Effect of chronic metabolic acidosis on bone density and bone architecture in vivo in rats

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1 Novartis Institute for BioMedical Research, Department of Musculoskeletal Diseases, Basel, Switzerland; 2 Department of Medicine, University of California, San Francisco, California; and 3 University Department of Medicine, Kantonsspital Bruderholz, Basel, Switzerland and Klinik St. Anna Hirslanden, Lucerne, Switzerland

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Gasser JA, Hulter HN, Imboden P, Krapf R. Effect of chronic metabolic acidosis on bone density and bone architecture in vivo in rats. Am J Physiol Renal Physiol 306: F517–F524, 2014. First published December 19, 2013; doi:10.1152/ajprenal.00494.2013.—Chronic metabolic acidosis (CMA) might result in a decrease in vivo in bone mass based on its reported in vitro inhibition of bone mineralization, bone formation, or stimulation of bone resorption, but such data, in the absence of other disorders, have not been reported. CMA also results in negative nitrogen balance, which might decrease skeletal muscle mass. This study analyzed the net in vivo effects of CMA’s cellular and physicochemical processes on bone turnover, trabecular and cortical bone density, and bone microarchitecture using both peripheral quantitative computed tomography and μCT. CMA induced by NH4Cl administration (15 mM/kg body wt/day) in intact and ovariectomized (OVX) rats resulted in stable CMA (mean Δ[HCO3−]p = 10 mmol/l). CMA decreased plasma osteocalcin and increased TRAP5b in intact and OVX animals. CMA decreased total volumetric bone mineral density (vBMD) after 6 and 10 wk (week 10: intact normal +2.1 ± 0.9% vs. intact acidosis −3.6 ± 1.2%, P < 0.001), an effect attributable to a decrease in cortical thickness and, thus, cortical bone mass (no significant effect on cancellous vBMD, week 10) attributed to an increase in endosteal bone resorption (nominally increased endosteal circumference). Trabecular bone volume (BV/TV) decreased significantly in both CMA groups at 6 and 10 wk, associated with a decrease in trabecular number. CMA significantly decreased muscle cross-sectional area in the proximal hindlimb at 6 and 10 wk. In conclusion, chronic metabolic acidosis induces a large decrease in cortical bone mass (a prime determinant of bone fragility) in intact and OVX rats and impairs bone microarchitecture characterized by a decrease in trabecular number.

osteoporosis; mineral density; metabolic acidosis; ovariectomy

CHRONIC METABOLIC ACIDOSIS (CMA) is one of the cardinal acid-base disorders, characterized by a primary decrease in body base content and diagnosed clinically by measuring decreased plasma [HCO3−] and blood pH. The disorder can be caused by increased production or decreased elimination of protons (such as in organic forms of metabolic acidosis or chronic renal failure) or by base losses (such as intestinal bicarbonate loss in diarrhea or bicarbonaturia in proximal tubular acidosis). Metabolic acidosis is regarded as the most common acid-base disorder due to the high worldwide prevalence of chronic diarrheal disease.

Bone serves as an important proton buffer in metabolic acidosis and, thus, exhibits a homeostatic role by attenuating the severity of metabolic acidosis. Bone exposed to acidic media in vitro undergoes physicochemical mineral dissolution leading to a fall in mineral sodium, potassium, carbonate, calcium, and phosphate. In this process, calcium released from the bone leads to hypercalciuria, the magnitude of which in humans is proportional to the acid load imposed on the body (20). Cellular events then ensue and sustain calcium loss from bone. These cell-mediated events are characterized both by increased osteoclastic and reduced osteoblastic activities, which are paralleled by alterations in the expression of a number of osteoclastic and osteoblastic genes (6). Osteoblasts can sense ambient acidity via proton sensor proteins such as OGR-1 (9). Downstream of this initiating event, osteoblastic cyclooxygenase (COX-2) mRNA and protein expression increases, leading to stimulated osteoblastic PGE2 secretion (18). PGE2 then stimulates in an autocrine fashion, osteoblastic RNA expression of the osteoclast activator, receptor activator of nuclear factor-κB ligand (RANKL), while expression of its soluble decoy receptor, osteoprotegerin is unchanged. This provides a key mechanism by which metabolic acidosis enhances osteoclastogenesis and osteoclast activity. Both RANKL expression and cell-mediated calcium efflux from bone are inhibited by cyclooxygenase inhibition, confirming the central role of acidosis-induced PGE2 stimulation (7). Metabolic acidosis also inhibits osteoid mineralization (4), and, therefore, metabolic acidosis is believed to induce an albeit poorly characterized bone disease with features of both osteoporosis and osteomalacia.

Several systemic acidosis-induced alterations exist that might decrease bone mass in addition to the described effects on bone cells and the mineralization process. Metabolic acidosis was demonstrated to result in negative nitrogen balance (1) and subsequently reported to induce muscle catabolism by increased, glucocorticoid-dependent proteolysis, which, in turn, might decrease the bone-anabolic physical load on the skeleton by ensuing sarcopenia (12). Metabolic acidosis was also demonstrated to result in low plasma 1,25(OH)2D3 levels, secondary hyperparathyroidism, growth hormone insensitivity, and increased glucocorticoid activities in humans (5, 16, 21), endocrine changes that are able, alone or in aggregate, to exert catabolic effects on bone.

At the level of cortical and trabecular bone density and microarchitecture, little information is available on the effects of experimental CMA in any species, despite reports on CMA’s in vitro effects on the underlying bone formation and resorption rates, whose net effects determine bone volume and bone density. Dynamic histomorphometry data in rats have shown that CMA impairs bone formation rates in both trabecular and cortical bone (17), while no change in formation rates was found by microradiography. Vice versa, although microradiography suggested increased cortical resorption (2),
The reported effects of CMA on bone resorption and formation have been independent of the effects of a functioning parathyroid gland (8, 17).

In rats, urinary hydroxyproline excretion, a biochemical marker of bone resorption, was reported as increased by CMA (17, 22), as was a bone formation marker (plasma alkaline phosphatase) (22), suggesting that both processes are coupled, possibly reflecting increased bone turnover.

The net effect of experimental CMA to decrease bone density has not been observed using histomorphometry (8, 17). Decreased bone density in the femur ash-to-volume ratio (8). Decreased bone density in association with osteomalacia (defective mineralization of bone) has been reported effects of CMA on bone resorption and formation (17, 22), as was a bone formation marker (plasma alkaline phosphatase) (22), suggesting that both processes are coupled, possibly reflecting increased bone turnover.

The characterization of net changes in cortical and trabecular bone density and microarchitecture remains a critical, unanswered question regarding the skeletal effects of CMA. Therefore, the aim of our study was to answer this question in vivo in rodents (Basel, Switzerland). All animal experimentation was conducted in accordance with accepted standards of humane animal care.

The net effect of experimental CMA to decrease bone density was not demonstrated by histomorphometry (17). The reported effects of CMA on bone resorption and formation have been independent of the effects of a functioning parathyroid gland (8, 17).

In rats, urinary hydroxyproline excretion, a biochemical marker of bone resorption, was reported as increased by CMA (17, 22), as was a bone formation marker (plasma alkaline phosphatase) (22), suggesting that both processes are coupled, possibly reflecting increased bone turnover.

The characterization of net changes in cortical and trabecular bone density and microarchitecture remains a critical, unanswered question regarding the skeletal effects of CMA. Therefore, the aim of our study was to answer this question in vivo in rats, since this is the species used initially to characterize the effects of CMA on bone formation and on resorption. In addition, we aimed to study the changes in bone density and microarchitectural properties of CMA with and without the effects of estrogen deficiency (bilateral ovariectomy), a model of severe bone loss, to assess the relative magnitude of CMA effects on bone.

**METHODS**

Six-month-old, skeletally mature virgin Wistar rats purchased from BRL (Füllinsdorf, Switzerland) were studied. Animals were housed at 25°C with a 12:12-h light-dark cycle with food and water being provided ad libitum (Purina, St. Louis, MO; containing 22.8% protein, 4.5% fat, 52.9% carbohydrate by weight, metabolizable energy 3.6 kcal/g). Studies described herein were performed according to the animal permit BS575 and were approved by the Kantonaes Veterinärarzt (Basel, Switzerland).

### Table 1. Blood and urinary acid-base composition after 6 wk

<table>
<thead>
<tr>
<th></th>
<th>Blood pH, U</th>
<th>[HCO₃⁻], mmol/l</th>
<th>Urinary pH, U</th>
<th>NH₄⁺/creatinine, mg/mmol</th>
<th>TA/creatinine, mg/mmol</th>
<th>HCO₃⁻, creatinine, mg/mmol</th>
<th>NAE/creatinine, mg/mmol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>7.436 ± 0.012</td>
<td>27.7 ± 1.2</td>
<td>6.03 ± 0.10</td>
<td>4.92 ± 0.34</td>
<td>1.51 ± 0.21</td>
<td>0.41 ± 0.12</td>
<td>6.02 ± 0.33</td>
</tr>
<tr>
<td>OVX</td>
<td>7.381 ± 0.021</td>
<td>27.6 ± 1.1</td>
<td>6.15 ± 0.11</td>
<td>3.95 ± 0.27</td>
<td>1.65 ± 0.24</td>
<td>0.37 ± 0.15</td>
<td>5.23 ± 0.29</td>
</tr>
<tr>
<td>Sham + NH₄⁺ Cl</td>
<td>7.210 ± 0.019*</td>
<td>17.1 ± 1.6</td>
<td>5.39 ± 0.12*</td>
<td>45.35 ± 3.72*</td>
<td>4.81 ± 1.11*</td>
<td>&lt;0.1*</td>
<td>50.16 ± 4.11*</td>
</tr>
<tr>
<td>OVX + NH₄⁺ Cl</td>
<td>7.182 ± 0.027*</td>
<td>17.9 ± 1.7</td>
<td>5.48 ± 0.13*</td>
<td>41.71 ± 4.39*</td>
<td>5.98 ± 1.21*</td>
<td>&lt;0.1*</td>
<td>47.69 ± 4.77*</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SD. *P at least <0.025 for the comparison to respective nonacidotic group. n values at week 6 are 20, 20, 10 and 13 for sham, OVX, sham + acidosis and OVX + acidosis, respectively.

### Table 2. Blood and urinary acid-base composition after 10 wk

<table>
<thead>
<tr>
<th></th>
<th>Blood pH, U</th>
<th>[HCO₃⁻], mmol/l</th>
<th>Urinary pH, U</th>
<th>NH₄⁺/creatinine, mg/mmol</th>
<th>TA/creatinine, mg/mmol</th>
<th>HCO₃⁻, creatinine, mg/mmol</th>
<th>NAE/creatinine, mg/mmol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>7.325 ± 0.011</td>
<td>27.7 ± 1.3</td>
<td>5.91 ± 0.12</td>
<td>4.06 ± 0.34</td>
<td>1.51 ± 0.21</td>
<td>0.50 ± 0.15</td>
<td>5.07 ± 0.40</td>
</tr>
<tr>
<td>OVX</td>
<td>7.326 ± 0.019</td>
<td>27.4 ± 1.1</td>
<td>6.34 ± 0.10</td>
<td>3.49 ± 0.27</td>
<td>1.65 ± 0.24</td>
<td>0.42 ± 0.14</td>
<td>4.72 ± 0.32</td>
</tr>
<tr>
<td>Sham + NH₄⁺ Cl</td>
<td>7.110 ± 0.017*</td>
<td>17.3 ± 1.4</td>
<td>5.31 ± 0.14*</td>
<td>43.30 ± 4.72*</td>
<td>5.11 ± 1.23*</td>
<td>&lt;0.1*</td>
<td>48.41 ± 4.65*</td>
</tr>
<tr>
<td>OVX + NH₄⁺ Cl</td>
<td>7.129 ± 0.022*</td>
<td>17.9 ± 1.5</td>
<td>5.32 ± 0.11*</td>
<td>39.71 ± 3.61*</td>
<td>6.75 ± 1.09*</td>
<td>&lt;0.1*</td>
<td>46.46 ± 4.27*</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SD. *P at least <0.025 for the comparison to the respective nonacidotic group. n values at week 10 are 20, 20, 8, and 8 for sham, OVX, sham + acidosis and OVX + acidosis, respectively.
an IRMA kit (Immutopics International, San Clemente, CA) and TRACP5b by ELISA kit (Rat TRAP.IDS, Immunodiagnostic Systems, Frankfurt, Germany).

Statistical analysis. Values given in this manuscript are presented as means ± SE. All statistical comparisons were made using an unpaired, two-sided t-test.

RESULTS

Acid-base and divalent ion analysis. As illustrated by Tables 1 and 2, NH₄Cl administration induced a large, significant decrease in plasma [HCO₃⁻] of ~10 mmol/l in association with a significant reduction in blood pH, consistent with chronic metabolic acidosis. The severity of acidosis did not differ among the ovariectomized and sham-operated animals and remained stable over time, as there were no significant differences in arterial plasma [HCO₃⁻] or blood pH among the groups, at either 6 or 10 wk. Metabolic acidosis induced a large and stable increase in renal net acid excretion. The increase in acid excretion was attributable mainly to an increase in urinary ammonium excretion.

As shown in Table 3, metabolic acidosis induced by NH₄Cl administration resulted in significant hypercalciumia and hyperphosphaturia, while there was no effect on renal magnesium excretion. The effect of acidosis on renal calcium, phosphate, and magnesium excretion was not different between OVX and sham-operated animals.

Bone marker analysis. Osteocalcin plasma concentrations (Fig. 1A) were significantly higher in nonacidotic OVX compared with nonacidotic sham-operated animals (11.01 ± 0.56 ng/ml vs. 9.64 ± 0.64 ng/ml; P = 0.03). Metabolic acidosis significantly decreased osteocalcin plasma concentrations in sham-operated, but nonsignificantly in ovariectomized animals (7.14 ± 0.50 and 9.86 ± 0.44 ng/ml, respectively) compared with nonacidotic sham-operated or ovariectomized animals.

Figure 1B illustrates that chronic metabolic acidosis significantly increased bone-specific, tartrate-resistant acid phosphatase (TRACP5b) both in sham-operated (increase from 4.16 ± 0.30 to 6.24 ± 0.78 U/l, P < 0.005) and in ovariectomized animals (increase from 6.24 ± 0.70 to 8.88 ± 0.69 U/l, P < 0.01). In addition, as expected, ovariectomy induced a significant increase in TRACP5b activity both in nonacidotic and in acidotic animals (P < 0.007 and P < 0.005, respectively).

Analysis of volumetric bone density and bone microarchitectural parameters. As illustrated by pQCT measurements in the proximal tibia metaphysis (Fig. 2, A–D, Table 4), metabolic acidosis significantly decreased total vBMD and decreased cortical bone mass, based on the significant decrease in cortical thickness at week 6 (not significant at week 10, possibly related to the decrease in n) in sham-operated and ovariectomized mice (Fig. 2A). The effect was exclusively attributable to the reduction in cortical thickness (Fig. 2B, Table 4), while no substantial detrimental effect in either acidotic group on cancellous density was observed (Fig. 2C). The reduction in cortical thickness can be attributed entirely to an increased endocortical resorption process, which resulted in a nominal enlargement of the endosteal circumference (Fig. 2D), while periosteal circumference was not affected significantly (Table 4).

The reduction in body weight gain in the acidotic, sham-operated, and ovariectomized animals is consistent with a loss in muscle mass (sarcopenia), as illustrated by the decrease in muscle cross-sectional area of the tibial muscle group, as determined by pQCT (Fig. 3, A and B).

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Table 3. Urinary divalent anion excretion at 6 and 10 wk

<table>
<thead>
<tr>
<th>Group</th>
<th>Week 6</th>
<th>Week 10</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ca²⁺/creatinine, mmol/mmol</td>
<td>Mg²⁺/creatinine, mmol/mmol</td>
</tr>
<tr>
<td>Sham</td>
<td>0.62 ± 0.09</td>
<td>1.24 ± 0.14</td>
</tr>
<tr>
<td>O VX</td>
<td>0.61 ± 0.08</td>
<td>1.28 ± 0.16</td>
</tr>
<tr>
<td>O VX + NH₄Cl</td>
<td>2.59 ± 0.34*</td>
<td>1.29 ± 0.13</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SD. *P at least <0.025 for the comparison between acidotic and respective nonacidotic groups. There were no significant differences between the acidotic groups or between values obtained at weeks 6 and 10.
The analysis of trabecular architecture by μCT (Fig. 4, A–D and Table 5) shows that metabolic acidosis significantly decreased trabecular bone volume (BV/TV) in both ovariectomized (10 wk) and sham-operated rats (6 and 10 wk, Fig. 4A). The effect is predominantly explained by a significant (sham-operated animals) decrease in trabecular number (Fig. 4B), while trabecular thickness remained essentially unchanged in both groups (Fig. 4C). As a result of these changes, trabecular separation increased significantly with CMA (Table 5), and trabecular connectivity deteriorated. The absolute and % changes of the parameters depicted in Fig. 4, A–D are listed in Table 5.

**DISCUSSION**

We report herein for the first time the simultaneous in vivo effects of CMA on cortical and cancellous bone mass, bone microarchitecture, and biochemical bone markers. The main three results were the following:

1. CMA significantly decreases bone mass in both OVX and intact rats by increasing endosteal (cortical) bone resorption leading to large decreases in cortical BMD by pQCT along with a modest contribution of decreasing BV/TV, as mediated primarily by decreasing the number of TbN.

2. CMA accelerates even ovariectomy-induced bone loss by further enhancing the reduction in cortical thickness as measured by pQCT.

3. On the basis of the biomarker results, CMA in vivo resulted in a persistent reduction in bone formation (decrease in plasma osteocalcin) and a persistent increase in bone resorption (plasma Trap5b).

Our results provide the first evidence that CMA can induce a significant reduction in cortical bone mass. This conclusion is based on the finding of CMA’s large and significant reduction in cortical thickness by pQCT, necessitating a large reduction in cortical bone mineral content. Importantly, although it has been reported previously that cortical resorption rate and cor-
Table 4. Effect of metabolic acidosis with and without ovariectomy on body weight and pQCT-derived cancellous and cortical bone parameters

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body Weight, g</th>
<th>Total BMD, mg/cm³</th>
<th>Cancellous BMD, mg/cm³</th>
<th>Cortical Thickness, mm</th>
<th>Endocortical Perimeter, mm</th>
<th>Periosteal Perimeter, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 6</td>
<td>Week 10</td>
<td>Week 6</td>
<td>Week 10</td>
<td>Week 6</td>
<td>Week 10</td>
</tr>
<tr>
<td>Sham n = 20</td>
<td>7.0</td>
<td></td>
<td>309.2 ± 8.5</td>
<td>508.9 ± 10.2</td>
<td>0.91 ± 0.04</td>
<td>0.90 ± 0.04</td>
</tr>
<tr>
<td>Sham + acidosis</td>
<td>1.0</td>
<td></td>
<td>275.4 ± 6.9</td>
<td>798.2 ± 9.4</td>
<td>0.3 ± 0.8</td>
<td>0.1 ± 1.1</td>
</tr>
<tr>
<td>OVX n = 20</td>
<td>9.4</td>
<td></td>
<td>338.5 ± 11.6</td>
<td>238.7 ± 13.3</td>
<td>0.66 ± 0.01</td>
<td>0.63 ± 0.01</td>
</tr>
<tr>
<td>Sham + acidosis</td>
<td>0.32</td>
<td></td>
<td>769.3 ± 9.6</td>
<td>665.9 ± 13.7</td>
<td>-2.3 ± 1.7</td>
<td>-2.9 ± 1.7</td>
</tr>
<tr>
<td>Sham + acidosis</td>
<td>0.03</td>
<td></td>
<td>21.7 ± 1.2</td>
<td>27.9 ± 2.5</td>
<td>-12.1 ± 0.6</td>
<td>-14.7 ± 1.2</td>
</tr>
<tr>
<td>OVX + acidosis</td>
<td>1.2</td>
<td></td>
<td>42.2 ± 1.3</td>
<td>13.6 ± 1.1</td>
<td>-3.4 ± 0.9</td>
<td>-3.6 ± 1.2</td>
</tr>
<tr>
<td>Sham + acidosis</td>
<td>0.91</td>
<td></td>
<td>30.9 ± 8.5</td>
<td>62.5 ± 10.1</td>
<td>-16.8 ± 1.0</td>
<td>-20.4 ± 0.9</td>
</tr>
<tr>
<td>Sham + acidosis</td>
<td>20.0</td>
<td></td>
<td>19.0 ± 1.4</td>
<td>20.3 ± 1.8</td>
<td>-34.3 ± 2.2</td>
<td>-52.5 ± 3.1</td>
</tr>
</tbody>
</table>

Absolute values and changes in % from baseline (week 0).

p-value (t-test)
- Sham vs. acidosis
- OVX vs. OVX + acidosis
- Sham vs. OVX

<table>
<thead>
<tr>
<th>P</th>
<th>Week 6</th>
<th>Week 10</th>
<th>Week 6</th>
<th>Week 10</th>
<th>Week 6</th>
<th>Week 10</th>
<th>Week 6</th>
<th>Week 10</th>
<th>Week 6</th>
<th>Week 10</th>
<th>Week 6</th>
<th>Week 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>0.071</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.025</td>
<td>0.434</td>
<td>0.044</td>
<td>0.451</td>
<td>0.071</td>
<td>0.079</td>
<td>0.251</td>
<td>0.086</td>
</tr>
<tr>
<td>OVX</td>
<td>0.160</td>
<td>0.081</td>
<td>0.001</td>
<td>0.008</td>
<td>0.468</td>
<td>0.979</td>
<td>0.038</td>
<td>0.122</td>
<td>0.144</td>
<td>0.262</td>
<td>0.634</td>
<td>0.573</td>
</tr>
<tr>
<td>Sham vs. OVX</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.661</td>
<td>0.708</td>
</tr>
</tbody>
</table>
The finding in rats that prolonged overt CMA results in a selective loss of cortical bone mass contrasts with the results of a recent randomized placebo-controlled trial in elderly normal humans, in which low-grade CMA induced by the Western diet was neutralized with prolonged K citrate treatment (13). In the human study, neutralization of the dietary acid load resulted in a large and significant improvement in total vBMD, net of placebo, but the improved vBMD was fully accounted for by increased trabecular vBMD. Whether the differing bone envelopes modulating total BMD changes reflect a species difference, an effect of the magnitude of induced CMA, or undefined factors remains to be determined.

Prior in vivo studies of CMA in rats have been limited to histomorphometric and microradiographic endpoints, yielding somewhat inconsistent results. In young thyroparathyroidectomized (TPTX) rats, a brief 2-wk duration of CMA failed to demonstrate an increase in trabecular metaphyseal or cortical resorption surface (17). However, trabecular resorption surface in the epiphysis did increase with CMA. In contrast, using older intact rats, a significant increase in cortical resorption surface was demonstrated after 300 days of CMA (2). In the TPTX study, using double tetracycline labeling, CMA caused a significant reduction in cortical bone formation rate and mineral apposition rate. This is in line with our finding that CMA reduced plasma osteocalcin levels, a biomarker of bone formation. In contrast, bone formation was not impaired by CMA in the 300-day study (2). In the longer study, formation rate was measured with a microradiographic assessment of cortical osteoid surface, a method that may not be sensitive enough to demonstrate the requisite subtle changes. Alternatively, it is possible that the suppression of bone formation with CMA is a transient phenomenon that is no longer seen at 300 days. There is no reported in vivo evidence of experimental CMA-induced osteomalacia but our μCT data, which shows a reduction in trabecular volumetric “material” density, suggest that mineralization may be impaired under acidic conditions.
The increase in endocortical resorption (endosteal circumference) and plasma TRAP5b levels in our study provide strong support for the cortical microradiographic results of Barzel and Jowsey (2), which demonstrated a significant increase in the cortical resorption surface. Our results, including the negative effect of CMA on osteocalcin, also provide evidence for a net adverse effect of CMA on bone formation rate and are in line with histomorphometric evidence of decreased bone formation rate shown in rat studies (17). The potential role of increased corticosterone secretion (in rodents) to contribute to the findings of the present study, or to hypercortisolism to explain potential CMA effects in human bone, requires further investigation (5, 12, 16, 21).

While hypercalcuria in CMA has been attributed largely to bone losses, the relative magnitude of the possible sources for hyperphosphaturia has not been clarified. The present finding of hyperphosphaturia in CMA rats is consistent with prior reports in human (16) and mouse mineral acidosis (24). However, the finding that intestinal Pi uptake into small intestinal apical vesicles is increased in parallel with increased small intestinal NaPiIIb transporter protein in mice with CMA suggests that intestinal P; hyperabsorption may play a major role in the hyperphosphaturia of acidosis. The relative roles of increments in skeletal and intestinal Pi loads in the hyperphosphaturia of CMA remain to be determined.

The finding in the present study that CMA induced a significant decrease in lower extremity muscle cross-sectional area is consistent with the results of studies in normal human subjects demonstrating CMA-induced negative nitrogen balance (1) and with results in rats demonstrating that CMA results in glucocorticoid-dependent proteolysis. Nitrogen balance in patients with chronic renal disease and CMA has also been shown to improve significantly with alkali supplements sufficient to correct CMA (14). The present results, however, provide the first report of CMA-induced reductions in muscle mass or dimensions in any species. Whether this finding reflects loss of myocyte size or a reduction in myocyte number or both, remains to be determined. The acidosis of chronic renal disease is often accompanied by osteopenia and whether correction of acidosis results in a clinical skeletal benefit should also be explored.

In conclusion, CMA is confirmed to simultaneously decrease bone formation and to potently enhance endosteal cortical bone resorption, leading to significant and large decreases in cortical bone mass, a key determinant of bone fragility. Bone microarchitecture is also impaired by CMA via a decrease in trabecular number leading to a decrease in trabecular density (BV/TV). In addition, assuming that the hindlimb is representative, CMA in rats results in significant and large decreases in muscle mass (i.e., sarcopenia).

ACKNOWLEDGMENTS

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DISCLOSURES

J. A. Gasser and P. Imboden are employees of Novartis Pharmaceuticals. H. N. Hulter and R. Krapf have no conflicts of interest, financial or otherwise.
IN VIVO EFFECTS OF ACIDOSIS ON BONE

AUTHOR CONTRIBUTIONS


REFERENCES