Skeletal Effects of Cyclosporin A Are Gender Related in Rats

REINHOLD G. ERBEN, KATRIN S. BRUNNER, BIANCA BREIG, JOHANNES EBERLE, MICHEL GOLDBERG, AND LORENZ C. HOFBAUER

Institute of Physiology (R.G.E., K.S.B., B.B., J.E., M.G.), Physiological Chemistry and Animal Nutrition, Ludwig Maximilians University, 80539 Munich, Germany; and Division of Gastroenterology and Endocrinology (L.C.H.), Philippus University, 35033 Marburg, Germany

The immunosuppressive drug cyclosporin A (CsA) is thought to be involved in the pathogenesis of postransplantation osteoporosis. To evaluate further the skeletal effects of CsA, we treated aged male and female sham-operated and gonadectomized rats with low doses of CsA for 4 months. Here, we show that CsA is antiresorptive and bone-sparing in aged female rats but increases bone resorption and reduces bone mass in aged male rats. However, even in male rats, CsA treatment, at clinically relevant doses, increased bone resorption only transiently and did not result in pronounced long-term cancellous bone loss. The gender-specific skeletal effects of CsA were not modulated by sex hormones or gonadectomy. CsA did not influence sex steroid metabolism in male or female rats. However, endogenous estradiol in sham-operated female rats (and especially, exogenous administration of 17β-estradiol in ovariectomized rats) markedly diminished blood levels of CsA, probably by increasing hepatic CsA metabolism. Although the mechanism for the gender-specific skeletal effects of CsA is still obscure, our findings may have important implications for clinical therapy with CsA. (Endocrinology 144: 40–49, 2003)

THE DISCOVERY OF the powerful immunosuppressive actions of the fungal cyclic peptide cyclosporin A (CsA) has revolutionized transplantation medicine (1). CsA inhibits the activation of T lymphocytes and thus, graft rejection, mainly via transcriptional suppression of the interleukin-2 gene (2, 3). However, a common and serious side effect of allogeneic organ transplantation is osteoporosis, and CsA may contribute to its pathogenesis (4). In most transplantation patients, CsA is coadministered with other immunosuppressive drugs that are known to adversely affect bone, such as glucocorticoids (5), making it difficult to address the question of the skeletal effects of CsA in clinical studies. Studies in heart transplant patients (6, 7) or patients receiving bone marrow transplantation (8) indicated a deleterious effect of CsA on bone mass, whereas other clinical trials have suggested that monotherapy with CsA may not be associated with bone loss after kidney transplantation (9–13). Moreover, a recent study in renal transplant recipients reported that CsA may counterbalance the adverse effects of glucocorticoids on the skeleton (14). Thus, this issue remains controversial.

Animal studies aimed at defining the skeletal effects of CsA have yielded conflicting results. The majority of rat studies have shown that CsA, in a broad dose range, induces a rise in both bone resorption and in bone formation, along with a negative bone balance, thus resulting in high turnover osteopenia in young growing (15, 16) and in aged male rats (17). In addition, an early study showed that CsA, at an oral dose of 15 mg/kg, aggravated cancellous bone loss in female ovariectomized (OVX) rats. However, this finding could not be reproduced in a later study by the same group using an identical experimental design (19). In contrast, some studies found deleterious effects on the rat skeleton only at the very high, toxic, oral dose of 30 mg/kg CsA (20), and other studies have shown no effect of CsA on bone resorption (21), or even inhibition of bone resorption by CsA (22). Interestingly, all studies reporting adverse skeletal effects of CsA only at toxic dosages, or even an antiresorptive effect of this drug, have been performed with female rats (20–22). However, all experiments with male rats have consistently reported high turnover osteopenia induced by CsA (15–17). Thus, whereas CsA seems to have little effect on the skeleton of female rats, it uniformly produces high turnover osteopenia in male rats. This prompted us to formulate the hypothesis that the skeletal effects of CsA may be gender related.

To test this hypothesis we treated aged male and female sham-operated (SHAM) rats, as well as OVX and orchiec-tomized (ORX) rats, with clinically relevant doses of CsA for 4 months. We chose the 4-month treatment period to assess the long-term effects of CsA on cancellous and cortical bone. In this paper, we show that CsA is antiresorptive and bone-sparing in aged female rats but increases bone resorption and reduces bone mass in aged male rats. However, even in male rats, CsA treatment, at clinically relevant doses, increased bone resorption only transiently and did not result in pronounced long-term cancellous bone loss.

Materials and Methods

Animal procedures

All animal procedures were approved by the Ethical Committee of the University of Munich and the local government authorities. Six-month-old female and 9-month-old male Fischer 344 rats were either SHAM,
O VX, or ORX under ether anesthesia. All experiments were designed using eight rats for each treatment group. CsA treatment was started at 5 d post surgery. Vehicle or CsA was given sc at doses of 1–5 mg/kg body weight (BW), three times per week. CsA was dissolved in medium chain triglycerides containing 0.5% absolute ethanol in the final solution. The concentration of CsA in the injection solutions was adjusted in such a way that all animals received the same injection volume per kilogram BW (330 µl/kg). Some ORX rats were sc treated, during the whole experimental period, with vehicle (Ricinus oil/benzyl benzoate) or testosterone undecanoate (TUD, 6 or 5 mg/kg, once a week). Some ORX rats received sc implanted placebo or estradiol-containing slow-release pellets (60-d release pellets with 0.05 mg 17β-estradiol each; Innovative Research of America, Sarasota, FL) or were sc injected with vehicle (Ricinus oil/benzyl benzoate) or 0.3 µg/kg 17β-estradiol, 5 times per week. The sc estradiol pellets were replaced once during the 4-month experimental period. All animals were sc injected with calcein (20 mg/kg BW) on d 9 and 4 before the end of the experiments. Urine was collected in metabolic cages, during a 14-h period, overnight at 1, 2, 3, and 4 months post surgery. All rats were killed 4 months after the start of the experiments and 24 h after the last administration of CsA, by exsanguination, from the abdominal aorta under ketamine/xylazine anesthesia (50/10 mg/kg ip).

Blood and urine analysis

Total calcium, potassium, and sodium in serum and urine were determined by flame photometry (EFOX 5053; Eppendorf, Hamburg, Germany). Serum alkaline phosphatase activity, serum urea, creatinine, phosphorus, and urinary creatinine, phosphorus, and magnesium were analyzed on a Hitachi 766 Autoanalyzer (Roche Molecular Biochemicals, Mannheim, Germany). Serum osteocalcin was measured by an RIA specific for rat osteocalcin (Biomedical Technologies, Stoughton, IN). Total serum testosterone was determined by RIA (Diagnostic Systems Laboratories, Inc., Sinsheim, Germany) and serum biochemistry in male SHAM and ORX rats treated with CsA for 4 months

Table 1. TW and serum biochemistry in male SHAM and ORX rats treated with CsA for 4 months

| Variable                  | SHAM | ORX*CsA | ORX | ORX*CsA | NS | P  \\
|---------------------------|------|---------|-----|---------|----|----
| BW (g)                    | 352 (12) | 356 (12) | 354 (12) | 342 (7) | 321 (5) | 324 (3) | 5 |
| Serum ALP (U/liter)       | 334 (22) | 328 (21) | 326 (21) | 310 (12) | 286 (11) | 273 (10) | NS |
| Serum Ca (mg/dl)          | 38.9 (1.9) | 40.7 (1.8) | 46.4 (2.2) | 48.0 (2.0) | 36.5 (1.0) | 39.8 (2.2) | NS |
| Serum P (mg/dl)           | 5.71 (0.54) | 6.00 (0.44) | 6.23 (0.22) | 6.52 (0.34) | 4.99 (0.45) | 5.64 (0.46) | NS |
| Urea (mg/dl)              | 12.6 (0.4) | 14.0 (0.5) | 14.2 (0.6) | 14.4 (0.7) | 13.3 (0.5) | 13.5 (0.5) | NS |
| Serum BGP (ng/ml)         | 12.6 (0.4) | 14.0 (0.5) | 14.2 (0.6) | 14.4 (0.7) | 13.3 (0.5) | 13.5 (0.5) | NS |
| Seminal vesicles weight   | 321 (9) | 326 (11) | 322 (11) | 325 (11) | 65 (2) | 59 (2) | NS |
| ORX*CsA, Two-way interaction; ALP: alkaline phosphatase; BUN: blood urea nitrogen; Ca: calcium, P: phosphorus; BGP: osteocalcin; NS: not significant. All values are means ± SD (in parentheses). |

Cancellous bone histology and histomorphometry

Bone specimens were fixed in 40% ethanol at 4 C for 48 h and embedded in methylmethacrylate, and quantitative cancellous bone histomorphometry was performed on midshaft sections from the proximal tibia and on median sections of the first lumbar vertebral (L1) bodies as described in detail elsewhere (24). Sections were prepared using an HM 360 microtome (Microtem, Walldorf, Germany). In brief, structural data were measured with an automatic image analysis system (VIDAS; Carl Zeiss, Oberkochen, Germany) on sections stained with von Kossa, and cellular and fluorochrome-based measurements were made with a semiautomatic system (Videoplan, Carl Zeiss) and a microscope with a drawing attachment, on unstained sections or sections stained with toluidine blue. The area within 0.5 mm from the growth plates was excluded from all measurements. Osteocalcin numbers were expressed using the mineralized bone perimeter (not covered by osteoid) as referent. The bone formation rate was calculated by multiplying the mineralizing perimeter (percentage of calcified double-labeled bone perimeter) times the mineral apposition rate.

Bone mineral density measurements

Bone mineral density of the tibial diaphysis was measured by peripheral quantitative computed tomography (pQCT) using an XCT Research M + pQCT machine (Stratec Medizintechnik, Pforzheim, Germany). The measurements were made with a collimator opening of 0.2 mm, on specimens embedded in methylmethacrylate. One slice in the mid-diaphysis of the tibia located 2 mm proximal to the tibiofibular junction

TABLE 1. Bone mineral density of the tibial diaphysis in male SHAM and ORX rats treated with CsA for 4 months

| Variable                  | SHAM | ORX*CsA | ORX | ORX*CsA | NS | P  \\
|---------------------------|------|---------|-----|---------|----|----
| BW (g)                    | 352 (12) | 356 (12) | 354 (12) | 342 (7) | 321 (5) | 324 (3) | 5 |
| Serum ALP (U/liter)       | 334 (22) | 328 (21) | 326 (21) | 310 (12) | 286 (11) | 273 (10) | NS |
| Serum Ca (mg/dl)          | 38.9 (1.9) | 40.7 (1.8) | 46.4 (2.2) | 48.0 (2.0) | 36.5 (1.0) | 39.8 (2.2) | NS |
| Serum P (mg/dl)           | 5.71 (0.54) | 6.00 (0.44) | 6.23 (0.22) | 6.52 (0.34) | 4.99 (0.45) | 5.64 (0.46) | NS |
| Urea (mg/dl)              | 12.6 (0.4) | 14.0 (0.5) | 14.2 (0.6) | 14.4 (0.7) | 13.3 (0.5) | 13.5 (0.5) | NS |
| Serum BGP (ng/ml)         | 12.6 (0.4) | 14.0 (0.5) | 14.2 (0.6) | 14.4 (0.7) | 13.3 (0.5) | 13.5 (0.5) | NS |
| Seminal vesicles weight   | 321 (9) | 326 (11) | 322 (11) | 325 (11) | 65 (2) | 59 (2) | NS |
| ORX*CsA, Two-way interaction; ALP: alkaline phosphatase; BUN: blood urea nitrogen; Ca: calcium, P: phosphorus; BGP: osteocalcin; NS: not significant. All values are means ± SD (in parentheses). |
was measured. A voxel size of 0.100 mm and a threshold of 710 mg/cm³ were used for calculation of cortical bone mineral density (BMD).

Cortical bone histology and histomorphometry

Two-hundred-micrometer-thick cross-sections of tibial shafts embedded in methylmethacrylate were taken, 2 mm proximal to the tibiofibular junction, with a precision band saw (Exakt, Norderstedt, Germany) and were subsequently ground to a final thickness of 20 μm with the help of the microgrinding system (Exakt). Quantitative bone histomorphometry was performed with an automatic image analysis system (VIDAS, Carl Zeiss) on sections stained with toluidine blue. In addition to the determination of total cross-sectional area, cortical bone area, and marrow area, the system measured cortical thickness on 90 radii originating from the center of gravity of the bone cross-section. Cortical thickness was expressed as the mean value of these measurements. The endocortical and perosteal bone formation rate was calculated by multiplying the mineralizing perimeter (percentage of calcein double-labeled bone perimeter) times the mineral apposition rate.

Statistical analyses

Statistics were computed using SPSS for Windows 9.0 (SPSS, Inc., Chicago, IL). The data were generally analyzed using two-way factorial ANOVA. Two-way factorial ANOVA evaluated the effects of CsA treatment and of gonadectomy and also determined whether there was a two-way interaction between the individual factors, i.e. whether the different treatment factors mutually influenced each other in a nonadditive way. A separate post hoc analysis of the data from the treatment groups for each gender and gonadal status involved one-way ANOVA followed by Dunnett’s multiple comparison test. In the experiments where OVX and ORX rats were treated with sex hormones, statistical comparisons were made using one-way ANOVA followed by Dunnett’s or Student-Newman-Keuls multiple comparison tests.

To formally test the hypothesis that the skeletal effects of CsA may be gender-related, we performed three-way ANOVA of the combined data from male and female SHAM and gonadectomized CsA-treated rats, evaluating the effects of the factors CsA, gonadectomy, and gender, as well as their mutual interactions. P values of less than 0.05 were considered significant for all statistical analyses. The data are presented as the mean ± SEM.

Results

Estradiol is a modifier of CsA blood levels

Although CsA treatment induced dose-dependent increases in serum urea and partially also in creatinine in SHAM and gonadectomized male or female rats, there were no overt signs of chronic toxicity, such as a reduction in BW (Tables 1 and 2). Serum minerals and serum PTH remained unaffected by CsA treatment (Tables 1 and 2, and data not shown). The trough blood levels of CsA found in CsA-treated male and female rats in these experiments (Fig. 1A and B) spanned the range of circulating blood levels of 200–400 ng CsA/ml that are usually maintained in early posttransplantation patients to prevent organ rejection (4). CsA blood levels were similar in SHAM and ORX male rats (Fig. 1A). Interestingly, however, estrogen-deplete OVX rats showed about 2-fold higher CsA blood levels, compared with female SHAM rats (Fig. 1B). To examine the effects of exogenous administration of sex hormones on CsA blood levels, we treated ORX and OVX rats for 4 months with supraphysiological doses of testosterone (8 mg/kg TUD, once weekly) and estradiol (sc slow-release pellets), respectively, as assessed by the weight of typical target organs for sex steroid action. At the end of the trial, the organ weight of the seminal vesicles in testosterone-supplemented ORX rats was 367 ± 24 mg vs. 197 ± 9 mg in SHAM controls, and the uterine weight

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<th>OVX</th>
<th>OVX-Ca</th>
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<td>Serum P (mg/dl)</td>
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<td>0.37</td>
</tr>
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<td>Serum BGP (mg/dl)</td>
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<td>0.55</td>
<td>0.55</td>
</tr>
<tr>
<td>BW (g)</td>
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<td>205</td>
<td>205</td>
</tr>
<tr>
<td>Serum ALP (mg/dl)</td>
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<td>183</td>
<td>183</td>
</tr>
<tr>
<td>Serum PTH (mU/ml)</td>
<td>0.32</td>
<td>0.32</td>
<td>0.32</td>
</tr>
<tr>
<td>Uterine weight (mg)</td>
<td>43</td>
<td>43</td>
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</tr>
</tbody>
</table>

TABLE 2. BW and serum biochemistry in female SHAM and OVX rats treated with CsA for 4 months.

Erben et al. • Skeletal Effects of CsA Are Gender Related

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was 305 ± 11 mg in OVX rats supplemented with estradiol slow-release pellets vs. 275 ± 16 mg in SHAM controls. Testosterone supplementation of ORX rats nonsignificantly lowered CsA blood levels by about 50%, compared with androgen-deplete ORX rats. D, When CsA-treated OVX rats are supplemented with estradiol-containing slow-release pellets, CsA blood levels are strongly diminished, to about 10% of those seen in estrogen-deplete OVX rats. Data represent mean ± SEM (n = 5–8 per group). *, P < 0.05 vs. OVX + vehicle; #, P < 0.05 vs. SHAM + vehicle, one-way ANOVA followed by Student-Neuman-Keuls test; NS, not significant.

CsA does not alter sex hormone levels in male and female rats

To evaluate possible changes in sex steroid hormone levels induced by CsA, we measured circulating levels of total testosterone and estradiol. Administration of CsA did not influence estradiol or testosterone levels in male or female SHAM or gonadectomized animals (Fig. 2, A and B) and had no influence on uterine or seminal vesicles weight in female and male SHAM or gonadectomized rats (Tables 1 and 2). Also, CsA did not alter sex hormone levels in ORX and OVX rats (Fig. 2, C and D) supplemented with physiological doses of testosterone (6 mg/kg TUD, once a week) or estradiol (0.3 μg/kg 17β-estradiol). Compared with SHAM animals, seminal vesicles weight and uterine weight were nearly iden-
tical in ORX and OVX animals receiving these doses of testosterone and estradiol, respectively (data not shown). Thus, our results clearly showed that long-term treatment with clinically relevant doses of CsA does not influence sex steroid synthesis or metabolism in male or female rats.

**CsA has gender-specific effects on biochemical markers of bone turnover**

When we assessed the influence of CsA on bone metabolism, we found gender-specific differences in male and female rats. After CsA treatment, urinary excretion of calcium increased, with a maximum at 2 months, in male SHAM rats, whereas renal calcium excretion in female SHAM rats was decreased (Fig. 3, A and B). The effects of CsA on urinary calcium excretion in ORX and OVX rats were very similar to those in sex-matched SHAM controls (data not shown). Similarly, urinary excretion of the bone resorption marker deoxypyridinoline was increased in CsA-treated male SHAM and ORX rats (Fig. 3, C and D), with a maximal response at 2 months after start of CsA treatment (Fig. 3C). In contrast, urinary deoxypyridinoline excretion was unchanged, or even decreased, in CsA-treated female SHAM and OVX rats at 2 (Fig. 3E) and 4 months (Fig. 3F). The serum levels of the bone formation marker osteocalcin were increased by CsA in both male and female SHAM and gonadectomized rats (Tables 1 and 2). We conclude that CsA transiently increased bone resorption in male SHAM and ORX rats but had mild antiresorptive effects in female SHAM and OVX rats.

**Long-term effects of CsA on cancellous and cortical bone are gender-related**

To examine the effects of CsA on cancellous and cortical bone mass, we performed bone histomorphometry and pQCT. Although there was a trend for decreased vertebral cancellous bone area in CsA-treated male SHAM and ORX rats, we found no significant effects of CsA on vertebral or tibial cancellous bone after the 4-month treatment period in male rats (Fig. 4, A and B; Fig. 5, A–D). In contrast, CsA treatment significantly increased tibial cancellous bone area in female SHAM and OVX rats but had no effect in the vertebrae (Fig. 4, C and D; Fig. 5, E–K). The bone-sparing effect of CsA was generally more pronounced in female SHAM rats. In fact, some female rats showed a pronounced increase in tibial cancellous bone area, in response to CsA treatment, with thickening of existing bone structural elements (Fig. 5, G and K). Osteoclast number tended to be nonsignificantly elevated, especially in CsA-treated male ORX rats, but was significantly reduced in CsA-treated female SHAM and OVX rats (Fig. 4, E and F). However, CsA

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**FIG. 3.** Gender-specific effects of CsA on biochemical markers of bone resorption. A, Urinary calcium/creatinine excretion (UrCa/Crea) is increased in CsA-treated male SHAM rats, with a maximum at 2 months after start of the treatment; whereas CsA, at the highest dose, suppresses urinary calcium excretion in female SHAM rats at the later time points (B). Similarly, urinary excretion of deoxypyridinoline is increased in male SHAM and OVX rats at 2 (C) and, to a lesser extent, at 4 months (D) post surgery. In contrast, deoxypyridinoline excretion is diminished by CsA administration at 2 (E) and 4 (F) months post surgery in female rats. Data represent mean ± SEM (n = 5–8 per group). *, P < 0.05; **, P < 0.01; ***, P < 0.001 vs. vehicle-treated SHAM group, one-way ANOVA followed by Dunnett’s test (A and B). Insets show results of two-way ANOVA (C–F).
treatment did not have significant effects on the cancellous bone formation rate in male and female rats (Fig. 4, G and H).

Sex steroid deficiency induced cortical bone osteopenia in both male and female rats (Fig. 6, A and B). In both genders, gonadectomy resulted in a widening of the marrow cavity, with a concomitant decrease in cortical thickness (Fig. 6, C–F). Relative to sex-matched SHAM rats, total cross-sectional area of the tibial shaft was decreased in male ORX rats but increased in female OVX animals (Fig. 6, G–H). Thus, reduced periosteal bone apposition contributed to the decrease in cortical bone mass in male ORX rats. In analogy to cancellous bone, the effects of CsA treatment on cortical bone showed a clear gender difference. CsA dose-dependently decreased total bone mineral density of the tibial shaft, measured by pQCT in male SHAM and ORX rats, but had no effect in female SHAM or OVX rats (Fig. 6, A and B). Histological analysis of cross-sections of the tibial shaft revealed that CsA induced a dose-dependent decrease in cortical thickness, attributable to an expansion of the marrow cavity in male SHAM and ORX rats, but had no effect in female rats (Fig. 6, C–F). Total cross-sectional area of the tibial shaft remained unchanged by CsA in male SHAM and ORX rats but showed a dose-dependent increase in female SHAM and OVX animals (Fig. 6, G–H). The latter effect was probably attributable to a stimulatory effect of CsA on periosteal bone formation in female rats (Fig. 6K). This effect did not quite reach statistical significance, however (P = 0.054). In male rats, CsA did not significantly influence periosteal bone formation (Fig. 6I) but up-regulated the endocortical bone formation rate (Fig. 6L), probably indicating increased endocortical bone remodeling in response to CsA. In contrast, endocortical bone formation remained unchanged in female SHAM and OVX rats (Fig. 6M). Thus, the effects of CsA on periosteal and endocortical bone surfaces differed in males and females.

To formally test the hypothesis that the skeletal effects of CsA are gender-related, we performed three-way ANOVA using the combined data from male and female CsA-treated animals. In the case that gender modulates the skeletal effects of CsA, one would expect a mutual influence of these factors, and thus, a significant statistical interaction between gender and the treatment effects of CsA. The results of this additional statistical analysis for some selected biochemical and bone parameters are shown in Table 3. Though we found no significant interaction between CsA and gender for serum creatinine, a parameter reflecting CsA toxicity, there was a significant interaction between CsA and gender for urinary deoxypyridinoline, a marker of bone resorption, in the cancellous bone area (B) and the proximal tibia (C) but not in the vertebra (D). Osteoclast numbers in vertebral cancellous bone tend to increase with increasing doses of CsA in male ORX rats (E) but are decreased in female SHAM and OVX rats (F), 4 months post surgery. CsA treatment has no influence on the bone formation rate in male (G) or female rats (H). Data represent mean ± SEM (n = 5–8 per group).

FIG. 4. Gender-specific effects of CsA in cancellous bone sites. After 4 months of CsA therapy, proximal tibial (A) and vertebral cancellous (B) bone area (B) are nonsignificantly decreased in male SHAM and ORX rats. In contrast, treatment of female rats with CsA increases bone area significantly in the proximal tibia (C), and nonsignificantly also in the vertebra (D). Osteoclast numbers in vertebral cancellous bone tend to increase with increasing doses of CsA in male ORX rats (E) but are decreased in female SHAM and OVX rats (F), 4 months post surgery. CsA treatment has no influence on the bone formation rate in male (G) or female rats (H). Data represent mean ± SEM (n = 5–8 per group). Insets show results of two-way ANOVA.
lous bone area showed borderline significance for this interaction term. These results clearly show that gender indeed modulates the effects of CsA on bone metabolism and cancellous and cortical bone mass.

**Discussion**

Our data indicate that endogenous estradiol in female SHAM rats, and especially exogenous administration of estradiol in OVX rats, strongly reduces CsA blood levels, probably by inducing hepatic CsA metabolism. In accordance with our findings, it has recently been shown that female rats clear CsA faster than male rats, after a single iv injection (26), mainly because of increased biotransformation (27), and that the synthetic estrogen ethynylestradiol stimulates hepatic CsA metabolism through increased expression of the cytochrome P-450-dependent enzyme CYP3A9 in female rats (28). Our findings may also explain why ovariectomy aggravates the neurotoxicity of CsA in female rats (29). In contrast to our study, a recent investigation by Molpeceres et al. (30) has suggested decreased CsA blood levels after ovariectomy of female rats. We do not have a good explanation for this discrepancy, but rats were treated with CsA for only 3 d in the latter study. In agreement with our results in rats, human data on gender-related differences in pha-
Macokinetics of CsA have suggested that females clear CsA faster than males (31), presumably because of higher CYP3A activity in women compared with men (32–34). However, more extensive research is required to define the role of gender and sex hormone status on CsA metabolism in humans.

Although high doses of CsA have been shown to reduce serum testosterone in male rats by a reduction in LH receptor number and by a decrease in the activity of steroidogenic cytochrome P-450-dependent hydroxylases in Leydig cells in the testes (35, 36), our results clearly show that long-term treatment with clinically relevant doses of CsA does not...
TABLE 3. Three-way ANOVA of selected biochemical and bone parameters in male and female SHAM and gonadectomized rats treated with CsA for 4 months

<table>
<thead>
<tr>
<th>Variable</th>
<th>CsA</th>
<th>GX</th>
<th>Gender</th>
<th>CsA*GX</th>
<th>CsA*Gender</th>
<th>GX*Gender</th>
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<tbody>
<tr>
<td>Serum creatinine</td>
<td>P &lt; 0.001</td>
<td>P = 0.193</td>
<td>P &lt; 0.001</td>
<td>P = 0.101</td>
<td>P = 0.682</td>
<td>P = 0.734</td>
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<tr>
<td>Serum osteocalcin</td>
<td>P &lt; 0.001</td>
<td>P = 0.001</td>
<td>P &lt; 0.001</td>
<td>P = 0.107</td>
<td>P = 0.072</td>
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<td>Urinary DPD/creatinine</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>P = 0.017</td>
<td>P = 0.582</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Tibial cancellous bone area</td>
<td>P = 0.007</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>P = 0.078</td>
<td>P = 0.049</td>
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<tr>
<td>L1 cancellous bone area</td>
<td>P = 0.864</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>P = 0.698</td>
<td>P = 0.085</td>
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<tr>
<td>Osteoclast number</td>
<td>P = 0.333</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>P = 0.922</td>
<td>P = 0.003</td>
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<tr>
<td>Total BMD of tibial shaft</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>P = 0.709</td>
<td>P = 0.615</td>
<td>P = 0.003</td>
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<tr>
<td>Cortical thickness of tibial</td>
<td>P = 0.056</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>P = 0.807</td>
<td>P = 0.001</td>
<td>P = 0.049</td>
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GX, Gonadectomy; DPD, deoxypyridinoline.

influence sex steroid synthesis or metabolism in male or female rats. In line with these data, most of the available clinical studies could not demonstrate a decrease in sex hormones in patients at therapeutic doses of CsA (37–40).

The current study has shown, for the first time, that long-term CsA administration increases cancellous bone mass and has no adverse effect on cortical bone in female rats but decreases cortical bone mass in male rats. CsA increased bone resorption in male SHAM and ORX rats as measured by urinary deoxypyridinoline, but it had mild antiresorptive effects in female SHAM and OVX rats as measured by urinary deoxypyridinoline and osteoclast numbers. In contrast, the bone formation marker serum osteocalcin was increased by CsA treatment in both male and female rats. The sex-specific skeletal effects of CsA were not modulated by sex hormones or gonadectomy. As mentioned above, some studies in female rats have reported unchanged, or even diminished, bone resorption (21, 22), whereas experiments in male rats have uniformly shown increased bone resorption (15, 17). Thus, although the role of differences in experimental design (such as strain of rats, dose range, or route of CsA administration) still needs to be clarified, our results may provide an explanation for these hitherto unresolved discrepancies, and a potential mechanism for the sexual dimorphism of skeletal CsA susceptibility. However, it is important to note that, even in male rats, the CsA-induced increase in bone resorption was transient, and the deleterious effects of long-term CsA treatment at clinically relevant doses on cancellous bone mass were less pronounced than anticipated from extrapolation of the results from short-term studies (15, 17). Therefore, it is likely that the major adverse effects of long-term immunosuppressive therapy on bone mass in posttransplantation patients is attributable to glucocorticoids, which depress osteoblastic bone formation and induce secondary hyperparathyroidism through inhibition of intestinal calcium absorption (5). This notion is corroborated by several clinical studies demonstrating no adverse effects of CsA monotherapy on BMD (9, 10, 12, 13), or even a beneficial effect of this drug, in posttransplantation patients (11) or in patients with severe rheumatoid arthritis resistant to methotrexate (41).

At present, the molecular and cellular mechanisms for the gender-specific skeletal effects of CsA are still unknown. It has been reported that CsA-induced bone loss does not occur in T-lymphocyte-deficient male nude rats (42), strongly suggesting a role for T lymphocytes or other immune cells in the pathogenesis of CsA-induced osteopenia. In this context, it is interesting that the inhibitory effects of CsA in whole-blood lymphocyte proliferation assays shows gender-related differences in rats and humans (43, 44). Therefore, the gender-specific skeletal effects of CsA may involve gender-specific differences in the response of T lymphocytes to this drug.

Currently, it is unknown whether the skeletal response to CsA may be different in women and men. Interestingly, a prospective clinical trial, over 18 months, in renal transplant recipients, reported an increase in lumbar spine BMD, relative to baseline values, in response to CsA monotherapy, which was significantly greater in women compared with men (11). Therefore, gender-related differences in the skeletal actions of CsA may also occur in humans. Although the mechanism for the gender-specific skeletal effects of CsA is still obscure, our findings may have important implications for the osteoporosis risk assessment in patients receiving CsA therapy and may help to devise effective therapeutic interventions to prevent posttransplantation osteoporosis.

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Address all correspondence and requests for reprints to: Reinhold G. Erben, M.D., D.V.M., Institute of Animal Physiology, University of Munich, Veterinaerstrasse 13, D-80539 Munich, Germany. E-mail: r.erben@lrz.uni-muenchen.de.

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