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Effect of the High-Affinity Estrogen Receptor Ligand ICI 182,780 on the Rat Tibia*

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ABSTRACT

We examined the specificity of the steroidal antiestrogen ICI 182,780 (ICI) on bone and reproductive tissues in adult and growing female rats. Using a 1.5-mg/kg dose (sc), we evaluated the effects of ICI on the bone, body weight, uterine weight, serum cholesterol, and serum estradiol in either adult and/or growing rats. ICI increased serum estradiol cholesterol in ovary-intact rats, had no effect on uterine weight in ovariectomized rats, and resulted in uterine atrophy in ovary-intact animals comparable with ovariectomy. In contrast, ICI had no effect on body weight. In bone, ICI significantly increased the rate of periosteal bone formation in long bones of growing and mature female rats. In contrast, ICI had no effect on longitudinal bone growth in rapidly growing rats. When ICI was administered to mature rats with or without ovaries, two-factor ANOVA revealed significant interaction ($P \leq 0.05$) between ovariectomy and ICI treatment for cancellous bone area and labeled bone perimeter. ICI increased skeletal indices of bone turnover in the cancellous bone of ovary-intact rats but reduced these indices of bone turnover in the cancellous bone of ovariectomized rats. The increase in bone turnover was associated with a reduction in cancellous bone area in the ovary-intact rats. A reduction in bone turnover was similarly associated with an increase in bone area in the ICI-treated ovariectomized rats. In summary, ICI exhibited complete estrogen antagonism in cortical and cancellous bone, partial agonism in cancellous bone, and no activity on tibial longitudinal growth rate of growing ovary-intact rats. The effects in adult rats were influenced by circulating levels of estradiol. ICI had no activity on body weight and complete antagonism on uterine weight. These results demonstrate that a ligand with high binding affinity to the estrogen receptor(s) can elicit an array of estrogen-mediated regulation of bone metabolism. (*Endocrinology* **139:** 3736–3742, 1998)

ANTIESTROGENS is the term originally applied to compounds which block the physiological response of reproductive tissues to estrogen. It was initially assumed that these agents would antagonize estrogen in all target cells. Estrogen analogs can act as antiestrogens by competing with estrogen for the receptor to produce an inactive ligand-receptor complex, but the analog-receptor complex can also display variable degrees of estrogen agonism (1). One class of estrogen analogs is the pharmaceutically-developed C7-alkyl substituted steroid analogs of estrogen (2–4). In the rodent, these antiestrogens possess both high affinity for the estrogen receptor and complete absence of uterotrophic activity. Although the ICI compound 164,384 has been reported to disable the ligand-receptor complex from binding to DNA (5), there is another report that the formed complex facilitates binding to DNA (6). It is the absence of estrogen agonism in reproductive tissues to which the terminology, pure antiestrogens, was initially referenced (2–5).

Because of their high binding affinity and their lack of estrogen agonism, these estrogen antagonists have great potential as clinical chemoagents for breast cancer. The ability of ICI 182,780 (ICI) to inhibit cell growth, in fact, has been reported in MCF-7, as well as Br10 human breast cancer cells (3). The effectiveness of the C7 alkyl-substituted compounds in chemotherapy contrasts with tamoxifen, a pharmaceutically-developed antiestrogen of a triphenylethylene structure. Tamoxifen is the chemoagent of choice for defense in breast cancer (7–10). Tamoxifen though possesses partial estrogen agonism in reproductive tissue. It is this degree of agonism that is thought to contribute to the acquired tolerance of breast tumor cells after extended tamoxifen therapy (4, 11). Antiestrogens with complete absence of estrogen agonism, on the other hand, may be more rapid, more potent tumorstatic agents with longer lasting results (4, 12).

In addition, the variable display of estrogen agonism by antiestrogens seems to be tissue-specific. A potential mechanism for the tissue-selectivity of antiestrogens involves differential transactivational gene transcription by the estrogen receptor (13). The model proposes that capacity of the ligand-bound estrogen receptor to activate gene transcription is mediated by two distinct regions within the receptor molecule. Analogs of different antiestrogen classes could confer conformational changes to the estrogen receptor, thereby affecting the interaction, either positively or negatively, between the ligand-bound estrogen receptor and DNA. Alternatively, a second type of estrogen receptor, the estrogen receptor-β, could account for tissue differences based on variable tissue distributions of receptors (14). Because of the tissue-selectivity and the variable degree of agonism in antiestrogens, it is important to investigate the tumorstatic estrogen analogs, in terms of their side effects in nontumor, estrogen-responsive tissues. There are reports that these

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compounds may have detrimental effects on the skeletal system (15).

Estrogen has similar effects on the rat and human skeletons, and the response of the laboratory animal model to estrogen antagonists has successfully predicted the outcome in women (16). Thus, we investigated the effects of the ICI on skeletal tissue of female rats that were growing or sexually mature and either with or without ovaries.

Materials and Methods

Animal procedures

Procedures used in all animal experiments were approved by the institutional animal care and use committee at the Mayo Clinic in accordance with the NIH Guide for the Care and Use of Laboratory Animals. All rats were fed laboratory chow *ad libitum*.

Exp 1. Forty 10-week-old female Sprague-Dawley rats (Harlan, Indianapolis, IN) were used to evaluate the effects of ICI on skeletal growth and modeling. Thirty of the rats were divided into three groups of ovary-intact rats (n = 10): one group was injected with ICI, the second group was injected with the sesame oil vehicle, and the third served as the baseline control group. The baseline group was killed at the start of the treatment. A fourth group (n = 10) was ovariectomized 1 week before the start of the study (OVX group). ICI powder (Zeneca Pharmaceuticals, Macclesfield, Cheshire, UK) was resuspended in 95% ethanol to a 100 mg/ml Stock Solution. Each day, an aliquot of the stock solution was resuspended in sesame oil and sonicated for the delivery of a 1.5-mg/kg BW dose of ICI, based on an averaged weekly weight of rats for the 3-week study. A vol of 0.1 ml was injected sc at the back of the neck. Ovariectomized rats were injected with vehicle. The fluorochrome tetracycline (20 mg/kg; Sigma Chemicals, St. Louis, MO) was injected at the base of the tail on the first day of treatment (day 1) and 2 days before death (day 19). Another fluorochrome calcein (20 mg/kg; Sigma Chemicals, St. Louis, MO) was similarly injected 9 days before death (day 13). Rats were anesthetized by CO_2 inhalation and killed by guillotine decapitation. Wet weights of uteri were recorded. Tibiae were removed and fixed by immersion in 70% ethanol.

Exp 2. Rats which were either ovariectomized or ovary-intact were treated with either ICI or vehicle. Forty female Sprague-Dawley rats (Harlan, Indianapolis, IN) at 6 months of age, were divided into 4 groups of approximately 10 (±2) rats each. Two groups were ovariectomized 1 week before the start of treatment, and the other groups remained ovary-intact. One ovariectomized group and one ovary-intact group were treated groups and injected with ICI; the other ovariectomized and ovary-intact group were treatment controls and injected with vehicle. A small group of ovary-intact rats (n = 6) was killed on the first day of treatment. The bone fluorochrome tetracycline (20 mg/kg; Sigma Chemicals, St. Louis, MO) was injected at the base of the tail on the first day (day 1) of the 8-week study and 2 days before death (day 54). The bone fluorochrome calcein (20 mg/kg; Sigma Chemicals, St. Louis, MO) was similarly injected 12 days before death (day 44). The averaged body weight of the rats was recorded weekly. An aliquot of a 5% wt/vol stock solution of ICI in sesame oil (Zeneca Pharmaceutical, Macclesfield, Cheshire, UK) was diluted further in sesame oil for delivery of a 1.5-mg/kg·day dose, based on the weekly averaged body weight. As in Exp 1, a 0.1-ml vol was injected sc at the back of the neck daily throughout the 8-week study. Rats were anesthetized by CO_2 inhalation and killed by guillotine decapitation. Blood was drained from the carcass, clotted, and centrifuged for serum isolation. Serum aliquots were stored at −80 C before assay. Wet weights of uteri were recorded. Tibiae were removed and fixed by immersion in 70% ethanol.

Exp 3. Skeletal effects were evaluated in 8-month old ovary-intact female Sprague-Dawley rats (Harlan, Indianapolis, IN), treated for 52 days with ICI (n = 7). Rats were injected sc with ICI (Zeneca Pharmaceutical, Macclesfield, Cheshire, UK) or sesame oil vehicle (1.5 mg/kg BW ICI). Rats were anesthetized by Ethrane inhalation and killed by exsanguination. Blood was obtained from the abdominal aorta. Tibiae were harvested and fixed, as in previous experiments, for cancellous bone histomorphometry.

Bone histomorphometry

Measurements for Exp 1 and 2 were performed with an SMI-Microcomp semiautomatic image analysis system (Southern Micro Instruments, Inc., Atlanta, GA), which consists of a Compaq computer with microcomp software interfaced with a microscope and image analysis software. Measurements for Exp 3 were executed as for Exps 1 and 2, except for the image analysis software (OsteoMetrics, Inc., Atlanta, GA). Skeletal indices are measured by registering the movement of a digitizing pen across a graphics tablet as a tracing is superimposed on an image of the section displayed on the video screen. As regions of interest are traced in the bone specimen, the computer software records the lengths of tracings and calculates the enclosed areas.

Cortical bone measurements. All cortical bone measurements of Exp 1 were made on unstained cross-sections obtained from the tibia-fibula synostosis using a low-speed saw equipped with a diamond wafer blade (Isomet, Buehler, Lake Bluff, IL). Cross-sections (150 μm) were ground to 15–20 μm on a roughened glass plate and permanently mounted. Fluorochrome labeling was visualized under reflected UV light. Cortical bone histomorphometry included cross-sectional area, medullary area, cortical area, periosteal bone formation rate, and periosteal mineral apposition rate and were performed as previously described (17), except for a bone growth period of 21 days.

Cancellous bone measurements. Proximal tibial metaphyses were dehydrated in 95% ethanol for 1 day, followed by 6 days in 100% ethanol before embedding, without demineralization, in a mixture of methylmethacrylate-2-hydroxyethyl-methacrylate (12.5:1). Parasagittal sections were cut from the middle of the proximal tibia (5 μm thick) with a Reichert Jung microtome.

All measurements, except for longitudinal growth rate, were conducted in a standard sampling site located in the secondary spongiosa of the metaphyseal region of the proximal tibia. This sampling site was located 1 mm from the most distal point of the epiphyseal growth plate, extended bilaterally, but excluded endocortical bone and encompassed a 2.88-mm^2 tissue area (TAr). Mean longitudinal growth rate was determined as the distance from the calcein label to the metaphyseal growth plate cartilage at five equidistant sites across the growth plate. The mean distance was divided by the growth period of 9 days.

The following indices were obtained or calculated from measurements performed in the metaphyseal sampling site, as previously described (17), and according to Parfitt *et al.* (18): cancellous bone area, cancellous bone perimeter, labeled bone perimeter, mineral apposition rate, bone formation rate, and osteoclast-covered perimeter. Indices of cancellous bone architecture, such as trabecular number, trabecular thickness and trabecular separation, were estimated according to standard formulas (19).

Serum measurements

Measurements of 17β-estradiol in Exp 3 were made using a double-antibody RIA that has a minimum detectable limit of 1.4 pg/ml (Diagnostic Products Corporation, Los Angeles, CA). Cholesterol measurements in Exp 2 were measured by the Immunochemical Core Facility at the Mayo Clinic using an automated procedure (Roche Diagnostic System, Los Angeles, CA).

Statistical analysis

Comparisons between multiple pairs of groups were accomplished by application of Fisher's protected least-significant difference (PLSD) *post hoc* test, after determination of significance by one-way ANOVA (Exp 1). Two-way ANOVA was performed to determine whether there is a significant effect of either ovarian status or ICI treatment or whether there is a significant interaction between the two factors (Exp 2). Subsequently, one-way ANOVA, followed by multiple-group comparisons with Fisher's PLSD, were conducted to determine the significance of ICI treatment in the ovary-intact and in the ovariectomized rats. Student's *t* test was performed for comparisons between ovary-intact control and ICI-treated rats (Exp 3). Statistical significance was considered at P values ≤ 0.05.

Results

Body growth, uterine weight, serum estradiol, and cholesterol

For Exps 1–3, ICI had no negative effects on the husbandry or overall health status of the female rats. ICI had no direct effect on the final body weights of the treated animals, when compared with the vehicle controls, regardless of ovarian status (Table 1).

Exp 1. Ovariectomy increased final body weight. Ovariectomy and ICI reduced uterine weight (Table 1).

Exp 2. Ovariectomy increased final body weight, but there was no interaction of ovariectomy and ICI, by two-way ANOVA, on final body weight (Table 1). Two-way ANOVA revealed no effect of ovariectomy, a significant effect of ICI, and a significant interaction between ovariectomy and ICI on serum cholesterol levels. ICI significantly increased serum cholesterol in ovary-intact (25%) but not in ovariectomized rats (2%) (see Table 3).

Exp 3. ICI increased circulating levels of 17β-estradiol almost 10-fold in the ovary-intact rats (18.8 ± 3.5 pg/ml ICI *vs.* 1.8 ± 0.8 pg/ml VEH, $P \leq 0.0001$). Serum 17β-estradiol was not detectable in ovariectomized rats.

Skeletal data

Exp 1. ICI had no effect on cross-sectional area, medullary area, and cortical area measured at the tibia diaphysis, whereas ovariectomy and ICI similarly increased rates of mineral apposition and bone formation measured at the same site (Table 2). Ovariectomy increased longitudinal growth at the proximal tibial metaphysis in the young rats, whereas ICI treatment of intact rats had no effect on longitudinal growth rate (Fig. 1).

Exp 2. Ovariectomy increased dynamic indices of bone formation (labeled bone perimeter, mineral apposition rate, and bone formation rate) in cancellous bone ($P \leq 0.01$-.0005), increased the percent of osteoclast-covered perimeter (Fig. 2), and decreased indices of cancellous bone volume (bone area, trabecular number), had no effect on trabecular thickness, and increased trabecular separation (Table 3). ICI had no effect on labeled bone perimeter and on bone formation rate but decreased mineral apposition rate. However, there was an interaction between ovarian status and ICI on the labeled bone perimeter, such that ICI increased labeled perimeter in ovary-intact rats and decreased this measurement in ovariectomized animals. Neither of these effects was significant in group comparisons after one-way ANOVA. As presented in Table 3 and Fig. 2, significant interaction between ovarian status and ICI was evident for cancellous bone area (BAr/TAr), bone-forming perimeters (LPm/TPm), and osteoclast-covered perimeter (OcPm), and for the architectural indices of trabecular number (TbN) and separation (TbSp), *i.e.* the direction of ICI's actions was dependent on ovarian status (Table 3 and Fig. 2). ICI decreased measurements related to bone volume and bone-forming surfaces in ovary-intact rats but increased these values in the ovariectomized rats.

Exp 3. ICI increased the percent of double-labeled perimeter (62% increase) in cancellous bone of ovary-intact rats but had no effect on mineral apposition rate (Fig. 3). ICI increased the percent of double-labeled perimeter (63.8 ± 11.3 mm ICI *vs.* 14.6 ± 4.1 mm vehicle, $P \leq 0.001$) and the rates of mineral apposition (1.28 ± 0.11 μm/day ICI *vs.* 0.97 ± 0.15 μm/day vehicle, NS) and bone formation (89.4 ± 12.7 mm^2/day × 10^{-3} ICI *vs.* 36.2 ± 10.2 mm^2/day × 10^{-3} vehicle, $P \leq 0.001$) at the periosteal surface of cortical bone of ovary-intact rats.

Discussion

ICI binds with high affinity to both ERα and ERβ (14). Early investigations failed to detect any transcriptional activity in the estrogen receptor-ICI complex, suggesting that the ICI-bound receptor is unproductive and implying that ICI functions as a pure estrogen antagonist. As a consequence, the observed skeletal response to ICI in the rat was unanticipated.

ICI's effects on the uterus, however, are consistent with previous reports. ICI resulted in uterine atrophy comparable with ovariectomy in ovary-intact rats and had no uterotrophic activity in the ovariectomized rat. These findings are in agreement with the results of Lundeen *et al.* (20), who reported that ICI, itself, displayed no uterotrophic activity in the ovariectomized rat but blocked uterine stimulation by 17α-ethynyl estradiol when administered together. These effects of ICI contrast with those of triphenylethylene and benzothiophene antiestrogens, which, besides being competitive inhibitors of estradiol, possess partial uterotrophic activity (20).

In addition, ICI contrasts with the nonsteroidal antiestro-

TABLE 1. ICI has no effect on body growth but reduces uterine weight comparable with ovariectomy

Measurement ⇒ Group ⇓	Exp 1			Exp 2			Exp 3		
	Initial body weight (g)	Final body weight (g)	Uterine weight (mg)	Initial body weight (g)	Final body weight (g)	Uterine weight (mg)	Initial body weight (g)	Final body weight (g)	Uterine weight (mg)
Baseline (intact) (n = 6–10)	229 ± 2	238 ± 2	402 ± 25^{a,b}	270 ± 10	276 ± 10^{a}	429 ± 21^{a,b}	ND	ND	ND
Intact control (n = 10–11)	227 ± 3	258 ± 3^{a}	472 ± 39^{a,b}	268 ± 5	299 ± 5^{a}	518 ± 38^{a,b}	272 ± 8	284 ± 6	595 ± 64^{b}
Intact + ICI (n = 10–11)	226 ± 2	252 ± 3^{a}	170 ± 11	272 ± 10	287 ± 6^{a}	175 ± 13	272 ± 7	284 ± 5	141 ± 9
OVX Control (n = 11–12)	223 ± 2	299 ± 3^{b}	152 ± 10	273 ± 7	348 ± 10^{b}	172 ± 10	NA	NA	NA
OVX + ICI (n = 9–12)	NA	NA	NA	268 ± 4	345 ± 5^{b}	156 ± 13	NA	NA	NA

Two-way ANOVA for Exp 2: the effects of ovariectomy and ICI treatment were significant, with significant interaction between the two factors for uterine weight ($P \leq .0001$). For final body weight, there was a significant effect of ovariectomy only ($P \leq .0001$), with no significant interaction between surgery and ICI treatment. One-way ANOVA; comparison to ovariectomized control rats: ^{a} $P \leq .0001$; comparison to ICI-treated intact rats: ^{b} $P \leq .0001$. Values are means ± SE. NA, Not applicable; ND, not determined.

TABLE 2. Exp 1. ICI stimulates radial growth in growing, ovary-intact rats

Measurement ⇒ Groups ⇓	Medullary area (mm^2)	Cortical area (mm^2)	Cross-sectional area (mm^2)	Periosteal mineral apposition rate (μm/day)	Periosteal bone formation rate (mm^2/day)
Baseline (intact) (n = 10)	0.91 ± 0.06	3.5 ± 0.1	4.4 ± 0.1	ND	ND
Intact + VEH (n = 11)	0.90 ± 0.04	3.6 ± 0.1	4.5 ± 0.1	1.78 ± 0.14	0.014 ± 0.001
Intact + ICI (n = 11)	0.85 ± 0.04	3.7 ± 0.1	4.5 ± 0.1	2.71 ± 0.44[a]	0.021 ± 0.003[a]
OVX + VEH (n = 11)	0.90 ± 0.03	3.7 ± 0.1	4.6 ± 0.1	2.79 ± 0.21[a]	0.021 ± 0.002[a]

Means ± SEM; n, Number of measured animals per group. Cortical bone formation rate over 21 days. One-way ANOVA: comparison to (intact + VEH) group: [a] $P \leq .05$.

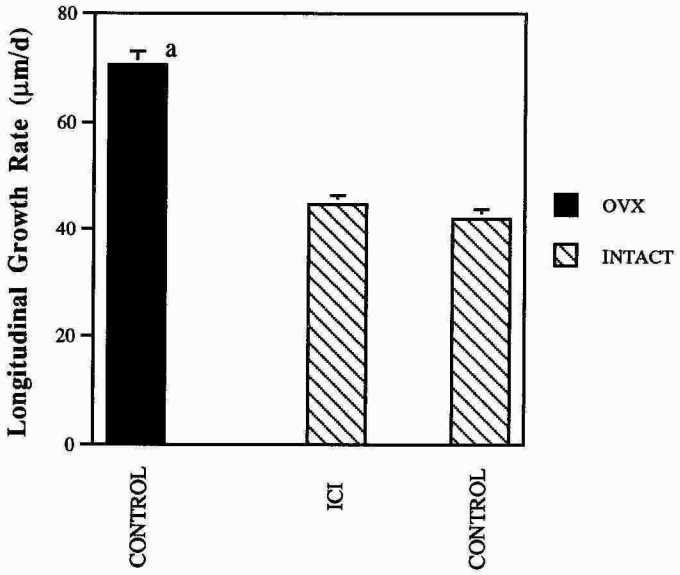

FIG. 1. Effect of ICI on longitudinal growth rate in growing rats (Exp 1). Estrogen deficiency with ovariectomy (*dark bar*) increased the longitudinal growth rate but ICI treatment of ovary-intact rats (*hatched bar*) had no effect on longitudinal growth rate. a, $P \leq 0.0001$ *vs.* ovary-intact group. Values are means ± SE; n = 8–11.

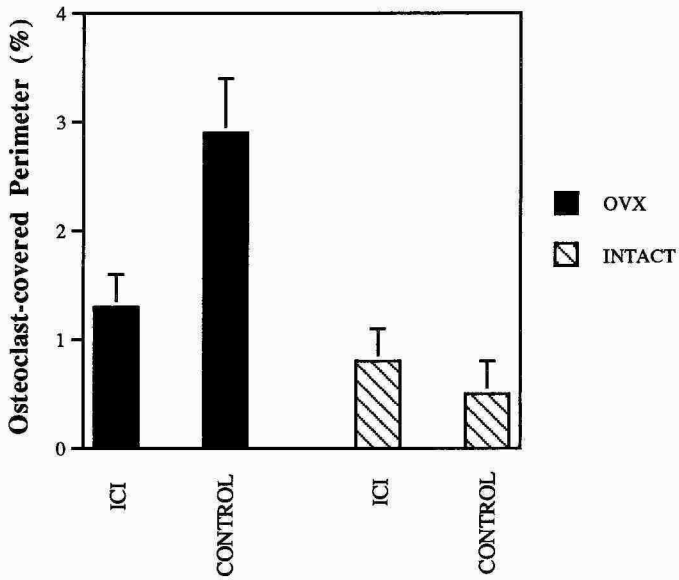

FIG. 2. Effect of ICI on osteoclast-covered bone perimeter in adult rats (Exp 2). ICI decreases the extent of osteoclasts on bone surfaces of the ovariectomized rats (*dark bar*), $P \leq 0.01$ by Fisher PLSD; but tends to increase osteoclasts in the ovary-intact rats (*hatched bar*), NS by Fisher PLSD. By two-way ANOVA, effect of ovariectomy: $P \leq 0.001$; effect of ICI: NS; interaction, $P \leq 0.02$. Values are means ± SE; n = 5–7.

gens in its effects on body weight. Ovariectomy increases body weight (21, 22), and estrogen replacement therapy prevents this change (21–23), which is similarly mimicked by tamoxifen (17, 24) and raloxifene (22). This weight gain is associated with increased food consumption, exhibits a predominant accumulation of fat, but cannot be completely prevented by matching the food intake of ovariectomized rats to the amount ingested by ovary-intact animals (21, 23). The change in body weight, therefore, suggests an alteration in metabolism. The exact mechanism of body weight increase is unclear, although it is under central regulation (25). ICI had no influence on the expected weight changes after ovariectomy. Our observation was similarly noted by Wakeling (3), which he attributed to the failure of ICI to cross the blood-brain barrier (26).

The effects of estrogen and estrogen deficiency on serum cholesterol in the rat model have been recently characterized (20). Triphenylethylene and benzothiophene antiestrogens have similar levels of estrogen agonism on bone and serum cholesterol (20). We investigated the effects of ICI on serum cholesterol, but results are inconclusive. Though ovariectomy did not increase serum cholesterol in this study, ICI increased serum cholesterol when given in the presence of ovaries. An effect of ovariectomy on serum cholesterol is not always seen in our laboratory (27) or in others (28). In one instance, we observed an increase in serum cholesterol in ovariectomized rats (22), whereas in another study, we saw no change (27). Estrogen's regulation of circulating cholesterol levels in the rat involves a complex array of processes, many of which remain to be defined (20). We and others, however, have seen a consistent reduction of circulating cholesterol in ovariectomized rats treated with estrogen, triphenylethylene, and benzothiophene antiestrogens (20, 22, 27, 28). In this study, ICI had no effect on circulating cholesterol in the ovariectomized rat when analyzed by one-way ANOVA.

The skeletal effects of ovariectomy and estrogen replacement have been well characterized in the rat (21, 23, 29–31). The ovariectomized reference groups in this study consistently displayed the skeletal effects of estrogen deficiency (21, 23, 29–32) at the examined sites in the tibia. The anticipated increases in radial and longitudinal bone growth and cancellous osteopenia were all manifested. The cancellous bone of the adult ovariectomized rat exhibited increased indicies of bone turnover (30, 31, 33).

Young ovary-intact rats were selected specifically to in-

TABLE 3. Exp 2. The effects of ICI treatment on cancellous bone histomorphometry and serum cholesterol are influenced by ovarian status

Measurement ⇒ Group ⇓	Cancellous bone area (% BAr/TAr)	Labeled bone perimeter (% LPm/TPm)	Mineral apposition rate (μm/day)	Bone formation rate (mm^2/day)	Trabecular thickness (μm)	Trabecular number (mm^{-1})	Trabecu separat (μm
Intact							
Control	14.4 ± 1.8	7.2 ± 0.9	0.67 ± 0.07	0.050 ± 0.009	45.7 ± 2.7	3.1 ± .2	346 ±
ICI	8.8 ± 1.5	11.5 ± 2.5	0.48 ± 0.04	0.058 ± 0.013	44.3 ± 2.6	2.0 ± .3	553 ±
Ovariectomy							
Control	5.9 ± 1.2	21.3 ± 4.1	0.73 ± 0.06	0.157 ± 0.031	43.0 ± 6.2	1.4 ± .2	833 ±
ICI	9.0 ± 1.2	14.6 ± 2.0	0.69 ± 0.03	0.103 ± 0.016	44.4 ± 3.0	1.9 ± .2	618 ±
2-way ANOVA							
Ovariectomy $P \leq$	.01	.01	.01	.0005	NS	.001	.01
ICI Treatment $P \leq$	NS	NS	.05	NS	NS	NS	NS
Interaction $P \leq$	.01	.05	NS	NS	NS	.005	.05
Group comparisons post 1-way ANOVA							
Intact ± ICI $P \leq$	.05	NS	.05	NS	NS	.005	NS
Ovariectomy ± ICI $P \leq$	NS	NS (.06)	NS	.05	NS	NS	NS

Values are means ± SEM; n = 6–12 animals per group. Two-way ANOVA was performed to evaluate significant effects of surgery, ICI treatment or sig the two factors. One-way ANOVA, followed by Fisher PLSD, was performed to evaluate significant effects of ICI treatment in rats per ovarian statu TPm, Total bone perimeter.

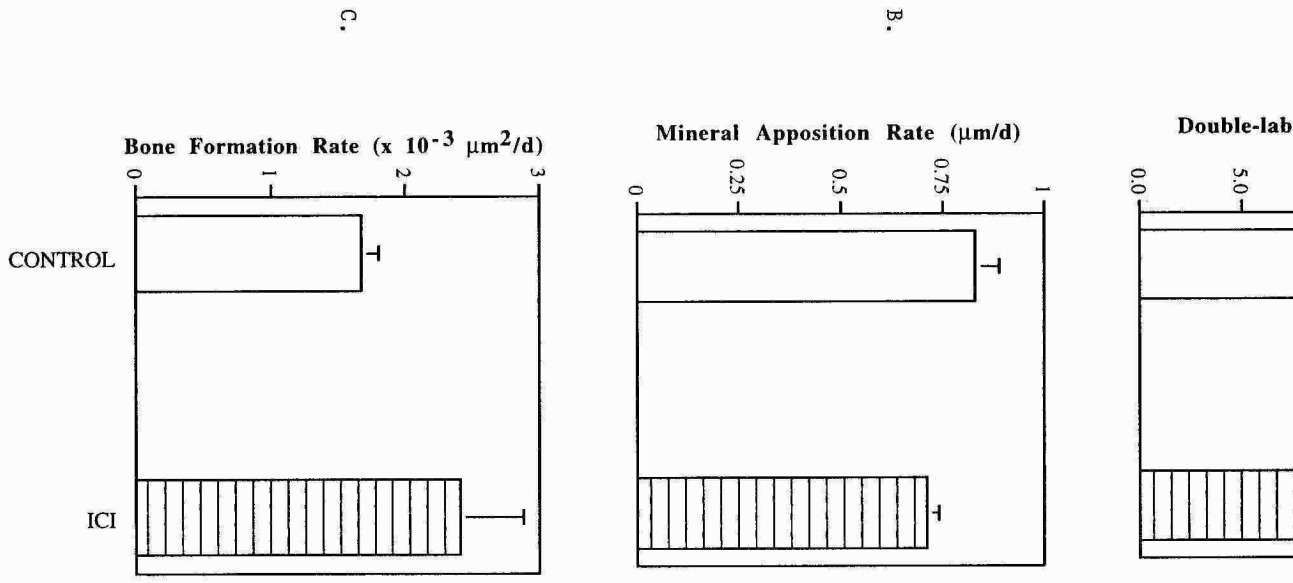

FIG. 3. Effect of ICI on mineral apposition and double-labeled perimeter in cancellous bone (Exp 3). As a result of the opposing trends in double-labeled perimeter (A) and mineral apposition rate (B), there was no significant increase in the bone formation rate (C), which is the product of the two indices. *Clear bar*, Ovary-intact vehicle controls; *hatched bar*, ICI-treated ovary intact rats. Values are means ± SE; n = 7.

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