

Measurement of steroid sex hormones in serum: a comparison of radioimmunoassay and mass spectrometry

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Abstract

Concern has been raised about the adequacy of radioimmunoassays to measure steroid sex hormones in population studies. We compared steroid sex hormone measurements in serum by radioimmunoassay with mass spectrometry. Four male and four female serum pools with known relative concentrations of steroid sex hormones were measured multiple times by both methods. Because measurements are expected to increase linearly with concentration for each sex, we examined whether the linear regressions of hormone measurements on concentration were the same for radioimmunoassay and mass spectrometry. Estradiol, estrone, androstenedione, testosterone, and dehydroepiandrosterone sulfate were measured in female pools; testosterone, dihydrotestosterone, androstenedione, and dehydroepiandrosterone sulfate were measured in male pools. Regression slopes for radioimmunoassay and mass spectrometry measurements were comparable for all hormones except androstenedione, which had a steeper slope when measured by mass spectrometry ($P \leq 0.02$). Intercepts for radioimmunoassay and mass spectrometry were similar and close to zero for estradiol, androstenedione, dehydroepiandrosterone sulfate, and in male samples, testosterone. For testosterone in female samples, estrone, and dihydrotestosterone, radioimmunoassay and mass spectrometry intercepts differed significantly. Standard deviations of individual measurements by radioimmunoassay and mass spectrometry differed by hormone and serum concentration; neither method consistently measured hormone concentrations with less variability. Our findings suggest that although absolute concentrations may differ for some hormones, radioimmunoassay and mass spectrometry can yield similar estimates of between subject differences in serum concentrations of most steroid sex hormones commonly measured in population studies. Relative power of studies using radioimmunoassay and mass spectrometry will depend on the hormones measured and their serum concentrations. © 2002 Elsevier Science Inc. All rights reserved.

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1. Introduction

Sex hormones play a key role in regulating growth, maturation, and reproduction and are believed to be of etiologic importance in several chronic diseases including breast and

prostate cancers, osteoporosis, and cardiovascular disease. Concerns have been raised, however, about the reliability and validity of steroid sex hormone measurements in biologic specimens using routine radioimmunoassay (RIA) techniques and the effect laboratory error may have on results of epidemiologic investigations [1–3]. Studies performed to evaluate the reproducibility of these assays generally have found that there is considerable variation in results from different laboratories, but measurements from a single laboratory are sufficiently reproducible to compare individuals [4,5]. The one study that attempted to evaluate the validity of these measurements used serum samples of known dilutions and compared the observed (measured) to the expected (calculated) hormone concentrations in the samples [5]. With the exception of estrone, mea-

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sured steroid sex hormone concentrations were reasonably close to the expected values, suggesting that valid comparisons of serum sex hormone concentrations among individuals can be made when all measurements are performed in the same laboratory. Differences between laboratories, however, preclude comparisons across laboratories for some steroid sex hormones.

These earlier studies sent panels of serum samples to multiple laboratories and compared results obtained on RIA performance among laboratories. We compared RIA measurements of steroid sex hormones in serum performed by a single laboratory for quality control of a clinical trial with those obtained by mass spectrometry (MS). The study used for this purpose was the Hormone Ancillary Study to the Dietary Intervention Study in Children (DISC). DISC was a multi-center randomized clinical trial conducted by the National Heart, Lung, and Blood Institute to evaluate the efficacy of a fat-modified diet to lower low-density-lipoprotein cholesterol in children and the safety of this diet to promote growth and development [6]. The Hormone Ancillary Study was conducted by the National Cancer Institute to evaluate the effect of the intervention on steroid sex hormones in boys and girls. Masked quality control samples were included with each batch of DISC serum samples to monitor RIA performance. Steroid sex hormone concentrations in quality control samples were also measured by gas chromatography–mass spectrometry (GC/MS) or high pressure liquid chromatography–mass spectrometry (HPLC/MS) at a separate laboratory.

2. Experimental procedures

To approximate the expected steroid sex hormone concentrations in DISC participants, who ranged from 8 to 18 years old over the course of the study, plasma collected from adults was diluted with plasma that was stripped of steroids by charcoal. Up to 500 ml of plasma in anticoagulant citrate dextrose solution was collected from healthy adult male (20–35 years old) and female (18–35 years old) volunteers between 8:00 and 10:00 am following a 12 h fast and immediately frozen at -80°C . The women all had regular menstrual cycles, were not pregnant, lactating, or taking oral contraceptives, and were between days 21 and 24 of their menstrual cycles on the day plasma was drawn.

Plasma from male volunteers, and separately from female volunteers, was pooled and defibrinated by Valley Biomedical Inc. (Winchester, VA) using Pentex™ bovine thrombin (Miles Inc., Kankakee, IL). The serum was filtered and part of each serum pool was then stripped of steroids, including steroid sex hormones, with charcoal using proprietary methods. To simulate the normal ranges of steroid sex hormone levels in DISC participants, the female and male serum pools were diluted with the same-sex stripped pool at two ratios each. Dilution ratios (unstripped:stripped) were 1:3 and 1:11 for females and 1:2 and 1:9 for males. A single individual measured all serum volumes used for dilutions,

and a second individual independently checked all volumes before mixing. Undiluted serum and serum from each dilution pool was aliquoted in glass vials and stored at -80°C until analyzed. Steroid sex hormones measured by RIA between January and August 1996 and by MS in July and October 1996 are compared in the current analysis.

Samples from serum pools were measured by RIA for quality control of steroid sex hormone assays performed as part of the DISC study. Quality control samples were labeled so as to be indistinguishable from DISC participants' samples and were randomly distributed among participants' samples within each assay batch. DISC female and male participants' serum samples from baseline, year-1, and year-3 clinic visits were analyzed separately in batches of size 50. Each assay batch included six quality control samples comprised of three replicates from each of two sex-specific serum pools. Because steroid sex hormone concentrations were expected to be lower in serum from earlier DISC visits when participants were younger, quality control samples from the two sex-specific pools of diluted serum were included in assay batches from baseline and year-1 visits, whereas quality control samples from serum pools with dilution ratios of 1:3 for females and 1:2 for males and from the undiluted pools were included in assay batches from year-3 visits. Samples from females were assayed for estradiol, estrone, androstenedione, and dehydroepiandrosterone sulfate (DHEAS) at all visits, and for testosterone at the year-3 visit only. Samples from males were assayed for testosterone, androstenedione, and DHEAS at all visits, and for dihydrotestosterone (DHT) at year-3 visits only. Because quality control samples from different dilution pools were measured at the three visits and all hormones were not always measured, the number of replicate steroid sex hormone measurements in serum from the six quality control pools varied. Three samples each of the male and female charcoal stripped serum used in making dilutions were measured by RIA separately.

All RIAs were performed by Esoterix Endocrinology, Inc. (Calabasas Hills, CA) using standard procedures. Estradiol and estrone were measured using a modification of the procedure developed by Wu and Lundy [7]. Serum samples were extracted with hexane:ethyl acetate, 80:20 (vol/vol). The extract was then washed with dilute base, concentrated and chromatographed on Sephadex LH20 micro columns (Sigma, St. Louis, MO). Estradiol and estrone were specifically eluted using benzene:methanol, 85:15 (vol/vol). Estradiol was quantified by RIA in duplicate using antiserum raised to an estradiol-6-oxime-BSA conjugate and estrone was quantified using antiserum raised to an estrone-6-oxime albumin conjugate. Androstenedione was first extracted from serum with hexane:ethyl acetate, 99:1 (vol/vol). The extract was then separated from the aqueous phase by centrifugation (2200 g for 2 min at room temperature), and aliquots were evaporated to dryness prior to quantification by RIA in duplicate using antiserum raised to an androstenedione-6-thioether-BSA conjugate. Testoster-

one was measured using a modification of the procedure developed by Furuyama et al. [8]. Samples were extracted with hexane:ethyl acetate, 90:10 (vol/vol), and the extracts were applied to aluminum oxide micro columns. The columns were washed with hexane containing 0.55% ethanol, and testosterone was specifically eluted using hexane containing 1.4% ethanol. Testosterone in eluates was quantified in duplicate by RIA using antiserum raised to a testosterone-3-oxime-BSA conjugate. To measure DHT, serum samples were first extracted with seven volumes of hexane:ethyl acetate. Extracts were then evaporated to dryness and re-dissolved in potassium permanganate to oxidize steroids containing conjugated ketones. DHT was then selectively re-extracted. Duplicate aliquots of each purified sample were measured by RIA using antiserum raised to a DHT-3-oxime-BSA conjugate. DHEAS was measured as DHEA after enzymolysis of the DHEAS. DHEA was quantified by RIA in duplicate using antiserum raised to dehydroepiandrosterone-7-oxime-BSA conjugate.

GC/MS was used to measure estradiol, estrone, androstenedione, testosterone, and DHT using an adaptation of a previously published method [9]. Non-labeled reference standards estradiol, estrone, testosterone, and DHT were purchased from Sigma (St. Louis, MO) and androstenedione was purchased from Steraloids (Wilton, NH). The Sigma reference standards were pre-weighed and were dissolved accordingly. Androstenedione was weighed on a Cahn electronic balance. The final concentration for all solutions was 10 mg/ml. Labeled steroids used were [16,16,17] $^2\text{H}_3$ estradiol (Merck, Darmstadt, Germany); [3,4] $^{13}\text{C}_2$ estrone (Euroisotop, Saint-Aubin Cedex, France); [7,7] $^2\text{H}_2$ androstenedione (synthesized by Dr. S. Wudy, Mass Spectrometry Facility, Children's Hospital Oakland Research Institute, Oakland, CA); [16,16,17] $^2\text{H}_3$ testosterone (Sigma, St. Louis, MO); [16,16,17] $^2\text{H}_3$ DHT (custom made by Sterling Winthrop Pharmaceuticals Research Division, Rensselaer, NY). Solutions were made following weighing of each crystalline steroid.

Mixtures of the reference standards and the labeled steroids were prepared to approximate the expected steroid concentrations in a 1:1 ratio for each of the six serum pools and the charcoal stripped serum that was used in making dilutions. These solutions were analyzed by the GC/MS SIM method described below to measure the response factor for each steroid relative to its internal standard. The response factors were essentially unity.

Steroids in each serum pool were quantified as follows. A solution of labeled internal standards was added to each sample (2 ml) to approximate each analyte steroid in a 1:1 ratio. Different standard mixtures were used for each pool, dependent on their predicted steroid concentrations. The serum samples were extracted twice with 4 ml methylene chloride/isooctane (1:2, vol/vol). The dried organic layers were taken up in 3 ml water and extracted using a C18 cartridge (Sep-pak, Waters Assoc.). The steroids were eluted with methanol which was dried under nitrogen. The

steroids were derivatized with pentafluoropropionic anhydride (PFPA) to form PFP derivatives of hydroxyls and 3-carbonyls. This was an adaptation of an earlier published method, which used heptafluorobutyric derivatives that had too great a mass for analysis by the current GC/MS instrument [9]. The extracts were dissolved in 100 μl acetonitrile and 25 μl PFPA was added. After heating for 15 min at 60°C, the derivatives were transferred into microvials for GC/MS injection. The samples were injected into a 15 m DB-1 column that interfaced with a mass selective detector (Hewlett-Packard 5970 MSD) run in the selected ion monitoring (SIM) mode. The following ions were monitored for unlabeled and labeled steroids, respectively: testosterone - m/z 580 and 583; androstenedione - m/z 432 and 434; DHT - m/z 436 and 439; estrone - m/z 416 and 418; and estradiol - m/z 564 and 567. The linearity of measurement of peak areas for analyte and internal standard was confirmed by preparing and analyzing mixtures of varying amounts of the analytes with constant amount of internal standard. Standard curves were drawn. While the analyses employing trideutero internal standards were linear over a wide range, those employing dideutero standards (estrone and androstenedione) were only linear over a limited range and quantification had to be achieved from standard curves.

Each derivatized sample was injected into the mass spectrometer three times on each of two days, yielding six measurements per sample. Since four samples were measured per serum pool, a maximum of 24 measurements for each steroid sex hormone was possible for each pool. Hormone levels below the assay limit of detection (LOD) and analytical problems resulted in fewer than 24 measurements being reported for some sex hormones, particularly in pools with the lowest hormone concentrations.

HPLC/MS was used to measure DHEAS. Samples were prepared by mixing varying but quantitatively appropriate amounts of serum from the male and female pools (25–300 μl) with a known amount of labeled DHEAS to achieve approximately the same concentration of analyte and internal standard. Three ml of water was added to each tube, and the mixture was sonicated at 50°C for 20 min to disrupt protein-steroid binding and equilibrate the labeled and unlabeled steroids. The sample was extracted using a sep-pak C18 cartridge, eluted with methanol, dried under nitrogen and redissolved in methanol:water (1:1). This sample was then injected into the HPLC instrument.

HPLC/MS was carried out by an adaptation of a previously published method [10] using a Micromass BioQ electrospray instrument interfaced to a Michrom microbore HPLC housing a 10 \times 2 mm C_{18} column. The solvent system was 10 mM ammonium acetate (65%), acetonitrile (35%) delivered at 60 $\mu\text{l}/\text{min}$. Post column, the eluant was split and 4 $\mu\text{l}/\text{min}$ entered the mass spectrometer. The internal standard [7,7] $^2\text{H}_2$ was synthesized according to the published method [10]. Quantification was achieved by operating the mass spectrometer in the selected ion monitoring (SIM) mode and monitoring the molecular anions of

Table 1
Means and standard deviations (SD) of serum steroid sex hormone measurements by radioimmunoassay (RIA) and mass spectrometry (MS)

| Hormone | Sex | RIA | | | MS | | |
|-------------------------|-------------------|------------------|---------------------|-------------------|-------|--------|--------|
| | | Low ^a | Medium ^b | High ^c | Low | Medium | High |
| Estradiol (ng/dl) | Female | | | | | | |
| | Mean ^d | 0.86 | 2.67 | 11.29 | 1.26 | 2.81 | 10.80 |
| | SD ^e | 0.26 | 0.34 | 0.68 | 0.18 | 0.40 | 1.39 |
| Estrone (ng/dl) | Female | | | | | | |
| | Mean | 1.09 | 1.99 | 6.07 | 4.13 | 4.13 | 8.98 |
| | SD | 0.43 | 0.53 | 0.91 | 1.11 | 0.67 | 2.30 |
| Androstenedione (ng/dl) | Female | | | | | | |
| | Mean | 13.88 | 28.44 | 105.33 | 15.16 | 39.82 | 140.85 |
| | SD | 1.97 | 4.65 | 6.56 | 2.00 | 2.63 | 6.91 |
| | Male | | | | | | |
| | Mean | 19.41 | 39.76 | 96.21 | 15.99 | 38.16 | 107.19 |
| | SD | 3.84 | 5.70 | 7.69 | 2.27 | 3.03 | 10.68 |
| DHEAS (μg/dl) | Female | | | | | | |
| | Mean | 10.24 | 37.01 | 145.71 | 13.38 | 43.61 | 147.88 |
| | SD | 0.58 | 3.18 | 14.53 | 3.23 | 9.59 | 9.31 |
| | Male | | | | | | |
| | Mean | 13.02 | 50.39 | 154.92 | 18.10 | 47.56 | 163.73 |
| | SD | 2.41 | 3.49 | 19.68 | 1.76 | 1.51 | 30.75 |
| Testosterone (ng/dl) | Female | | | | | | |
| | Mean | 3.38 | 7.89 | 41.24 | 5.77 | 12.75 | 41.66 |
| | SD | 0.60 | 2.05 | 3.07 | 1.29 | 0.69 | 1.21 |
| | Male | | | | | | |
| | Mean | 41.54 | 130.80 | 371.83 | 50.31 | 132.92 | 393.40 |
| | SD | 2.63 | 16.56 | 41.12 | 1.03 | 4.15 | 38.79 |
| DHT (ng/dl) | Male | | | | | | |
| | Mean | 4.01 | 12.62 | 36.29 | 2.09 | 7.19 | 33.44 |
| | SD | 0.97 | 1.64 | 6.66 | 0.67 | 1.50 | 3.73 |

^a Low serum pool dilution ratio (unstripped:stripped) for females = 1:11 and for males = 1:9.

^b Medium serum pool dilution ratio (unstripped:stripped) for females = 1:3 and for males = 1:2.

^c High serum pools were undiluted serum from female and male volunteers.

^d Mean of batch means.

^e SD of a single measurement from variance components analysis.

DHEAS and its dideutero analog. Standard curves were prepared by HPLC/MS of analyte/standard mixtures where the analyte amount varied and the internal standard remained constant. Duplicate serum samples were analyzed on six different days and two or three injections were made from each extract yielding a maximum of 12 to 18 measurements of DHEAS in each serum pool.

Because serum pools were created by dilution, steroid sex hormone measurements in samples from each set of sex-specific pools should increase linearly with concentration for each assay method. We examined whether the linear regressions of steroid sex hormone measurements on known relative concentration were the same for RIA and MS for each hormone and sex. Because the underlying relationship is a simple linear one, but the error variance is not homogeneous, weighted regression was used. With $i = 0,1$ used to denote the two methods (RIA and MS), and $j = 0,1,2,3$ used to denote the four known relative steroid sex hormone concentrations (stripped serum plus 3 sex-specific pools), the analytic model for method i is

$$\bar{Y}_{ij} = \alpha_i + \beta_i \bar{C}_j + \varepsilon_{ij}$$

where \bar{Y}_{ij} is the average measurement obtained using concentration $0 \leq \bar{C}_j \leq 1$ and ε_{ij} is a normal variate with mean 0 and variance σ_{ij}^2/n_{ij} . Assigned to the average measurement is a weight obtained as the inverse of the estimated error variance, i.e. n_{ij}/σ_{ij}^2 , where σ_{ij}^2 is the observed variance of batch means for method i and concentration j . Sex hormone measurements by MS on the same day were treated as a batch for statistical purposes.

Separately for RIA and MS, we first evaluated whether the relationship of each steroid sex hormone's measurements with concentration was linear by using a goodness of fit test to determine if addition of a second order polynomial of concentration significantly improved how well the model described the data. Equality of slopes between RIA and MS was then tested. If the hypothesis of equal slopes was not rejected, a model that forced an equal slope was fit and equality of intercepts for RIA and MS was tested.

Because the numbers of samples measured in each batch by RIA and on each day by MS varied, the means of the batch means of sex hormone measurements in each serum pool were calculated for RIA and MS. A nested components

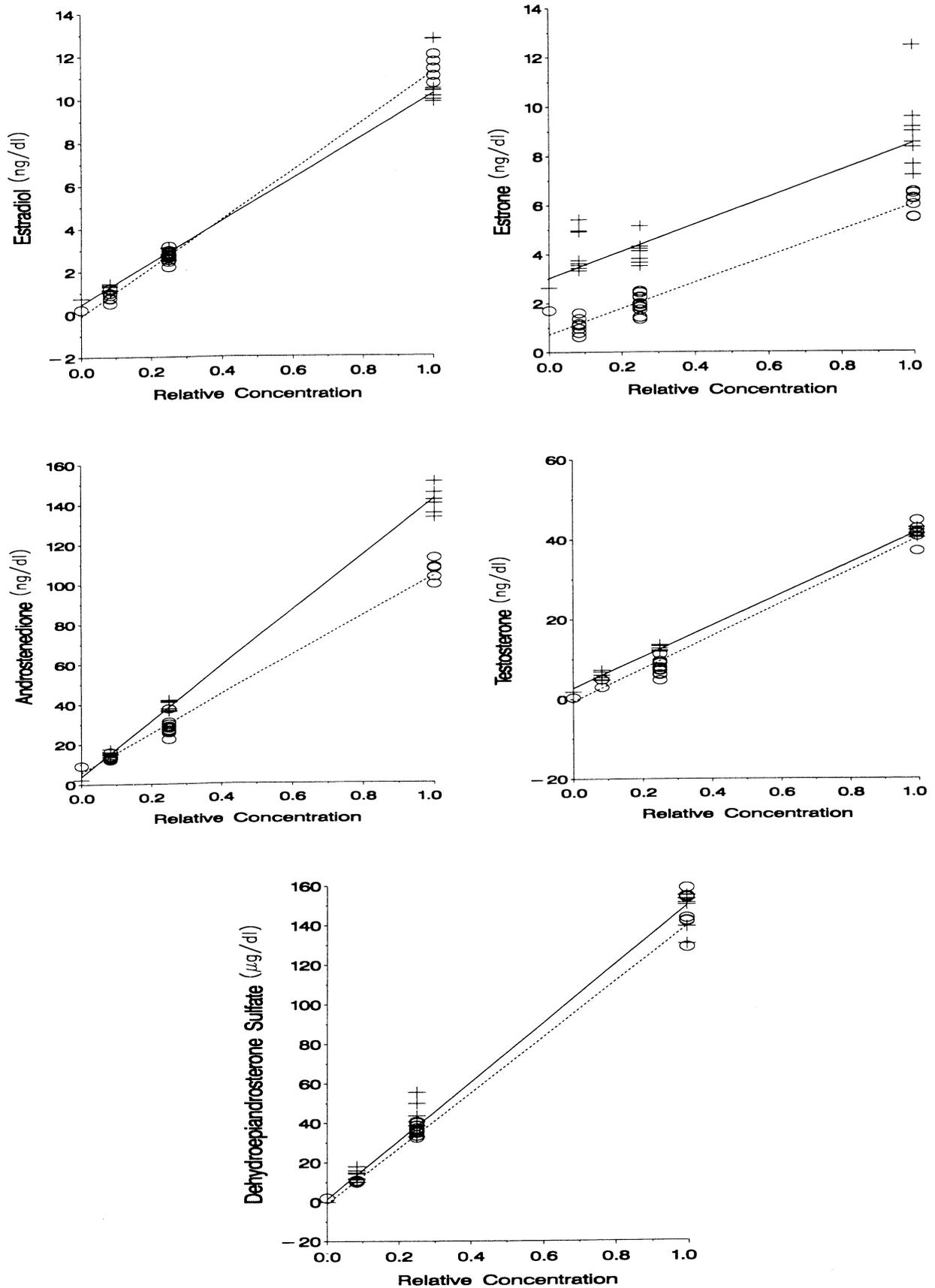


Fig. 1. Weighted linear regressions of measured steroid sex hormone concentrations on known relative concentrations in serum from females. RIA batch means (o) and fitted lines (---), and MS batch (daily) means (+) and fitted lines (—).

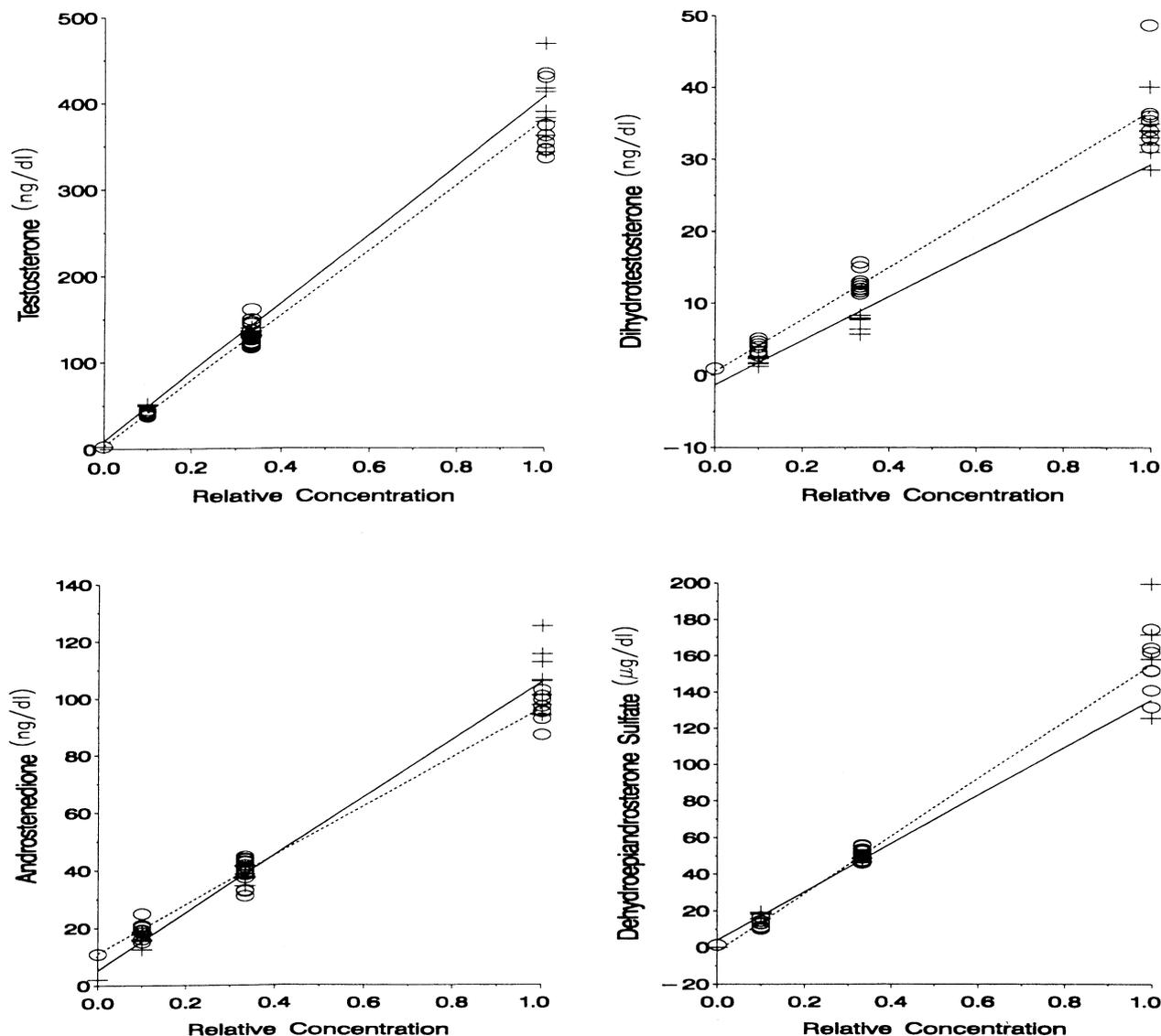


Fig. 2. Weighted linear regressions of measured steroid sex hormone concentrations on known relative concentrations in serum from males. RIA batch means (○) and fitted lines (---), and MS batch (daily) means (+) and fitted lines (—).

of variance analysis was performed to estimate the variation of each method. For the RIA, components were estimated for batch, sample within batch, and replicate within sample. For MS, components were estimated for day and sample within day. The sum of the components is an estimate of the variation for a single measurement.

3. Results

Means and standard deviations of individual measurements of serum steroid sex hormones by RIA and MS are shown in Table 1. In Figs. 1 and 2, batch means of steroid sex hormone concentrations measured by RIA and MS are plotted against their known relative serum concentrations and estimated regression lines are shown. Estimates of in-

tercepts and slopes from these regressions are provided in Table 2.

Estradiol measurements by both RIA and MS increased linearly over the known relative concentrations in female serum. The slope from the regression for RIA was slightly steeper compared to MS, but the difference was not quite significant ($P = 0.07$). Assuming a common slope, the intercepts were similar ($P = 0.08$) and not significantly different from zero. Variation of estradiol measurements by RIA was greater at low serum concentrations, but at high concentrations, the standard deviation of the MS assay was approximately twice that of the RIA.

Estrone measurements by both assay methods also increased linearly over its relative concentrations in female samples. Although regression slopes for RIA and MS were comparable, their intercepts were different ($P = 0.02$). The

Table 2

Estimates of intercepts and slopes from weighted linear regressions of steroid sex hormone measurements by radioimmunoassay (RIA) and mass spectrometry (MS) on the known relative hormone concentrations in serum from females and males

| Hormone | Sex | RIA | | | | MS | | | |
|-----------------|--------|-----------|------|--------|-------|--------------------|------|---------------------|-------|
| | | Intercept | SE | Slope | SE | Intercept | SE | Slope | SE |
| Estradiol | Female | −0.10 | 0.11 | 11.31 | 0.31 | 0.46 | 0.10 | 9.75 | 0.57 |
| Estrone | Female | 0.72 | 0.20 | 5.29 | 0.45 | 3.02 ^a | 0.47 | 5.48 | 1.43 |
| Androstenedione | Female | 5.74 | 1.06 | 98.03 | 3.72 | 3.82 | 0.98 | 138.60 ^b | 3.83 |
| | Male | 11.03 | 1.02 | 85.40 | 2.39 | 5.23 | 0.84 | 100.44 ^b | 3.32 |
| DHEAS | Female | −0.85 | 1.16 | 139.79 | 11.75 | 1.41 | 4.76 | 147.73 | 15.86 |
| | Male | −2.14 | 1.88 | 157.03 | 7.43 | 4.01 | 1.61 | 131.57 | 6.06 |
| Testosterone | Female | −0.59 | 0.75 | 40.79 | 2.18 | 2.79 ^a | 0.53 | 38.97 | 1.07 |
| | Male | 3.73 | 7.06 | 377.46 | 55.59 | 9.33 | 2.93 | 398.28 | 26.41 |
| DHT | Male | 0.53 | 1.36 | 36.06 | 5.02 | −1.29 ^a | 0.87 | 30.55 | 3.83 |

^a RIA and MS intercepts are significantly different at $P \leq 0.05$ assuming a common slope.

^b RIA and MS slopes are significantly different at $P \leq 0.05$.

intercept for RIA was small but significantly ($P = 0.01$) greater than zero. The intercept for MS was almost four times larger and also greater than ($P = 0.001$) zero. Standard deviations of estrone measurements by MS were larger than RIA at all concentrations.

In both male and female serum, regressions of measured androstenedione concentration on known relative concentration were linear, but the slopes for MS were significantly ($P \leq 0.02$) greater than RIA. The intercepts from regressions for RIA and MS did not differ for either sex. Both were small, but for male samples, the RIA and MS intercepts were significantly ($P = 0.003$) greater than zero. Neither RIA nor MS consistently measured androstenedione in serum with less variation.

Regressions of RIA and MS measurements of DHEAS on its known relative concentration were linear in both male and female serum, and in female samples, slopes were similar. While the slope for the RIA using male serum was steeper than MS, the difference was not quite significant ($P = 0.06$). Assuming a common slope for each sex, the intercepts were comparable, small, and not different from zero. A consistent pattern of differences in variation of DHEAS measurements by RIA compared to MS was not apparent.

Testosterone analyses were performed after removal of one RIA sample from the male medium concentration serum pool because it was an influential outlier. There was no evidence of departure from linearity in the relationship of MS measurements of testosterone and its known relative concentration in male or female serum. For RIA measurements of female but not male samples, there was a suggestion ($P = 0.03$) of curvature in the data. This discrepancy may reflect differential performance of the RIA at different serum concentrations of testosterone; apart from blank samples, the highest testosterone concentrations in female samples were lower than the lowest male samples. The slopes from linear regressions for RIA and MS were similar for both male and female samples. Intercepts also were similar and close to zero for male samples. Intercepts for female

samples, however, were different ($P = 0.03$). The intercept for RIA was small and close to zero, whereas the intercept for MS was larger and greater than ($P = 0.003$) zero. Measurements of testosterone by RIA tended to be more variable compared to MS.

DHT measurements by RIA and MS increased linearly over its known relative concentration in male serum and the assay slopes were comparable. However, intercepts were different ($P = 0.04$). The intercept for RIA was near zero, but the intercept for MS was significantly ($P < 0.04$) less than zero. Variation of DHT measurements was larger for RIA, particularly at high concentrations.

4. Discussion

We compared RIA and MS measurements of steroid sex hormones in DISC quality control serum samples by estimating the intercepts and slopes from weighted linear regressions of measured concentrations on known relative concentrations and by calculating standard deviations of measurements. Our RIA and MS assays for estradiol in female serum, testosterone in male serum, and DHEAS in serum from both sexes had common slopes and intercepts and would be expected to yield similar measurements of absolute levels of these hormones at the relevant concentrations. For estrone and testosterone in female serum and DHT in male serum, our RIA and MS assays had common slopes but different intercepts. Therefore, the absolute hormone concentrations in samples measured by our RIA and MS assays would not be expected to be the same, but differences in concentrations between samples should be comparable. For androstenedione, our RIA assay had a less steep slope and would be expected to yield smaller measured differences in concentration between samples compared to our MS assay. In our analysis, variation of RIA and MS measurements differed by steroid sex hormone and serum concentration. Variation in our RIA measurements of

estradiol and estrone was less than or not different from MS, whereas variation in our RIA measurements of testosterone and DHT tended to be larger than or not different from MS. Consistent differences in variation of androstenedione and DHEAS measurements were not apparent.

The DISC quality control samples that we used to compare RIA and MS measurements of steroid sex hormones were made by diluting serum collected from volunteers with serum that had been stripped of steroids with charcoal. Dilutions were chosen to cover the wide range of steroid sex hormone concentrations expected in our study participants who were 8–18 years of age. Results of RIAs revealed that concentrations of sex hormones in the samples were, in fact, within the normal ranges for the laboratory.

We could not collect the large volumes of serum needed to create quality control pools from young children. To achieve the low concentrations of steroid sex hormones in children's serum, we diluted serum collected from adults with serum that had been stripped of steroids with charcoal. Stripping may not have removed all steroids from serum used to create our samples and the artificial nature of our samples could have introduced interferences into assays. Additionally, the relative concentrations of different steroids that could potentially cross-react with antibodies used in our RIAs may not have been the same in our samples as found in undiluted serum from children. However, concentrations of the measured steroid sex hormones in our quality control samples were similar to their concentrations in participants' samples. Furthermore, because we used serial dilutions, measured concentrations of hormones in the quality control samples should increase linearly with concentration, and we could evaluate assay specificity by testing for linearity. If we had used undiluted serum samples from several individuals with unknown relative hormone concentrations, assay specificity would have been difficult to evaluate.

The antibodies used in our RIAs had high affinity and specificity for the steroid sex hormones we measured. Furthermore, sex hormones of interest were selectively extracted from serum, and when necessary to improve assay specificity, further separated using column chromatography or centrifugation prior to RIA. Different laboratory techniques could have produced divergent results from ours.

In conclusion, results of this study indicate that although absolute concentrations may differ for some hormones, RIA and MS can yield similar estimates of between subject

differences in serum concentrations of most steroid sex hormones commonly measured in population studies. Relative power of studies using RIA and MS will depend on the hormones measured and their concentrations in participants' serum.

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