Prepubertal OVX increases IGF-I expression and bone accretion in C57BL/6J mice

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Osteoporosis is a major health concern in humans, and this disease is characterized by a low bone mineral density (BMD), which leads to increased bone fragility and risk of fractures. BMD is an important determinant of bone strength, and low BMD is the result of increased bone resorption and decreased bone formation. It has been well established that the pubertal growth period is a critical period in time when rapid increase in bone accretion takes place. We and others (19, 32, 48) have demonstrated in both humans and mice that 40–50% of bone accretion occurs during puberty. Understanding the mechanisms involved in regulating bone accretion during this period of rapid growth is of considerable importance in the prevention and treatment of osteoporosis.

In terms of the potential messenger molecules that contribute to rapid skeletal growth during puberty, it has been suggested that the growth hormone (GH)/insulin-like growth factor (IGF) axis is a key regulator of early bone development (34, 55). Recent studies using transgenic mouse models lacking IGF-I or GH have provided convincing evidence for a key role for GH/IGF axis in the regulation of skeletal growth that occurs during puberty (28). It is well accepted that increased IGF-I during sexual maturation is regulated by increased GH production and/or action as demonstrated by reduced IGF-I and bone density in mice lacking GH (28). In addition, we and others recently demonstrated that local and systemic IGF-I are required for attainment of optimal bone density during the prepubertal and pubertal growth periods in mice (9, 10, 22, 26, 28, 38, 56, 58). Although the important roles of GH and IGF-I in regulating bone accretion are well established, the molecular mechanisms that contribute to increased GH effect during the period of sexual maturation are not known.

In terms of potential messenger molecules that regulate the GH/IGF axis and bone accretion during puberty, it has been generally assumed that sex steroids contribute to higher GH/IGF action during the pubertal growth period (18). In support of these assumptions, several studies demonstrate a positive correlation between serum estrogen and BMD during puberty in females (16, 49, 57, 59), thus concluding that estrogen must be a major stimulator of bone development during this period of rapid growth. Although several studies make this conclusion, there is no direct evidence that estrogen is the key molecule responsible for the increased bone accretion and increased GH during puberty. If estrogen is a key player in mediating the increased actions of GH during puberty (49), then we would expect that mice lacking estrogen receptor to exhibit reduced serum IGF-I levels compared with control mice at the end of the pubertal growth period. However, it has been found that mice lacking both estrogen receptor-α and -β exhibited only a 10% decrease compared with corresponding wild-type mice at 140 days of age that was not statistically significant (20, 21). In addition, there are also data that suggest increased estrogen during puberty may inhibit periosteal bone formation (49). Based on these data and conflicting reports on the role of estrogen in bone accretion, we hypothesized that the increase in GH/IGF action during puberty may be mediated by factors other than estrogen. To test whether pubertal surge in estrogen is necessary for the increased IGF-I and corresponding skeletal changes that occur during sexual maturation, we evaluated the consequence of ovariectomy (OVX) in prepubertal mice on skeletal changes and GH/IGF axis during puberty.
MATERIALS AND METHODS

Animals

Eighteen-day-old female C57BL/6J mice were purchased from Jackson Laboratories. They were allowed to acclimate for 3 days prior to surgery. Mice were fed 4% mouse/rat diet ad libitum (Harlan Teklad, Madison, WI) and had free access to water. The experimental procedures performed in this study were in compliance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Animal Studies Subcommittee at the Jerry L. Pettis Memorial Veterans Affairs Medical Center.

Experimental Design

Experiment 1. At 3 wk of age mice were ovariectomized (OVX group; n = 6) or sham operated (n = 7).

Experiment 2. At 3 wk of age mice were OVX or sham-operated. During surgery, the OVX mice were administered a timed-released estrogen (OVX+E; 17β-estradiol pellet, 21-day release at 0.05 mg/pellet; Innovative Research of America, Sarasota, FL) or placebo (OVX+placebo; same pellet used for estrogen group, but lacking the 17β-estradiol) implanted subcutaneously on the lateral side of the neck between the ear and the shoulder. The dose was chosen based on previous work in which similar doses have been used for studies on bone phenotype and/or gene expression in mice (17, 24). We used a total of 27 mice with nine mice per treatment group. In both experiments, at 3 wk following surgery (6 wk of age), animals were euthanized, and serum and tissues were collected for various end-point measurements.

Surgery

Mice were anesthetized with isoflurane (3–4%) for surgery. The back and sides of the mice were shaved and cleaned with 70% ethanol and betadine. The ovaries were removed from the mice by dorsal incision into the region between the dorsal hump and the base of the tail. A ligature was placed, and the ovary removed. The muscle incision and the skin were closed with sutures. The procedure was repeated for the second ovary. Uterine weight was measured at the end of the experiment to determine effectiveness of OVX and estrogen treatment.

Bone Densitometry by Dual X-Ray Absorptiometry

Bone mineral content (BMC) and areal BMD were measured by dual X-ray absorptiometry, using the PIXImus instrument (Lunar-Corp, Madison, WI) prior to surgery (3 wk of age) and 3 wk postsurgery (6 wk of age). The precision for the BMC and BMD was ±1% for repeat measurements of the same bones several times (23). Animals were anesthetized by a ketamine/xylazine (50/5 mg/kg body weight) injection prior to measurement.

Volumetric BMD and Geometric Parameters

Volumetric BMD (vBMD) and geometric parameters at the mid-diaphysis of the femur were determined by peripheral quantitative computed tomography (Norland Stratec XCT; Stratec Medizintechnik, Madison, WI) at 6 wk of age. Analysis of the scans was performed using the manufacturer-supplied software program (Stratec Medizintechnik Bone Density Software, version 5.40 C). Total BMD and geometric parameters were estimated with Loop analysis. The threshold was set at 230 to 630 mg/cm³. For femur analysis, nine scans per bone were measured, and the data presented are the average of the fourth, fifth, and six scan (mididiaphysis region). The coefficient of variation for total BMD, periosteal circumference, and endosteal circumference for repeated measurements of four mouse femurs (2–5 measurements) were < 3%, < 1%, and < 2%, respectively (23). The longitudinal lengths of the femurs were measured with a caliper.

Histological Measurements

For mice in experiment 2, mineral apposition rate (MAR) and bone formation rate (BFR) were determined between 3 and 6 wk of age as previously described (14, 36) using a double calcein (15 mg/kg body wt) label. Specifically, calcein was administered at 3 wk of age immediately following surgery and again 3 days before euthanasia (about 6 wk of age). Due to the age of the mice and rapid longitudinal growth rate of the femurs, measurements were taken at the location of the third trocanter.

Serum IGF-I RIA

Serum IGF-I was measured by RIA in both experiments using rabbit polyclonal antisemur and recombinant IGF-I as standard and tracer, respectively. IGF binding proteins (IGFBPs) were removed from serum prior to RIA by acid gel filtration protocol (25).

Serum IGFBP-5 RIA

Serum IGFBP-5 was measured by a RIA that was previously validated in our laboratory. Recombinant human IGFBP-5 was used as a standard and tracer. Antibodies against recombinant human IGFBP-5 were raised in guinea pigs as described previously (27). IGFBP-5 antiserum that showed cross-reactivity with mouse IGFBP-5 was selected for RIA. Mouse serum samples were diluted 1:10 before assay. The inter- and intraassay coefficient of variation for this assay is < 10%. The sensitivity of the assay is 10 ng/ml. None of the other IGFBPs showed significant cross-reactivity in this assay.

Type I Collagen C-Telopeptide Assay

Type I collagen C-telopeptide serum levels were analyzed for experiment 2 by means of an ELISA described previously (40). The assay is specific for COOH-terminal peptide fragments of mouse type-I collagen that are released from bone resorption. Sensitivity of the C-telopeptide ELISA is < 0.1 ng/ml, with an average intra- and interassay coefficient of variation of <12% (40). Samples were run in duplicate, and the operator was blinded regarding type and age of animal.

Procollagen Peptide Assay

Serum procollagen peptide (PCP) concentrations for both experiments were determined using a rat/mouse NH₂-terminal propeptide of type I procollagen enzyme immunoassay kit according to the manufacturer’s instructions (Immunodiagnostic Systems, Fountain Hills, AZ).

RNA Extraction

RNA was extracted from the tissues using a Lipid Tissue Mini Kit (Qiagen, Valencia, CA) according to the manufacturer’s protocol. For bone sample collection, muscle and tissue were removed, and bone marrow was flushed from the bone. Following RNA extraction, residual DNA was removed from up to 10 μg of RNA with a DNA-free kit (Ambion, Austin, TX). RNA quality was determined using a 2100 Bioanalyzer (Agilent, Palo Alto, CA) and RNA was quantified using a NanoDrop Spectrophotometer (Wilmington, DE).

Gene Expression Analysis

Quantitative real-time RT-PCR analysis was used to determine the expression levels of IGF-I and PPIA (peptidylprolyl isomerase A; endogenous control) as previously described (8). Primers used were validated as previously described (8). CT values for gene of interest minus CT values for control gene were determined, and comparisons of the CT values were used for relative quantification of gene expression (3).
Statistical Analysis

Data were analyzed by ANOVA, and post hoc analysis was performed using Newman-Keuls analysis. Data were analyzed using Statistica 6 software (StatSoft, Tulsa, OK). Data are presented as means ± SE, and a significant difference was determined at \( P \leq 0.05 \).

RESULTS

Growth and Skeletal Parameters

Experiment 1. Successful OVX was determined by a 67% reduction in uterine weight, thus demonstrating the effectiveness of our OVX surgeries. Interestingly, in our initial experiment, we observed a 12% increase in body weight \( (P = 0.01) \) and an 18% reduction in percent total body fat \( (P = 0.02) \) in the OVX mice compared with sham mice (Table 1). In addition, BMC increased 12% in OVX mice compared with sham mice \( (P = 0.02) \); Table 1). Interestingly, bone length and bone size were also increased (4%, \( P = 0.01 \) and 3%, \( P = 0.08 \), respectively) in the OVX mice (Table 1). We did not observe a difference in vBMD between OVX and sham mice (Table 1).

Experiment 2. Because OVX increased bone accretion in our C57BL/6J female mice, we performed a second experiment to confirm our findings, as well as further test the role of estrogen by replacing estrogen in the OVX mice. In addition to reduced uterine weight with OVX (sham = 45 ± 6 mg; OVX + placebo = 25 ± 1 mg), we were able to successfully restore the reduced estrogen levels using our 21-day release estrogen implants (OVX + E = 108 ± 5 mg) as demonstrated by the lack of reduction in uterine weight in the OVX + E group (Fig. 1). Although this was a super physiological dose, as demonstrated by the 237% increase in uterine weight in the OVX + E group, we chose this dose to ensure optimal estrogen levels to evaluate the potential role of estrogen in mediating growth and bone parameters. Our results from experiment 2 revealed similar increases in body weight (18%), BMC (11%), and reduction (7%) in percent total body fat \( (P < 0.001) \) in OVX + placebo mice compared with sham mice (Fig. 1), thus confirming our findings in experiment 1. Estrogen replacement (OVX + E) partially returned body weights to that of sham (Fig. 1). In contrast, estrogen treatment (OVX + E) resulted in a greater reduction in percent body fat and greater increase in BMC compared with OVX + placebo (Fig. 1). Similar to experiment 1, femur length (4%) and bone size (3%) were increased in OVX mice \( (P < 0.05; \text{Fig. } 2) \). In addition, estrogen treatment suppressed the OVX-induced increase in these bone parameters (Fig. 2). Although we did not observe a change in vBMD in OVX + placebo mice, we did see an increase in OVX + E mice (Fig. 2B). This increase in OVX + E mice is caused by increased cortical thickness that is reflected by the significant reduction in the endosteal circumference in the OVX + E mice compared with sham \( (P < 0.01; \text{Fig. } 2D) \). Overall, the changes in vBMD and bone size were similar in cortical and trabecular bone (data not shown). However, we did observe a slight increase in vBMD in trabecular bone at the distal end of the femur (248 vs. 227 mg/cm³, \( P = 0.03 \)).

Table 1. OVX increased growth, BMC, and bone size in female mice during puberty

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>OVX</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>15.9±0.58</td>
<td>17.8±0.57</td>
<td>0.01</td>
</tr>
<tr>
<td>Percent fat, %</td>
<td>18.9±1.11</td>
<td>15.43±0.56</td>
<td>0.02</td>
</tr>
<tr>
<td>BMC, mm</td>
<td>0.2550±0.006</td>
<td>0.2865±0.010</td>
<td>0.02</td>
</tr>
<tr>
<td>Femur length, mm</td>
<td>13.3±0.11</td>
<td>13.8±0.12</td>
<td>0.01</td>
</tr>
<tr>
<td>Peri circ, mm</td>
<td>4.65±0.02</td>
<td>4.79±0.07</td>
<td>0.08</td>
</tr>
<tr>
<td>Endo circ, mm</td>
<td>3.4±0.03</td>
<td>3.5±0.06</td>
<td>&gt;0.10</td>
</tr>
<tr>
<td>vBMD, mg/cm³</td>
<td>415±8.7</td>
<td>410±7.0</td>
<td>&gt;0.10</td>
</tr>
</tbody>
</table>

Data are means ± SE from experiment 1. BMC: bone mineral content; Peri circ, periosteal circumference; Endo circ, endosteal circumference; vBMD, volumetric bone mineral density.
Estrogen Suppresses IGF-I During Puberty in Female Mice

If sex hormones are important mediators of pubertal rise in serum IGF-I, serum IGF-I levels should have decreased in OVX mice. On the contrary, we observed a 30–50% increase in serum, liver, and bone IGF-I in both experiments (P < 0.05; Fig. 3, A and B). Consistent with the inhibitory role of estrogen in growth and bone parameters, estrogen treatment blocked the OVX-induced increase in serum and liver IGF-I (Fig. 3B). Unfortunately, bone samples from experiment 2 were not available for IGF-I expression analysis. Similar to circulating IGF-I, serum concentrations of IGFBP-5, which is known to parallel IGF-I in the circulation (27, 31, 41), increased 37–77% (P < 0.01) in OVX mice, and this increase was abolished by estrogen treatment (Fig. 4).

Based on the reduced IGF-I levels, we hypothesized that estrogen may be acting through GH. Since GH is a known regulator of acid labile subunit (ALS) expression in the liver (2, 29), we determined ALS expression in experiment 2 and did not observe an effect of OVX or estrogen treatment (1.35 ± 0.54-fold change; P = 0.54).

Dynamic Histomorphometric Analysis

The mechanism behind the significantly reduced BMC and bone size was evaluated by performing dynamic histomorphometry in experiment 2. At the periosteum of the femoral middiaphysis at the site of the third trocanter, we observed an increase in MAR (40%), BFR (54%), and BFR/bone surface (BFR/BS; 49%) at the periosteum in OVX+placebo mice compared with sham (P < 0.05; Fig. 5). Consistent with a role for estrogen in inhibiting BMC and bone size, estrogen treatment blocked the OVX-induced increase in these parameters, thus suggesting that estrogen plays a role in inhibiting osteoblast function during pubertal growth in female C57BL/6J mice. At the endosteum, we observed a 59, 56, and 61% increase in MAR (P = 0.04), BFR (P = 0.15), and BFR/BS (P = 0.22), respectively, in OVX compared with sham mice (Fig. 5). Estrogen treatment increased MAR (129%) compared with sham (P = 0.001) and increased BFR (77%), and BFR/BS (100%) compared with OVX+placebo (P ≈ 0.001; Fig. 5).

Serum Markers of Bone Formation and Bone Resorption

Consistent with increased bone formation in the OVX mice, we observed a 30–50% increase in serum PCP in the OVX mice compared with sham (experiment 2: sham = 202 ± 13 ng/ml, OVX+placebo = 266 ± 19 ng/ml; P < 0.05). In addition, estrogen treatment blocked the OVX-related increase in PCP levels (experiment 2: OVX+E = 224 ± 9 ng/ml). To determine whether lack of estrogen during puberty was associated with increased bone resorption, we evaluated serum C-telopeptide in the mice from experiment 2. As expected,
OVX mice had elevated C-telopeptide; however, estrogen treatment did not block this increase (sham/H11005 6.1/H11006 ng/ml, OVX/H11001 placebo/H11005 9.1/H11006 0.67 ng/ml, OVX/H11001 E/H11005 8.5/H11006 0.86 ng/ml).

DISCUSSION

Estrogen Is Not a Key Mediator of the GH/IGF Surge During Pubertal Growth

The GH/IGF axis is a major regulator of growth and bone development during prepubertal and pubertal growth, a period in which 40–50% of bone accretion occurs (19, 28, 32). Specifically, the surge in GH and IGF-I levels during the pubertal growth period are associated with a 15% increase in bone size and 35–40% increase in BMC in mice (32). The role of GH and IGF-I as regulators of bone development during these periods of rapid growth have been well characterized in several human and mouse models lacking GH or IGF-I. For example, in mice, we demonstrated a 65 and 90% reduction in BMC in mice lacking GH or IGF-I, respectively, during puberty (28). In terms of the potential messenger molecules that regulate the surge in the GH/IGF axis, it is well accepted that the pubertal surge in estrogen is responsible for increased GH, which in turn stimulates IGF-I and thus bone growth. Several studies demonstrate increased growth and GH with estrogen treatment during puberty or a positive correlation between bone development and increased pubertal growth, which are associated with increased estrogen (7, 19, 28, 32, 37, 47, 57).

If estrogen is indeed the major factor involved in regulating the pubertal surge in GH/IGF axis and increased bone accretion, then we would expect OVX prior to the pubertal growth period to result in decreased rise in IGF-I levels, bone size, and bone mass; however, this was not the case. Surprisingly, we demonstrated that lack of estrogen during puberty resulted in increased bone mass, bone size, and IGF-I levels in mice. In support of these findings, in the ER knockout mouse model, there was little effect on bone size and bone mass (20), thus suggesting that other factors besides estrogen may regulate the GH/IGF surge during pubertal growth in mice.

If the pubertal surge in GH/IGF is not caused by sex hormones produced by the ovary, this raises the question of what other potential factors could contribute to this surge. In this regard, the neurally mediated hypothesis predicts that gonadal-independent activation of signals from the central nervous system (CNS) could initiate puberty. The CNS, hypothalamus, and other signals that stimulate release of GH-releasing hormone include neurotransmitters, neuropeptides, appetite, leptin, physical activity, and sleep (50, 51). It remains to be determined whether one or more of these factors contribute to the pubertal surge in GH/IGF.

Lack of Pubertal Surge in Estrogen Results in Increased Bone Size

It is known that during puberty, testosterone is growth promoting in males, whereas in females, estrogen limits skeletal growth by cessation of longitudinal growth and periosteal expansion (6). Therefore, two mechanisms are involved in regulating sex differences observed in bone accretion during puberty. Increased testosterone in males increases periosteal bone formation as demonstrated by reduced axial and appendicular bone size and reduced mineral in orchidectomized male mice. The GH/IGF surge in males is not caused by sex hormones produced by the ovary, thus raising the question of what other potential factors could contribute to this surge. In this regard, the neurally mediated hypothesis predicts that gonadal-independent activation of signals from the central nervous system (CNS) could initiate puberty. The CNS, hypothalamus, and other signals that stimulate release of GH-releasing hormone include neurotransmitters, neuropeptides, appetite, leptin, physical activity, and sleep (50, 51). It remains to be determined whether one or more of these factors contribute to the pubertal surge in GH/IGF.
rats during puberty (45, 59). In addition, loss of the androgen receptor results in reduced periosteal BFR in mice (52). Increased estrogen in females inhibits periosteal bone formation as demonstrated by increased periosteal perimeter and BFR in OVX rats between 2 and 4 mo of age (5, 16, 42, 54). Our findings are consistent with these previous reports in rats and demonstrate that OVX in female mice during puberty results in male-like skeletal characteristics and bone development. In contrast, when OVX was performed in older rats, the effect on the periosteal and endosteal parameters were variable (15, 30, 42, 43). In addition, estrogen treatment of orchidectomized mice increases periosteal expansion (53). These findings suggest that estrogen action on bone growth may be age- and gender-dependent.

In terms of the changes at the endosteum, we observed an increase in endosteal circumference in the OVX mice, which may be due to increased bone resorption as reflected by higher levels of C-telopeptide in the OVX mice. The endosteal circumference in the OVX+E group was significantly lower than the sham group, which is most likely due to the high dose of estrogen. Estrogen is known to reduce production of cytokines that stimulate osteoclast function, which may contribute to decreased bone resorption and thus decreased endosteal circumference. However, our finding that increased C-telopeptide was not reversed by estrogen treatment suggest the decreased marrow space in our model may be due to increased bone formation. This hypothesis is consistent with previous reports in mice in which estrogen treatment in OVX mice increased bone formation at the endosteum (1, 4). Further studies are needed to confirm the mechanism by which estrogen reduced the endosteal circumference in our model.

Our observation that estrogen treatment did not completely restore values to those of sham mice could be explained. First, estrogen may exert a biphasic effect with a high dose being less active than an optimal dose. Second, estrogen when given intermittently might have a different effect compared with constantly elevated levels as in this case. Finally, nonestrogen factors produced by the ovary may partially contribute to increased body weight seen in OVX mice. A recent study evaluating bone development in progesterone receptor knockout mice demonstrates increased cortical bone mass in knockout mice (33), thus demonstrating that progesterone, in addition to estrogen, may also antagonize pubertal bone development. Additional studies are needed to identify which other ovarian factors besides estrogen are also critical in inhibiting bone accretion during pubertal growth.

Estrogen Regulation of Longitudinal Growth

In humans, the role of estrogen in inducing growth plate fusion and cessation of longitudinal bone growth in girls is well known (13, 37). High doses of estrogen treatment given
to tall girls is known to promote bone maturation, induce growth plate fusion, and thereby reduce their final height. The increase in bone length in our ovariectomized mice is consistent with the known effect of estrogen as an inhibitor of longitudinal growth in girls. In girls, fusion of growth plate occurs during Tanner stage V when estrogen concentrations are very high (13, 37). Furthermore, men with aromatase deficiency do not fuse their growth plates and continue to grow after sexual maturation, while estrogen treatment induces growth plate fusion in patients with aromatase deficiency, thus suggesting that aromatase-mediated conversion of testosterone to estrogen is involved in growth plate fusion in males, too (13, 49). In addition, the observed increase in bone length and size also contribute to increased BMC observed in these mice.

Consistent with our findings, previous studies in rats have demonstrated estrogen inhibition of longitudinal bone growth. Interestingly, these studies also demonstrated that in these rats, trabecular bone volume increased (44, 46). Our peripheral quantitative computed tomography analysis demonstrate that changes in cortical and trabecular parameter were similar; however, we did see an increase in the vBMD in the trabecular bone of the estrogen-treated mice. These findings suggest that estrogen may have different effects on cortical vs. trabecular bone in mice. Additional studies using micro-CT or histomorphometric analysis are needed to confirm these findings.

In terms of the timing and dose of estrogen treatment, estrogen has a biphasic effect on growth as demonstrated by estrogen treatment in girls with Turner’s syndrome in which low doses of estrogen are stimulatory and high doses are inhibitory of development (35). Therefore, discrepancies between studies could be due to different doses and/or levels of estrogen. Nevertheless, we and others have shown direct evidence in rats and mice that removal of estrogen stimulates growth, bone size, and IGF-I, and estrogen replacement inhibits these parameters. Further studies in humans are needed to determine whether estrogen plays a similar inhibitory role in bone size and IGF-I action in females during puberty.

Mechanism of Estrogen Inhibition of Bone Accretion During Puberty

Interestingly, we found that estrogen is a negative regulator of bone mass, bone size, and IGF-I. Based on our similar findings in two experiments and that we were able to reverse the effect of OVX with estrogen treatment, we are confident that during pubertal growth in female C57BL/6J mice, estrogen is a negative regulator of IGF-I and bone accretion. Consistent with our hypothesis that estrogen inhibitory effects are mediated by IGF-I, it was previously demonstrated in rats that estrogen treatment suppressed IGF-I mRNA expression in bone (46). Although it is possible that the reduced IGF-I is due to reduced levels of GH, our findings that ALS expression in the liver were not altered suggest that serum GH was not altered in our OVX and OVX+E mice. Therefore, the inhibitory effects of estrogen on IGF-I and bone may be directly through IGF or mechanisms other than GH.

Model of Estrogen Action During Puberty in Mice

If the pubertal surge in estrogen is involved in regulating the activation of GH/IGF axis and subsequent musculoskeletal growth, then the loss of this estrogen surge should impair pubertal growth. However, that was not the case. To our surprise, we found that removal of ovaries prior to the pubertal growth period increased body weight and bone and muscle mass, and decreased fat content (features similar to that of male mice). Furthermore, our findings that the serum IGF-I and IGFBP-5 levels and liver and bone IGF-I mRNA expression were increased in OVX mice compared with sham mice suggest that ovarian hormones play a negative role in regulating IGF-I expression during the pubertal growth period. Our second experiment confirmed the findings in our first experiment and demonstrated that estrogen is indeed a negative regulator of growth and bone formation in female mice during puberty. However, if estrogen is the sole mediator of ovarian effects during puberty, then the phenotype of estrogen receptor knockout mice should resemble those of OVX mice, but that was not the case (20). Based on these observations, we raise the possibility that estrogen, as well as other factors from the ovary may mediate the negative effects of ovary on the musculoskeletal growth during puberty (Fig. 6). Overall, based on our findings that OVX mice had increased BMC, bone size, bone length, BFR, and IGF-I and these changes were reversed with estrogen administration, we conclude that estrogen is an inhibitor of IGF-I and bone accretion during puberty in female C57BL/6J mice. Further experiments are needed to determine whether other factors produced by the ovary and/or CNS/hypothalamic signals are also involved in regulating the GH/IGF axis and bone accretion during this rapid growth period.

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REFERENCES


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