Letters to the Editor


Jacklyn W.S. Ng1
Deborah C. Holt2
Patyian Andersson2
Philip M. Giffard2

* Address correspondence to this author at:
Menzies School of Health Research
Charles Darwin University
Darwin, Northern Territory, Australia

2 Division of Global and Tropical Health
Menzies School of Health Research
Charles Darwin University
Darwin, Northern Territory, Australia

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Clinical Use of Reference Intervals Derived from Some CALIPER Studies Questioned

To the Editor:

The availability of accurate and appropriate reference intervals is imperative for proper screening, diagnosis, and monitoring of disease. It is therefore with great interest that we read the recent reports (1, 2) by the CALIPER (Canadian Laboratory Initiative for Pediatric Reference Intervals) group. The efforts of the CALIPER project to provide these reference intervals for a diverse pediatric population is highly commendable, particularly in view of the substantial challenges of establishing them for this demographic. Apart from the rigors of selecting and collecting samples to establish reference intervals, the analytical quality of the data has to be ensured. It is with regard to these aspects of these studies that we would like to raise certain important concerns.

Steroid concentrations are known to change substantially with the time of day when the blood sample is drawn. These samples were drawn over a 24-h period; thus, the within-participant variation in concentrations with time was not taken into account. This limitation is probably the most significant criticism of the CALIPER studies. With regard to the study of Bailey et al. (2), diurnal variation in cortisol is present in healthy children from an early age. Hospitalized patients, on the other hand, are stressed and consequently show a lessening of the diurnal effect. The lack of a substantial diurnal effect raises concerns about the validity of the published intervals, for both the immunoassay and the mass spectrometry data. Furthermore, thyroid-stimulating hormone (TSH) and free triiodothyronine (FT3) also have a circadian rhythm. We are concerned about the lack of a linear relationship between the free thyroxine (FT4) concentration and the logarithm of TSH concentration obtained with the immunoassays and platform used in this study.

The CALIPER studies used samples collected in SST™ serum-separator tubes (BD) to analyze steroid hormones by liquid chromatography–tandem mass spectrometry (LC-MS/MS) (1) and steroid and thyroid hormones by immunoassay (2). We used SST and plain red-top tubes with our laboratory LC-MS/MS assays for steroid and thyroid hormones in assessing ionization inhibition (or lack thereof). We collected paired samples from volunteers into SST and plain tubes (study approved by the Institutional Review Board of the NIH, Clinical Protocol No. 93-CC-0094). The sample analyte and internal standard (IS) areas for thyroid hormones [electrospray ionization (ESI)] and steroid hormones [atmospheric pressure partial ionization (APPI)] were compared with respect to the 2 tube types. We then measured the percentage difference in area between the results obtained with the 2 tube types (Table 1). It is clear that ESI is greatly affected by tube type, whereas APPI is affected only minimally. Atmospheric pressure chemical ionization (APCI) is not a “soft” (low-energy) ionization source, and APCI results would closely resemble those obtained with the ESI source.

Kyriakopoulou et al. in the CALIPER group (1) measured steroids by tandem mass spectrometry with an APCI source. Both ESI and APCI show large and variable sample-to-sample ionization-inhibition effects when they are used for measuring steroid concentrations (4). Consequently, IS peak heights and areas vary substantially between samples, potentially affecting the quality of experimental results. Such effects are minimal with APPI. We have obtained differences in IS peak heights of up to 30-fold for patient urine cortisol samples analyzed with ESI, compared with 2- to 3-fold differences obtained with APPI. Of interest would be for the authors to comment on the variation in IS peak heights and areas they obtained with their current APCI method. The between-day CVs for the mass spectrometry assays reported by Kyriakopoulou et

1 Nonstandard abbreviations: CALIPER, Canadian Laboratory Initiative for Pediatric Reference Intervals; TSH, thyroid-stimulating hormone; FT3, free triiodothyronine; FT4, free thyroxine; LC-MS/MS, liquid chromatography–tandem mass spectrometry; IS, internal standard; ESI, electrospray ionization; APPI, atmospheric pressure partial ionization; APCI, atmospheric pressure chemical ionization.
al. in their Table 4C (1) also raise concerns because these CVs are lower than the within-day CVs for 4 of the 8 analytes.

In general, immunoassay results obtained for cortisol agree fairly well with mass spectrometry results obtained for the concentration interval of 5–20 µg/dL (138–552 nmol/L). As a rule, mass spectrometry methods are superior to immunoassay methods for evaluating hypofunction in either thyroid or steroid disorders. The inability of immunoassays to accurately detect the low concentrations of steroid hormones (such as testosterone) found in a pediatric population has been well described.

Finally, Bailey and coworkers (2) indicate that all CALIPER measurements were performed within a 12-week period after sample collection. A previous CALIPER substudy described a decrease in parathyroid hormone of up to 27.2% that was evident after 2 months of storage at −80 °C (5). We ask whether the authors considered this decrease when they evaluated the reference intervals for parathyroid hormone and, if so, what steps they took to avoid this effect.

We believe that such globally important reference intervals should be developed with the most accurate and reliable methods available. The collection of samples for steroid analysis was not optimally timed in either of these studies, and the reference intervals published should be interpreted with caution.

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References


Verena Gouden²
Steven J. Soldin²,³*

² Department of Laboratory Medicine
National Institutes of Health Clinical Center
Bethesda, MD
³ Department of Medicine
Georgetown University
Washington, DC

* Address correspondence to this author at: Department of Laboratory Medicine National Institutes of Health Clinical Center
Bldg. 10, Rm. 2C-249
Bethesda, MD 20892
Fax 301-402-1885
E-mail soldinsj@cc.nih.gov

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