These results are consistent with previous observations (2, 3). The lower values for the ambient sample set for PRA indicates that sample collection and handling does affect assay results. Our findings suggest that the enzyme activity of renin is affected by sample handling, but its immunoreassay detection is not influenced by ambient temperature. The apparent increase in renin activity from the refrigerated samples, therefore, does not suggest that 2 h on ice increases renin by cryoactivation of prorenin. Rather, renin enzyme activity appears to be decreased by processing at room temperature.

In addition, our results show a good correlation between PRA and immunoreactive renin. However, immunoreactive renin has advantages of less assay variation than PRA and is not limited by substrate concentrations.

In conclusion, prolonged exposure to refrigerated temperatures should be avoided to prevent cryoactivation of prorenin to renin, which leads to falsely increased renin results. Samples for measurement of immunoreactive renin may be collected at either 4°C for 2 h or at ambient temperature before assay without affecting assay results. However, collection of samples for assay of PRA should be avoided because it will lead to lower assay values.

References

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Rapid Enantiomeric Differentiation of Urinary Metabolites in a Patient with Bacterial Overgrowth Syndrome

To the Editor:

Patients with short bowel syndrome (SBS) are prone to periods of acute life-threatening metabolic acidosis attributable to the accumulation of l-lactate caused by bacterial overgrowth (1–3). The malabsorption of carbohydrates in the gut leads to large amounts of d- and l-lactate produced by intestinal flora (3). Because only l-lactate is routinely analyzed in most laboratories, an unrecognized l-lactisic acidosis can have severe neurological consequences if not diagnosed and treated promptly.

A 4-year-old boy with SBS after surgical correction for a small intestinal volvulus was admitted with a history of periodic metabolic acidosis. During a bout of fever, he developed a severe metabolic acidosis (pH 7.19; reference range, 7.35–7.45; PaCO₂, 21.8 mmHg; reference range, 27–40 mmHg; base excess, −20.0; reference range, −4 to 2 mmol/L), was drowsy with indistinct speech, and was hyperventilating. Initially, he was treated orally (40 mL) and subsequently intravenously (40 mL) with buffered bicarbonate, and glucose (20 mL of a 200 g/L solution), after which he could be aroused. Serum t-lactate was 2.8 mmol/L. The possibility of bacterial overgrowth was considered, and routine qualitative urinary organic acid analysis (gas chromatography–mass spectrometry) (4) revealed increased excretion of lactate, 3-hydroxypropionate, phenyllactate, and 4-hydroxyphenylacetate, a pattern characteristic of bacterial overgrowth syndrome. A subsequent enantiomeric analysis of urinary organic acids using enantioselective multidimensional capillary gas chromatography–mass spectrometry (enantio-MDGC-MS) (5) demonstrated a d:l-lactate ratio of >95:5, indicating d-lactic acidosis. We also found increased concentrations of 2R,3S- and 2S,3S-2-hydroxy-3-methylpentanoic acids in the same chromatographic run. Increased d-phenyllactate and 4-hydroxyphenyllactate were also found, confirming the results of others (6). Enantio-MDGC-MS also detected d-alloisoleucine and d- and l-alanine with d:l ratios of ∼50:50, also confirming the bacterial origin of the d-form (7). The high concentration (9.1 mmol/L) of d-lactate in plasma supported the diagnosis, and large numbers of Lactobacillus were cultured from stool samples. The patient was treated with a course of antibiotics (isocillin; 300 000 units, three times a day) and subsequent intestinal wash-out. Analysis of urine samples when the patient was clinically well showed a d:l-lactate ratio of 50:50.

d-Lactate is a very rare metabolite in healthy individuals, but it is detected frequently in patients with SBS and bacterial overgrowth. Most routine clinical chemistry laboratories do not assay d-lactic acid; thus, a diagnosis of d-lactic acidosis may be delayed. In this patient, routine urinary organic acid analysis provided
the first indication that bacterial metabolites were present. Because the patient had an increased d-lactate concentration in serum, it was decided that in this initial investigation, the reporting of the d:l-lactate ratios would suffice. In addition, the patient responded to antibiotic therapy, thus providing further support for the diagnosis.

Our studies in this patient also confirmed the findings of Bongaerts et al. (8) in that d-lactate is present in the urine of SBS patients even when they are clinically well, is related to food intake, and can show a strong circadian rhythm. We also found traces of d-lactate in urines of “normal” healthy individuals, suggesting a “normal” bacterial colonization. Enantio-MDGC-MS supported the diagnosis. This method has been used for studies of inborn errors of metabolism where enantiodifferentiation is important (9, 10), and it is fast, accurate, and reproducible. The application of enantio-MDGC-MS in further studies of bacterial metabolism or analysis of serum or plasma samples may provide useful information for unraveling complex metabolic pathways.

References

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