and functional sensitivity was calculated to be the dose in the equation with a total CV of 20%. We found the functional sensitivity, using reagent lots 26 and 29, to be 0.012 mIU/L. This value is considerably lower than that of Rawlins and Roberts (1), although the same reagent lots were used. For the two newer in-date lots, functional sensitivity was calculated to be 0.022 mIU/L. Both determinations are consistent with the manufacturer's claim of 0.019 mIU/L for the ADVIA Centaur TSH-3.

In summary, we believe that the data support our functional sensitivity claim for the third-generation ADVIA Centaur TSH-3 method and that this claim has been demonstrated in other studies (4–6).

References

1. Rawlins ML, Roberts WL. Performance characteristics of six third-generation assays for thyroid-stimulating hormone. Clin Chem 2004;50: 2338–44.
2. Bayer HealthCare. ADVIA Centaur assay manual. TSH-3 instructions for use, revision K 2004-03. Tarrytown, NY: Bayer HealthCare, 2004.
3. Ognibene A, Drake CJ, Jeng KY, Pascucci TE, Hsu S, Luceri F, et al. A new modular chemiluminescent immunoassay analyser evaluated. Clin Chem Lab Med 2000;38:251–60.
4. Vogeser M, Weigand M, Fraunberger P, Fischer H, Cremer P. Evaluation of the ADVIA Centaur TSH-3 assay. Clin Chem Lab Med 2000;38: 331–4.
5. Kelly A, DeCarlo F, McCann L, Sasse E. Third generation TSH functional sensitivity and patient comparisons for the Abbott Architect and Bayer-Chiron Centaur [Abstract]. Clin Chem 2000;46(Suppl 6):A126.
6. Gruson D, DeNayer P, Phillippe M. Evaluation of the analytical and clinical performance of a third generation TSH assay on Bayer Advia Centaur analyzer [Abstract]. Clin Chem 2004; 50(Suppl 6):A97.

David Waskiewicz[1*]
Alan Burkhardt[2]
Kenneth Emancipator[2,3]

[1] Bayer HealthCare
Diagnostics Division
Walpole, MA

Bayer HealthCare
[2] Diagnostics Division
and
[3] Diabetes Care Division
Tarrytown, NY

*Address correspondence to this author at: Bayer HealthCare, Diagnostics Division, 333 Coney St., Walpole, MA 02032. Fax 508-660-4500; e-mail david.waskiewicz.b@bayer.com.

Dr. Roberts responds:

To the Editor:

We appreciate the information provided by Waskiewicz et al. in their letter. They are correct that the study by Ognibene et al. (1) refers to a second-generation thyrotropin (TSH) assay on the ADVIA Centaur and not to a third-generation TSH assay. We regret this error. The study by Vogeser et al., cited as Ref. 4 by Waskiewicz et al., did not actually include an estimate of the functional sensitivity, but rather imprecision was 22.3% at a TSH concentration of 0.014 mIU/L and 3.9% at 0.26 mIU/L (2). These are not sufficient data to estimate functional sensitivity.

The major issue is why our study yielded a higher functional sensitivity than theirs did. They indicate that each pool was tested with all reagent lots in one run. Our study used each of two reagent lots sequentially, which might in part account for the higher imprecision (3). The instrument in their study was used for various patient sample evaluations in support of Centaur customers. The instrument in our study was used for routine testing of patient samples in a reference laboratory setting with ~10 000 patient results reported monthly, and TSH-3 was one of the analytes being routinely reported. The differing environments and use of the ADVIA Centaur analyzers in these 2 studies may have contributed to differences in imprecision. We maintain that our experimental conditions are more representative of what will be encountered in routine clinical testing.

It is unclear whether authors of previous studies have performed imprecision studies in a research setting or in a clinical testing environment. To our knowledge, no one has re-

ported on the effects of increasing workload on assay imprecision, but this may be a factor affecting the precision of some analyzers. A better understanding of which variables are most important and how they affect assay imprecision could lead to better assay performance during routine clinical use. In the study by Waskiewicz et al., the functional sensitivity of lots 38 and 41 of TSH-3 reagent was 0.022 mIU/L, whereas that of lots 26 and 29 (the ones used in our study) was 0.012 mIU/L. It would be interesting to field-test lots 38 and 41 to see whether the increased functional sensitivity exhibited by these two lots in a controlled setting would also be evident in routine clinical testing.

References

1. Ognibene A, Drake CJ, Jeng KY, Pascucci TE, Hsu S, Luceri F, et al. A new modular chemiluminescent immunoassay analyser evaluated. Clin Chem Lab Med 2000;38:251–60.
2. Vogeser M, Weigand M, Fraunberger P, Fischer H, Cremer P. Evaluation of the ADVIA Centaur TSH-3 assay. Clin Chem Lab Med 2000;38: 331–4.
3. Rawlins ML, Roberts WL. Performance characteristics of six third-generation assays for thyroid-stimulating hormone. Clin Chem 2004;50: 2338–44.

William L. Roberts

*Department of Pathology
University of Utah
Health Sciences Center
Salt Lake City, UT*

Address for correspondence: ARUP Laboratories, 500 Chipeta Way, Salt Lake City, UT 84108. Fax 801-584-5207; e-mail william.roberts@aruplab.com.

The Biological Variation of C-Reactive Protein in Polycystic Ovarian Syndrome

To the Editor:

An inverse relationship between increased C-reactive protein (CRP) concentrations and insulin sensitivity has occurred in individuals with polycystic ovarian syndrome (PCOS) (1) and is thought to contribute to an

increased risk of coronary heart disease *(2)*. However, no data currently exist on the biological variability of CRP and insulin resistance within the same individuals with PCOS, information that is essential to assess the full relationship between the two measures. We describe a study to establish whether a PCOS patient's CRP concentration remains within narrow biological limits or varies more widely over a given time period, as well as to correlate its variability to that of insulin resistance.

Twelve overweight [mean (SD) body mass index (BMI), e.g., 33.2 (6.3) kg/m^2] Caucasian women, diagnosed with PCOS [median (range) age, 28 (18–31) years], and 11 weight-matched Caucasian women [controls; mean (SD) BMI, 29.9 (3.3) kg/m^2], with regular menses (every 28–30 days) and without PCOS [median (range) age, 30 (19–33) years], participated in the study. The BMI in the PCOS group was not significantly greater ($P = 0.151$) than that of the control group. Diagnosis of PCOS was based on evidence of hyperandrogenemia [defined as free androgen index >8; mean (SD) index: PCOS group, 21.85 (7.95); controls, 4.68 (2.05)], with a history of oligomenorrhea and hirsutism or acne. Mean (SD) concentrations of testosterone and sex hormone–binding globulin (SHBG) in the PCOS group compared with the control group were 4.69 (0.76) vs 2.66 (0.87) nmol/L (P <0.001) and 22.87 (5.06) vs 64.51 (7.65) nmol/L, respectively (P <0.001). Fasting venous blood was collected into serum gel tubes (Becton Dickinson) and 1 fluoride oxalate tube at the same time each day (0800–0900) on 10 consecutive occasions at 4-day intervals. Samples were separated by centrifugation at 2000*g* for 15 min at 4 °C, and 2 aliquots of the serum were stored at −20 °C within 1 h of collection. Plasma glucose was analyzed in singleton within 4 h of collection. The serum samples were split before assay. All participants gave informed written consent before entering the study, which had been approved by the Hull and East Riding Local Research Ethics Committee.

Serum CRP was measured by the high-sensitivity method on a DPC Immulite analyzer (Euro/DPC), using the manufacturer's recommended protocol. The interassay CV was 4% using the study samples. Serum insulin was assayed by a competitive chemiluminescent immunoassay, supplied by Euro/DPC. The assay was performed on a DPC Immulite 2000 analyzer (Euro/DPC), according to the manufacturer's recommended protocol. The CV of this method was 8%, calculated as below, for study samples. The detection limit was 2 milliunits/L, and there was no stated cross-reactivity with proinsulin. Plasma glucose was measured with a Synchron LX 20 analyzer (Beckman-Coulter), according to the manufacturer's recommended protocol. The CV for this assay was 1%, with a mean glucose value of 5.3 mmol/L during the study period. Fasting glucose in the PCOS group [mean (SD), 4.98 (0.58) mmol/L] was not significantly different from the control group [4.81 (0.32) mol/L]. Fasting insulin was much higher in the PCOS group than in the control group [mean (SD), 23.56 (8.54) vs 7.70 (1.83) μmol/L; P <0.001].

Statistical analysis was performed using SPSS for Windows NT, Ver. 9.0 (SPSS Inc.). We analyzed biovariability data by calculating both intra- and interindividual analytical variances (SD$_A^2$, SD$_I^2$, SD$_G^2$, respectively), according to the methods of Fraser and Harris *(3)*. The insulin resistance was calculated by use of the Homeostasis Model Assessment (HOMA) method [resistance = (insulin × glucose)/22.5] *(4)*.

Before analysis, all serum samples were thawed and thoroughly mixed. The duplicate samples (i.e., 2 per visit) were randomized and then analyzed in a single continuous batch with a single batch of reagents. The distribution of CRP was found to be log-gaussian (by Kolmogorov–Smirnov) in both the women with PCOS and the control group and consequently was logarithmically transformed before statistical analysis.

The CRP concentration in the PCOS group was greater than in the control group [median (range), 3.54 (0.80–61.35) mg/L vs 1.07 (0.18–9.24) mg/L; $P = 0.0001$, Mann–Whitney test; Fig. 1)]. For the group with PCOS, the analytical variance contributed 0.2% to the total test variance, intraindividual variance contributed 30.2%, and interindividual variance contributed 69.6%. For the control group, the analytical variance contributed 1% to the total test variance; intraindividual variance, 36.8%; and interindividual variance, 62.2%. After accounting for analytical variation, the mean intraindividual variation was similar in both the group with PCOS and the control group (mean, 1.63 vs 1.76). In contrast, as reported previously for the same individuals *(5)*, the HOMA-IR was not only greater in the group with PCOS [mean (range), 5.85 (1–42.1) units vs 1.67 (0.48–3.49) units; $P = 0.001$], but
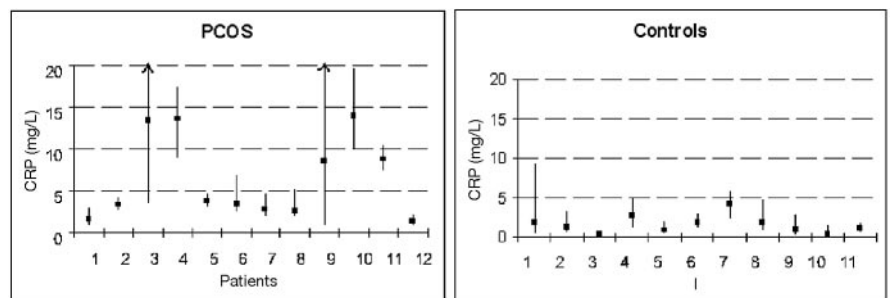

Fig. 1. Median and range of CRP concentrations in the PCOS and control groups.

was also more variable than in the control group (mean, 1.19 vs 0.23). The interindividual variation in CRP in its natural log for both groups was similar, at 0.5. The critical difference between 2 consecutive CRP samples in an individual patient with PCOS, calculated using the formula $2.77(CV_I)$ *(3)* on the log-derived data, was -64% or $+179\%$ of any initial concentration of CRP. This indicates that a subsequent sample must increase by $>179\%$ or decrease by $>64\%$ to be considered significantly different from the first.

This is the first study to examine the biological variation of CRP in women with PCOS, and it shows that although the mean concentration of CRP is higher in individuals with PCOS compared with healthy controls, the intraindividual variation of CRP is similarly large in both groups. Indeed, the potential utility of CRP as a marker of cardiovascular risk may be limited by the magnitude of this variability in both health and disease, as there can be substantial overlap between PCOS and control individuals. Our control group data are in accord with results demonstrated by previous studies suggesting a similarly wide intraindividual variability in CRP of $\sim$30%–40% *(6–9)*. In contrast, Ockene et al. *(10)* have suggested that high-sensitivity CRP has a degree of measurement stability similar to that of total cholesterol, therefore providing evidence of potential clinical utility of high-sensitivity CRP screening as a tool for vascular risk prediction. This issue of clinical usefulness, therefore, has yet to be resolved.

The lack of concordance between the variability of CRP concentration and the variability of insulin resistance in PCOS, compared with the controls, indicates that despite the known inverse relationship, the magnitude of CRP changes in the same individual does not closely mirror that of insulin resistance. Therefore, an increased CRP concentration cannot be used as a direct surrogate marker to establish the presence of insulin resistance in this group. There has also been the assumption that, in patients with PCOS, CRP values may reflect the presence of the metabolic syndrome *(11, 12)*; indeed it still might, but insulin resistance alone would not be the sole cause or the main factor. It seems more likely that CRP may reflect or be a marker of many factors that contribute to the syndrome.

References

1. Kelly CC, Lyall H, Petrie JR, Gould GW, Connell JM, Sattar N. Low grade chronic inflammation in women with polycystic ovarian syndrome. J Clin Endocrinol Metab 2001;86:2453–5.
2. Tarkun I, Arslan BC, Canturk Z, Turemen E, Sahin T, Duman C. Endothelial dysfunction in young women with polycystic ovary syndrome: relationship with insulin resistance and low-grade chronic inflammation. J Clin Endocrinol Metab 2004;89:5592–6.
3. Fraser CG, Harris EK. Generation and application of data on biological variation in clinical chemistry. Crit Rev Clin Lab Sci 1989;27:409–37.
4. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412–9.
5. Jayagopal V, Kilpatrick ES, Holding S, Jennings PE, Atkin SL. The biological variation of insulin resistance in polycystic ovarian syndrome. J Clin Endocrinol Metab 2002;87:1560–2.
6. Clark GH, Fraser CG. Biological variation of acute phase proteins. Ann Clin Biochem 1993; 30(Pt 4):373–6.
7. Campbell B, Badrick T, Flatman R, Kanowski D. Limited clinical utility of high-sensitivity plasma C-reactive protein assays. Ann Clin Biochem 2002;39:85–8.
8. Kluft C, de Maat MP. Determination of the habitual low blood level of C-reactive protein in individuals. Ital Heart J 2001;2:172–80.
9. Macy EM, Hayes TE, Tracy RP. Variability in the measurement of C-reactive protein in healthy subjects: implications for reference intervals and epidemiological applications. Clin Chem 1997;43:52–8.
10. Ockene IS, Matthews CE, Rifai N, Ridker PM, Reed G, Stanek E. Variability and classification accuracy of serial high-sensitivity C-reactive protein measurements in healthy adults. Clin Chem 2001;47:444–50.
11. Campbell B, Flatman R, Badrick T, Kanowski D. Problems with high-sensitivity C-reactive protein. Clin Chem 2003;49:201; author reply, 202.
12. Lee WY, Park JS, Noh SY, Rhee EJ, Sung KC, Kim BS, et al. C-reactive protein concentrations are related to insulin resistance and metabolic syndrome as defined by the ATP III report. Int J Cardiol 2004;97:101–6.

Li Wei Cho[1*]
Vijay Jayagopal[1,3]
Eric S. Kilpatrick[2]
Stephen L. Atkin[1]

[1] *Department of Medicine*
University of Hull
Hull, United Kingdom

[2] *Department of Clinical Biochemistry*
Hull Royal Infirmary
Hull, United Kingdom

[3] *Department of Medicine*
York Hospital
York, United Kingdom

*Address correspondence to this author at: The Michael White Centre for Diabetes and Endocrinology, 220-236 Anlaby Road, Hull Royal Infirmary, Hull HU3 2RW, United Kingdom. Fax 44-1482-675395; e-mail l.cho@hull.ac.uk.

Pseudocholinesterase Activity in Organophosphate Poisoning after Storage of Unseparated Blood Samples at Room Temperature for 3 Weeks

To the Editor:

Suppressed pseudocholinesterase activity is a well-established laboratory finding in patients with serious organophosphate poisoning *(1)*. Recently, a 48-year-old man with suspected ingestion of methyl parathion died, and the postmortem examination was not indicative. After 3 weeks, an overlooked specimen was discovered that had been collected from the patient $\sim$1 h after the suspected poisoning. The determination of pseudocholinesterase activity was requested. The blood sample, which showed complete hemolysis, was separated by centrifugation, and the pseudocholinesterase activity was determined. The result of 4.21 kU/L indicated the presence of only minor organophosphate poisoning without suppression of pseudocholinesterase activity.

Data regarding pseudocholinesterase activity in unseparated blood after storage at room temperature are rare, however, with the longest reported duration (48 h) showing only negligible differences *(2)*. Because one would expect enzyme activities to be extensively changed after storage for 3 weeks at room temperature, we performed a limited study with