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

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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 reported 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.

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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/m²] 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/m²], 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 different from 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 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 2000g 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) mmol/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 intraindividual and interindividual analytical variabilities (SDA², SDI², SDI×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...
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

\[
2.77(CVI) = \frac{\log(CRPM) - \log(CRPN)}{\log(2)}
\]

calculated using the formula

in an individual patient with PCOS, between 2 consecutive CRP samples. The interindividual variation in CRP control group (mean, 1.19 vs 0.23). was also more variable than in the group. That, in patients with PCOS, CRP 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


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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 ~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