Short Communication

Reproducibility of Serum Pituitary Hormones in Women

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Abstract

Endogenouspituitaryhormonesarecommonlyusedin clinical and epidemiologic studies and some of them are thought to influence the risk of several diseases in women. In most studies, endogenous levels of pituitary hormones are usually assessed at a single point in time, assuming that this single measurement represents the long-term biomarker status of the individual. Such an assumption is rarely tested and may not always be valid. This study examined the reproducibility of the following pituitary hormones: adrenocorticotropic hormone (ACTH), growth hormone, follicle-stimulating hormone (FSH), luteinizing hormone (LH), thyroid-stimulating hormone (TSH), and prolactin, measured using the Luminex xMap method in sera of healthy premenopausal and postmenopausal women. The study included 30 premenopausal women with three yearly samples and 35 postmenopausal women with two repeated yearly samples randomly selected from an existing prospective cohort. Analysis of intraclass correlation coefficients suggested higher reproducibility in postmenopausal women compared with premenopausal women for the following hormones: FSH (0.72 and 0.37, respectively), LH (0.83 and 0.44, respectively), and growth hormone (0.60 and 0.35, respectively). The intraclass correlation coefficients were relatively high and similar between postmenopausal and premenopausal women for ACTH (0.95 and 0.94, respectively), TSH (0.85 and 0.85, respectively), and prolactin (0.72 and 0.69, respectively). This study found that serum concentrations of FSH, LH, and growth hormone are stable in postmenopausal women and that ACTH, TSH, and prolactin are stable in both premenopausal and postmenopausal women, suggesting that a single measurement may reliably categorize average levels over at least a 2-year period.

Introduction

The anterior lobe of the pituitary gland secretes several hormones playing critical roles in body growth (growth hormone), function of adrenal glands [adrenocorticotropic hormone (ACTH)], regulation of thyroid secretion [thyroid-stimulating hormone (TSH)], secretion of breast milk (prolactin), and development and function of the reproductive system [follicle-stimulating hormone (FSH) and luteinizing hormone (LH)].

Endogenous levels of pituitary hormones are commonly used in epidemiologic studies and some of them are thought to influence the risk of several diseases in women. For example, prolactin and growth hormone have been shown to be associated with risk of breast cancer (1-3); gonadotropins (FSH and LH) are thought to play a role in ovarian cancer (4, 5); and ACTH and prolactin may play a role in osteoporosis (6). The majority of the previous studies relied on a single measurement, assuming that the intr-individual variability in hormone levels is smaller than the inter-individual variability. However, data on the intr-individual variability of pituitary hormones are limited. With large intr-individual variability, a single measurement may include a large degree of measurement error and, subsequently, observed associations and risk estimates could be substantially attenuated. Therefore, it is important to assess how well a single hormone measurement reflects longer-term levels before conducting and evaluating studies of these associations.

The objective of the present study was to assess the reproducibility of a number of serum markers over a 2- to 3-year period measured using the Luminex xMap method in premenopausal and postmenopausal women. The markers analyzed for the current report include the following pituitary hormones: ACTH, growth hormone, FSH, LH, TSH, and prolactin.

Materials and Methods

Subjects. Participants were selected from women participating in the New York University Women’s Health Study prospective cohort. Between March 1985 and June 1991, 14,274 women, 35 to 65 years old, were...
enrolled at a mammography screening center in New York. The cohort was restricted to women who in the preceding 6 mo were neither pregnant nor treated with hormones (7, 8).

**Blood Sampling.** At the time of enrollment and at annual screening visits thereafter, subjects were asked to provide 30 mL of nonfasting peripheral venous blood, drawn using collection tubes without anticoagulant. Serum aliquots were stored at −80°C for future biochemical analyses. Fifty-one percent of the cohort members donated blood on more than one occasion, making a reproducibility study feasible.

**Reproducibility Study Design.** Subjects were selected at random among the New York University Women’s Health Study participants who fulfilled the criteria listed below: repeated blood donations (at least two yearly samples for postmenopausal women and at least three yearly visits for premenopausal women); no diagnosis of any cancer (except nonmelanoma skin cancer) or cardiovascular disease; and no use of any exogenous sex hormones at the time of any of the selected blood donations. Subjects who had been included as cases or controls in any previous nested case-control study were not eligible. Women were classified as postmenopausal if they reported (a) absence of menstrual cycles in the previous 6 mo; (b) a total bilateral oophorectomy; or (c) a hysterectomy without total oophorectomy and their age was 52 years or older. Women were classified as premenopausal if they reported at least one menstrual cycle during the past 6 mo before enrollment. In addition, for each premenopausal woman, the phase of menstrual cycle was calculated from the date of next menstruation, which was obtained from mail-back calendars distributed at the time of blood drawing. Based on the number of days before the first day of the next menstrual period, a subject was considered in luteal (0-11 d), ovulatory (12-16 d), or follicular (≥17 d) phase of the cycle at the time of blood donation (9). The annual serum samples of a given subject were collected usually 1 y apart during the same month, thereby limiting the potential effect of seasonal variation in hormone levels.

Among the women meeting eligibility criteria, we randomly selected 35 postmenopausal women with two yearly samples and 30 premenopausal women with three yearly samples (with two of the samples taken during the same phase of cycle). For quality control, random duplicate samples of five premenopausal and five postmenopausal women were selected and analyzed on the same well-plate as the matching samples to assess intrabatch coefficients of variation. All samples were labeled to ensure blinding of the laboratory personnel.

**Luminex Assay Specifications and Procedures.** Serum 1-mL aliquots, which had not previously been thawed, were packed in dry ice and sent to the laboratory where they were stored at −80°C until they were assayed in a single batch. Hormones were analyzed using the xMap technology, which combines the principle of a sandwich immunoassay with fluorescent-bead–based technology allowing multiplex analysis of up to 100 different analytes in a single microtiter well (10). Serum ACTH, growth hormone, FSH, LH, TSH, and prolactin were measured using Human Pituitary LINCOplex kits provided by Linco/Millipore Research. The xMap serum assays were done in a 96-well microplate format according to the protocols. A filter-bottom, 96-well microplate (Millipore) was blocked for 10 min with PBS/bovine serum albumin.

To generate a standard curve, 5-fold dilutions of appropriate standards were prepared in serum diluent. Standards and patient sera were pipetted at 50 μL/well and mixed with 50 μL of the bead mixture. The microplate was incubated for 1 h at room temperature on a microtiter shaker. Wells were then washed twice with washing buffer using a vacuum manifold. Phycoerythrin-conjugated secondary antibody was added to the appropriate wells and the wells were incubated for 45 min in the dark with constant shaking. Wells were washed twice; assay buffer was added to each well; and samples were analyzed using the Bio-Plex suspension array system (Bio-Rad Laboratories). The samples were analyzed in a single measurement because in Luminex platform, reaction-to-reaction coefficient of variation is measured based on analysis of 100 beads, each representing a separate reaction. Analysis of data was done using four-parameter curve fitting (11). The interbatch coefficients of variation were 7.4% for ACTH, 14.9% for growth hormone, 4.9% for FSH, 6.7% for LH, 3.0% for TSH, and 8.2% for prolactin. The intrabatch coefficients of variation were 10.8% for ACTH, 5.4% for growth hormone, 7.2% for FSH, 6.3% for LH, 6.9% for TSH, and 7.0% for prolactin.

**Statistical Analysis.** All analyses were done on natural-logarithm–transformed values to reduce the positive skewness of the raw data. The temporal reproducibility was estimated by the intraclass correlation coefficient, which is defined as the proportion of the

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**Table 1. Geometric mean levels (10-90 percentile) of serum pituitary hormones at baseline and during repeated visits by menopausal status**

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Premenopausal women, n = 30</th>
<th></th>
<th>Postmenopausal women, n = 35</th>
<th>Baseline visit</th>
<th>Year 2 visit</th>
<th>Year 3 visit</th>
<th>Baseline visit</th>
<th>Year 2 visit</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH, mIU/mL</td>
<td>5.8 (2.8-12.4)</td>
<td>5.9 (2.7-11.6)</td>
<td>6.3 (2.2-14.8)</td>
<td>64.2 (30.0-111.4)</td>
<td>64.7 (28.9-110.9)</td>
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<tr>
<td>LH, mIU/mL</td>
<td>5.0 (2.0-14.9)</td>
<td>4.7 (1.8-11.5)</td>
<td>4.6 (1.4-10.0)</td>
<td>29.7 (17.9-44.9)</td>
<td>27.1 (16.6-46.0)</td>
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</tr>
<tr>
<td>TSH, mIU/mL</td>
<td>2.2 (1.2-3.8)</td>
<td>2.0 (0.8-4.1)</td>
<td>2.2 (1.0-4.9)</td>
<td>2.0 (0.8-3.4)</td>
<td>1.9 (0.9-3.8)</td>
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</tr>
<tr>
<td>PRL, ng/mL</td>
<td>14.4 (6.0-23.1)</td>
<td>13.6 (7.0-25.6)</td>
<td>13.8 (7.7-25.0)</td>
<td>10.7 (5.6-18.7)</td>
<td>9.1 (4.5-16.8)</td>
<td></td>
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</tr>
<tr>
<td>GH, ng/mL</td>
<td>0.9 (0.2-5.9)</td>
<td>1.1 (0.3-6.1)</td>
<td>0.9 (0.2-4.5)</td>
<td>0.5 (0.1-3.5)</td>
<td>0.4 (0.1-1.8)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>ACTH, pg/mL</td>
<td>10.4 (7.3-35.2)</td>
<td>10.3 (3.7-35.2)</td>
<td>8.4 (3.4-35.2)</td>
<td>11.2 (5.1-29.6)</td>
<td>11.2 (4.3-8.2)</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Abbreviations: PRL, prolactin; GH, growth hormone.
total variability that is due to between-subject variability. The variance components were estimated with a random effects one-way ANOVA model using the SAS procedure MIXED. Exact 95% confidence intervals for the intraclass correlation coefficients were calculated as described by McGraw and Wong (12). All analyses were done using SAS 9.1 (SAS Institute).

Results

Table 1 presents geometric means (10-90%) of pituitary hormones by menopausal status. As expected, postmenopausal women had higher mean levels of FSH and LH and lower levels of prolactin and growth hormone compared with premenopausal women. Levels of TSH and ACTH were comparable between premenopausal and postmenopausal women.

Geometric mean levels of pituitary hormones were fairly stable from visit to visit (Table 1). There were slight decreases in ACTH and LH levels in premenopausal women at year 3 visit compared with visits at year 1, but the differences were not statistically significant.

Analysis of intraclass correlation coefficients (Table 2) suggested that reproducibility for FSH, LH, and growth hormone differ by menopausal status. We observed high to moderate correlations across donations for these hormones in postmenopausal women (intraclass correlation coefficients of 0.72, 0.83, and 0.60 for FSH, LH, and growth hormone, respectively). In premenopausal women, intraclass correlation coefficients for samples collected in all phases of the cycle had substantially lower temporal reproducibility (intraclass correlation coefficients of 0.37, 0.44, and 0.35 for FSH, LH, and growth hormone, respectively). However, restricting the analyses to repeated samples in the same phase of menstrual cycle improved the reproducibility (intraclass correlation coefficients of 0.62, 0.64, and 0.42 for FSH, LH, and growth hormone, respectively). The intraclass correlation coefficients were relatively high and very similar between postmenopausal and premenopausal women in all phases of cycle for ACTH (0.95 and 0.94, respectively), TSH (0.85 and 0.85, respectively), and prolactin (0.72 and 0.69, respectively). Adjustment for body mass index, age, race/ethnicity, medication use, alcohol consumption, and smoking status at baseline did not change the intraclass correlation coefficients substantially (data not shown).

Discussion

The importance of assessing the reliability of exposure measurement before planning the epidemiologic studies is based on the fact that poor reliability may reduce the effective sample size (13), resulting in a loss of statistical power and a bias toward unity in relative risk estimates (14). The issue of reliability is even more important for cohort studies using prospectively collected biological samples, where utilization of the valuable specimens for only reliable exposure measurements should be given priority.

The results of the study show that the stability of pituitary hormone serum levels, measured using the Luminex xMap method, varies by menopausal status. In postmenopausal women, the levels of all six pituitary hormones studied (ACTH, growth hormone, FSH, LH, TSH, and prolactin) were very stable from visit to visit over a 2- to 3-year period. In premenopausal women, three hormones (FSH, LH, and growth hormone) showed low reproducibility if the phase of menstrual cycle is not taken into account. However, measurements in the same phase of menstrual cycle yielded improved reproducibility of these hormones, whereas ACTH, TSH, and prolactin showed high reproducibility regardless of the phase of menstrual cycle.

To date, a limited number of studies had assessed the reproducibility of serum levels of pituitary hormones, with the exception of prolactin. Two studies have shown that prolactin is moderately to highly reproducible over at least a 2-year period in postmenopausal women (9, 15). One study found low reliability of serum prolactin in postmenopausal women (16). In premenopausal women, studies have shown moderate reliability of prolactin with intraclass correlation coefficients ranging from 0.40 (16) to 0.64 (17). The results of our study are consistent with the conclusion that a single measurement of prolactin is sufficient to characterize the serum prolactin level in both postmenopausal and premenopausal women.

The study results confirm the previous reports that a single measurement is sufficient to characterize the serum FSH level in postmenopausal women (18, 19) but not in premenopausal women (18) unless the phase of menstrual cycle is taken into account. A similar conclusion can be made about the reproducibility of LH in serum. We are not aware of previous studies that assessed the temporal reproducibility of TSH, growth hormone, and ACTH. Our results suggest that TSH and

Table 2. Intraclass correlation coefficients (95% confidence intervals) for repeated measures of pituitary hormones by menopausal status

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Premenopausal women (all phases of cycle), n = 30; 3 visits</th>
<th>Premenopausal women (same phase of cycle), * n = 30; 2 visits</th>
<th>Postmenopausal women, n = 35; 2 visits</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH</td>
<td>0.37 (0.15-0.60)</td>
<td>0.62 (0.35-0.80)</td>
<td>0.72 (0.52-0.85)</td>
</tr>
<tr>
<td>LH</td>
<td>0.44 (0.21-0.65)</td>
<td>0.64 (0.38-0.81)</td>
<td>0.83 (0.68-0.91)</td>
</tr>
<tr>
<td>TSH</td>
<td>0.85 (0.74-0.92)</td>
<td>0.81 (0.65-0.91)</td>
<td>0.85 (0.72-0.92)</td>
</tr>
<tr>
<td>PRL</td>
<td>0.69 (0.52-0.82)</td>
<td>0.58 (0.29-0.78)</td>
<td>0.72 (0.52-0.85)</td>
</tr>
<tr>
<td>GH</td>
<td>0.35 (0.13-0.58)</td>
<td>0.42 (0.08-0.67)</td>
<td>0.60 (0.34-0.78)</td>
</tr>
<tr>
<td>ACTH</td>
<td>0.94 (0.90-0.97)</td>
<td>0.93 (0.86-0.97)</td>
<td>0.95 (0.91-0.98)</td>
</tr>
</tbody>
</table>

Abbreviation: ICC, intraclass correlation coefficient; 95% CI, 95% confidence interval.

*Limited to 30 premenopausal women with 2 visits at the same phase of menstrual cycle.
ACTH have moderate to high reproducibility over a 2- to 3-year period in both premenopausal and postmenopausal women, whereas growth hormone has lower reproducibility in premenopausal women.

Our study had several limitations. The study population included women only, so the results may not be generalized to males. The study assessed relatively short-term reliability using samples collected 2 to 3 years apart and did not investigate the effects of seasonal variability on reproducibility. Studies of long-term reliability would be of great interest.

In conclusion, this study using the Luminex xMap method found that serum concentrations of FSH, LH, and growth hormone are stable in postmenopausal women and that ACTH, TSH, and prolactin are stable in both premenopausal and postmenopausal women, suggesting that a single measurement may reliably categorize average levels over at least a 2-year period.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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References