A Concomitant Decrease in Cortical and Trabecular Bone Mass in Isolated Hypogonadotropic Hypogonadism and Gonadal Dysgenesis

Ki Hyun Park¹, Sang Joon Lee², Jin Young Kim², Jeong Yeon Kim¹, Sang Wook Bai¹, and Jae Wook Kim¹

Abstract

To assess the impact of hypogonadism on bone mineral density, we performed a cross-sectional study of 70 amenorrheic women, comprising 22 cases of gonadal dysgenesis and 48 cases of isolated hypogonadotropic hypogonadism (IHH). Bone mineral density was measured by DEXA at four sites: the femur neck, Ward's triangle, trochanter, and lumbar spine (L2-4). The results were compared to those of a control group consisting of 60 age-matched, normal-cycling women. Bone mineral densities around age 20 were already significantly lower at all four sites in patients with IHH and gonadal dysgenesis when compared with controls, suggesting that these patients failed to achieve peak bone mass during pubertal development. In patients with IHH, the initial BMD around age 18–20 were significantly lower at all four sites and the decrease in bone density continued rapidly during the early twenties up to age 25, and then it slowed markedly thereafter. Bone biochemical marker, ICTP and osteocalcin were significantly negatively correlated with age and remained increased until age 40, which was reminiscent of menopausal bone loss pattern such as high bone turn-over in the early twenties, followed by slow bone loss in the late twenties. In patients with gonadal dysgenesis, bone biochemical marker, ICTP and osteocalcin were also significantly negative correlated with age and remained increased until age 40, but no significant changes in BMD were noted as a function of age, which may be attributed to the small sample size and slow bone loss. These findings suggest that the initiation of prompt and timely therapeutic intervention as early as possible in the menarchal period and throughout the remainder of life, particularly during the period associated with rapid bone loss.

Key Words: Bone, density, hypogonadism

INTRODUCTION

Premenopausal amenorrheic women associated with hypogonadism are at risk of developing osteoporosis. Significant bone loss induced by amenorrhea results in osteoporotic fractures in relatively young patients and also predisposes these women to further osteoporosis in later life. In particular, it has been known that a deficiency of sex steroids during pubertal maturation, during which 50% of adult bone density is acquired, critically decreases the peak bone mass and consequently increases the risk of fractures, especially in later life. Patients with irreversible gonadal failure, such as gonadal dysgenesis and isolated hypogonadotropic hypogonadism (IHH) hypothalamic amenorrhea, may enter the fracture threshold zone earlier, even before the age of menopause, while those with reversible hypogonadism, such as functional hypothalamic amenorrhea and hyperprolactinemia, may enter menopause with an already low bone density, and consequently may reach the fracture threshold more quickly due to the accelerated bone loss associated with menopause.

Stephan et al. reported that bone biochemical indices of bone remodelling paradoxically remained increased in patients with gonadal dysgenesis despite low bone loss during this period or during their twenties and thirties. Emans et al. suggested that young women 18 years and older with high serum osteocalcin levels were predictive of low medullary bone contents and medullary bone mineral density.

In this investigation, we performed a cross-sectional study of 70 patients with IHH and gonadal dysgenesis to assess the changes in bone mineral density by age and also to investigate the relationship between the changes in BMD and bone turn-over markers.
MATERIALS AND METHODS

Subjects

A total of 70 women of reproductive age (age range; 18 to 39 yr) were seen over a 3-year period at our reproductive endocrinology clinic at Yonsei University Medical Center for disorders associated with amenorrhea. The diagnostic categories studied were gonadal dysgenesis (N=22) and IHH (n=48). Patients with IHH showed fair to good ovarian-pituitary response to pulsatile GnRH infusion (5 μg, every 2 hours with interval with Zyklomat (Ferring GMBH, Kiel, Germany) for 1 to 2 weeks. Serum levels of pituitary hormones such as GH, ACTH, TSH and Prolactin were well preserved on initial single bolus, standard anterior pituitary function test using 100 μg of GnRH, 400 μg of TRH and 0.8 IU/Kg regular insulin. The karyotype analysis in 22 patients with gonadal dysgenesis indicated 46 XX in 4 patients, 45 XO in 10 patients, 4 mosaics and 4 partial deletion of the X-chromosome. Controls had no calcium metabolism or reproductive function disorders and were menstruating normally before the study. No subject had taken any medication that might affect bone mineral density. Ages were well matched between the study subjects and controls. A questionnaire covering activities, habits, smoking, alcohol use and dietary intake was used to assess associated risk factors. The women's height and weight were measured and their body-mass indexes (BMI) were calculated by dividing their weight in kilograms by the square of their height in meters. Table 1 provides the clinical data on patients and normal controls.

Bone density measurement

Bone mineral densities of the spine (lumbar vertebrae 2-4) and femur (femur neck, trochanter, and Ward's triangle) were measured with DEXA (Dual X-ray absorptiometry, Lunar Corp., Madison, WI, USA). The intra- and interassay coefficients of variation in the measurement of bone mineral density of the spine were 0.36% and 1.14%, respectively. Those of the femur were 0.85% and 2.27% (mean of the three areas). To obtain a Z-score of each patient's bone mineral density in each respective area, the difference between the bone mineral densities of the patient and the mean bone mineral density of normal controls was divided by the SD of the bone mineral densities of the age-matched normal controls. We compared the difference in bone mineral density of each patient group with that of the normal controls. The changes in bone mineral density were also assessed as a function of age in patients with gonadal dysgenesis (n=22) and in those with IHH (n=48).

Hormone assays

Fasting venous blood and urinary samples were collected in the morning. Blood samples were centrifuged and the serum was stored at −20°C until analyzed. All samples were run in duplicate. Serum estradiol-17β, gonadotropins, prolactin, thyroxin, and thyroid-stimulating hormone were estimated by radioimmunoassay at the Central Endocrine Laboratory of Yonsei University Medical Center.

Biochemical markers of bone turnover

The analyses were performed on blood collected from patients after an overnight fast. Serum osteocalcin was assayed with RIA kits in which rabbit antibovine osteocalcin antibody was used (INCSTAR Co., Stillwater, MN, USA). The intra- and interassay CV were 3% and 5% at 4.5 ng/ml and the detection limit was 0.3 ng/ml. As new collagen turnover markers, serum type 1 collagen C-terminal crosslinks (ICTP) were markers of type 1 collagen degradation. The intra- and inter-assay CV of ICTP were 5% and 8% at 4.0 μg/ml.

Statistical evaluation

Data from individuals were used to determine the mean and SD for the parameters studied, and unpaired t-test and Mann-Whitney test for non-parametric analysis was used to determine the significant differences both from the control values and between the groups. Relationships between the Z scores and various indices were tested with single linear regression and p<0.05 was regarded as significant for all analyses.

RESULTS

Table 1 presents the clinical data and basal hor-
Table 1. Clinical Data of Patients and Normal Controls

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (yr)</th>
<th>17β-estradiol (pg/ml)</th>
<th>LH (mU/mL)</th>
<th>FSH (mU/mL)</th>
<th>Prolactin (μg/L)</th>
<th>BMI (kg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IHH (N=48)</td>
<td>25.6±3.2</td>
<td>6.5±2.2*</td>
<td>4.8±3.0</td>
<td>5.1±10.9</td>
<td>14.9±10</td>
<td>20.3±2.6</td>
</tr>
<tr>
<td>Gonadal dysgenesis (N=22)</td>
<td>22.2±3.7</td>
<td>5.8±3.2*</td>
<td>40.6±2.2*</td>
<td>58.7±2.7*</td>
<td>18.7±12.1</td>
<td>21.4±3.0</td>
</tr>
<tr>
<td>Controls (N=60)</td>
<td>28.5±3.2</td>
<td>65±8.8</td>
<td>11.5±1.6</td>
<td>6.8±1.2</td>
<td>23.5±5.8</td>
<td>22.2±2.1</td>
</tr>
</tbody>
</table>

IHH, isolated hypogonadotropic hypogonadism. Values are expressed as mean±SD.
* p<0.01, compared to controls.

Table 2. Bone Mineral Density in Various Groups of Amenorrhea (grams per cm²)

<table>
<thead>
<tr>
<th>Group</th>
<th>Femur neck</th>
<th>Ward's triangle</th>
<th>Trochanter</th>
<th>Lumbar spine</th>
</tr>
</thead>
<tbody>
<tr>
<td>IHH (N=48)</td>
<td>0.67±0.068*</td>
<td>0.56±0.11*</td>
<td>0.54±0.09*</td>
<td>0.83±0.13*</td>
</tr>
<tr>
<td>Gonadal dysgenesis (N=22)</td>
<td>0.65±0.10*</td>
<td>0.56±0.12*</td>
<td>0.53±0.10*</td>
<td>0.87±0.12*</td>
</tr>
<tr>
<td>Controls (N=60)</td>
<td>0.92±0.10</td>
<td>0.84±0.12</td>
<td>0.72±0.10</td>
<td>1.13±0.20</td>
</tr>
</tbody>
</table>

IHH, isolated hypogonadotropic hypogonadism. Values are expressed as mean±SD of bone mineral densities of patients and controls. Significance was determined by unpaired t test.
* p<0.001, compared to controls.

Table 3. Change in BMD by Age in IHH (gram/cm²)

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>N</th>
<th>Femur neck</th>
<th>Ward's triangle</th>
<th>Trochanter</th>
<th>Lumbar spine</th>
</tr>
</thead>
<tbody>
<tr>
<td>18〜20</td>
<td>4</td>
<td>0.76±0.09†</td>
<td>0.72±0.16‡</td>
<td>0.61±0.18†</td>
<td>0.95±0.09†</td>
</tr>
<tr>
<td>21〜25</td>
<td>21</td>
<td>0.65±0.06*</td>
<td>0.53±0.12*‡</td>
<td>0.52±0.10*</td>
<td>0.81±0.11*‡</td>
</tr>
<tr>
<td>26〜30</td>
<td>17</td>
<td>0.64±0.10*</td>
<td>0.54±0.13*</td>
<td>0.53±0.10*</td>
<td>0.79±0.76*</td>
</tr>
<tr>
<td>&gt;30</td>
<td>6</td>
<td>0.62±0.07*</td>
<td>0.46±0.13*</td>
<td>0.48±0.06*</td>
<td>0.77±0.10*</td>
</tr>
<tr>
<td>Control</td>
<td>60</td>
<td>0.92±0.10</td>
<td>0.84±0.12</td>
<td>0.72±0.10</td>
<td>1.13±0.20</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD of bone mineral densities of patients. Significance was determined by unpaired t test and Mann-Whitney test.
* p<0.001, compared to controls.
† p<0.01, compared to controls.
‡ p<0.05, compared to controls.
§ p<0.01, compared to age 20 group.

monal profile and clearly shows that the hormonal profiles were compatible with the clinical diagnoses.

Change in bone mineral density

Bone mineral densities measured in the amenorrhea groups are given in Table 2, along with the control values. Bone mineral densities were significantly lower in patients with IHH and gonadal dysgenesis at all four sites compared to normal controls, when tested by unpaired t-test (p<0.001).

In the IHH group, the relationship between age and bone mineral densities was investigated. The initial BMD at age 18〜20 was significantly lower at
all four sites and the decrease in bone density continued rapidly during the early twenties up to age 25 and then slowed markedly thereafter (Table 3). A 14% decrease ($p<0.01$) in bone density at both the femur neck and lumbar spine and a 26% decrease ($p<0.01$) at Ward's triangle were observed in the early twenties. After age 25, the bone mineral densities tended to decrease with age, but the decrease was not statistically significant ($p>0.05$).

In patients with gonadal dysgenesis, the initial bone mineral density was significantly lower at all four sites in the early 20s compared to normal controls, but in contrast with the IH group, there were no significant changes as a function of age.

**Biochemical markers of bone turnover**

In 40 patients including cases with IH (n=22) and gonadal dysgenesis (n=18), bone biochemical marker data were available for statistical analysis. Bone biochemical markers, ICTP and osteocalcin were significantly negatively correlated with age and remained high until age 40 (Fig. 1 and 2).

In IH, patient age was negatively correlated with serum levels of osteocalcin ($n=22$, $r^2=0.17$, $p<0.05$) and ICTP ($n=22$, $r^2=0.18$, $p<0.001$), but both sustained relatively high levels through the whole age range compared to normal controls (Fig. 1). In patients with gonadal dysgenesis, serum osteocalcin and ICTP were negatively correlated with patient age and both of them (osteocalcin and ICTP) were also sustained increased until the thirties ($n=18$, $r^2=0.09$, $p<0.05$) (Fig. 2). Serum osteocalcin was negatively correlated with the Z values of the spine ($n=40$, $r^2=0.16$, $p<0.01$), femur neck ($n=40$, $r^2=0.16$, $p<0.01$), and Ward's triangle ($n=40$, $r^2=0.17$, $p<0.01$), while no correlation was observed with that of the trochanter ($n=40$, $r^2=0.32$, $p>0.05$). Serum ICTP (cross-linked carboxyterminal telopeptide of type 1 collagen) had a significant negative correlation with the Z values of the spine ($n=40$, $r^2=0.19$, $p<0.001$), femur neck ($n=40$, $r^2=0.203$, $p<0.001$) and Ward's triangle ($n=40$, $r^2=0.14$, $p<0.001$) but not the trochanter ($n=40$, $r^2=0.04$, $p>0.05$).

The osteocalcin level was positively correlated with the serum levels of ICTP ($n=40$, $r^2=0.26$, $p<0.001$).

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**DISCUSSION**

It has been reported that patients with IH, gonadal dysgenesis and other forms of IH amenor-rhea of prepubertal onset have a marked decrease in both the cortical and trabecular bone envelope compared to age-matched controls. In this study, it was clearly demonstrated that patients with IH and gonadal dysgenesis were associated with significant low bone mass in both cortical and trabecular bone around age 20. It is generally considered that osteoporosis or osteopenia can result from low peak
bone mass or accelerating bone loss. Hypogonadotropic hypogonadism of prepubertal onset, gonadal dysgenesis or delayed puberty do not achieve peak bone mass as a result of absent or delayed gonadal activation during pubertal development.\textsuperscript{13-16} Cann et al. found that bone mineral density was low at the radial cortical bone and spinal trabecular bone in his small subgroup of patients with IHH in a series of 38 amenorrheic women aged 16–49 and suggested that reduced hypothalamic stimulation of the pituitary gland during the adolescent growth phase might decrease the production of certain factors, such as growth hormone, that are known to stimulate cortical bone formation.\textsuperscript{17} The increased insulin-like growth factors found at puberty may reflect a synergistic interaction of sex steroids and growth hormone.\textsuperscript{14} Normal functioning of the growth hormone-somatomedin axis is altered secondarily due to the lack of gonadal activation.\textsuperscript{19} In addition, Gilson et al. demonstrated a close relationship between sex steroids and peak bone mass in experiments using maturing rabbits.\textsuperscript{15}

In patients with IHH, bone mineral density was significantly lower at all four sites tested. The initial bone mineral density in four patients at age 20 was already significantly lower than that of normal controls in both the spine where trabecular bone predominates and in the femur where cortical bone predominates. The decrease in bone densities continued rapidly during the early twenties up to age 25 and then slowed after 25. In these patients who were in their early twenties, a 14% decrease in bone density was observed at both the femur and spine and a 26% decrease was noted at Ward’s triangle. Thereafter, the rate of bone loss began to decrease. Even though this group was small, this finding suggests that patients lose bone rapidly during their early twenties. The majority of skeletal growth during puberty in this group was thus considered to be deranged, with bone loss continuing at a high rate into the early twenties. These findings were very informative in regard to the management of bone loss in this particular diagnostic group. Only a few studies have addressed the rate of bone loss as a function of age in patients with IHH. In this patient group, bone turnover markers such as serum osteocalcin and ICTP were negatively correlated with bone mineral density and age. So these findings were reminiscent of postmenopausal bone loss pattern such as rapid bone loss during the early twenties up to age 25, and then it slowed markedly thereafter.

It has been reported that IHH is associated with a low, normal or high bone turn-over rate.\textsuperscript{18,19} It was suggested that increased serum osteocalcin after age 18 was predictive of low bone mineral content.\textsuperscript{8} If our results are extrapolated to a menopausal bone loss pattern, we see a shift from the early twenties (osteoclast-dependent, high bone turn-over) to the late twenties (osteoblast-dependent bone loss pattern, low bone turn-over status).\textsuperscript{20} These findings suggest the initiation of prompt and timely therapeutic intervention from the menarchal period throughout the rest of life, especially during the early twenties, which was associated with rapid bone loss in our study.

In patients with gonadal dysgenesis, the initial bone mineral density was significantly lower at all four sites in their early 20s compared to normal controls, but in contrast with IHH, there were no significant changes noted as a function of age. Bone biochemical markers, ICTP and osteocalcin were significantly negatively correlated with age and remained high until age 40. These findings were compatible with Stepan’s study of patients with Turner’s syndrome, which showed a high bone remodeling rate despite slow bone loss status.\textsuperscript{5} Brown et al. colleagues examined iliac crest biopsies from 8 girls with Turner’s syndrome aged 9–19 years.\textsuperscript{21} The predominant feature was the increased percentage of bone surfaces undergoing resorption, leading them to conclude that estrogen deficiency early in life has a significant role in the maintenance of the skeleton although there has been a long-standing controversy as to whether the osteopenia is caused by lifelong estrogen deficiency or by primary defect in the bone structure.\textsuperscript{22,23}

In patients with gonadal dysgenesis, the treatment modality should be equivalent to that of IHH. In other words, pharmacologic induction of puberty through appropriate treatment with exogenous sex steroids or other equivalents in the hope of establishing a reasonably appropriate hormonal milieu for pubertal bone growth and timely intervention of hormone replacement to make up low bone mass or to prevent further bone loss.
REFERENCES


