1994 Biochemical markers of renal osteodystrophy in pediatric patients undergoing CAPD/CCPD. Kidney Int. 45:253–258.
3. Pletka PG, Strom TB, Hampers CL, et al. 1976 Secondary hyperparathyroidism in human kidney transplant recipients. Nephron. 1976; 17:371–381.
4. Sherrard DJ, Hercz G, Pei Y, et al. 1993 The spectrum of bone disease in end-stage renal failure—an evolving disorder. Kidney Int. 43:436–442.
5. Pei Y, Hercz G, Greenwood C, et al. 1993 A plastic osteodystrophy in diabetic patients. Kidney Int. 44:159–164.
6. Hercz G, Pei Y, Greenwood C, et al. 1993 A plastic osteodystrophy without aluminum: the role of "suppressed" parathyroid function. Kidney Int. 44:860–866.
7. Wang M, Hercz G, Sherrard DJ, Maloney NA, Serge GV, Pei Y. 1995 Relationship between intact 1-84 parathyroid hormone and bone histomorphometric parameters in dialysis patients without aluminum toxicity. Am J Kidney Dis. 26:836–844.
8. Pei Y, Hercz G, Greenwood C, et al. 1995 Risk factors for renal osteodystrophy: a multivariant analysis. J Bone Miner Res. 10:149–156.
9. Mathias RS, Salusky IB, Harmon WH, et al. 1993 Renal bone disease in pediatric patients and young adults treated by hemodialysis in a childrens hospital. J Am Soc Nephrol. 12:1938–1946.
10. Goodman WG, Ramirez JA, Belin TR, et al. 1994 Development of adynamic bone in patients with secondary hyperparathyroidism after intermittent calcitriol therapy. Kidney Int. 46:1160–1166.
11. Salusky IB, Kuizon BD, Belin T, et al. 1998 Intermittent calcitriol therapy in secondary hyperparathyroidism: a comparison between oral and intraperitoneal administration. Kidney Int. 54:907–914.
12. John MR, Goodman WG, Gao P, Cantor TL, Salusky IB, Jüppner H. 1999 A novel immunoradiometric assay detects full-length human PTH but not amino-terminally truncated fragments: implications for PTH measurements in renal failure. J Clin Endocrinol Metab. 84:4287–4290.
13. Slatopolsky E, Finch J, Clay P, et al. 2000 A novel mechanism for skeletal resistance in uremia. Kidney Int. 58:753–761.
14. Brossard JH, Cloutier M, Roy L, Lepage R, Gascon-Barre M, D'Amour P. 1996 Accumulation of a non-(1–84) molecular form of parathyroid hormone (PTH) detected by intact PTH assay in renal failure: importance in the interpretation of PTH values. J Clin Endocrinol Metab. 81:3923–3929.
15. Lepage R, Roy L, Brossard JH, et al. 1998 A non-(1–84) circulating parathyroid hormone (PTH) fragment interferes significantly with intact PTH commercial assay measurements in uremic samples. Clin Chem. 44:805–809.

What Are "Normal" Testosterone Levels for Women?

To the editor:

The cross-sectional study by Laughlin *et al.* (1), comparing endogenous sex hormones levels with hysterectomy and oophorectomy status, chronological age, and years since menopause in older women, reinforces the possible adverse endocrine sequelae of oophorectomy in older women. However, we question some aspects of the data analysis and, hence, the clinical significance and interpretation of some of the said findings.

The inference from the manuscript is that, in women with intact ovaries, there is an increase in total testosterone levels after the time of menopause and with increasing age reaching premenopausal levels concomitant with falling androstenedione levels. That testosterone should rise while androstenedione falls is incongruous because adrenal androgen precursor levels fall linearly with age (2) and, following the menopause, peripheral conversion of androstenedione becomes a major source of circulating testosterone (3). We would appreciate the authors proposing a hypothesis for this incongruity.

The reason for adjusting total testosterone and bioavailable testosterone for body mass index (BMI) in postmenopausal women is not justified. Because weight may vary significantly with increasing age and years since menopause, and the authors suggest that sex hormone-binding globulin concentrations were inversely related to BMI, one is left to wonder if without adjustment for BMI whether any variation occurred. The authors do not report a relationship between either testosterone or androstenedione and BMI, making the need for the adjustment most curious. Adjustment for BMI is not clinically informative, because the absolute bioavailable circulating levels are the values of physiological significance in terms of direct androgen action and as precursors for extragonadal estrogen biosynthesis in tissues such as bone. Indeed, was a difference seen for bioavailable testosterone not adjusted for BMI? Non sex hormone-binding globulin-bound testosterone includes albumin-bound testosterone, which may account for up to 20% of total testosterone (4). In view of the age of the subjects, if one is to adjust for BMI, surely one should also adjust for variations in serum albumin. Caution in adjusting for covariates in reporting clinical findings has recently been highlighted (5). Presentation of the unadjusted data from this study would be very informative.

The authors report an assay sensitivity of 0.07 nmol/L for testosterone, however, standard RIAs for testosterone are notoriously inaccurate at the lower end of the female range. We are surprised that such distinct differences were observable in values below 0.5 nmol/L. Furthermore, the actual RIA used for measuring total testosterone is not stated. The most pronounced difference in total testosterone increase was reported to occur between the 6th and 7th decades, yet for the 6th decade there were only 29 women with intact ovaries and seven after oophorectomy. Hence, we caution a 35% increase on a mean value of less than 0.5 nmol/L in so few women becoming a generalizable fact. Furthermore, reporting of values adjusted for BMI gives us little feel for what the actual normal values were, and this is exacerbated in Table 2 in which values were adjusted for both age and BMI.

Although ovarian stromal hypertrophy and hyperplasia may sometimes persist or develop after menopause, probably secondary to elevated LH levels and individual sensitivity, resulting in increased testosterone production (6), other researchers have not found this to be the general rule (7). That ". . . levels in women more than 70 yr of age or 20 yr post menopause were comparable to premenopausal levels" is also questionable. In Fig. 3 in the article, the *dotted lines* indicate the mean testosterone level for premenopausal women as being between 0.6–0.7 nmol/L. However, according to Sinha-Hikim *et al.* (8), who defined the range of total and free testosterone levels during the normal menstrual cycle in healthy women, the mean testosterone level across the cycle was 1.20 ± 0.69 nmol/L and the mean free testosterone was 12.8 ± 5.59 pmol/L. These levels were measured using an equilibrium dialysis method, and the sensitivity of the total testosterone assay was increased to 0.008 nmol/L. The results from that study are sound and have been used as references in many other studies. Furthermore, these testosterone levels are in agreement with those reported in other literature (9, 10).

If we accept the data of Sinha-Hikim *et al.* (8) as reference levels, then the testosterone levels in the "intact postmenopausal women" in the study by Laughlin *et al.* (1) are, in fact, very low and do not actually increase back to premenopausal levels (1.20 ± 0.69 nmol/L). Although in *Results* the authors state (referencing a 1997 paper) these premenopausal values as being from the same laboratory as used for this study, they are inconsistent with the widely accepted range. Were these also BMI adjusted? Either way, the comparison is not meaningful. Of note, the actual citation for the testosterone levels for premenopausal women in Fig. 3 is that of an article by S. E. Bulun and E. R. Simpson, a study of levels of aromatase cytochrome P450 transcripts in adipose tissue of women. The bioavailable values reported are ~10-fold greater than the normal range for free testosterone. Reporting of free testosterone would have been far more meaningful.

The role of androgens in women is becoming increasingly more recognized and established. Certainly, the use of androgens, particularly testosterone, has been shown to influence life aspects, such as mood, women's general well being and restoration of sexual desire. However, there is limited data establishing normal androgen values for women of differing ages, to enable us to define those with "androgen deficiency." It is, therefore, necessary to highlight the incongruencies and shortcomings of the paper by Laughlin *et al.* (1), and the need for larger prospective studies to establish the variations in testosterone levels in women with age.

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References

1. Laughlin G, Barrett-Connor E, Kritz-Silverstein D, Von Muhlen D. 2000 Hysterectomy, oophorectomy, and endogenous sex hormone levels in older women: the Rancho Bernado Study. J Clin Endocrinol Metab. 85:645–651.
2. Zumoff B, Rosenfeld RS, Strain GW. 1980 Sex differences in the 24-hour mean

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plasma concentrations of dehydroisoandrosterone (DHA) and dehydroisoandrosterone sulfate (DHAS) and the DHA to DHAS ratio in normal adults. J Clin Endocrinol Metab. 51:330–334.
3. Judd HL, Lucas WE, Yen SSC. 1994 Effect of oophorectomy on circulating testosterone and androstenedione levels in patients with endometrial cancer. Am J Obstet Gynecol. 118:793–798.
4. Luthold WW, et al. 1993 Serum testosterone fractions in women: normal and abnormal clinical states. Metabolism. 42:638–643.
5. Assmann S, Pocock S, Enos L, Kasten L. 2000 Subgroup analysis and other-(mis)uses of baseline data in clinical trials. Lancet. 355:1064–1069.
6. Procope B. 1968 Studies on the urinary excretion, biological effects and origin of estrogens in postmenopausal women. Acta Endocrinol (Copenh). 135:1–86.
7. Labrie F, Belanger A, Cusan L, Gomez J-L, Candas B. 1997 Marked decline in serum concentrations of adrenal C19 sex steroid precursors and conjugated androgen metabolites during aging. J Clin Endocrinol Metab. 82:2396–2402.
8. Sinha-Hikim I, et al. 1998 The use of a sensitive equilibrium dialysis method for the measurement of free testosterone levels in healthy, cycling women and in human immunodeficiency virus-infected women. J Clin Endocrinol Metab. 83:1312–1318.
9. Judd HL, Yen SSC. 1973 Serum and androstenedione and testosterone levels during the menstrual cycle. J Clin Endocrinol Metab. 36:475–481.
10. Abraham GE. 1974 Ovarian and adrenal contribution to peripheral androgens during the menstrual cycle. J Clin Endocrinol Metab. 39:340–346.

Postmenopausal Testosterone

To the editor:

Drs. Davis and Tran raise important issues that can be summarized to three main areas: 1) the effect of adjusting results for body mass index (BMI); 2) the validity of the testosterone measurements and their relation to premenopausal levels; and 3) a plausible biological explanation for the findings.

1) The effect of adjusting results for BMI.

The relation between hormone levels and chronological and menopausal age was analyzed 1) without adjusting for covariates, 2) adjusting for BMI, and 3) adjusting for BMI, smoking, exercise, and alcohol consumption. In the interest of conserving space, only the BMI-adjusted results were presented. The association (or absent association) with age did not differ in unadjusted analyses, and the total and bioavailable testosterone levels were virtually identical to the BMI-adjusted values shown in the figures. The minimal impact of BMI on bioavailable testosterone in this study may reflect the narrow range of BMI among the Rancho Bernardo cohort (95% confidence interval, 23.8–24.5 kg/m^2), in whom BMI did not vary across age groups.

2) The validity of the testosterone measurements and their relation to premenopausal levels.

Total testosterone levels were measured by a RIA developed in the steroid research laboratory of Samuel Yen, M.D., during the 1970s using an antibody produced at the University of California–San Diego. An important feature of steroid hormone measurements in this laboratory is sample purification by solvent extraction and celite column chromatography before RIA. The testosterone assay sensitivity of 0.07 nmol/L is comparable with published values (0.03–0.05 nmol/L) for assays using similar sample purification steps (1–3). Because of the high sensitivity and specificity of this method, measured values are lower than direct serum measurements with commercial kits. However, the testosterone levels in this study were similar to levels in other large studies of postmenopausal women using comparable assay methods (1–3).

The authors also question the validity of the testosterone level cited for premenopausal women. Levels for postmenopausal women were compared with a recently published value (0.63 ± 0.23 nmol/L) from the Yen laboratory for 32 women sampled in the early follicular phase of the menstrual cycle (4). A 1979 study (5) using the same assay reported a 24-h mean level of 0.69 ± 0.04 nmol/L for early follicular phase women, therefore, the assay/laboratory has good internal consistency. (The 1973 Judd and Yen article cited by Davis and Tran used an earlier, less specific assay.) Although the sensitivity of the Sinha-Hikim total testosterone assay is very high, total testosterone levels were apparently measured directly in the dialysate. Equilibrium dialysis would not eliminate cross-reacting substances, which may account for higher (1.04 ± 0.76 nmol/L) early follicular phase testosterone levels with this assay compared with the Yen assay. In our view, steroid hormone assays may yield differing results for a variety of reasons. Direct comparisons are valid only when all samples are measured with the same assay techniques, as was the case in our paper.

Drs. Davis and Tran correctly point out that the finding of an increase in testosterone levels with postmenopausal aging was, in large part, based on lower levels among only 29 intact women in the 50- to 59-yr age range. As stated in the article, "although age-specific levels were based on relatively few women for the youngest decade, the similarity of hormone patterns after stratification by decade and by years since menopause supports the validity of the age-related associations." Among the intact women, 125 were less than 20, 136 were 20–30, and 119 were more than 30 yr menopausal. Testosterone levels were 25% higher among intact women more than 20 yr postmenopause compared with intact women less than 20 yr postmenopause.

3) A plausible biological explanation for the findings.

The increase in circulating testosterone levels with postmenopausal aging is likely to be ovarian in origin. Testosterone levels did not increase with age among the oophorectomized women, and the metabolism and interconversion of androgens in women is not altered after menopause (6). In recent studies, ovarian vein testosterone levels were related to the degree of stromal hyperplasia in 18 postmenopausal women (7) and correlated positively with age in 52 women aged 42–69 yr (8). Postmenopausal ovarian androgen production is thought to be at least partially gonadotropin driven. Gonadotropin receptors have been identified in the stroma of menopausal ovaries (9), and peripheral steroid levels in postmenopausal women increase after hCG stimulation (10) and decline after administration of long-acting GnRH agonists (11, 12). Thus, stimulation by elevated gonadotropins could account for increased ovarian testosterone synthesis in older women. Although postmenopausal gonadotropin levels tend to decline with advancing age (13), they remain well above premenopausal levels. It is possible that prolonged exposure to high gonadotropin stimulation may up-regulate ovarian responses to LH. To our knowledge, no published studies address this possibility.

The absence of a postmenopausal increase in androstenedione levels with age similar to the increase in testosterone is not surprising. As shown in previous studies and confirmed in this one, the postmenopausal ovary contributes 40% of circulating testosterone, but only 10–15% of circulating androstenedione. Thus, an increase in ovarian androstenedione production would be masked by the many-fold greater adrenal contribution.

Our conclusion that testosterone levels return to the premenopausal range in older, intact postmenopausal women needs clarification. The internal (unpublished) normal laboratory values for this assay are 0.72 ± 0.34, 1.02 ± 0.48, and 0.93 ± 0.38 nmol/L for the early follicular, mid-cycle, and luteal phases, respectively. Because testosterone levels do not undergo cyclic changes in postmenopausal women, our results suggest that older, as well as younger, postmenopausal women are relatively testosterone deficient when compared with younger cycling women, with levels similar to the early follicular phase. We appreciate this opportunity to expand and clarify our findings. Clearly, interpretation of cross-sectional data are subject to error. Prospective studies are needed to define with certainty the pattern of changing testosterone levels in older women.

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References

1. Hankinson SE, Willett WC, Manson JE, et al. 1998 Plasma sex steroid hormone levels and risk of breast cancer in postmenopausal women. J Natl Cancer Inst. 90:1292–1299.
2. Cauley JA, Lucas FL, Kuller LH, et al. 1999 Elevated serum estradiol and

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