Serum Concentrations of Steroids, Parathyroid Hormone, and Calcitonin in Postmenopausal Women During the Year Following Hip Fracture: Effect of Location of Fracture and Age

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Background. Hip fracture in the aged is a major health problem, especially considering the increasing proportion of the elderly in the population. This study examines changes in circulating levels of hormones, which are purported to affect bone metabolism, in response to hip fracture in postmenopausal women.

Methods. Patients consisted of women ages 65 and older who had surgery within 2 days of fracture. Serum samples were obtained at 3, 10, 60, 180, and 360 days postfracture. Healthy women without hip fractures from the same age range served as a control group (n=17). Hormones were determined by radioimmunoassay. Subjects with fractures in the neck region of the femur (n=78) were compared to subjects with fractures in the trochanteric region (n=88).

Results. Estrone concentration (47.6 ± 5.7 pg/mL; mean ± SEM) at 3 days postfracture was elevated (p<.001) compared to control levels of 20.7 ± 4.6 pg/mL. By 2 months, levels had declined to control levels. Androstenedione and the adrenal hormones, DHEAS and cortisol, displayed similar responses. Parathyroid hormone (PTH) levels were not significantly different from the control concentration at 3 days following fracture, but increased (p<.001) during the year following fracture. Calcitonin concentrations were much higher (p<.001) 3 days postfracture (42.1 ± 3.7 pg/mL) compared to controls without fracture (9.8 ± 3 pg/mL). Except for testosterone, no differences could be attributed to fracture location. Only PTH, with concentrations higher in the older age groups (p<.001), showed an age-related response.

Conclusions. Following hip fracture, there are some dramatic responses in hormones that purportedly are mechanistically important in bone metabolism. These changes include transient increases in steroid hormones, chronic elevations in calcitonin, and rising levels of PTH during the year after fracture.

Hip fracture in the aged is a major health problem, especially considering the increasing proportion of the elderly in the population. Women account for approximately 78% of the fractures, with about 90% accounted for by women over 65 years of age (1). It is estimated that 18–33% of hip fracture patients die within a year of their fracture (2). We have demonstrated that 40% of those patients who do survive never return to their prefracture level of function (3).

Women are especially prone to hip fracture, most likely due to loss of ovarian function and the subsequent decline in circulating estrogens (4). Other hormones, both steroids and protein, may also play a role in bone metabolism. Androgens are purported to affect bone turnover and increase bone mass (1). In contrast, cortisol has a negative effect on bone (5). The peptide hormone calcitonin inhibits bone loss (6), whereas parathyroid hormone (PTH) is associated with increased bone turnover with net resorption (7).

This study examines changes in circulating levels of these hormones, which are purported to affect bone metabolism, in response to hip fracture in postmenopausal women. These fractures occur primarily in the neck or trochanteric region of the femur. Fractures in the neck region are intracapsular and interfere with the regional blood supply and often result in healing complications (8). For this reason, we sought to determine if the location of fracture affected any response. The magnitude or duration of any changes in level of these hormones in the blood may be important in the healing process and may suggest medical interventions. We have also examined these hormonal responses as a function of age.

Methods. Patients initially consisted of 205 community dwelling women ages 65 and older who entered one of two area hospitals with nonpathological proximal femur fractures and had surgery within 2 days of fracture between 1992 and 1996. Patients were excluded from this study if no blood samples were obtained or if they were currently on hormone replacement therapy. Because black women are known to have higher bone mass than white women (9) and because too few black subjects were available for separate analyses, they were also excluded from the analyses. A total of 169 subjects (mean age = 80.7 years; range = 65–96 years) were included in this study. Institutional Review Board approval was received from both the University of Maryland and Union Memorial Hospital and informed consent was obtained from the patients. Serum sam-
ples were obtained at 3, 10, 60, 180, and 360 days postfracture and stored at -70°C. For “day 3” samples, the actual range was 2-5 days, whereas “day 10” samples had a range of 7-13 days. All samples were collected between 12:00 noon and 3:00 pm, thus attenuating diurnal effects. Healthy women without hip fractures from the same age range also had blood drawn and served as an age-matched control group (n=17; mean age = 81.8 years, range = 69–98 years).

Hormone analyses were determined by radioimmunoassay (RIA) using commercial reagent kits available from the sources listed below. Sensitivities and coefficient of variations (CV) were confirmed or determined by our laboratory. All assays utilized 125I tracers and were counted on a LKB-Wallace Gamma Counter with a counting efficiency of >90% (Model 1272). All samples were analyzed in duplicate. For all assays, the intra-assay CV was <12% and interassay CV was <16%. All samples from a given patient were analyzed in a single assay run, precluding inter assay variations within a patient.

The estrone RIA was purchased from Diagnostic Systems Laboratories (Webster, TX) and is a direct competitive binding assay. The antibody cross-reactivity with 17β-estradiol is 1.25%. The sensitivity of the assay is 1.2 pg/mL. Estrone was chosen because it is the predominant estrogen in circulation during menopause (10) and more reliably measured than estradiol at this time (11).

The androstenedione assay (Diagnostic Products Corporation, Los Angeles, CA) is a competitive immunoassay using antibody-coated tubes following solvent extraction. Hexane:ethyl acetate (1:10) was used for extraction rather than ethyl ether as described in the manufacturer’s protocol. This change was made primarily for safety reasons after consultation with the manufacturer. Recovery is >95%. Sensitivity of the assay is 40 pg/mL. The highest cross-reactivity is with androsterone (6.3%).

The total testosterone assay (Diagnostic Products) is a direct competitive immunoassay using antibody-coated tubes. Sensitivity is 4 ng/dL.

The assay for DHEAS (Diagnostic Products) is also a direct competitive binding immunoassay with antibody-coated tubes with a sensitivity of 1 ng/dL.

Cortisol (Incstar, Stillwater, MN) is assayed using an antibody-coated tube displacement assay. The sensitivity is 20 ng/dL.

The calcitonin assay (Diagnostic Systems) is a direct competitive binding assay with a sensitivity of 14 pg/mL. There is little (<0.1%) cross-reactivity with calcitonin gene–related peptide.

The PTH method (Incstar) is an immunoradiometric assay using two distinct polyclonal antibodies. The first antibody, specific for PTH 39–84, is bound to polystyrene beads, and the second antibody, directed against the PTH 1–34 region, is labeled with 125I; thus, the assay detects only the intact form of PTH. The assay sensitivity is 0.07 pg/mL.

For analysis, the subjects were divided into groups by two sets of categories. The first was by fracture type. Those subjects with fractures in the neck region of the femur (n=78) were compared to subjects with fractures in the trochanteric region (n=88). Three patients were not included in this analysis because their fracture types were not in one of these two groups. To describe change over time in the various assays, longitudinal analyses using mixed models were employed (12). The concentration over time was a repeated measure for each patient. All data were analyzed assuming a compound symmetry structure for the covariance matrix. The mixed models were used to obtain least-squares means and associated standard errors (13). Serum concentrations of each hormone from patients without fractures were compared to all 3-day postfracture samples using a Student’s t test. The second categorical division of the study population was defined by age groups. The groups were divided as follows: 65–74 years of age (n=35), 75–84 years of age (n=79), and 85 years and older (n=55). These data were also analyzed by mixed models, with time and age as the factors.

Regression analyses following log transformations were determined for some sets of data as described in the results section.

Results

Initial analyses revealed no interactions between fracture type and age group. Thus, if differences between age groups were observed, these differences occurred regardless of fracture type. Also, there were no interactions between time following fracture and age group.

Hormonal Levels Following Hip Fracture as a Function of Location of Hip Fracture

Figure 1A illustrates estrone concentrations in the plasma following hip fracture and associated surgical procedures. For all patients combined, estrone concentration (47.6 ± 5.7 pg/mL; mean ± SEM) at 3 days postfracture is elevated (p<.001) compared to control levels of 20.7 ± 4.6 pg/mL. By 2 months, levels had declined (p<.001) to levels not significantly different from controls. Those patients with fractures in the neck region of the hip appeared to have higher levels compared to those with fractures in the trochanter region. However, this did not reach statistical significance (p=.069). Similarly, androstenedione (Figure 1A) levels were elevated (p<.01) compared to controls immediately following fracture; however, by 2 months, levels had declined to control levels (p<.001, Figure 1B). Patients with neck region fractures had androstenedione levels not significantly higher throughout the year compared to those with trochanteric fractures. For testosterone, there was a significant fracture type by time interaction (p=.02, Figure 1C). Testosterone levels of patients with trochanteric fractures declined significantly by 2 months postfracture, whereas testosterone of patients with neck region fractures remained at a constant level over the time period studied. The mean initial level of testosterone was lower in the patient group compared to that of the controls; however, this difference was not statistically different.

Figure 2 illustrates changes in the adrenal hormones DHEAS and cortisol following hip fracture. For both, concentrations 3 days after fractures were significantly elevated in comparison to the controls (p<.001), and both hormones declined by 2 months, remaining at the control levels for the year after fracture. There was no evidence for any difference in cortisol or DHEAS in those patients with fractures in the neck region compared to patients with fractures in the trochanteric region.

PTH levels (Figure 3A) were not significantly different from the control concentration at 3 days following fracture. However, PTH concentration tended to rise (p<.001) during the year following fracture from an overall mean of 49.7 ± 2.9 pg/mL at 3 days to 62.1 ± 3.0 pg/mL at 12 months. There was no significant difference between fracture types.

Calcitonin concentrations were much higher (p<.001) 3 days postfracture (42.1 ± 3.7 pg/mL) compared to controls without
Hormonal Changes Following Hip Fracture

fracture (9.8 ± 3 pg/mL). Calcitonin declined with time (p=.004), but never reached the control levels even 1 year following the fracture. No differences could be attributed to fracture location.

Hormonal Concentrations as a Function of Age Groups

Figure 4 illustrates changes in the concentrations of estrone, androstenedione, and testosterone during the year following fracture for each of the age groups. For these steroids, there was no difference in the pattern of plasma concentration of these steroids with age. The decline in concentration with time was still apparent. Neither the DHEAS nor cortisol response was affected by age (Figure 5).

PTH increased (p<.001) from an overall mean of 40.8 ± 4.5 pg/mL for those subjects 65–74 years old to 52.0 ± 3.0 pg/mL for those 75–84 years old to 61.2 ± 3.6 pg/mL for those 85 and

Figure 1. Serum concentrations of estrone (A), androstenedione (B), and testosterone (C) in the year following hip fracture. In this and Figures 2 and 3, solid circles represent patients with femoral neck fractures (n=78), open triangles represent patients with femoral trochanteric fractures (n=88), and open squares represent controls (n=17). Vertical bars represent ± standard error of the means. Main effects from ANOVA are indicated on each graph. Interactions (INT) are only indicated if they are significant. Control versus overall mean of day 3 is significantly different for estrone (p<.001) and androstenedione (p<.01).

Figure 2. Serum concentrations of DHEAS (A) and cortisol (B) in the year following hip fracture. See Figure 1 for details. Control versus overall mean of day 3 is significantly different for both DHEAS and cortisol (p<.001).
old (Figure 6A). Calcitonin showed no significant difference among age groups (Figure 6B).

Correlation coefficients were determined for selected pairs of variables to determine if values for one hormone were related, either positively or negatively, to values of another hormone within a patient. Analyses were performed for each individual time period. Table 1 shows the results for the 3-day time period when serum concentrations were usually the highest and the 6-month period when most steroids returned to control values. Log transformations were performed on each variable because most values were concentrated at one end of the scale. DHEAS correlated well with cortisol \((r = .39, p < .001)\), consistent with the concept that they are cosecreted by the adrenal gland. Other steroid correlations were also significant at 3 days, consistent with the precursor-metabolite relationship. At 6 months, when concentrations of these hormones were at a baseline level, the correlations were weak, but still significant except for estrone versus androstenedione. At 3 days, DHEAS and estrone were correlated significantly with calcitonin, but the correlation coefficient was lower \((r = .22, p = .04)\) than for the correlations between the steroids. By 6 months, when baseline levels were reached, these correlations were no longer significant. This might be anticipated because there is no expected relationship between steroid and calcitonin production even though there were similarities in their patterns of serum concentration over the year after fracture. Also, there was no significant correlation between PTH and DHEAS.

**DISCUSSION**

Increased occurrence of hip fractures in postmenopausal women is attributed to accelerated loss of bone mass secondary to decreases in estrogen production by the quiescent ovaries. Though estrogen is produced during menopause, cyclicity in cir-
culturating levels cease and concentrations drop even below follicular phase levels of the cycle (10,14). Androgen levels remain the same (15) or are depressed during menopause (4,16). Most estrogens are produced during menopause via peripheral conversion of circulating precursors, such as DHEA and its sulfated form or androstenedione, which originate primarily in the adrenal glands. However, there remains some production of estrogens and significant amounts of androgens by the ovaries (10,17). Aromatization of androgens to estrogens have been shown to be correlated with age (18). This enzymatic activity occurs in peripheral tissue, most notably adipose tissue (19), although other tissues, including bone, have aromatase activity (20,21).

Both estrogens and androgens have been implicated in preventing bone loss. The role of estrogens on bone are well documented and reviewed (1,22). Estrogen replacement in menopause has been demonstrated to slow bone loss and reduce the incidence of hip fracture (23). Estrogens may act by increasing calcium absorption from the gut and decreasing urinary excretion of calcium (22). Estrogens also have a direct stimulating effect on osteoblasts (24) and also affect osteoclasts, inhibiting their activity (25,26). Androgens may also be important in preventing bone loss. Hypogonadism is an important risk factor for osteoporosis in men (27). In a recent clinical trial, patients with osteoporosis demonstrated a positive calcium balance with the synthetic androgen, nandrolone decanoate (28). In another study, estradiol and methyl testosterone was administered to menopausal women and was more effective than estrogen alone in increasing vertebral bone mineral density and maintaining femoral bone density after 12–24 months of therapy (29). Androgen may have a direct inhibiting effect on osteoclasts (30). In part, androgen’s effect may be due to local conversion to estrogens by bone (20).

Following hip fracture, both estrone, the major circulating hormone in postmenopausal women, and androstenedione increased dramatically at 3–10 days following hip fracture. By 2 months, the levels returned to control levels and remained there throughout the year following fracture. We found that testosterone demonstrates a similar pattern in the trochanteric, but not the neck, fracture group. In this study population, the immediate postfracture testosterone levels were not higher than the control values. The sex steroid precursor DHEAS and cortisol (a non-precursor), both of which arise from the adrenal, also showed similar short-term increases after fracture. It is reasonable to assume that the rise in estrone and androstenedione are the result of an adrenal stress reaction and the subsequent increased pro-

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**Table 1. Correlation Coefficients (r) and p Values for Selected Pairs of Hormones**

<table>
<thead>
<tr>
<th></th>
<th>3 Days</th>
<th>6 Months</th>
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<tr>
<td></td>
<td>n</td>
<td>r</td>
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<tr>
<td>DHEAS × Cortisol</td>
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<td>.39</td>
</tr>
<tr>
<td>DHEAS × ΔA</td>
<td>104</td>
<td>.31</td>
</tr>
<tr>
<td>E₁ × ΔA</td>
<td>101</td>
<td>.47</td>
</tr>
<tr>
<td>T × ΔA</td>
<td>105</td>
<td>.47</td>
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<tr>
<td>DHEAS × PTH</td>
<td>106</td>
<td>-.12</td>
</tr>
<tr>
<td>DHEAS × CAL</td>
<td>106</td>
<td>-.22</td>
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<tr>
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<td>.20</td>
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<tr>
<td>ΔA × CAL</td>
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**Note:** All correlations were performed on log transformed data. ΔA = androstenedione; E₁ = estrone; T = testosterone; CAL = calcitonin.
duction of adrenal DHEA. The initial stress is probably due, not only to the fracture, but to the subsequent surgery which is usually accomplished within 48 hours of the fracture. Similar stress induced responses of these hormones have been described for acute illness (31). Whether there are physiologic effects of the short-term elevations in these hormones is not known. However, we have a preliminary report of increases in markers of bone metabolism within the first 10 days of fracture (32).

Fractures in the neck region are intracapsular and interfere with the regional blood supply and often result in healing complications (8). Because of this, we compared the steroid levels by fracture type. We found that those patients with femoral neck fractures tended to have somewhat higher levels of sex steroid throughout the year after fracture compared to those with fracture in the trochanteric region; however, this was statistically significant only for testosterone. The adrenal hormones DHEAS and cortisol, though they showed the initial stress response, showed no evidence of different concentrations as a function of fracture type. When steroid concentration patterns in the year after fracture were examined by age groups, there were no significant differences in response.

Although estrogens and androgens have a positive effect on bone mass, glucocorticoids have a contrary effect. Osteoporosis is a well-known manifestation of Cushing’s disease (7). Glucocorticoid therapy for rheumatoid arthritis and other inflammatory diseases has rapid bone loss as a serious side effect (33). Corticoid-induced bone loss is due to demineralization, primarily of trabecular bone, and is more severe for vertebrae and ribs than for long bones (5). Many mechanisms for glucocorticoid-induced bone loss have been described, but no primary mechanism is known (7). It is unknown if the transient increase in cortisol observed in our study is of sufficient duration to have an adverse effect on bone.

We found good correlation between the various adrenal and sex steroids. For example, for a given patient with high DHEAS, the androstenedione is also high. This supports the argument for the precursor-metabolite relationship. This relationship has been directly demonstrated by administration of oral DHEA to postmenopausal women resulting in a rapid increase in testosterone and androstenedione along with elevated estrogens occurring within 2 weeks (34). Good correlation between adrenal hormones and sex hormones have been demonstrated previously in women with acute illness (31).

PTH is a peptide secreted by the parathyroid gland. It responds inversely to changes in calcium levels in the circulation and acts to remove calcium from bone and subsequent bone resorption. PTH accelerates bone turnover, but bone resorption occurs at a faster rate than bone formation (7). Its primary action may be on osteoblasts which secrete second messengers that, in turn, stimulate osteoclast activity (35). Paradoxically, small intermittent doses may stimulate bone formation (36). Several recent publications describe a rise in PTH concentration during menopause (37–40) and in osteoporotic women (41). Following hip fracture, we find no immediate change in PTH concentration; however, it appears to rise slowly, but significantly, peaking at 1 year after fracture. This may be of importance, because of PTH’s negative effect on bone. Benhamou and colleagues (43) report no increase in PTH following hip fracture; however, they followed 12 patients for only a 2-month period. Secondary hyperparathyroidism in the elderly occurs in response to decreased serum calcium levels. This is associated with low dietary calcium intake and intestinal absorption as well as low vitamin D levels due to poor diet and decreased exposure to sunshine. In a limited sample of patients (n=85) from our study population, it was found that 75% had 25-hydroxyvitamin D levels below the normal range (44). Yet, in that study, there was no correlation between vitamin D and PTH.

Calcitonin is a peptide from the parafollicular cells of the thyroid and is regulated primarily by serum calcium levels. Its main action is through osteoclastic inhibition of bone resorption (42). A reduction in calcitonin in postmenopausal women (45) and lower production rates in osteoporotics regardless of age have been described (46). Furthermore, with advancing age there is a decreased responsiveness of calcitonin to a given calcium load (45). Although calcitonin concentrations in our postmenopausal controls were low, calcitonin was elevated immediately following fracture, and these levels remained elevated throughout the following year. There were no differences in calcitonin concentration among the different age groups in our study. Calcitonin is currently being used to treat osteoporosis (6). Whether the elevated calcitonin levels reported in this study are of functional significance is not known. The levels we report, though high, are still within the normal premenopausal range (46).

This study illustrates that, following hip fracture, there are some dramatic responses in hormones, both steroid and peptide, that purportedly are mechanistically important in bone metabolism. These changes include transient increases in steroid hormones, chronic elevations in calcitonin, and rising levels of parathyroid hormone during the year after fracture. In subsequent reports, we will examine the relationship between these hormones and changes in bone densities, bone metabolic markers, and other functional changes (e.g., gait, weight-bearing) occurring during the year after hip fracture.

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