Letters to the Editor

Authors’ Disclosures or Potential Conflicts of Interest: Upon manuscript submission, all authors completed the author disclosure form. Disclosures and/or potential conflicts of interest:

Employment or Leadership: None declared.
Consultant or Advisory Role: None declared.
Stock Ownership: None declared.
Honoraria: R. Senior, speaker fees from Bracco Milan Italy, Philips Healthcare.

Research Funding: Institutional funding was received from the NIHRC Cardiovascular Biomedical Research Unit, Royal Brompton and Harefield National Health Service (NHS) Foundation Trust.

Expert Testimony: None declared.

Conflicts of Interest:

Authors’ Disclosures or Potential Conflicts of Interest: None declared.

References


To the Editor:

Steroid metabolism is known to show diurnal variation for both cortisol and testosterone starting as early as 2–3 months of age (1). Cortisol shows significant diurnal variation, with higher values in the morning than in the evening, as has been documented by several groups. The measurement of midnight cortisol concentration is performed routinely as a screening test for Cushing syndrome, and dysregulation of diurnal variation is associated with disease pathology. Brambilla et al. (2) showed diurnal variation in testosterone for men 30–40 years of age; concentrations were 20%–25% lower at 1600 than at 0800 and the difference declined with age, showing a 10% difference at 70 years of age. Less information is available for other steroids, particularly where measurement has been performed with the newer, specific LC-MS/MS methodologies.

Our objective was to demonstrate and define the extent of steroid diurnal concentration fluctuations that potentially necessitate strict adherence to time of blood sample draw and requirement of separate time-dependent reference intervals with our recently reported multistroiderol profile using minimal sample volume (3). Briefly, we used an Agilent 6490 triple-quadrupole MS coupled with an atmospheric pressure photoionization source and Agilent 1200 Infinity series HPLC, including isotope dilution with deuterium-labeled internal standard for each analyte. A protein crash method was used for sample preparation. We added 75 μL acetonitrile containing inter-
nal standards to 50 μL plasma. The sample was then spun down to collect protein into a pellet. Supernatant (75 μL) was diluted with 250 μL water, and an aliquot was injected onto a Poroshell 120 EC-C18 column. After washing, the steroids were eluted with a methanol gradient. We used multiple reaction monitoring (MRM) to measure the steroid hormones cortisol, cortisone, progesterone, androstenedione, 11-deoxycortisol (11DOC), 17α-hydroxyprogesterone (17OHP), and corticosterone.

The use of volunteer samples reported in this study was approved by the National Institute of Diabetes and Digestive and Kidney Diseases/National Institute of Arthritis and Musculoskeletal and Skin Diseases institutional review board (approved protocol NCT00428987). Morning samples (minimum n = 20) were collected between 7:30 AM and 8 AM, and paired midnight samples were taken between 11:30 PM and midnight. Healthy participants were not taking any medications or supplements, had stable body weight (within 3%) for at least 30 days before testing, and were generally healthy with no indication of current or past disease during a history and physical examination by a medical provider. Additionally, all women were premenopausal and in the early follicular phase during inpatient admission, on the basis of their menstrual cycle histories. Paired-sample Student t-tests were performed between AM and PM values for all the measured steroid hormones. Statistical analyses were performed with GraphPad Prism version 6 and MedCalc version 14.8.1. Significant values were determined at P < 0.05.

Our findings revealed significant diurnal fluctuations for 11DOC, corticosterone, cortisone, androstenedione, and 17OHP, all of which showed significantly higher values in the morning than in the evening (Fig. 1). Progesterone showed no significant difference for values taken at the 2 different times of day for men (P = 0.1039) and women (P = 0.2089). Additionally, women displayed no significant difference in AM vs PM testosterone values (P = 0.6771), whereas males did display a significant difference (P < 0.001).

The literature has clearly documented variation in testosterone and...
Employment or Leadership: interest:

Disclosures and/or potential conflicts of interest: Upon manuscript submission, all authors completed the author disclosure form. Disclosures and/or potential conflicts of interest:

Research Funding: None declared.

Honoraria: None declared.

Stock Ownership: None declared.

Previously published online at DOI: 10.1373/clinchem.2014.232546

Access to Data and Analysis: All authors confirmed that they have access to the data and analysis. None declared.

Author Contributions: All authors contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Authors’ Disclosures or Potential Conflicts of Interest: Upon manuscript submission, all authors completed the author disclosure form. Disclosures and/or potential conflicts of interest:

References


Mass Spectrometric Immunoassay Raises Doubt for the Existence of Parathyroid Hormone Fragment 7–84

To the Editor:

Parathyroid hormone (PTH) plays an important role in the regulation of systemic calcium and phosphate homeostasis and bone remodeling, and its measurement in serum or plasma is used to diagnose hyperparathyroidism. Traditionally, PTH has been quantified using 2-site immunochemical assays. Previous comparisons of immunoassays demonstrated significant proportional biases as well as sample-specific discrepancies between immunoassays, due to differences in calibration, interferences, or both (1–3).

Biochemically, PTH is a heterogeneous collection of molecules with posttranslational modifications that include phosphorylation, oxidation, and proteolytic cleavage. Different assays variably detect these modified PTH molecules. Previous experiments used HPLC to fractionate serum and identify a peak that cross-reacts with many PTH immunoassays. This peak did not coelute with intact PTH (1–84), but did coelute with PTH fragment (7–84) (4). It has been proposed and generally accepted that this fragment (and to a lesser extent other PTH fragments) interferes with most second-generation immunoassays (those that target amino acid residues proximal to the amino terminus).

We aimed to evaluate whether PTH fragments interfere with 2 commonly used US Food and Drug Administration (FDA)-approved second-generation clinical immunoassays: the Roche Elecsys/Cobas

* Address correspondence to this author at: Department of Laboratory Medicine Room 2C306 NIH Bldg 10 9000 Rockville Pike Bethesda, MD 20892 E-mail steven.soldin.nih.gov

© 2014 American Association for Clinical Chemistry

1 Nonstandard abbreviations: PTH, parathyroid hormone; FDA, US Food and Drug Administration.