Hyperparathyroidism Is Augmented by Ovariectomy in Nagase Analbuminemic Rats

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ABSTRACT The role of albumin in bone metabolism was studied in Nagase analbuminemic (NA) rats. Serum calcium (Ca), inorganic phosphate (Pi) and magnesium (Mg) concentrations did not differ between female NA and control Sprague-Dawley (SD) rats at the time of ovariectomy (ovx), although serum ionized Ca was significantly lower in NA rats than in SD rats. Serum parathyroid hormone (PTH) and osteocalcin (OC) concentrations and urinary Ca excretion were significantly greater in NA rats than in SD rats, suggesting hyperparathyroidism and the resultant enhanced bone turnover in NA rats. Paradoxically, ovx increased serum PTH and OC in NA rats but not in SD rats. Ovx-induced exacerbation of hyperparathyroidism was confirmed by significantly greater conversion of 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D in ovx NA rats even after normalization to vitamin D–binding protein. Bone mineral density (BMD) in proximal tibia increased similarly in a time-dependent manner in sham-operated NA and SD rats. However, ovx ablated the time-dependent increase of BMD in SD rats and significantly decreased BMD in NA rats by 2 wk after ovx, resulting in a significantly lower BMD in ovx NA rats than in ovx SD rats. In summary, NA rats, which are analbuminemic with compensatory increases in lipid and protein synthesis, developed hyperparathyroidism, possibly due to an increase in serum Pi and a reduction of ionized Ca, and ovx induced a greater BMD reduction in NA rats than in SD rats, probably by exacerbating hyperparathyroidism. J. Nutr. 130: 1543–1547, 2000.

KEY WORDS: • albumin • protein deficiency • hyperparathyroidism • osteocalcin • 1,25-dihydroxyvitamin D • rats

Albright originally called attention to the importance of albumin in the pathogenesis and treatment of osteoporosis (Albright et al. 1941, Albright and Reifenstein 1948). Serum albumin correlates significantly with skinfold and body mass index (Thiebaud et al. 1997), providing a valuable biochemical marker for nutritional status (Craig 1987, Laccy 1991). It has also been shown that serum albumin correlates significantly with age-adjusted bone mineral density (BMD)2 (Rico et al. 1992, Thiebaud et al. 1997), whereas low serum albumin was the strongest independent variable correlated with hip fractures (Punnonen et al. 1986, Thiebaud et al. 1997). In fact, elderly persons who have osteoporotic fracture are often undernourished as evidenced by hypoalbuminemia (Cooper et al. 1989, Rico et al. 1992, Thiebaud et al. 1997). Albumin also plays an important role as a major calcium (Ca)-binding protein in serum (Payne et al. 1979). Therefore, it should have more direct effect on bone metabolism; the reduction in serum albumin results in an increase in free Ca (Ladensen et al. 1979), which may suppress bone turnover by inhibiting secretion of parathyroid hormone (PTH).

It was reported that a pair of siblings with analbuminemia showed severe juvenile osteoporosis; in these subjects, human serum albumin replacement therapy prevented the progression of osteoporosis (Kallee 1996). Although they developed hyperparathyroidism, the exact mechanism for the development of osteoporosis is yet to be determined.

Nagase analbuminemic (NA) rats, which were established from Sprague-Dawley (SD) rats (Nagase et al. 1979), lack serum albumin due to a block of albumin mRNA splicing by a 7-bp deletion in intron HI of the albumin gene (Esumi et al. 1979), which may suppress bone turnover by inhibiting secretion of parathyroid hormone (PTH).

We investigated the effect of albumin deficiency and the resulting compensatory increase of protein synthesis on bone metabolism by comparing the basal state of bone metabolism and its responses to ovariectomy (ovx) in NA and control SD rats.

MATERIALS AND METHODS

Rats. Female 9-wk-old NA rats and female SD rats, which served as a control group, were purchased from Keari Co. (Osaka, Japan).
Rats were caged individually with free access to food and water, as previously described (Inaba et al. 1999). They were maintained on a 12-h light:dark cycle and fed a nonpurified diet (CE Diet, Clea, Japan) containing 24.8% protein, 1.25% Ca and 1.06% inorganic phosphate (Pi). The experiment started when the rats were 10 wk old, following a 7-day acclimation period. All animal procedures were approved by the Institute’s Animal Care Committee. NA rats (n = 70) and SD rats (n = 70) were divided into two groups, one group undergoing a sham operation (sham) and the other group undergoing ovx. Under anesthesia with ether, bilateral ovx was performed in 12-wk-old NA (n = 35) and SD rats (n = 35) by using a dorsal approach with a small single midline dorsal skin incision. The other NA and SD rats (n = 21 each) were subjected to a sham operation by exposing but not removing the ovaries. Success of ovx was confirmed at necropsy by failure to detect ovarian tissue and by the observation of marked atrophy of uterine horns.

The rats were weighed biweekly and checked for general health and food intake. To facilitate 24-h collection of urine, the rats were placed in individual metabolic cages with free access to the same food and water. After being deprived of food overnight, but with free access to water, the rats (n = 7/treatment) were killed by aortic puncture at the indicated times. The blood obtained was centrifuged at 1,200 g for 10 min and the serum was stored at −80°C until analysis.

**Biochemical variables.** Serum levels of total protein, albumin, α₁, α₂, β- and γ-globulins, glucose, cholesterol, triglyceride, serum urea nitrogen (SUN), creatinine (Cr), alkaline phosphatase (ALP), total Ca, and Pi were measured with a Hitachi model 7450 autoanalyzer (Hitachi, Tokyo, Japan) and ionized Ca with NOVA CRT-8 (NOVA Biomedical, Tokyo, Japan). Urinary Cr and Ca were also measured with the autoanalyzer, and urinary excretion of Ca was corrected for that of Cr. Serum PTH was measured by immunoradiometric assay (Allegro Intact PTH, Nichols Institute, San Juan Capistrano, CA), which measures only active intact PTH but not degradation products resulting from its cleavage (Finch et al. 1992). Serum osteocalcin (OC), also known as bone Gla-protein, was measured with a commercial RIA kit (Yamasa Shoyu, Choshi, Japan), using rat OC as a standard (Inaba et al. 1999, Nakatsuka et al. 1991).

**Assay of vitamin D metabolites and vitamin D-binding protein (DBP).** Serum levels of vitamin D metabolites and DBP were determined at 3 wk after ovx. Vitamin D metabolites were assayed for serum levels by Mitsubishi Kagaku Bio-Clinical Laboratories (Tokyo, Japan), as described previously (Inaba et al. 1997). Samples were dried with nitrogen and then resuspended in 300 μL of methanol/isopropanol/n-hexane (1:6:93, v/v/v) just before injection. Vitamin D metabolites were separated by HPLC (Inaba et al. 1991 and 1997) with a 0.46 × 30.0 cm APS-Hypersil NH₂ column (Shandon, Cheshire, UK). Methanol/isopropanol/n-hexane (1:6:93) comprised the elution solvent, which was passed through a 0.5-μm filter (Millipore, Medford, MA) just before use. The column was eluted at a flow rate of 2 mL/min and 4-mL fractions were collected. Absorbance was measured at a wavelength of 264 nm by a JASCO 875 UV (Japan Spectroscopic, Tokyo, Japan). After vitamin D metabolites were separated, levels of 25-hydroxyvitamin D (25-OH-D) and 25,26-dihydroxyvitamin D [24,25-(OH)₂D] were measured by an established competitive protein binding assay using vitamin D–deficient rat serum. Levels of 1,25-dihydroxyvitamin D [24,25-(OH)₂D] were measured by radioreceptor assay using vitamin D receptor extract from bovine thymus (Yamasa Shoyu, Choshi, Japan), as described previously (Inaba and DeLuca. 1989, Inaba et al. 1997). Serum DBP, determined by rocket immunoelectrophoresis, was expressed relative to that of a control sample (Masada et al. 1989).

**Bone densitometry.** BMD was measured in the proximal tibia with dual energy X-ray absorptiometry (DCS-600 Aloka, Tokyo) every 2 wk until the age of 18 wk (Oikawa et al. 1999).

**Statistical analysis.** Values are expressed as means ± SD unless otherwise indicated. Statistical analysis was performed by two-way ANOVA, followed by Fisher’s paired least-significant difference (PLSD) test. Time effect was tested by ANOVA for repeated measurement and post-hoc tests. The assessment of differences between NA and SD rats was performed by Student’s t test. A level of P < 0.05 was regarded as significant.

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**RESULTS**

**Initial characteristics of NA rats.** A typical electrophoretic analysis of serum protein in NA rats indicated a serum albumin concentration of only 1.8 g/L (total protein, 63.0 g/L; albumin, 2.8%; α₁-globulin, 54.7%; α₂-globulin, 12.4%; β-globulin, 23.3%; and γ-globulin, 6.8%) with an albumin/γ-globulin (A/G) ratio of 0.03, in contrast to 36.4 g/L (total protein, 68.0 g/L; albumin, 35.5%; α₁-globulin, 22.3%; α₂-globulin, 6.3%; β-globulin, 13.7%; and γ-globulin, 4.2%) with an A/G ratio of 1.15 in SD rats. Body weight did not differ between NA and SD rats at the age of 12 wk (Table 1). There were no differences in serum concentrations of total Ca, Pi and Mg, but serum ionized Ca was significantly lower in NA rats than in SD rats. Furthermore, NA rats had significantly greater serum concentrations of intact PTH and OC and urinary excretion of Ca than SD rats. No significant difference in SUN or serum Cr concentrations was found between NA and SD rats, negating the possibility of uremic secondary hyperparathyroidism in NA rats. NA rats also had significantly greater serum concentrations of total cholesterol and triglycerides compared with SD rats.

**Body weight changes in SD and NA rats.** Body weights of SD and NA rats in the sham group increased significantly by 34 and 28%, respectively, during the 8-wk experimental period (Fig. 1). Weight gain was significantly greater in the ovx groups than in the sham groups.

**Biochemical variables of Ca metabolism in SD and NA rats.** Serum total Ca did not differ significantly between NA and SD rats in either the sham or ovx groups (Fig. 2). However, although serum Pi did not differ significantly between NA and SD rats before ovx or sham operation, serum Pi in NA rats became greater than that in SD rats after surgery. Significant greater urinary Ca excretion was observed in NA rats compared with SD rats, both at the time of ovx and after 3 wk [urinary Ca/Cr (μmol/mmol), 1.54 ± 0.31 vs. 1.12 ± 0.34 at the time of ovx, P < 0.05; 1.40 ± 0.17 vs. 0.92 ± 0.43 at 3 wk after ovx, P < 0.05]. Serum PTH (Fig. 3A) and OC (Fig. 3B) levels were significantly higher in NA rats than in SD rats at the time of surgery, and 1 and 3 wk afterwards. Ovx induced greater serum concentrations of total cholesterol and triglycerides compared with SD rats.

**TABLE 1**

Profiles of Nagase analbuminemic (NA) rats and control Sprague-Dawley (SD) rats

<table>
<thead>
<tr>
<th></th>
<th>SD</th>
<th>NA</th>
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<tbody>
<tr>
<td>Body weight, g</td>
<td>241.4 ± 8.13</td>
<td>225.3 ± 4.90</td>
</tr>
<tr>
<td>Proximal tibia bone mineral density, mg/cm²</td>
<td>105.4 ± 2.4</td>
<td>100.6 ± 4.3*</td>
</tr>
<tr>
<td>Serum urea nitrogen, mmol/L</td>
<td>8.74 ± 0.71</td>
<td>9.24 ± 0.50</td>
</tr>
<tr>
<td>Serum creatinine, mmol/L</td>
<td>61.9 ± 5.30</td>
<td>64.5 ± 11.5</td>
</tr>
<tr>
<td>Serum total cholesterol, mmol/L</td>
<td>1.81 ± 0.26</td>
<td>4.71 ± 0.05*</td>
</tr>
<tr>
<td>Serum triglyceride, mmol/L</td>
<td>1.10 ± 0.24</td>
<td>3.60 ± 0.80*</td>
</tr>
<tr>
<td>Serum calcium, mmol/L</td>
<td>2.54 ± 0.03</td>
<td>2.57 ± 0.08</td>
</tr>
<tr>
<td>Serum ionized calcium, mmol/L</td>
<td>2.74 ± 0.02</td>
<td>2.60 ± 0.16*</td>
</tr>
<tr>
<td>Serum phosphate, mmol/L</td>
<td>2.24 ± 0.21</td>
<td>2.31 ± 0.08</td>
</tr>
<tr>
<td>Serum magnesium, mmol/L</td>
<td>0.86 ± 0.20</td>
<td>1.00 ± 0.17</td>
</tr>
<tr>
<td>Serum osteocalcin, mg/mL</td>
<td>5.83 ± 0.54</td>
<td>9.37 ± 1.40*</td>
</tr>
<tr>
<td>Serum intact parathyroid hormone, pg/mL</td>
<td>1.46 ± 0.15</td>
<td>2.35 ± 0.48*</td>
</tr>
<tr>
<td>Urinary excretion of calcium/creatinine, μmol/mmol</td>
<td>1.12 ± 0.34</td>
<td>1.54 ± 0.31*</td>
</tr>
<tr>
<td>Urinary excretion of deoxypyridinoline/creatinine, μmol/mmol</td>
<td>107.9 ± 22.7</td>
<td>92.9 ± 14.5</td>
</tr>
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</table>

Values are mean ± standard deviation, n = 7; * significantly different from SD rats, P < 0.05.
a significant increase in serum PTH and OC in NA rats but not in SD rats.

**Serum vitamin D metabolite and DBP concentrations.** Serum 1,25-(OH)₂D levels, determined at 3 wk after ovx, were significantly greater in ovx NA rats by 51 and 96% compared with sham NA rats and ovx SD rats, respectively (Fig. 4A). In contrast, serum 24,25-(OH)₂D and 25-OH-D were significantly higher in ovx SD rats than in either ovx NA rats or sham SD rats. Even after serum vitamin D concentrations were corrected for DBP, the ratios of 1,25-(OH)₂D/DBP, 24,25-(OH)₂D/DBP and 25-OH-D/DBP, which are considered to be indices of free vitamin D metabolites, showed essentially the same patterns (Fig. 4B).

**BMD changes in proximal tibia in sham and ovx rats.** BMD in proximal tibia did not differ significantly between NA and SD rats at the time of ovx (Fig. 5). Although BMD increased steadily over time in sham NA and SD rats, there was no significant difference between the two groups. However, ovx ablated the time-dependent increase of BMD in SD rats. Of great interest was that in ovx NA rats, BMD decreased significantly by 2 wk after ovx. Ovx NA and SD rats had significantly lower BMD at all time points after surgery than their respective sham-operated rats. Furthermore, ovx NA rats had significantly lower BMD than ovx SD rats at all times after ovx.

**DISCUSSION**

This study was designed originally to examine the effect of serum albumin deficiency, frequently observed in malnourished patients, on bone metabolism using genetically analbuminemic NA rats. NA rats are characterized by hyperlipidemia and normal total protein levels with an absolute deficiency of albumin (Joles et al. 1997). Because NA rats genetically lack the ability to synthesize albumin, a compensatory increase in the synthesis of proteins and lipids appears to cause an increase in serum cholesterol and triglyceride concentrations (Takahashi et al. 1983), as described in nephrotic patients (Kaysen 1992). The plasma protein concentration is nearly normal in NA rats because albumin is replaced by several high-molecular-weight proteins, including α₂-macroglobulin and α₁-inhibitor 3 as in rats with nephrotic syndrome (Stevenson et al. 1998).

Although serum albumin is a valuable biochemical marker of nutritional state, albumin should have a more direct effect on bone metabolism because of its role as a major Ca-binding protein; hypoalbuminemia should result in an increase in free Ca, which may suppress bone turnover by inhibiting PTH secretion from the parathyroid gland. However, because serum total Ca levels did not differ significantly between NA and SD rats, NA rats have other substances acting as Ca-binding proteins. Serum levels of ionized Ca at the time of ovx were significantly lower in NA rats than in SD rats, although serum total Ca and Pi did not differ significantly between NA and SD rats, suggesting that the major Ca-binding substance in the
serum of NA rats might have higher affinity for Ca than albumin. A significant reduction in serum ionized Ca in NA rats may contribute in part to the enhancement of bone turnover by inducing hyperparathyroidism. Furthermore, a significant increase in conversion of 25-OH-D to 1,25-(OH)₂D, in association with a significant reciprocal suppression of 24,25-(OH)₂D conversion in NA ovx rats compared with SD ovx rats, clearly supported the hypothesis that ovx paradoxically augmented hyperparathyroidism in NA rats as evidenced by increased synthesis of 1,25-(OH)₂D. Vitamin D binds mainly to DBP and albumin (Bikle and Gee 1989). DBP, which has striking homology with albumin (Gibbs and Dugaiczyk 1987), is a nutritional marker (Polberger et al. 1990) like albumin. Therefore, because it was possible that serum DBP level may be different in NA and SD rats, a free index was calculated using DBP-adjusted serum levels of vitamin D metabolites (Bikle and Gee 1989, Nyomba et al. 1989, Woloszczuk 1985). However, even after normalization of serum vitamin D metabolites to DBP, an activation step of vitamin D from 25-OH-D to 1,25-(OH)₂D remained significantly enhanced in NA rats (Fig. 4B). Although the development of hyperparathyroidism in NA rats may be explained in part by a significant reduction in serum ionized Ca, it cannot be the sole factor involved. Serum Pi did not differ significantly between NA and SD rats at the time of ovx when NA rats had a greater serum concentration of PTH. Because PTH has a strong phosphaturic action, Pi entry into the circulation likely was greater in NA rats. Furthermore, serum Pi levels became greater in NA rats than in SD rats thereafter in spite of higher PTH levels (Fig. 2B), clearly indicating the occurrence of increased Pi load as a major contributing factor to the development of hyperparathyroidism in NA rats (Fig. 3A). One possibility that would explain this is demonstrated in nephrotic patients, in whom it has been shown that intestinal Pi absorption is not reduced despite a significant reduction in Ca absorption (Farrington et al. 1983). The same phenomenon may occur in NA rats, thus contributing to the development of hypophosphatemia. Alternatively, in the absence of albumin, the transport of albumin-bound substances, including Ca, from the

FIGURE 3 Paradoxical increases in serum levels of (A) intact parathyroid hormone (PTH) and (B) osteocalcin (OC) in Nagase analbuminemic (NA) rats, but not in Sprague-Dawley (SD) rats, after ovariectomy (ovx). Values are means ± standard deviation, n = 7. Means at a time not sharing a letter are significantly different, P < 0.05. *Significantly different from wk 0, P < 0.05.

FIGURE 4 Serum concentrations of (A) vitamin D metabolites and (B) their free indices in Nagase analbuminemic (NA) and Sprague-Dawley (SD) rats 3 wk after ovariectomy (ovx). Free indices of vitamin D metabolites were calculated by normalizing serum levels of vitamin D metabolites to vitamin D–binding protein. Values are means ± standard deviation, n = 7. Means not sharing a letter are significantly different, P < 0.05.

FIGURE 5 Reduction of bone mineral density (BMD) in proximal tibia after ovariectomy (ovx) in Nagase analbuminemic (NA) rats and in Sprague-Dawley (SD) rats. Values are means ± standard deviation, n = 7. Means at a time not sharing a letter are significantly different, P < 0.05. *Significantly different from wk 2, P < 0.05.
blood plasma into extravascular space and vice versa is severely disturbed, which may cause hyperparathyroidism in NA rats.

Contrary to previous results indicating the suppressive effect of ovx on parathyroid function (Pioli et al., 1992), ovx enhanced hyperparathyroidism in NA rats. Because estrogen has a direct effect on suppression of bone resorption and therefore, bone resorption is enhanced by estrogen deficiency (Parfitt 1988, Wronska et al., 1988), ovx was expected to suppress serum FTH by increasing bone-derived Ca entry into the blood stream. Unexpectedly, NA rats showed a significant increase of serum FTH following ovx. Although plasma lipid levels are expected to decrease by estrogen replacement as in postmenopausal women (Heikkinen et al., 1999), a paradoxical rise in plasma lipid level was reported in ovx NA rats given estrogen replacement (Takahashi et al., 1983), suggesting that NA rats respond paradoxically to estrogen deficiency not only in Ca metabolism but also in lipid metabolism.

In summary, the present study demonstrated that, in NA rats with analbuminemia and a compensatory increase in protein synthesis, development of hyperparathyroidism may be due to an increased Pi load and a lower ionized Ca concentration. Moreover, the augmentation of hyperparathyroidism observed in NA rats due to ovx could result in the greater reduction of BMD in ovx NA rats than in ovx SD rats. However, in the hypoalbuminemia resulting from impaired synthesis of generalized protein in liver such as liver cirrhosis, the extent of the influence of hypoalbuminemia on bone metabolism is not known from this study.

In conclusion, hypoalbuminemia may cause hyperparathyroidism by increasing the level of serum Pi and suppressing ionized Ca concentration. Hence, the NA rats readily lose bone following ovx.

LITERATURE CITED


Esumi, H., Takahashi, T., Sato, S., Nagase, S. & Sugimura, T. (1983) Plasma lipid synthesis of generalized protein in liver such as liver cirrhosis, the extent of the influence of hypoalbuminemia on bone metabolism is not known from this study.

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