Effects of Long-term Treatment with Caffeine on the Ultrastructure of the Golden Hamster Parathyroid Gland and Tibia

By

Marjan JAMALI¹, Daisuke HAYAKAWA¹, Huayue CHEN¹, Shoichi EMURA², Yuki OZAWA¹, Hirotaka TAGUCHI¹, Ryuichiro YANO¹ and Shizuko SHOUMURA¹

¹Department of Anatomy, Gifu University, School of Medicine, Gifu, Japan
²College of Medical Sciences, Gifu University, Gifu, Japan

Summary: The ultrastructure of the parathyroid gland and the SEM appearances of the tibia were studied in hamsters with and without administration of caffeine. Caffeine was treated orally each day at either 2.5 mg (low dose) or 10 mg (high dose) per 100 g body weight for a period of 17 or 32 days. Statistical analysis showed no significant differences among all groups examined regarding the serum calcium level. Transmission electron microscopy of the parathyroid gland revealed that the volume densities occupied by the mitochondria, Golgi complexes and rough endoplasmic reticulum of caffeine-treated groups were found significantly higher when compared with controls. The number of secretory granules observed close to the cell membrane per total amount of these granules revealed significant increase in all caffeine-treated animals. The bone mineral content (BMC) values were closely related to body weight. In the high dose caffeine-treated hamsters increment of the mean BMC and body weight values was significantly lower than those of the controls after 32 days. In the scanning electron microscopic studies of the tibia, no alteration in the morphometric parameters was demonstrated.

It is considered that the synthesis and release of parathyroid hormone is stimulated following caffeine consumption. Our data suggest that although chronic administration of caffeine in the hamster may slightly increase bone turnover as evidenced by the BMC decrease, bone morphometry was not altered. Thus the osteoporotic changes were not proved in this study.

Materials and Methods

Animals

A total number of 42 adult, female hamsters were used. Hamsters were 3 months old and approximately 137 g at the beginning of the experiment, and were divided into 2 groups of 21 animals each. In each group 7 animals were served as controls drinking 0.5 ml/100 g body weight distilled water (DW), 7 animals received 2.5 mg/100 g body weight caffeine in 0.5 ml DW and 7 animals received 10 mg/100 g body weight caffeine in 0.5 ml DW orally, once a day. The amount of caffeine...
used in the present experiment is equivalent to 340 mg and 1360 mg/70 kg$^{0.75}$ respectively in humans, when conversion is based on the metabolic body weight (kg$^{0.75}$), (Yeh et al., 1986). The period of treatment was 17 days or 32 days for each group containing 21 hamsters.

**Serum Calcium Measurement**

The animals were anesthetized with sodium pentobarbital and 3 ml samples of cardiac blood were withdrawn into heparinized syringes for analysis on the sacrificing day that was the 18th and 33rd. All samples were centrifuged at 3000 x g for 10 minutes to obtain plasma fractions. The serum calcium concentration was determined by spectrophotometry.

**Transmission Electron Microscopy of Parathyroid**

The parathyroid glands were removed and immersed in a mixture of 2.5% glutaraldehyde and 2% osmium tetroxide in Millonig's buffer at pH 7.4 for 1 hour, dehydrated through ascending concentrations of acetone and embedded in Epon 812. Thin sections were cut on Porter-Blum MT-1 ultramicrotome, mounted on 200 mesh grids, stained with uranyl acetate and lead salts, and examined with a Hitachi H-800 electron microscope at 100 kV. From the parathyroid glands of each animal 20 medium-power (x 6000) electron micrographs were selected and enlarged at a final magnification of 22000. The total number of micrographs analyzed was 840.

The volume densities (volume of the particular structure per unit volume of tissue) of rough endoplasmic reticulum, mitochondria, Golgi complexes, secretory granules and large vacuolar bodies were determined using point-counting method (Weibel, 1979).

**BMC and BMD of the Whole Body**

The bone mineral content (BMC) and bone mineral density (BMD) of the whole body were measured twice for each group of animals. The measurements were done by (DXA) Dual Energy X-ray Absorptiometry (Toyo Medic QDR Type 2000) on the day before starting the caffeine administration that was the day 0 or the day before sacrificing that was the 17th or 32nd day of the experiment.

**Scanning Electron Microscopy of Tibia**

The specimens were taken from the proximal end of the tibia, just below the metaphysis and processed for SEM. The samples were placed in 5.25% sodium hypochlorite solution overnight to render them anorganic, dehydrated with acetone and vacuum-dried (Wink, 1982). According to previous observations, the bone turnover is more pronounced on the endosteal surface than on the periosteal surface (Wink, 1986), therefore in this study we observed the endosteal surfaces of the samples. Lateral portions of the sampled bones were glued on specimen stubs, endosteal surface up, ion sputter coated, and viewed at 15 kV using JEOL JSM-T300 scanning electron microscope. Osteocyte lacunae and vascular canal entrances were counted in 10 micrographs (×500) taken in sequence along the middle of each bone segment (Wink, 1982). The percent area of vascular canal entrances were measured using point-counting method. The total area of each micrograph was approximately 45000 μm$^2$.

**Statistics**

All of variance data were presented as the mean ± SEM. One way analysis of variance data (ANOVA) was used to detect significant differences among the means in the three examined groups with the same duration of treatment. Fisher's protected least significant difference comparison test was used to determine differences between pairs of mean. Significance was accepted at p < 0.05.

**Results**

**Serum Calcium Level**

The mean serum calcium levels (mg/100 ml) of the control and caffeine-treated groups are shown in the Table 1. There were no significant differences among all the groups examined, regarding the serum calcium level.

**Fine Structure of the Parathyroid Gland**

The description of the ultrastructure of the control hamster's parathyroid glands given in this paper agrees with previous reports (Isono et al., 1990; Table 1. Serum calcium levels in different groups given as (mg/100 ml)

<table>
<thead>
<tr>
<th>Group</th>
<th>Days of measurement</th>
<th>Number of animals</th>
<th>Serum calcium</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>18</td>
<td>7</td>
<td>11.03 ± 0.12</td>
</tr>
<tr>
<td>Low dose</td>
<td>18</td>
<td>7</td>
<td>11.57 ± 0.24</td>
</tr>
<tr>
<td>High dose</td>
<td>18</td>
<td>7</td>
<td>11.39 ± 0.24</td>
</tr>
<tr>
<td>Control</td>
<td>33</td>
<td>7</td>
<td>11.23 ± 0.21</td>
</tr>
<tr>
<td>Low dose</td>
<td>33</td>
<td>7</td>
<td>11.27 ± 0.13</td>
</tr>
<tr>
<td>High dose</td>
<td>33</td>
<td>7</td>
<td>11.20 ± 0.10</td>
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control: 0.5 ml/100 g body weight distilled water, low dose: 2.5 mg/100 g body weight caffeine in 0.5 ml distilled water, high dose: 10 mg/100 g body weight caffeine in 0.5 ml distilled water. The values are mean ± SEM.
Chen et al., 1999) (Fig. 1a). Parathyroid glands of the controls and of caffeine-treated hamsters comprised cells of similar shape and electron density of the cytoplasmic matrix. The volume density of the Golgi complexes associated with some prosecretory granules was estimated to be significantly elevated in all caffeine-treated groups (Fig. 1b). Cisternae of the rough endoplasmic reticulum (RER) were sometimes arranged in parallel arrays, distributed in the cytoplasm. The significant increment of RER was seen in the caffeine-treated animals comparing to the controls (Table 2). Mitochondria varied in number and size and were quite abundant in the caffeine-treated animals. Secretory granules varied from round to rod-shaped and measured from 150 to 350 nm in diameter (Fig. 1a). In the caffeine-treated animals the number of these granules was decreased while in these cells the number of the granules located in a peripheral position adjacent to the plasma membrane was considerably increased when compared with the controls (Fig. 2). Large vacuolar bodies of 350–750 nm in diameter, were sometimes observed in the cytoplasm. There were no significant differences among the six groups concerning the large vacuolar bodies.

**Weight, BMC and BMD of the Whole Body**

Table 3 sets out the mean values for weight (g), bone mineral content (BMC, g) and bone mineral density (BMD, g/cm²) of the whole body.

<table>
<thead>
<tr>
<th>Table 2. Volume density of cell components</th>
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<tr>
<td>Group</td>
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<tr>
<td>----------------</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Low dose</td>
</tr>
<tr>
<td>High dose</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Low dose</td>
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<tr>
<td>High dose</td>
</tr>
</tbody>
</table>

The volume densities are presented as percentage of cell volume.

*ap < 0.05: Significant differences from control group (of the same treatment days animals).

*b p < 0.05: Significant differences from low dose group (of the same treatment days animals).

The values are mean ± SEM.

<table>
<thead>
<tr>
<th>Table 3. Weight (g), BMC (g) and BMD (g/cm²) of whole body</th>
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<tbody>
<tr>
<td>Group</td>
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<tr>
<td>-----------------</td>
</tr>
<tr>
<td>Control&lt;sup&gt;17&lt;/sup&gt;</td>
</tr>
<tr>
<td>Low dose&lt;sup&gt;17&lt;/sup&gt;</td>
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<tr>
<td>High dose&lt;sup&gt;17&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control&lt;sup&gt;17&lt;/sup&gt;</td>
</tr>
<tr>
<td>Low dose&lt;sup&gt;32&lt;/sup&gt;</td>
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<tr>
<td>High dose&lt;sup&gt;32&lt;/sup&gt;</td>
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<td>Control&lt;sup&gt;17&lt;/sup&gt;</td>
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<tr>
<td>Control&lt;sup&gt;32&lt;/sup&gt;</td>
</tr>
<tr>
<td>Low dose&lt;sup&gt;32&lt;/sup&gt;</td>
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<tr>
<td>High dose&lt;sup&gt;32&lt;/sup&gt;</td>
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</table>

*p < 0.05: Significant difference from control group (of the 32 days caffeine-treated animals).

The values are mean ± SEM.
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In the 17 days caffeine-treated hamsters, no significant differences were revealed. The BMC changes were closely related to the body weight on the first or the second measurements. The increment of the body weight and BMC of 32 days caffeine-treated animals was significantly lower than those of the controls and attained significance in the high dose group.

**SEM Observations of Endosteal Surfaces of Tibia**

The endosteal bone surfaces of the caffeine-treated and control animals showed no significant differences in the number of osteocyte lacunae and the number or percent area of vascular canal entrances (Fig. 3, Table 4).

### Discussion

PTH transport pathway in the parathyroid cell comprises of synthesis of polypeptides in the RER followed by their transport via the vesicles to the Golgi region where secretory proteins are packed into membrane-bound granules which are released into the extra cellular fluid (Wild and Setoguti, 1995).

Depression of serum calcium level results in immediate PTH release (Blum et al., 1974). In our experiments we did not find any changes in serum calcium. The similar results were reported in previous chronic administration of caffeine studies (Yeh et al., 1986; Glajchen et al., 1988). A caffeine-induced urinary loss of calcium is expected after caffeine consumption which results a short-term hypocalcemia that in turn activates parathyroid glands. Therefore during the time chosen for measurement that was 24 hours after the last caffeine treatment, the serum calcium might be already compensated through PTH release.

The ultrastructural indications of activity include an increase in volume densities occupied by RER, Golgi complexes and mitochondria. All these features can be used as indicators of increased protein synthesis (Shoumura et al., 1990). Similar changes were found after application of progesterone or testosterone (Brunner et al., 1992). The significant increase in surface area of RER and Golgi complexes were found 24 hours after application of either progesterone or testosterone, indicating that elevation of capacity for PTH synthesis and packaging requires hours, however PTH release is modulated within seconds (Habener, 1981).

One of the cellular processes by which PTH is released from the gland into the extracellular fluid is believed to involve exocytosis. In active chief cells, secretory granules have a tendency to gather beneath the plasma membrane (Shoumura et al., 1989). In the caffeine-treated animals, the number of secretory granules close to the plasma membrane increased significantly, while the total number of secretory granules in the 17 days group was decreased compared with those of the controls. It is probable that the number of secretory granules in the chief cells does not appear to correlate with the functional states of the parathyroid gland (Shoumura et al., 1989).

Unfortunately, demonstration of PTH concentration level in hamsters using radioimmunoassay methods has not been possible so far.

The failure in the weight gain observed in high dose group after 32 days of caffeine treatment would imply that the caffeine was exerting its metabolic effect (Spurlock et al., 1996). The weight loss is consistent with human studies showing an increase in metabolic rate and free fatty acid mobilization with caffeine administration (Acheson et al., 1980).

Thirty-two days, high dose administration of caffeine resulted in low BMC in hamsters, though the BMD changes are not consistent with the final weight and BMC results. BMD is obtained by dividing BMC by bone area (BA) or width (BW). Recent studies have shown that BMD is not a
measure of true density because absorptiometry provides no information about the depth of the bone. Moreover areal BMD is unable to distinguish between osseous and non-osseous areas within the bone envelope. It has been shown that in measurements of the whole body, BMD was significantly related to BA (BW). The use of BMD would artificially overestimate relative bone mineral for individuals with bones larger than average and underestimate it for those with smaller bones (Prentice et al., 1994; Warner et al., 1998). There are other studies proving that a change in BMC correlated positively with a change in weight but weight loss causes an increase in BMD, arising from a reduction in bone area (Tothill et al., 1997). Thus the BMC deficiency in this study may be related to the weight loss following caffeine consumption.

Glaichen et al. (1988) after administration of 2.5 and 10 mg/100 g caffeine in drinking water for the period of 8 weeks reported that the serum osteocalcin in high dose caffeine-treated animals was significantly increased compared to the low dose and control groups. They concluded that caffeine administration may slightly increase bone turnover. On the other hand the bone histomorphometric analysis failed to reveal significant differences in bone remodeling activity parameters including osteoclast number. In our bone morphometric study, no significant differences were seen in the measured parameters among all groups examined. It seems that the period of treatment chosen for these experiments was not long enough to reveal the osteoporotic changes by caffeine.

We conclude that chronic caffeine ingestion results in changes of the morphometric parameters of parathyroid glands and enhances their secretory activity. The osteoporotic changes were not seen during this study. Further research with longer period is needed to determine the probable long-term heavy caffeine ingestion effects on bone structure that can be as a result of parathyroid hyperactivity.

References


22) Yeh JK, Aloia JF, Semla HM and Chen SY. Influence of injected caffeine on the metabolism of calcium and the re-

Explanation of Figures

Plate I

Fig. 1. Parathyroid chief cells of the 17 days, control hamster (a). Golgi complex (G) and secretory granule (arrow) are seen. In the parathyroid chief cells of the 17 days, high dose caffeine-treated hamsters (b), well-developed Golgi complexes (G) associated with prosecretory granules (P) and cisternae of the granular endoplasmic reticulum (ER) are observed. Secretory granules are located in the peripheral cytoplasm (arrows).
Fig. 2. The percentage of Peripheral SG/Total SG: The number of secretory granules close to the cell membrane per total number of secretory granules.
Plate III

Fig. 3. Endosteal surfaces of the tibia from the control (a) and high dose caffeine-treated hamsters (b) sacrificed on the 33rd day. The large and small vascular canal entrances are shown by (V) and arrow respectively. The arrow heads show the osteocyte lacunae. The bone samples (c to e) are from the 17 days treated hamsters; (c) is the control, (d) is the low dose and (e) is the high dose caffeine-treated hamsters. The vascular canal entrances (arrows) and the osteocyte lacunae (arrow heads) are observed.