

Recombinant Cell Ultrasensitive Bioassay for Measurement of Estrogens in Postmenopausal Women

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A recent analysis of data from nine studies provided convincing evidence that plasma estradiol measurements predict the risk of breast cancer in normal postmenopausal women. However, the median values detected by the various assays used in this study varied by 5-fold. These and other published data in normal postmenopausal women suggest that assays measuring low plasma estradiol concentrations suffer from problems of sensitivity, specificity, and precision. Availability of a practical, low-cost, specific, precise, and ultrasensitive estrogen assay might allow enhanced prediction of the risk of breast cancer and provide an objective means of selecting postmenopausal women for breast cancer prevention. A recombinant cell ultrasensitive bioassay (RCUB) for estrogen was recently validated for use in prepubertal children. We postulated that the RCUB might also prove useful for measurement of postmenopausal levels and designed the present study to examine this possibility.

Thirty normal postmenopausal volunteers provided blood samples for measurement of estrogen by RCUB and, for comparison, by RIA. The estrogenic activity measured by RCUB

[mean \pm SD, 11.9 \pm 10.9 pmol/liter (SI units, 3.23 \pm 2.96 pg/ml)] was significantly lower than estradiol levels measured by RIA [43.7 \pm 44.0 pmol/liter (11.9 \pm 12.0 pg/ml)] in our volunteer subjects ($P = 0.00001$). Nonetheless, plasma estradiol levels measured by bioassay were significantly correlated with the estrogenic activity measured by RIA ($r = 0.84$) and by gas chromatography/tandem mass spectrometry ($r = 0.85$). To obtain biological evidence of the validity of the RCUB, we related plasma estrogen levels to body weight and body mass index and found highly significant correlations ($r = 0.54$ and $r = 0.53$, respectively). Surprisingly, 28 of 30 postmenopausal women were found to have estrogen levels in the prepubertal range with the RCUB. The levels detected by RCUB were similar to those previously reported using an ultrasensitive but less practical yeast bioassay. These results provide validation for the RCUB in postmenopausal women and suggest that it might prove useful for selection of women for drug therapy to prevent breast cancer. (*J Clin Endocrinol Metab* 90: 1407–1413, 2005)

BREAST CANCER IS the second leading cause of cancer mortality among women in the United States, with 43,500 deaths yearly (1). The association between breast cancer and estrogen has been recognized for more than 100 yr. Sir George Beatson first demonstrated that bilateral oophorectomy resulted in the remission of breast cancer in premenopausal women (2). Subsequent evidence has implicated both endogenous and exogenous estrogen in the pathogenesis of breast cancer. Based on a variety of animal, epidemiological, and clinical studies, the International Agency for Research on Cancer has recently classified estradiol as a carcinogen (3).

Recent data from a pooled analysis of nine separate studies provided convincing evidence that measurement of plasma estradiol predicts the risk of breast cancer in postmenopausal women (4–14). However, the values reported with the different assays used in this pooled analysis were highly vari-

able, with median levels ranging from 21.6–100.9 pmol/liter (5.9–27.5 pg/ml) (4). A review of all published levels of estradiol in normal postmenopausal women additionally exemplifies the magnitude of variation among different assays (Refs. 4–25 and Fig. 1). When considering all of these reports, median estradiol levels varied by 13-fold, with the lowest value 7.3 pmol/liter (2 pg/ml) as measured by ultrasensitive yeast bioassay (USYB) (23).

The discordance among assays likely reflects difficulties inherent in measurement of estradiol at the low levels that circulate in postmenopausal women. Potential assay problems include lack of sensitivity, specificity, and precision. Major differences in methodology likely contribute to the variability. Notably, certain assays directly measure estradiol without prior purification, whereas others use various chromatographic separation techniques before detection with radiometric, chemiluminescence, or fluorescence techniques.

Lack of sensitivity may be the major limiting factor in the interpretation of estrogen measurements in postmenopausal women. Available estradiol assays can accurately detect premenopausal levels of 180–2200 pmol/liter (50–600 pg/ml) but are not sufficiently sensitive to detect postmenopausal estradiol levels of 3.7–37 pmol/liter (1–10 pg/ml). For example, the most sensitive RIA reported [sensitivity 5.1 pmol/liter (1.4 pg/ml)] could detect estradiol in only 110 of 222

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Abbreviations: BMI, Body mass index; ER, estrogen receptor; ERE, estrogen-responsive element; GC/MS/MS, gas chromatography, tandem mass-spectrometry; RCUB, recombinant cell ultrasensitive bioassay; USYB, ultrasensitive yeast bioassay.

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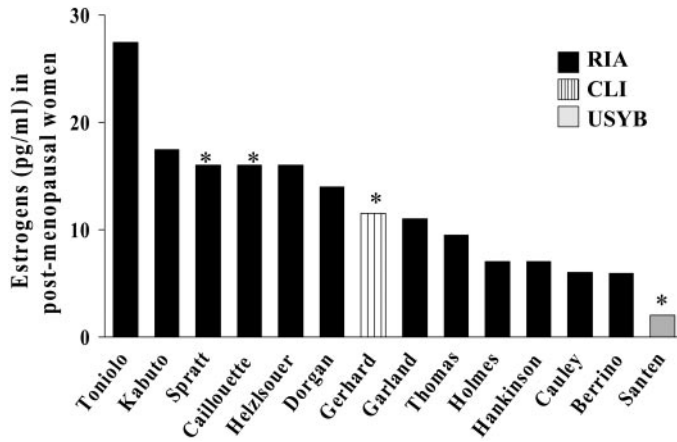


FIG. 1. Review of estrogen levels previously reported in normal, postmenopausal women using several different assay methods. The asterisks above the bars indicate that the values are means, all others are median values. The data for nine of the bars are taken from a study pooling data from nine previous reports (4). The other bars represent individual reports including Spratt *et al.* (24), Caillouette *et al.* (16), Gerhard *et al.* (51), Holmes *et al.* (20), and Santen *et al.* (23). To convert picograms per milliliter to picomoles per liter, multiply values by 3.67. CLI, Chemiluminescence assay.

(49.6%) samples from postmenopausal women with osteoporosis (26). Other less sensitive assays detected only 2 of 26, 5 of 26, and 0 of 222, respectively (27–29). Specificity also seems to be a problem because the difference between median levels of 101 pmol/liter (27.5 pg/ml), as reported in one assay (10), and 7.3 pmol/liter (2 pg/ml), measured in another (23), likely represents cross-reactivity with substances other than estradiol.

Several years ago, a USYB was developed to circumvent these problems and to allow measurement of estradiol in prepubertal girls and boys (18). With this assay, one could detect the difference between estradiol levels of 2.2 pmol/liter (0.6 pg/ml) in prepubertal girls and 0.29 pmol/liter (0.08 pg/ml) in prepubertal boys (30). However, this ultrasensitive bioassay, although highly sensitive and specific, is very labor intensive. It involves culturing of yeast cells, frequent retransfection of reporter constructs, drawing of blood in containers without rubber stoppers, extraction of serum, and a minimum of 48 h to perform the assay. For these reasons, the yeast assay has been set up and validated in the laboratory of only one investigator.

Recently, a new method named the “recombinant cell ultrasensitive bioassay” (RCUB), with a sensitivity of less than 3.67 pmol/liter (1 pg/ml), was developed to measure total biologically active estrogen levels in prepubertal children (25, 31). In this assay, HeLa cells that do not naturally express estrogen receptors (ERs) were stably transfected with human ER α along with an estrogen-responsive element (ERE) in the promoter region of the luciferase reporter gene. This assay is more sensitive than radioimmunoassay- and chemiluminescence-based assays, does not require serum extraction, and has been validated for use in children.

Three factors provided an impetus to validate the RCUB for use in postmenopausal women: 1) the recent report that plasma estradiol levels correlate with breast cancer risk; 2) the high variability among other commonly used assays (4);

and 3) the lack of sufficient sensitivity of RIA and chemiluminescence assays. In the current study, we measured plasma estrogen levels by RCUB in 30 postmenopausal women and compared the results both with a sensitive RIA and with a gas chromatography, tandem mass-spectrometry (GC/MS/MS) assay (32). The RCUB detected 3-fold lower median levels of estrogen than RIA and somewhat lower than with the GC/MS/MS assay. The levels correlated positively with weight and body mass index (BMI). These data validate the RCUB for use in postmenopausal women and lay the groundwork for a test that might better predict the risk of breast cancer in these patients.

Subjects and Methods

Review of published estradiol assays

After a literature search on Medline, we selected studies in which plasma estradiol levels were measured in normal postmenopausal women. Studies were excluded if women were selected on the basis of osteoporosis, hypoestrogenic state, severe vaginal atrophy, presence of breast cancer, and age over 70 yr (19, 21, 22, 26–29, 33). Median levels from these studies are shown when available and mean levels (signified by asterisk on Fig. 1) when not. The major database is taken from the collaborative study reporting pooled information from nine separate studies (4). The mean ages and BMIs from the collaborative estrogen study (4) were very similar to those in the patients reported here. For example, in the nine pooled studies, the mean age ranged from 58.1–71.8 yr vs. 64.8 \pm 8.8 (SD) yr in the current study. The mean BMIs in the pooled studies ranged from 22.3–26.6 kg/m² and were only slightly higher in the current study at 27.8 \pm 7.4 (SD) kg/m².

Subjects

Thirty women without a history of breast cancer volunteered to enter the study. They were considered postmenopausal if they met one of the following criteria: 1) regular menses had ceased for at least 12 months before study entry; 2) the subject had undergone bilateral oophorectomy; and 3) the volunteer was more than 57 yr of age if status post hysterectomy and at least one ovary was present (or the status of the ovaries was unknown). Exclusion criteria included the use of hormone therapy including shots, pills, or patches within the past 3 months before study entry. All subjects were recruited at the University of Virginia Clinics and underwent a complete physical examination.

Approval by the Human Investigation Committee (Institutional Review Board)

After this study was designed, the protocol was submitted to the Human Investigation Committee at the University of Virginia, and written approval was obtained. The University of Virginia Human Investigation Committee ensures that all appropriate measures regarding the treatment of human subjects are followed. Written informed consent, using the consent form approved by the Human Investigation Committee, was then obtained from each volunteer by one of the investigators (S.W.) before entering the subjects into the study.

Blood collection and serum separation

A single sample was collected into dry glass Vacutainer tubes. After clotting and centrifugation, serum was removed, transferred to secondary glass tubes, and frozen at -20 C until assay.

Estrogen assays

The RCUB was recently validated for use in prepubertal children, and the method was fully described (25, 31). Briefly, HeLa cells (a human uterine cervical carcinoma cell line) were stably transfected with an expression plasmid of human ER α and a luciferase reporter gene driven by an ERE. A key element in this assay is that the standard curve for each sample consists of stripped serum from the same patient, to which

incremental amounts of estradiol are added. A total of 800 μl of each patient's serum are stripped to remove steroids by incubation with 30 mg of C18 Oasis HLB batch for 10 min, followed by centrifugation (13,000 rpm, 10 min), and supernatant recovery recovered. A known amount of estradiol is added to the stripped serum to obtain a set of estradiol standards, with concentrations ranging from 10^{-12} to 10^{-7} M. It should be noted that a standard curve is individually constructed for each sample with that patient's stripped serum to eliminate the variability due to serum components. The stripped serum containing incremental amounts of estradiol and nonstripped serum is incubated for 4 h at 37 C with the transfected cells to permit an equilibrium between free estrogens and estrogens bound to SHBG. The culture medium is removed at the end of incubation with the test compounds. Luminescent buffer (DMEM without phenol red, 3×10^{-4} M luciferin) is then added to each well. Immediately afterward, luciferase activity is measured using a Wallac MicroBeta Trilux luminometer (PerkinElmer, Boston, MA). The intraassay and interassay coefficients of variation did not exceed 10% and 20%, respectively (25, 31).

RIA

Plasma estradiol measurement involved a previously published and highly sensitive RIA, used previously to detect the level of estradiol suppression in women receiving aromatase inhibitors (34). This assay is maximized for sensitivity based on the use of a high-affinity antibody, extraction of 4 ml of serum, and the use of ^{125}I -labeled estradiol tracer. Specificity is enhanced by purification on a Celite column. This assay can distinguish 18 pmol/liter (5 pg/ml) of estradiol from blank with 95% confidence limits. The coefficient of variation is 4.3% within assay and 11.4% between assay. This assay correlates with the GC/MS/MS assay with an *r* value of 0.98.

GC/MS/MS assay

Estradiol was measured by GC/MS/MS by an assay performed by Taylor Technology, Inc. (Princeton, NJ), according to a method presented in abstract form (32). Deuterated estradiol was added to 1.0-ml samples of unknown serum to provide assessment of recovery. Estradiol and the stable isotope internal standards were extracted using Bond Elut Certify solid-phase cartridges. Estradiol was eluted from the cartridges with ethyl acetate. The analytes underwent two separate derivatizations: 1) reaction with pentfluorobenzoyl chloride; and 2) reaction with *N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide. The derivatized analytes were separated by gas chromatography using a DB-17 fused silica capillary column and detected by tandem mass spectrometry using negative ion chemical ionization. The instruments included a Finnigan MAT mass spectrometer and a Varian 3400 gas chromatography system. The limit of detectability was 2.2 pmol/liter (0.6 pg/ml) when using a 1.0-ml sample. The precision was 8.44% within assay and 7.88% between assay at a concentration of 6.6 pmol/liter (1.8 pg/ml); 1.89% within assay and 3.72% between assay at 90 pmol/liter (24.6 pg/ml); and 2.66% within assay and 2.7% between assay at a concentration of 223 pmol/liter (60.8 pg/ml).

Statistics

All data were expressed as mean \pm SD. Comparisons between groups were made using the two-tailed Student's *t* test. Differences were considered significant at $P < 0.05$. Standard regression analyses were used to determine the statistical significance of correlations between estrogen levels and body weight, BMI, and age. All values are expressed as pg/ml to conform to the data previously published from multiple sources. To convert from picograms per milliliter to picomoles per liter, multiply by 3.67.

Results

Patient characteristics

The 30 postmenopausal women who volunteered for this study had a mean age of 64.8 ± 8.8 yr (mean \pm SD). Twenty subjects were Caucasian (67%), and 10 were African-American

(33%). The average weight and BMI were 72.7 ± 19 kg and 27.8 ± 7.4 kg/m², respectively.

Basal estrogen levels and comparison between assays

Measurement of estrogen by RCUB revealed levels averaging 11.9 ± 10.9 pmol/liter with median of 7.7 pmol/liter (3.23 ± 2.95 pg/ml with median of 2.1 pg/ml), whereas those measured by RIA were statistically significantly higher [mean 43.7 ± 44.0 pmol/liter, median 34.4 pmol/liter (mean 11.9 ± 12.0 pg/ml, median 9.4 pg/ml)] ($P < 0.0001$). Levels measured in 28 of 30 postmenopausal women were within the range previously reported for prepubertal girls [*i.e.* 13.0 ± 8.1 (SD) pmol/liter (3.53 ± 2.2 SD pg/ml)] (25). Figure 2 illustrates that nearly all values were higher with the RIA method than by RCUB (*i.e.* 28 of 30), and none were lower. Figure 3A shows that levels measured with RCUB were significantly correlated with those measured by RIA ($r = 0.84$, $P < 0.001$). The values obtained on the same samples by the GC/MS/MS assay were 26.6 ± 17.7 pmol/liter [mean \pm SD (7.26 ± 4.82 pg/ml)], and the correlations are shown in Fig. 3B.

Correlation of biological parameters with estrogen levels

To gain biological evidence of the validity of the RCUB, we correlated estrogen levels with BMI and body weight—two parameters reported previously to influence plasma estradiol levels. As shown in Fig. 3, C and D, the RCUB results correlated significantly with BMI ($r = 0.53$; $P < 0.002$) and weight ($r = 0.54$; $P < 0.001$). The RIA correlated to a somewhat greater extent at $r = 0.70$ and $r = 0.74$, respectively (data not shown). The correlation of age with estrogen levels was negative with both of the assay methods (-0.04 and -0.20 , respectively), but the association was relatively weak.

Discussion

Recent studies have demonstrated that plasma estrogen levels can predict the risk of breast cancer in postmenopausal women, but median values between assays were highly vari-

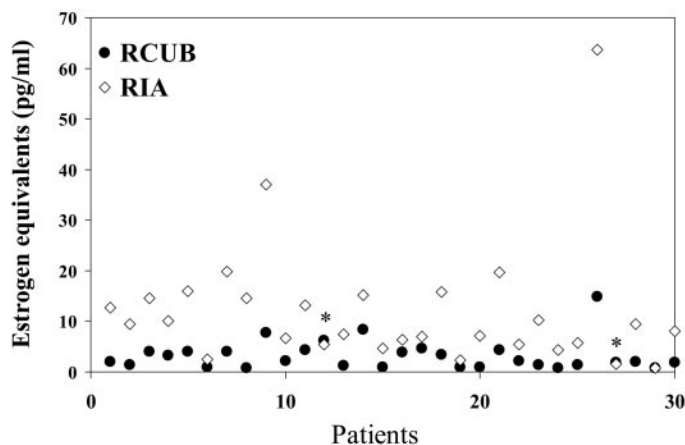


FIG. 2. Distribution and comparison of estrogen levels between the RCUB method and RIA assays in 30 postmenopausal women. To convert picograms per milliliter to picomoles per liter, multiply values by 3.67.

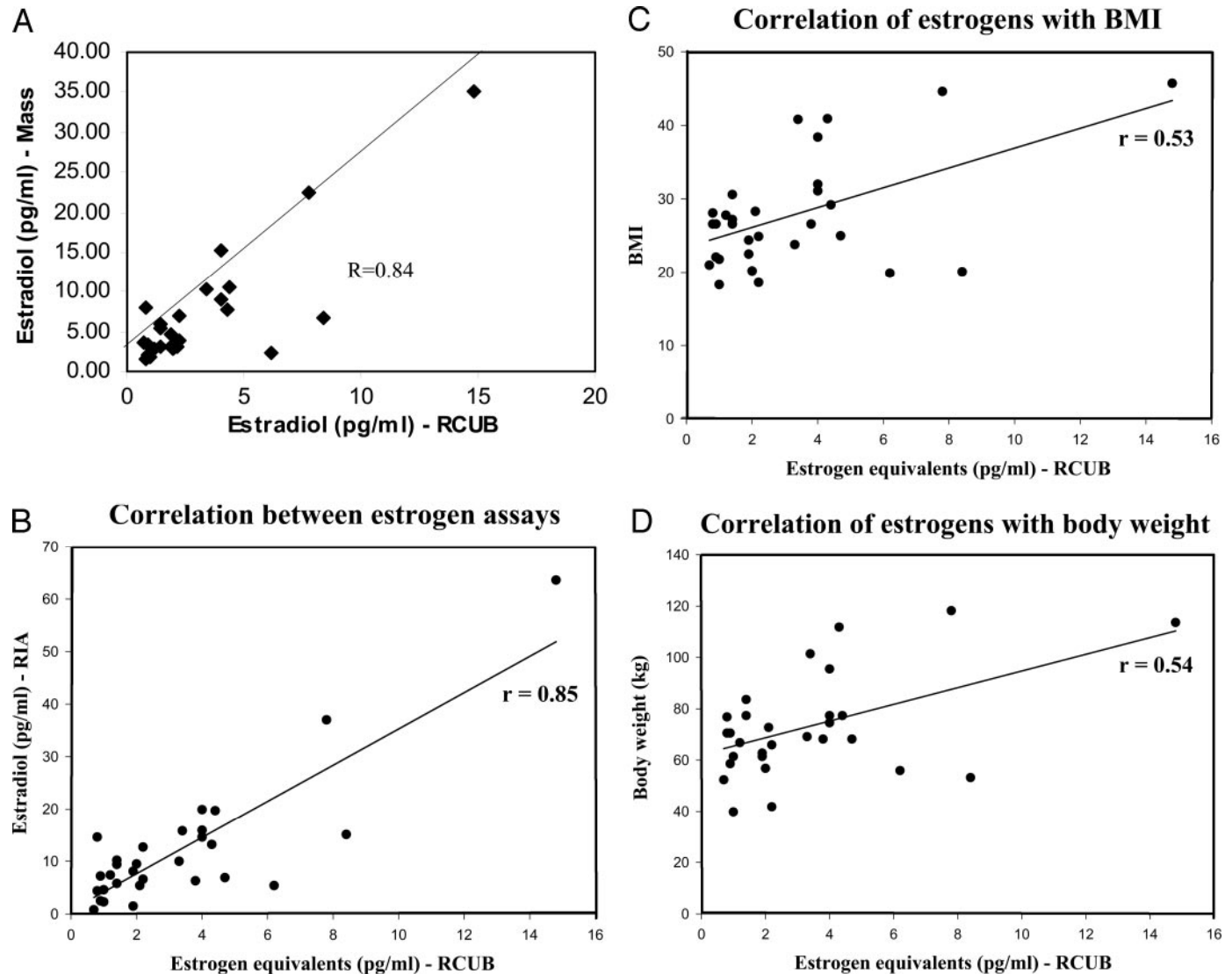


FIG. 3. A, Correlation of RCUB and RIA assays. B, Correlation of RCUB and GC/MS/MS assay. C, Correlation of estrogens measured by RCUB and BMI. D, Correlation of estrogens measured by RCUB with body weight in kilograms. To convert picograms per milliliter to picomoles per liter, multiply values by 3.67.

able (4). A review of published estradiol assays suggests major problems with sensitivity and specificity as an explanation for the widely divergent median values observed among normal postmenopausal women (4–15, 17–26, 31, 34–36). These considerations prompted us to evaluate an assay for postmenopausal women that was initially developed as an ultrasensitive assay for the measurement of estradiol in prepubertal children (25). This assay, called the RCUB, exhibited a high level of sensitivity and precision when used in children. In the present study, the data obtained with the RCUB in postmenopausal women correlated well with RIA measurements ($r = 0.85$), with GC/MS/MS measurements ($r = 0.84$), as well as with BMI ($r = 0.53$) and body weight ($r = 0.54$). Mean levels [11.7 pmol/liter (3.2 pg/ml)] were in the range reported previously using the USYB in normal postmenopausal volunteers [7.3 pmol/liter (2 pg/ml)] as well as in postmenopausal breast cancer patients [7.2 pmol/liter (1.95 pg/ml)] (23, 34).

The levels detected by RCUB were 2- to 13-fold lower in normal postmenopausal women than reported previously with either radiometric assays, RIAs, or chemiluminescent assays and 3-fold lower than with a direct comparison with an RIA, as described in this report. The finding of substantially lower plasma estrogen levels with the USYB and RCUB methods is not totally surprising. The recently developed ultrasensitive, two-site radiometric assays for LH, FSH, TSH, PTH, and GH detect lower mean levels than less sensitive one-site methods (37–49). The lower reported values likely represent greater specificity as well as improved sensitivity. This conclusion is best illustrated by a historical analysis of PTH assays. Initial RIAs recognized the midmolecule of PTH and detected levels of 200–400 ng/ml in normal subjects (43). The measurement of intact PTH with two-site radioimmuno-radiometric assays reported levels of 10–65 pg/ml (44). Later, it was found that intact assays measured both the inactive 7–84 form of PTH as well as the active 1–84 form

(45–47). With an assay designed to measure the latter, normal values fell to 5–45 pg/ml.

On the basis of the analogy of PTH assays, early RIA methods in postmenopausal women detected high levels of estradiol ranging from 73–101 pmol/liter (20–27.5 pg/ml) (10, 14, 21, 24). On the other hand, current RIAs with better sensitivity detect mean levels of 29 pmol/liter (8 pg/ml) (34), whereas the ultrasensitive bioassays report levels approximating 7.3 pmol/liter (2 pg/ml) (23, 34). Although there is no definitive proof for our conclusions regarding sensitivity and specificity (as for PTH), the available evidence suggests that the RIA- and chemiluminescence-based assays lack sufficient sensitivity and specificity for the true measurement of estradiol in postmenopausal women. This conclusion is additionally supported by the fact that the GC/MS/MS assay measured substantially lower mean levels than the RIA used in this study. Furthermore, a study to be published demonstrates that the GC/MS/MS assay detected lower mean estradiol levels than in six of seven highly sensitive and specific RIA (Lee, J., S. Cummings, M. Dowsett, and R. J. Santen, personal communication, in preparation).

The data reported with the RCUB assay have been verified with the “gold standard” GC/MS/MS assay, and correlations of $r = 0.84$ were found. The mean values with the GC/MS/MS assay are slightly higher than with the RCUB but much lower than with RIA methods. It should be noted that the results obtained with the RCUB are very similar to those measured in postmenopausal women with the yeast recombinant bioassay (18, 23, 34). Therefore, it is possible that the two bioassays are more specific for estradiol than even GC/MS/MS and that the latter assay measures a minor amount of nonestradiol material. Because GC/MS/MS is considered the gold standard assay, this tentative conclusion cannot be experimentally verified at the present time.

An appropriate question at the present time is whether the RCUB is sufficiently practical for widespread use as a means to predict the risk of breast cancer. It is clear that the USYB has been difficult to perform and requires frequent retransfection of yeast with the ER and ERE (18, 34). On the other hand, the RCUB method uses cells that are stably transfected and can be used long term (25, 31). With the establishment of a centrally located reference laboratory, the RCUB assay should be practical, available, and suitable to implement on a wider scale. Ultimately, the cost, applicability, practicality, and availability of the GC/MS/MS assay will need to be compared with that of the RCUB to determine which assay might be preferable for wide-scale application. The cost of the GC/MS/MS assay for estradiol used in this study exceeded that for the RIA method. However, with an increased volume of samples to be measured, the volume/cost relationship regarding the GC/MS/MS assay might allow the costs to approach that for RIA. An additional consideration is that the metabolism of estradiol to other products and the metabolic clearance rate of estradiol might differ in postmenopausal as opposed to premenopausal women, both as a result of aging and of the confounding effects of taking other medications. This could lower the circulating levels of estradiol relative to actual production rates. Measurement of estradiol metabolites with GC/MS/MS might be useful in future studies to examine these issues.

The RCUB seems to be measuring estradiol exclusively, although this has not been directly determined experimentally. It has been shown that the transfected ER and ERE constructs respond not only to estradiol but also to estrone and several environmental estrogens (25, 31). However, much larger concentrations than present in plasma of postmenopausal women are needed to stimulate the reporter gene. Estradiol induces a one- to two-log greater response in the ER/ERE reporter system than does estrone or estriol. Environmental estrogens and estrone-sulfate produce even weaker responses. The major proof that the assay is measuring estradiol exclusively is that the results are similar to, if not slightly lower than, the levels measured by the GC/MS/MS techniques. On the basis of these data, it would seem that the RCUB measures predominantly estradiol itself in postmenopausal women. Under other circumstances, the RCUB assay can be used to measure environmental estrogens, but not under the conditions used for postmenopausal samples (31).

The RCUB, GC/MS/MS, and yeast assays detect lower levels of estradiol in plasma than do most RIAs. It is appropriate to consider what objective evidence provides biological validation that these levels are correct. For the yeast assay, substantial biological evidence is available. Estradiol levels are higher in prepubertal girls aged 7.5 ± 2.1 yr [mean levels, 2.2 ± 0.22 pmol/liter (0.6 ± 0.6 pg/ml)] than in boys aged 9.5 ± 2.1 yr [0.29 ± 0.73 pmol/liter (0.08 ± 0.2 pg/ml)] (18, 30). The levels are higher in older prepubertal girls with an age range of 5–12 yr [12.4 ± 10.6 pmol/liter (3.4 ± 2.9 pg/ml)] compared with girls with Turner’s syndrome [6.2 ± 4.4 pmol/liter (1.7 ± 1.32 pg/ml)], and the levels increase significantly with pubertal stage, age, bone age, and time from pubertal onset in boys (30). In postmenopausal women with breast cancer, basal levels of estradiol averaged 7.2 pmol/liter (1.95 pg/ml) and fell to 0.26 pmol/liter (0.07 pg/ml) during treatment with the potent aromatase inhibitor, letrozole (34). This degree of suppression paralleled the 95% suppression detected by the more direct, isotopic kinetic (ρ) value technique. It should be noted that direct comparisons reveal lower values with the ultrasensitive assay as compared with RIA. In the present study, mean values were 11.7 pmol/liter (3.2 pg/ml) with the RCUB and 40 pmol/liter (11 pg/ml) with RIA. With the USYB, mean estradiol levels were 7.2 pmol/liter (1.95 pg/ml) compared with 29 pmol/liter (8 pg/ml) by RIA (34).

The use of sensitive plasma estradiol measurements to predict response to a breast cancer prevention therapy would seem to be a logical next step (4). This measurement could provide information analogous to that of low-density lipoprotein cholesterol for the prediction of heart disease and selection of patients for statin therapy. Aromatase inhibitors are clinically available to lower estrogen production, and preliminary results suggest that the aromatase inhibitors prevent breast cancer (50). One would predict that the ability to detect very low levels of estradiol would substantially enhance the power of predicting the risk of breast cancer. By analogy, assays capable of detecting low-density lipoprotein cholesterol levels less than 150 mg/dl would be more predictive of heart disease than those with a cut-off of sensitivity of 150 mg/dl. Estradiol assays with a detection limit of 3.7

pmol/liter (<1 pg/ml) should provide more powerful predictive information than those with a detection limit of 5–10 pg/ml. With the availability of an ultrasensitive assay, these considerations can now be experimentally tested.

The concept of using a plasma estradiol measurement clinically to screen for the risk of breast cancer was supported by a recent prospective study of women entering a raloxifene trial for osteoporosis (33). In the placebo group, women with estradiol levels greater than 9.9 pmol/liter (2.7 pg/ml) had a 6.8-fold higher rate of breast cancer development (3.0% per 4 yr: 95% confidence interval, 1.8–4.1%) than that of women with undetectable estradiol levels (0.6% per 4 yr: 95% confidence interval, 0–1.1; $P = 0.005$ for trend). More importantly, the women in the highest quintile of estradiol levels experienced the greatest reduction in breast cancer incidence (76% reduction) in response to raloxifene compared with those in the lowest quintile (no reduction). These results additionally support the use of plasma estradiol levels for this purpose and the need to use only ultrasensitive assays.

Because of the least-squares correlation analysis method, the strength of correlations of estradiol with several parameters is unduly influenced statistically by patients with high estradiol levels. Exclusion of these high values considerably lowers the strengths of the correlations. However, even after eliminating these high values, the correlations between assays and with BMI and body weight are similar to those found using other assay methods (Lee, J., and S. Cummings, manuscript in preparation). Larger sample sizes, which include women with a wider range of body mass indices and plasma estradiol levels, should strengthen the correlations found in this study. Planned studies will involve much larger sample sizes, and attention will be directed toward obtaining women with a wider range of body mass indices.

In summary, we report the use and validation of a RCUB estradiol method for use in postmenopausal women. The assay meets currently accepted criteria for precision and demonstrates a correlation with biological parameters. The RCUB method seems to have greater applicability than the USYB because of the stability of transfected cells and the ability to measure estradiol in unextracted serum. The assay is, however, not completely specific for estradiol and seems to measure other biologically active estrogens as well. However, this may be advantageous if one wishes to determine the total level of biologically active estrogen in plasma. The surprisingly low estrogen values obtained with the RCUB methods favorably compare to levels found with an USYB.

Acknowledgments

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