consuming cola beverages (groups III and IV) show a significant decrease of 25-hydroxyvitamin D.

Figure 3. Bone mineral density measured by DEXA. Animals consuming cola beverages (groups III and IV) show a significant decrease of bone mineral density.
Table 3. Bone mineral density (BMD), calcium in femoral ashes, and femoral cortical width of animals in the different groups of study 1

<table>
<thead>
<tr>
<th></th>
<th>Group I (Sham)</th>
<th>Group II (Ovariectomized control)</th>
<th>Group III (Soft drink A)</th>
<th>Group IV (Soft drink B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Femoral BMD (g/cm²)</td>
<td>0.20 ± 0.02</td>
<td>0.18 ± 0.01&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.16 ± 0.01&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>0.16 ± 0.01&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pelvic BMD (g/cm²)</td>
<td>0.22 ± 0.01</td>
<td>0.20 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.19 ± 0.02&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.19 ± 0.01&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lumbar spine BMD (g/cm²)</td>
<td>0.15 ± 0.01</td>
<td>0.14 ± 0.01</td>
<td>0.13 ± 0.01</td>
<td>0.13 ± 0.01</td>
</tr>
<tr>
<td>Calcium in femoral ashes (mg/g of dry tissue)</td>
<td>1.52 ± 0.55</td>
<td>1.36 ± 0.40&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>0.83 ± 0.52&lt;sup&gt;gh&lt;/sup&gt;</td>
<td>0.79 ± 0.66&lt;sup&gt;eh&lt;/sup&gt;</td>
</tr>
<tr>
<td>Femoral cortical width (mm)</td>
<td>0.70 ± 0.06</td>
<td>0.67 ± 0.05&lt;sup&gt;ij&lt;/sup&gt;</td>
<td>0.58 ± 0.06&lt;sup&gt;jk&lt;/sup&gt;</td>
<td>0.60 ± 0.05&lt;sup&gt;jk&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± SD; <sup>a</sup><sup>,p</sup><sup></sup><sup>0.006 vs group I; <sup>b</sup><sup>,p</sup><sup></sup><sup>0.02 vs groups III and IV; <sup>c</sup><sup>,p</sup><sup></sup><sup>0.01 vs group II; <sup>d</sup><sup>,p</sup><sup></sup><sup>0.002 vs group I; <sup>e</sup><sup>,p</sup><sup></sup><sup>0.001 vs groups III and IV; <sup>f</sup><sup>,p</sup><sup></sup><sup>0.01 vs group I; <sup>g</sup><sup>,p</sup><sup></sup><sup>0.02 vs group II; <sup>h</sup><sup>,p</sup><sup></sup><sup>0.0002 vs group I; <sup>i</sup><sup>,p</sup><sup></sup><sup>0.03 vs group I; <sup>j</sup><sup>,p</sup><sup></sup><sup>0.03 vs groups III and IV; and <sup>k</sup><sup>,p</sup><sup></sup><sup>0.03 vs group II.</sup></sup></sup></sup></p>

Table 4. Initial weight, final weight, ionized calcium, and calcium in femoral ashes of animals of study 2

<table>
<thead>
<tr>
<th></th>
<th>Group A (soft drink)</th>
<th>Group B (pair-fed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial weight (g)</td>
<td>304 ± 13</td>
<td>303 ± 14</td>
</tr>
<tr>
<td>Final weight (g)</td>
<td>336 ± 22</td>
<td>289 ± 25&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ionized calcium (mmol/L)</td>
<td>1.28 ± 0.07</td>
<td>1.44 ± 0.28&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Calcium in femoral ashes (mg/g of dry tissue)</td>
<td>0.85 ± 0.49</td>
<td>1.32 ± 0.51&lt;sup&gt;i&lt;/sup&gt;</td>
</tr>
<tr>
<td>Femoral cortical width (mm)</td>
<td>0.57 ± 0.04</td>
<td>0.64 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± SD; <sup>a</sup><sup>,p</sup><sup></sup><sup>0.01 vs group A; <sup>b</sup><sup>,p</sup><sup></sup><sup>0.02 vs group b; and <sup>i</sup><sup>,p</sup><sup></sup><sup>0.001 vs group A.</sup></sup></p>

Table 3 for these latter bones was near statistical significance. There are reports suggesting that in rats, femurs are more susceptible to develop hypodensity than other bones (5,6).

For the animals of study 2 only calcium values in femoral ashes and of femoral cortical width are available. DEXA bone density measurements were not done in these animals. However, in study 1 we found excellent concordance between bone density measured by DEXA and values of calcium in femoral ashes as previously reported (21).

We believe that the most plausible causal link between cola soft drink consumption and disorders of bone and calcium metabolism is the phosphoric acid content of this kind of beverages. Phosphate contents of the two most popular commercial brands of cola soft drinks are 19.7 and 16.1 mg/dL, respectively (22). Pathophysiologic explanations of mineral metabolism disorders caused by phosphate intake have been extensively discussed elsewhere. Briefly, exogenous phosphate loads are believed to inhibit renal 1α-hydroxylase and to reduce 1α, 25-dihydroxyvitamin-D synthesis (23,24). This in turn blocks intestinal absorption and renal tubular reabsorption of calcium, leading to hypocalcemia (24). Secondary hyperparathyroidism is developed, but apparently is not sufficient to prevent sustained hypocalcemia. This sequence of events explains the picture consistently found in human studies and animal models of heavy cola soft drink consumption, characterized by hypocalcemia, secondary hyperparathyroidism, and reduced 1α, 25-dihydroxyvitamin-D level (10,11). However, inhibition of renal 1α hydroxylase explains low 1α, 25-dihydroxyvitamin-D level but does not explain the previously reported reduction of 25-hydroxyvitamin-D level (11) confirmed in the present study. Therefore, these data suggest that exogenous phosphate loads interfere with earlier metabolic steps in vitamin D synthesis but additional studies are required to test this hypothesis.

In addition to phosphate content, there are other reasons why cola beverages may impair bone and mineral metabolism. Caffeine consumption is suspected of increasing fracture risk and reducing bone mineral density (7,17). Regular cola soft drinks can provide up to 18 mg of caffeine per 100 mL of the beverage (1). Acid loads may also adversely affect calcium and bone metabolism. Acidemia increases bone resorption and calcium mobilization (18,19) and also reduces renal 1α hydroxylase activity and 1α, 25-dihydroxyvitamin-D production (20). Heavy intake of cola beverages has been associated with metabolic acidosis in immature rats (11). Additionally, in humans, intake of carbonated beverages may displace other nutriments such as milk from the diet, decreasing calcium intake (25), which can lead to lower bone mineral density. Additional research is required to understand the precise contribution of each of these factors in the reduction of bone mineral density.

An unexpected finding was the reduction of alkaline phosphatase seen in animals receiving cola soft drinks. Increased levels of alkaline phosphatase are considered a marker of bone remodeling. An explanation for this finding may be the caffeine content of the beverages. Caffeine has been reported to lower alkaline phosphatase synthesis (26) and to inhibit the formation of a competent extracellular matrix during the osteoblast differentiation sequence, associated with inhibition of mineralization and low alkaline phosphatase activity (27).

Experimental animals of the present study that were allowed to drink cola beverages ad libitum dramatically increased their liquid intake. The fact that rats tend to increase their liquid consumption threefold when they have free access to cola soft drinks has been previously noted (28). Con-
comitantly, animals in the present report substantially decreased solid food consumption to only about 59% of the amount consumed by control rats, leading them to malnutrition characterized by decreased levels of serum albumin and serum creatinine. These findings raised the possibility that the observed bone disorders could be more related with decreased food intake or malnutrition than with soft drink consumption per se. The second part of the study ruled out this possibility, because in the face of a similar solid-food intake and more severe malnutrition features, paired-fed rats not consuming cola soft drinks did not show bone or mineral metabolism derangement. However, the volume of soft drinks ingested by the rats corresponds roughly to a daily intake approximately 40% of the animal’s body weight. Human soft-drink consumers can scarcely reach these huge consumption amounts. In a previous study, we reported that the child ranked first in cola beverage consumption in that survey only drank slightly more than 1 L of cola per day (3).

Additional studies are required to ascertain the minimal amounts of soft-drink ingestion needed to produce deleterious effects such as those described in the present report. However, these data demonstrate that heavy intake of cola soft drinks has the potential of reducing femoral mineral density in an animal model. The precise ingredient or combination of ingredients involved in bone mass reduction, minimal time of exposure, and minimal dose of soft drink required to cause the described effect remains to be clarified.

References


