Endogenous sex steroid, GH and IGF-I levels in normal elderly men: Relationships with bone mineral density and markers of bone turnover

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ABSTRACT. There are studies concerning the association among endogenous sex steroids, growth hormone (GH), insulin-like growth factor-I (IGF-I) and bone mineral density (BMD) in both men and women. However, little is known concerning the association of these parameters with markers of bone turnover in healthy elderly men. We studied the association of BMD (dual energy X-ray absorptiometry of spine, hip and forearm) and markers of bone turnover (bone-specific alkaline phosphatase, serum C-terminal propeptide of type I collagen, and serum osteocalcin reflecting formation, urine deoxypyridinoline and calcium excretion in relation to creatinine excretion reflecting resorption) with endogenous sex steroids, GH and IGF-I in 14 elderly normal men (age range 60-79 years). There was a negative correlation between age and dehydroepiandrosterone sulphate (DHEAS) (r=-0.60, p=0.022) and a positive correlation between GH and IGF-I (r=0.53, p=0.048). Serum estradiol concentrations correlated with BMD at distal 1/3 radius (r=0.41, p=0.1) and mid-radius (r=0.47, p=0.08), and negatively correlated with age (r=-0.45, p= 0.09). There was no correlation of estradiol with bone turnover markers, testosterone, free testosterone, DHEAS, GH and IGF-I. Serum GH and IGF-I levels showed no correlation with BMD (all sites) and bone turnover markers. Serum total testosterone concentrations positively correlated with BMD at distal 1/3 radius (r=0.47, p=0.09), femoral neck (r=0.56, p=0.037) and Ward's triangle (r=0.49, p=0.07). These data suggest that serum estradiol and testosterone levels are associated with BMD in elderly men, possibly indicating their contribution to skeletal maintenance in old age. However, correlations of IGF-I, GH and DHEAS with BMD and bone turnover markers are lacking in the group studied.

(J. Endocrinol. Invest. *24:* 408-414, 2001)

INTRODUCTION

Estradiol deficiency is a well-known cause of postmenopausal osteoporosis (1). It has been shown that androgens have an anabolic effect on bone in both hypogonadal (2) and eugonadal men (3). Recent clinical observations of severe osteoporosis in one man with defective estrogen receptor (4) and in two men with aromatase deficiency (5, 6) suggest that estrogens are also of importance for the maintenance of the male skeleton. Moreover, estradiol levels were decreased in male idiopathic osteoporosis (7). Also, insulin-like growth factor-I (IGF-I) has been shown to be important in the differentiation, maturation, and recruitment of osteoblast (8). The demonstration of reduced IGF-I in men with idiopathic osteoporosis (9) and the correlation of IGF-I with lumbar spinal bone mineral density (BMD) (10) have supported the clinical relevance of this phenomenon.

Although many studies have examined the association between endogenous sex steroids, IGF-I and BMD in both men and women, little is known concerning the association of these parameters with markers of bone turnover in healthy elderly men. In the present study, we present data concerning the association of BMD and markers of bone turnover with endogenous sex steroids, GH and IGF-I levels in a group of elderly normal men.

Key-words: Estradiol, testosterone, IGF-I, bone mineral density, bone turnover, male.

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Accepted October 27, 2000.

Table 1 - *Clinical and laboratory features of the study population (no.=14).*

Variable	Median	Range
Age (yr)	69.5	60-79
Height (m)	1.66	1.54-1.77
Weight (kg)	78.5	60-95
Calcium (mg/dl)	9.45	8.6-10.2
Phosphorus (mg/dl)	3.2	2.3-3.9
Total ALP (IU/l)	197.5	20.1-240
FSH (mIU/ml)	6.2	3.2-10.8
LH (mIU/ml)	4.2	3.2-7.2
Intact PTH (pg/ml)	37.9	19.8-61.5

ALP: alkaline phosphatase; FSH: follicle-stimulating hormone; LH: luteinizing hormone; TSH: thyroid-stimulating hormone; PTH: parathyroid hormone.

SUBJECTS AND METHODS

Study population

A total of 14 Caucasian men aged over 60 were enrolled. None of the subjects had a history of hyperthyroidism, glucocorticoid or anticonvulsant use, diabetes mellitus, gastrointestinal disease or surgery, malignancy or any known metabolic bone disease. All patients had normal gonadal, adrenal, thyroid and hepatic functions. Smoking status was determined by history. A subject who had been smoking more than one packet a year was considered to be a smoker. There were no ex-smokers. Five subjects were current smokers. Complete physical examination revealed no pathologic findings. Lumbar vertebral radiograms revealed no osteoporotic fractures. The clinical and laboratory features of the participants are given in Table 1. Informed consent was obtained from all participants.

Serum and urine measurements

Serum concentrations of total calcium, phosphorus, alkaline phosphatase, blood urea nitrogen, creatinine, aspartate aminotransferase and alanine aminotransferase were measured by automated techniques. Intact parathyroid hormone [PTH, normal range: 12-72 pg/ml, inter-assay coefficient of variation (CV): 7.2%], follicle stimulating hormone (FSH, normal range 0.7-11.1 mIU/ml, inter-assay CV: 6.3%), luteinizing hormone (LH, normal range 0.8-7.6 mIU/ml, inter-assay CV: 6.7%) and growth hormone (GH, normal range 0.06-5 ng/ml, inter-assay CV: 5.4%) were assessed by immunometric assay (Immulite 2000, Diagnostic Products Co., Los Angeles, CA, USA). Estradiol (normal <56 pg/ml, inter-assay CV: 16%, lowest detection limit 10 pg/ml), dehydroepiandrosterone sulphate (DHEAS, normal range 80-560 µg/dl, interassay CV: 9.8%) and total testosterone (normal range 200-810 ng/dl, inter-assay CV: 7.5%) were measured by radioimmunoassay (Diagnostic Systems Laboratories Inc., Webster, TX, USA). Serum IGF-I (normal range 100-494 ng/ml, inter-assay CV: 3.7%) was measured by immunoradiometric assay (Diagnostic Systems Laboratories Inc., Webster, TX, USA). Serum free testosterone (normal range 8.69-54.69 pg/ml, inter-assay CV: 7.9%) was measured by radioimmunoassay (Diagnostic Systems Laboratories Inc., Webster, TX, USA).

Bone-specific alkaline phosphatase (B-ALP) was determined by ELISA (Alkphase-B, Metra Biosystems, Inc., CA, USA). Normal range for B-ALP was 11.6-30.6 U/l and the minimum detection limit was 0.7 U/l. The inter-assay CVs at 12 U/l and at 35 U/l were 5.2% and 5.0%, respectively. Serum C-terminal propeptide of type I collagen was determined by ELISA (Prolagen-C, Metra Biosystems, Inc., CA, USA). Normal range of the assay was 69-147 ng/ml and the minimum detection limit was 0.2 ng/ml. The inter-assay CVs at 80.8 ng/ml and 296.7 ng/ml were 7.0% and 5.0%, respectively. Serum osteocalcin was measured by ELISA (Novocalcin, Metra Biosystems, Inc., CA, USA). Normal range was 3.7-10.0 ng/ml, and the minimum detection limit was 0.45 ng/ml. The inter-assay CVs at 6.2 ng/ml and 16.5 ng/ml were 9.8% and 7.6%, respectively. Serum B-ALP, C-terminal propeptide of type I collagen and osteocalcin are markers of bone formation (11). To determine deoxypyridinoline (DPD) and calcium excretion in relation to creatinine excretion, second void morning urine samples were obtained at approximately 9:00 h after an overnight fast. Urine calcium and creatinine were measured by autoanalyser (Boehringer-Mannheim, Germany). Urine DPD was measured by chemiluminescent enzyme immunoassay (Immulite Pyrilinks-D, Diagnostic Products Corp., CA, USA).The normal range of DPD excretion as expressed per mmol of creatinine was 3.0-7.4 nmol.

Expressed in relation to urinary creatinine, fasting urine calcium and DPD are markers of bone resorption (11).

Bone mineral density measurements

BMD was measured at lumbar vertebrae (L_{1-4}), hip (femoral neck, Ward's triangle, trochanter, intertrochanter and total hip), the forearm (midshaft, distal 1/3 and ultradistal radius, and total radius) by dual-energy X-ray absorptiometry (DEXA) (Hologic Inc., Model QDR-4500A, Bedford, MA, USA). The

Table 2 - *Clinical features, sex steroids, GH and IGF-I levels of smokers and non-smokers.*

Variable	Smokers (no.=5)	Non-smokers (no.=9)
Age (yr)	65 (60-75)	73 (64-79)
Height (m)	1.71 (1.65-1.77)	1.62 (1.54-1.75)*
Weight (kg)	80 (65-95)	78 (60-84)
Testosterone (ng/dl)	420 (336-1600)	440 (310-633)
Free testosterone (pg/ml)	11.9 (8.7-14.2)	12.7 (8.6-21.4)
DHEAS (µg/dl)	84.9 (30-162)	92.1 (36-182)
GH (ng/ml)	0.09 (0.05-0.8)	0.38 (0.05-1.7)
IGF-I (ng/ml)	134 (83.8-467)	168.3 (97-476)
Estradiol (pg/ml)	22.8 (19.8-42.9)	23.4 (12-46.5)

*p=0.04 by Mann-Whitney U test; DHEAS: dehydroepiandrosterone sulphate; GH: growth hormone; IGF-I: insulin-like growth factor-I; data are shown as median (range).

scanner was a highly collimated X-ray system (switched pulse, dual energy, 140 kV peak).The *in vivo* precision was <1% for all sites where the measurements were made. BMD was expressed in units of g/cm^2.

Statistical analysis

Bivariate correlations of the variables were analyzed by Spearman rank correlation test. The correlation coefficients (r values) and the statistical level of significance (p values) were calculated. If the r value was lower than 0.25, we considered that there was no correlation between the variables. The comparison of the median values of the variables between smokers and non-smokers were done by Mann-Whitney U test and p value less than 0.05 was considered significant.

RESULTS

All subjects had normal FSH, LH and total/free testosterone levels indicating normal gonadal function (Table 1). Serum calcium, phosphorus, total ALP and intact PTH concentrations were also nor-

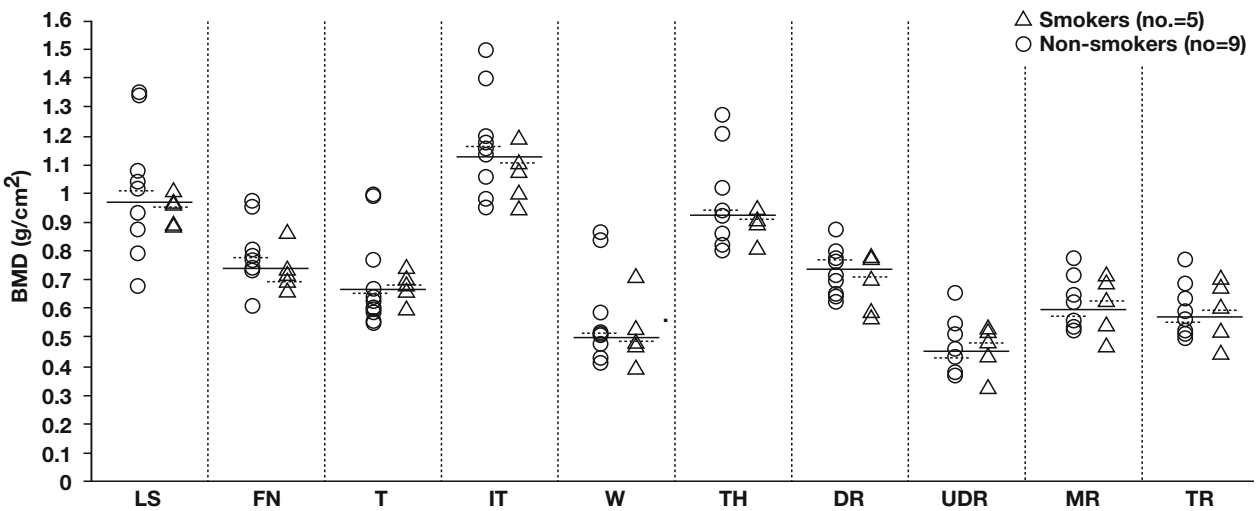

Fig. 1 - *Bone mineral density (BMD) values of the subjects.*
LS: lumbar spine, FN: femoral neck, T: trochanter, IT: intertrochanter, W: Ward's triangle, TH: total hip, DR: distal (1/3) radius, UDR: ultradistal radius, MR: midshaft radius, TR: total radius. Horizontal solid lines represent the median value of the whole group, horizontal dashed lines represent the median values of smokers and non-smokers. P: NS for all sites between smokers and non-smokers by Mann-Whitney U test.

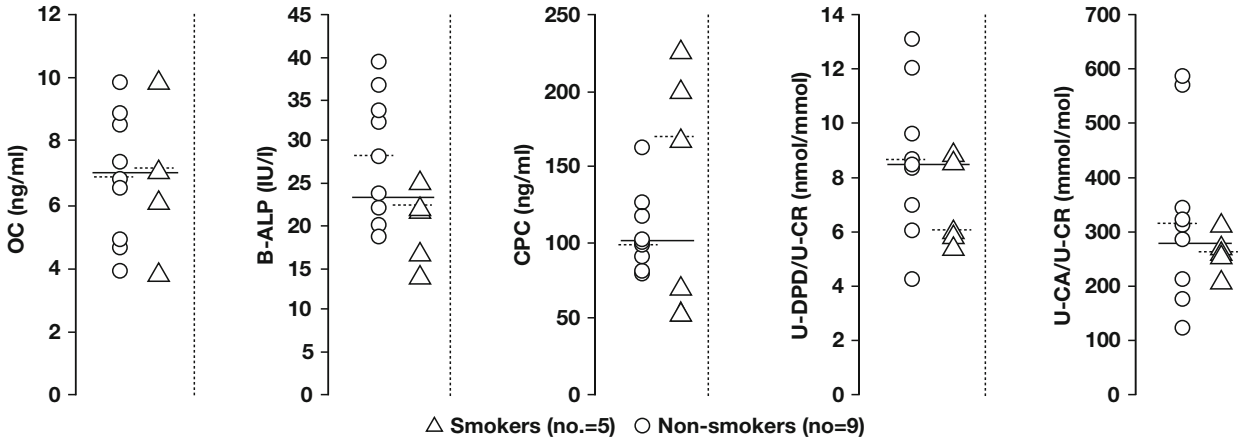

Fig. 2 - *Bone turnover markers of the subjects.*
OC: osteocalcin, B-ALP: bone-specific alkaline phosphatase, CPC: C-terminal propeptide of type I collagen, U-DPD: urinary deoxypyridinoline, U-CA: urinary calcium, U-CR: urinary creatinine. Horizontal solid lines represent the median value of the whole group, horizontal dashed lines represent the median values of smokers and non-smokers. P: NS for all markers between smokers and non-smokers by Mann-Whitney U test.

mal (Table 1). Smokers (no.=5) and non-smokers (no.=9) were comparable with respect to age, weight, levels of endogenous sex steroids, GH and IGF-1, but smokers were significantly taller than non-smokers (p=0.04) (Table 2).
The individual BMD values and bone turnover markers of the study group are presented in Figures 1 and 2, respectively. Smokers and non-smokers had comparable BMDs and bone turnover markers (Fig. 1 and 2, respectively).

Correlation analyses

Weight was positively correlated with trochanteric (r=0.52, p=0.05) and ultra-distal radius BMD (r=0.63, p=0.015). Age was negatively correlated with estradiol (r=-0.45, p=0.09) (Fig. 3A) and DHEAS (r=-0.60, p=0.022) (Fig. 3B), and a positive correlation was found between GH and IGF-I (r=0.53, p=0.048) (Fig. 3C).
Estradiol was positively correlated with BMD at distal 1/3 radius (r=0.41, p=0.1) and at mid-radius (r=0.47, p=0.08) (Fig. 4A). Serum total testosterone was positively correlated with BMD at distal 1/3 radius (r=0.47, p=0.09), Ward's triangle (r=0.49, p=0.07), and femoral neck (r=0.56, p=0.037) (Fig. 4B). Free testosterone was negatively correlated with urinary calcium/creatinine ratio (r=-0.56, p=0.038) (Fig. 4C). There was no correlation of estradiol with bone turnover markers, testosterone, free testosterone, DHEAS, GH and IGF-I. Serum GH and IGF-I levels showed no correlation with BMD (all sites) and bone turnover markers (p>0.2 for all, details not shown).

DISCUSSION

Our study provides cross-sectional data regarding the associations between endogenous sex steroids, GH, IGF-I, BMD and bone turnover markers in healthy elderly men. We found no significant correlations between GH, IGF-I and bone density/metabolism. In previous studies, IGF-I was found to decline in both serum and bone with aging (12, 13). It has also been reported that IGF-I has an important role in the genesis of male osteoporosis (10). Kurland *et al.* (9) found low levels of IGF-I in middle-aged osteoporotic men compared with controls. The cross-sectional design of our study, limited number of subjects, lack of clinically and radiologically apparent osteoporosis, lack of assessment of bone levels of IGF-I might have led us to overlook IGF-I and bone metabolism interaction. Although GH and sex steroids appear to have independent effects on bone mass, sex steroids can influence circulating levels of GH and IGF-I, suggesting that an interaction between these hormones may be important for the acquisition and maintenance of bone mass (14). However, we could not find any associations between GH, IGF-I and endogenous sex steroids.
The importance of sex steroids in the development and maintenance of female skeleton is unquestioned. However, studies of sex steroids, gonadal function, and their interaction with bone metabolism in elderly men are scarce. To our knowledge, there has been no extensive investigation concerning the association of endogenous sex steroids with bone metabolism in elderly men. Our results suggest that serum estradiol

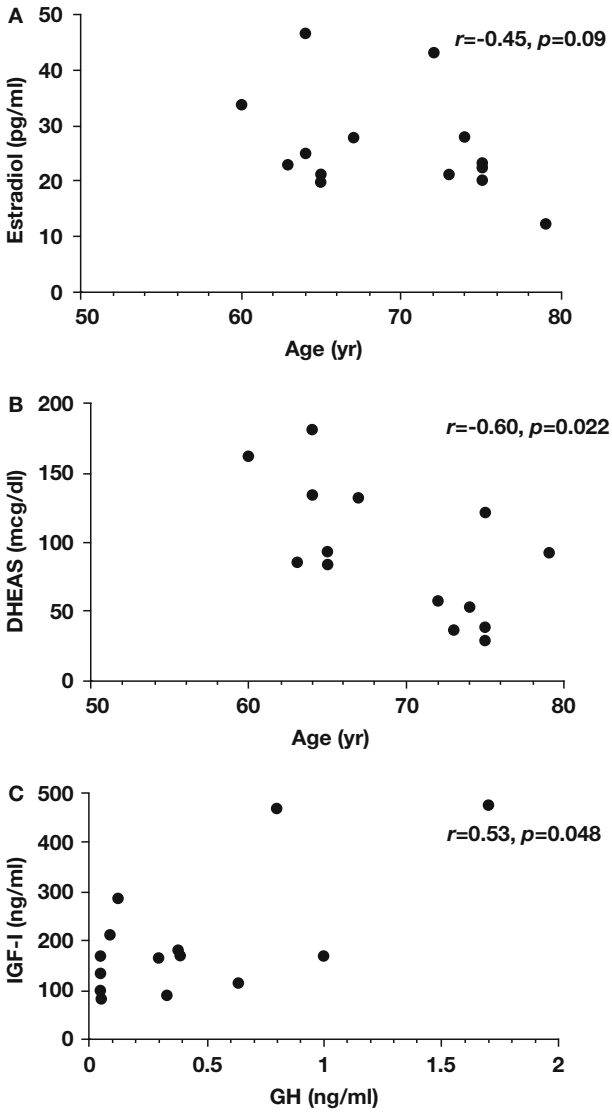

Fig. 3 - *Correlations: A) age* vs *estradiol, (B) age* vs *dehydroepiandrosterone sulphate (DHEAS), (C) growth hormone (GH)* vs *insulin-like growth factor-I (IGF-I). r: Spearman correlation coefficient.*

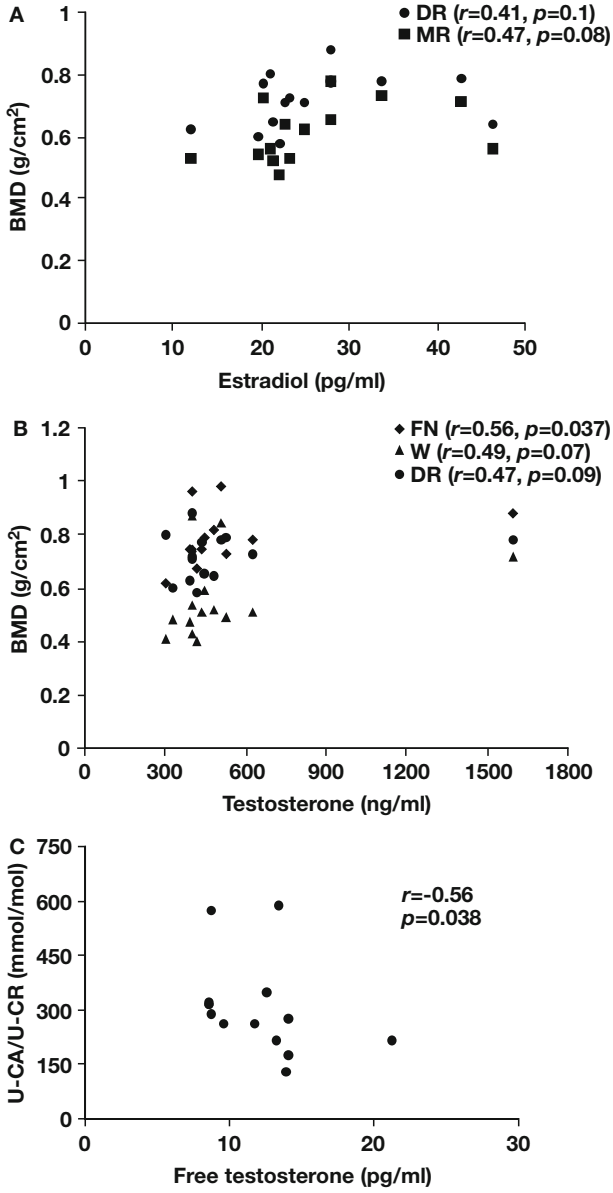

Fig. 4 - *Correlations: A) estradiol and B) testosterone* vs *bone mineral density (BMD), C) urinary calcium/creatinine (U-CA/U-CR)* vs *free testosterone. r: Spearman correlation coefficient; DR: distal (1/3) radius; MR: Mid-shaft radius; FN: femoral neck; W: Ward's triangle.*

concentrations show no association with bone formation and resorption as reflected by turnover markers. We found correlation of estradiol with forearm BMD. In a previous population-based study, bioavailable estradiol was correlated with BMD at forearm, spine and hip (15). Similarly, Slemenda *et al*. (16) found associations between estradiol and BMD in men aged over 65 (*r* values ranged between 0.21-0.35). Therefore, these observations and our data suggest that estradiol has some degree of contribution in skeletal maintenance in elderly men.

In a previous study by Center *et al*. (17), low serum free testosterone was found to be an independent predictor of low spinal BMD in elderly men, whereas low estradiol predicted low BMD at both spine and hip. These results also indicate the relatively more important role of estradiol compared with testosterone in the maintenance of male skeleton. We showed that serum total testosterone concen-

trations were positively correlated with forearm and hip BMD but not with spinal BMD. In contrast to our observations, Drinka *et al.* (18) failed to demonstrate a relation between testosterone levels and BMD at both axial and appendicular sites. Despite the correlation between BMD and testosterone, we could not show any association between testosterone and bone turnover markers. The only negative correlation existed between serum free testosterone and urinary calcium/creatinine ratio. We found a negative correlation between age and DHEAS, but DHEAS had no correlation with BMD and bone turnover markers. This finding is in accordance with the observations of Greendale *et al.* (15), who were able to demonstrate a positive association of DHEAS and BMD in women but not in men. The role of DHEAS in the maintenance of male bone mass has received limited investigation. However, in two studies (19, 20) no association was found between DHEAS and BMD in men.

Limitations of the study

There are two main limitations of this study. Firstly, the number of subjects is limited (no.=14), and we would have had lower *p* values in Spearman rank correlation tests if we had enrolled more subjects. Secondly, as in other reported studies, a single determination of sex steroids was made. This introduces a null bias, because sex steroids are not static in men and women. For example, testosterone is converted to estrogen in both men and women. Thus, it is difficult to judge which hormones are influencing the skeletal system directly and which are upstream sources of substrate to form the bone active hormones. In conclusion, our data suggest that serum estradiol and testosterone levels are associated with BMD in elderly men, possibly indicating their contribution to skeletal maintenance in old age. However, further studies with larger number of subjects are required to assess the interaction of endogenous sex steroids, GH and IGF-I with bone metabolism in aging men.

REFERENCES

1. Richelson L.S., Wahner H.W., Melton L.J. III
   Relative contributions of aging and estrogen deficiency to postmenopausal bone loss.
   N. Engl. J. Med. 1984, *311:* 1273-1275.
2. Greenspan S.L., Neer R.M., Ridgway E.C., Klibanski A.
   Osteoporosis in men with hyperprolactinemic hypogonadism.
   Ann. Intern. Med. 1986, *104:* 777-782.
3. Anderson F.H., Francis R.M., Peaston R.T., Wastell H.J.
   Androgen supplementation in eugonadal men with osteoporosis: effect of six months treatment on markers of bone formation and resorption.
   J. Bone Miner. Res. 1997, *12:* 472-478.
4. Smith E.P., Boyd J., Frank G.R., Takahashi H., Cohen R.M., Specker B., Williams T.C., Lubahn D.B., Korach K.S.
   Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man.
   N. Engl. J. Med. 1994, *331:* 1056-1061.
5. Carani C., Qin K., Simoni M., Faustini-Fustini M., Serpente S., Boyd J., Korach K.S., Simpson E.R.
   Effect of testosterone and oestradiol in a man with aromatase deficiency.
   N. Engl. J. Med. 1997, *337:* 91-95.
6. Morishima A., Grumbach M.M., Simpson E.R., Fisher C., Qin K.
   Aromatase deficiency in male and female siblings caused by a novel mutation and the physiological role of estrogen.
   J. Clin. Endocrinol. Metab. 1995, *80:* 3689-3698.
7. Gillberg P., Johansson A.G., Ljunghall S.
   Decreased oestradiol levels and free androgen index and elevated sex hormone-binding globulin levels in male idiopathic osteoporosis.
   Calcif. Tissue Int. 1999, *64:* 209-213.
8. Jones J.I., Clemmons D.R.
   Insulin-like growth factors and their binding proteins: biological actions.
   Endocr. Rev. 1995, *16:* 3-32.
9. Kurland E.S., Rosen C.J., Cosman F., McMahon D., Chan F., Shane E., Lindsay R., Dempster D., Bilezikian J.P.
   Insulin-like growth factor-I in men with idiopathic osteoporosis.
   J. Clin. Endocrinol. Metab. 1997, *82:* 2799-2805.
10. Ljunghall S., Johansson A.G., Burman P., Kampe O., Lindh E., Karlsson F.A.
    Low plasma levels of insulin-like growth factor I (IGF-I) in male patients with idiopathic osteoporosis.
    J. Intern. Med. 1992, *232:* 59-64.
11. Delmas P.D.
    Biochemical markers of bone turnover: methodology and clinical use in osteoporosis.
    Am. J. Med. 1991, *91* (Suppl. 5B): 59S-63S.
12. Canalis E., McCarthy T., Centrella M.
    Growth factors and the regulation of bone remodeling.
    J. Clin. Invest. 1988, *81:* 277-281.
13. Rosen C., Donahue L.R., Hunter S.J.
    Insulin-like growth factors and bone: the osteoporosis connection.
    Proc. Soc. Exp. Biol. Med. 1994, *206:* 83-102.
14. Holmes S.J., Shalet S.M.
    Role of growth hormone and sex steroids in achieving and maintaining normal bone mass.
    Horm. Res. 1996, *45:* 86-93.

15. Greendale G.A., Edelstein S., Barrett-Connor E.
Endogenous sex steroids and bone mineral density in older women and men: the Rancho Bernardo Study.
J. Bone Miner. Res. 1997, *12:* 1833-1843.

16. Slemenda C.W., Longcope C., Zhou L., Hui S.L., Peacock M., Johnston C.C.
Sex steroids and bone mass in older men: positive associations with serum estrogens and negative associations with androgens.
J. Clin. Invest. 1997, *100:* 1755-1759.

17. Center J.R., Nguyen T.V., Sambrook P.N., Eisman J.A.
Hormonal and biochemical parameters in the determination of osteoporosis in elderly men.
J. Clin. Endocrinol. Metab. 1999, *84:* 3626-3635.

18. Drinka P.J., Olson J., Bauwens S., Voeks S., Carlson I., Wilson M.
Lack of association between free testosterone and bone density in elderly men.
Calcif. Tissue Int. 1993, *52:* 67-69.

19. Murphy S., Khaw K., Cassidy A., Compston J.E.
Sex hormones and bone mineral density in elderly men.
Bone Miner. 1993, *20:* 133-140.

20. Barrett-Connor E., Kritz-Silverstien D., Edelstein S.L.
A prospective study of dehydroepiandrosterone sulphate and bone mineral density in older men and women.
Am. J. Epidemiol. 1993, *137:* 201-206.