Some areas, especially guanine- and cytosine-rich ones, may not be well captured and sequenced (see *PKD1* exon 1[2]), mutations to the complex autosomal dominant tubulointerstitial kidney disease gene, *MUC1*, are not detected by gene-panel screening, and the disease gene may not have been included on the panel. Interpretation of the pathogenicity of in-frame changes (variants of unknown significance) can sometime be difficult. The presence of large databases displaying the frequency of variants on more than 100,000 "normal" alleles of different ethnicity (http://gnomad.broadinstitute.org/) are invaluable, because alleles associated with monogenic disease are rare. Variant evaluation programs, assessing the chemical difference of a substitution, and the conservation of the residue in orthogs and domain structures can be helpful, especially if multiple programs are tested (http://marrvel.org/). Another aid to interpretation is sufficient clinical data to describe the phenotype, and databases of detected pathogenic variants, including for specific diseases, can also be of value. Ultimately, it is important to report results using developed criteria, with an emphasis on not over-interpreting the available data, while trying to provide a clear diagnosis.[9] Finally, it is important to remember that a genetic diagnosis, despite legislative progress, may result in discrimination, so appropriate genetic counseling and patient agreement are important before such testing.

DISCLOSURE

The author declared no competing interests.

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Bones and the sex hormones

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Sex hormones act in multiple ways to maintain a strong skeleton. In men, estrogen regulates cortical bone turnover, but testosterone maintains trabecular turnover. In normal men, sex hormone–binding protein is an independent risk factor for fractures. This led Aleksova and colleagues to measure the sex hormones and their binding protein in men receiving dialysis. Both higher sex hormone–binding globulin and higher total testosterone were associated with prevalent nonvertebral fractures, adding another layer of complexity to renal osteodystrophy.

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Sex hormones play a major role in the growth and maintenance of the skeletal system. Androgens mediate the periosteal growth in both genders. Because men have higher androgen levels they have greater cortical thickness and peak bone mass. The periosteum continues to expand throughout life, more so in men. Because cortical bone is placed further away from the neutral axis, there is greater resistance to bending. This helps to compensate for aging bone loss.[1]

In the past it was assumed that estrogen regulated bone metabolism in women, whereas testosterone performed these functions in men. An interventional study in normal men, all treated with gonadotropin blockade and aromatase inhibition and then randomized into 4 groups (receiving placebo, estrogen, testosterone, or both), showed that estrogen accounted for more than 70% of the effect of sex steroids on bone resorption. Since then, numerous studies in men have shown that bioavailable estrogen is more closely associated with bone density than bioavailable testosterone. But the site-specific actions of sex hormones are different in men than in women. Estrogen maintains cortical bone (which is 80% of the bone mass) in both men and women. In women, estrogen is the main hormone that maintains the cancellous bone, with small additional actions from testosterone, whereas in men the testosterone is the major hormone in the cancellous bone with minor contributions from estrogen[2] (Figure 1).

On a cellular basis regarding bone remodeling, androgens stimulate osteoblast precursors through triggering interleukin-1β. Both androgens and

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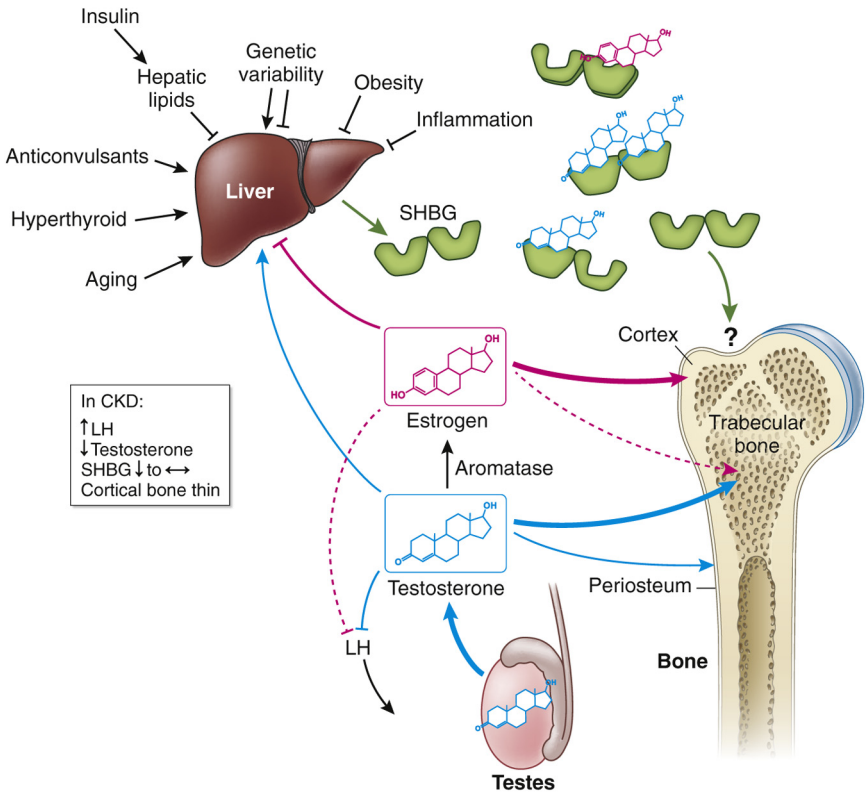

Figure 1 | Sex hormones and the bone, in men. Testosterone is secreted from the testes and bound to sex hormone–binding globulin (SHBG) or albumen. SHBG is made mainly in the liver and is influenced by many factors, including the sex hormones themselves. Aromatase converts some of the free testosterone to estrogen (which also can bind to the SHBG). The free hormones then activate receptors on the bone cells. It is unclear whether SHBG has a receptor on the bone cells. Estrogen acts mainly on the cortex with some effects on the trabecular bone, and testosterone acts on the trabecular bone. Testosterone also increases periosteal expansion. CKD, chronic kidney disease; LH, luteinizing hormone.

estrogens are known to suppress osteoblast apoptosis and increase osteoclast apoptosis. Androgens also reduce osteoclast formation and survival through indirect mechanisms involving the mesenchymal cells. Directly, estrogen decreases nuclear factor kappa-B ligand and thus inhibits osteoclast differentiation. Estrogen also increases osteoprotegerin production, skewing the osteoprotegerin and nuclear factor kappa-B ligand ratio and opposing osteoclast formation. Furthermore, estrogen decreases bone resorbing cytokines such as interleukin-1, interleukin-6, and tumor necrosis factor alpha. Two other major players to highlight are Wnt/β-catenin signaling pathway and sclerostin. Wnt signaling is necessary for osteoblast maturation. Also, Wnt signals osteoblastic cells to increase osteoprotegerin production. Estrogen increases osteoblast production of transforming growth factor beta, which results in increased Wnt production by osteoclasts. This Wnt pathway in bone is inhibited by sclerostin and Dickkopf-related protein 1, and estrogen decreases osteocyte secretion of sclerostin.[2] Altogether, sex hormones predominantly reduce bone resorption and contribute to bone formation to maintain bone homeostasis in adult life.

Testosterone is mostly produced by the Leydig cells under the influence of pulsatile gonadotropin-releasing hormone from the hypothalamus and luteinizing hormone (LH) secretion from the pituitary. LH is the primary regulator of testicular testosterone production. Sex hormone–binding globulin (SHBG) influences the availability of the sex hormones by binding tightly to 50% to 60% of circulating testosterone and 20% to 40% of estradiol. In the serum, total testosterone refers to the sum of strongly protein-bound, weakly albumin-bound, and free testosterone that is not bound to any protein. The majority of the circulating testosterone is protein bound, and only approximately 0.5% to 3% is free. The weakly albumin-bound testosterone easily dissociates and is accessible to tissues; therefore the free and albumin-bound testosterone is considered bioactive.

SHBG is produced in the liver. It rises with aging, anticonvulsants, and medical conditions including liver cirrhosis and hyperthyroidism. Lower levels of SHBG are noted in obesity, diabetes, and inflammation. Genetic variability also impacts SHBG levels. Insulin indirectly decreases SHBG via an effect on hepatic lipids. Testosterone inhibits SHBG production, whereas estrogen increases it.[3]

SHBG forms dimers that change shape when sex hormones bind, changing the binding affinity, so the estimations of free level that assume linear binding will not be accurate.[4] It is possible that SHBG itself may cause cell signaling, or that it can facilitate entry of sex hormones into the cell. A receptor for SHBG has been identified on some cell membranes that can bind unliganded SHBG. If a steroid hormone binds to the SHBG-receptor complex, there can be signaling involving generation of cyclic adenosine monophosphate. However, it is not clear if this occurs in any of the bone cells. Whether sex steroids enter the cell while bound to the SHBG is still undetermined.

Because SHBG plays a major role in the regulation of tissue availability of sex hormones, there has been growing research looking into the role of SHBG on male bone density and strength. A study of 950 men from Australia, using liquid gas chromatography mass spectrometry (LCMS), found that during 5 years of observation, increases in SHBG were associated with decreased bone density and increased fracture risk.[5] An international community-based cohort study of 5487 older men initially reported that high SHBG independently increased fracture risk in advanced age