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D-Lactic Acidemia and Aciduria in Pediatric and Adult Patients with Short Bowel Syndrome

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D-Lactate produced by abundant intestinal lactobacilli during acidic episodes in short bowel (SB) patients is commonly regarded as a main factor in the pathogenesis of SB syndrome-associated (d-lactic) acidosis. Since we had observed that gram-positive bacteria, mainly lactobacilli, were abundant even in the absence of acidosis, we studied serum concentrations and urinary excretions of d- and l-lactate in young and adult SB patients, especially during nonacidotic periods. Serum l-lactate and urinary l-lactate excretion were similar in adults and children. Serum d-lactate and urinary d-lactate excretion were higher in SB children than in SB adults. Food consumption affects d-lactate production and alters d-lactic acidemia and aciduria. We conclude that d-lactate is frequently present in serum of SB patients even in the absence of acidosis. High serum concentrations and urinary excretions may reflect dietary factors in these patients.

Indexing Terms: d-lactic acid/lactic acid/circadian rhythm/metabolic acidosis

At the start of this study two young patients (ages 1 and 3 years) with short bowel syndrome (SB) had frequent clinical symptoms of metabolic acidosis (e.g., hyperventilation), but the bacterial metabolite d-lactate, which is commonly regarded as the main pathogenic factor responsible for SB-associated (d-lactic) acidosis (1), could not be detected in blood or in urine. Surprisingly, a few weeks later the metabolite was detected, but the patient was not acidic.

The most common etiology of SB-associated metabolic acidosis is massive resection of the small intestine (1–6). Because of the resulting malabsorption of carbohydrates (5), large amounts of d- and l-lactate are produced by intestinal flora. With bacterial overgrowth, these metabolites are produced not only from mono- and disaccharides such as glucose and lactose (5, 7) but also from starch, due to fermentative activity of certain abundant intestinal gram-positive rod-shaped bacteria, mainly lactobacilli (8). Both d- and l-lactate are absorbed from the intestinal lumen into blood. However, only l-lactate can be metabolized by the human body, whereas d-lactate accumulates (d-lactic acidemia) and is excreted in urine (d-lactic aciduria) (9). Therefore, SB-associated metabolic acidosis is thought to be caused by prolonged d-lactic acidemia (1–3, 5, 6).

Publications on d-lactic acidosis have concerned short periods of illness, starting at the beginning of symptoms. However, even in symptom-free SB patients, we have always observed a fecal flora that consisted mainly of gram-positive rod-shaped bacteria (manuscript in preparation). Relevant information on the d-lactate status of symptom-free SB patients was still lacking. For example: Can d-lactate be detected in blood and urine of symptom-free patients? Why is d-lactate sometimes not detected during acidic periods? Are d-lactate concentrations different in SB children and SB adults? Does food consumption affect d-lactate accumulation?

The aim of this longitudinal study was to address these questions by measuring serum concentrations and urinary excretions of both d- and l-lactate in SB children (mainly during nonacidotic episodes), in adult symptom-free SB patients, and in healthy controls.

Materials and Methods

Definitions

Acidemia and aciduria are defined as the occurrence of acid in blood and urine, respectively, at concentrations greater than the reference values. Since in serum and urine of healthy persons d-lactate is usually not measurable, d-lactic acidemia and d-lactic aciduria are defined by the presence of detectable d-lactate in blood and urine, irrespective of clinical symptoms.

Patients

The study was performed in accordance with the Helsinki Declaration of 1975, as revised in 1983, and approved by the Ethics Committee of the University Hospital Nijmegen St. Radboud. The study included 8 SB patients: 2 female children (CH-1 and CH-2), 4 women (AD-2, AD-3, AD-4, AD-5), and 2 men (AD-1 and AD-6); it was performed mainly from December 1987 to December 1992, but completed in June 1993. The children entered the study in December 1987 at the ages of 1 and 3 years, respectively. All patients were studied longitudinally, albeit over variable periods of time. More extensive clinical details on the patients will be presented elsewhere (manuscript in preparation). The essential facts were that, in all mentioned patients, 80–85% of the small intestine had been resected, and all had received oral nutrition.

During the study the SB children regularly had episodes of acidosis. CH-2 was on an essentially lactose-free but otherwise normal oral diet, with soya milk replacing dairy products and with micronutrient supplementation. CH-1 was also on a same lactose-free oral diet, but...
only for the first 2 years of life. All SB adults were long-term patients, and none showed clinical symptoms of metabolic acidosis during the study. They had a normal caloric diet enriched with carbohydrates and proteins that contained a normal or slightly reduced quantity of fat, depending on the frequency of bowel movements. Consumption of lactose and dairy products was limited by occurrence of diarrhea and (or) other complaints indicating lactose intolerance. AD-4 and AD-6 were on wholly oral feeding, whereas the other adults received additional parenteral nutrition.

Biochemical Assays

The quantitative analysis of total lactate in urine and blood, as part of the analysis of organic acids, was performed by capillary gas chromatography or capillary gas chromatography–mass spectrometry. Extraction and derivatization of the urine and serum samples were performed essentially as described previously (10): After ethyl acetate extraction of the acidified samples, the organic acids were converted to their trimethylsilyl derivatives and analyzed. Gas chromatography was carried out on an HP 5880 gas chromatograph (Hewlett Packard, Palo Alto, CA) with flame-ionization detection, and with a 25 m × 0.25 mm (i.d.) fused silica CP Sil 8CB capillary column (Chrompack, Bergen op Zoom, The Netherlands). The following temperature program was used: 70°C for 4 min, then from 70°C to 230°C at 7°C/min. The injection port temperature was 240°C and the detector temperature was 260°C. Mass spectrometric analysis was performed on a Trio-2 gas chromatograph–mass spectrometer (VG Masslab, Altrincham, UK) in the electron impact mode, equipped with a CP Sil 8CB capillary column as described above, and with the same temperature programming. The ion source temperature was 180°C, the transfer line temperature was 280°C, and the electron energy was 70 eV.

For quantification of L-lactate in urine, an enzymatic assay (Lactate UV-kit; Boehringer Mannheim, Mannheim, Germany) with L-lactate dehydrogenase (L-LDH; EC 1.1.1.27) (Boehringer Mannheim) was performed. For quantification of D-lactate, L-LDH was replaced with D-LDH (EC 1.1.1.28) (Boehringer Mannheim). The enzymatic determination of D- and L-lactate in serum was performed with the same assay, but after deproteination of the sample with 6 g/L perchloric acid.

Urinary Lactate Excretion was determined by the alkaline picrate method (11).

**Results**

**Serum Lactate Concentrations**

The reference value for total lactate in serum is <2000 μmol/L. Since D-lactate is normally not found in serum of healthy persons, the reference value for L-lactate in serum is also <2000 μmol/L. Serum total lactate concentrations were 780–5000 μmol/L in SB children and 2800–9700 μmol/L in SB adults. Table 1 presents median values and ranges of enzymatically measured concentrations of both L- and D-lactate in serum. Serum L-lactate concentrations ranged from 620 to 2130 μmol/L (median value 1500) in SB children, from 1370 to 3825 μmol/L (median value 1750) in SB adults, and from 620 to 3825 μmol/L (median value 1730) for all SB patients. Thus, the median serum L-lactate concentration did not essentially differ for the SB children and the SB adults. Although the greatest serum L-lactate concentrations of CH-1 and CH-2 were nearly identical, the median values reflect that seven of eight serum L-lactate concentrations for CH-2 were in the range 1475–2130 μmol/L, whereas seven of nine serum L-lactate concentrations for CH-1 were in the range 865–1500 μmol/L. Serum D-lactate concentrations were <10–3800 μmol/L (median 500) in SB children, 50–785 μmol/L (median 485) in SB adults, and <10–3800 μmol/L (median 485) for all SB patients. Thus the median serum D-lactate concentration also did not differ for the SB children and the SB adults. Although in SB patients L-lactate concentrations were consistently higher than D-lactate concentrations, in the SB children the opposite was often true.

**Urinary Lactate Excretion in SB Children**

Reference values of total lactate excretion are ≤60 mmol/mol creatinine; for children <1 year, ≤140 mmol/mol creatinine. Since D-lactate is normally not present in urine of healthy persons, the reference values for urinary L-lactate excretion are identical to those for total lactate. At the start of this study, the urinary total lactate excretions measured in samples of both SB children were within normal limits.

Both children were routinely seen in the outpatient clinic once a month, CH-2 in the morning and CH-1 in the early afternoon. Small urine samples passed by CH-2 early in the morning (but not the first passing) never showed significantly increased excretion of lactate; in these samples total lactate excretion ranged from 0 to 310 mmol/mol creatinine (median 60), and D-lactate excretion from 0 to 180 mmol/mol creatinine (median 60). Total lactate excretion values in small samples produced by CH-1 in the afternoon ranged from 40 to 4245 mmol/mol creatinine (median 450); several times D-lactate excretion (up to 2060 mmol/mol creatinine) measured in these samples was considerably higher than the measured L-lactate excretion (≤50 mmol/mol creatinine).

<table>
<thead>
<tr>
<th>Patient</th>
<th>No. blood samples examined</th>
<th>L-Lactate*</th>
<th>D-Lactateb</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH-1</td>
<td>9</td>
<td>1240</td>
<td>470</td>
</tr>
<tr>
<td>CH-2</td>
<td>8</td>
<td>1880</td>
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<tr>
<td>AD-3</td>
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<td>785</td>
</tr>
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<td>AD-4</td>
<td>1</td>
<td>1370</td>
<td>140</td>
</tr>
<tr>
<td>AD-5</td>
<td>1</td>
<td>3825</td>
<td>175</td>
</tr>
<tr>
<td>AD-6</td>
<td>3</td>
<td>1750</td>
<td>485</td>
</tr>
</tbody>
</table>

* Reference value for serum L-lactate is <2000 μmol/L.

b In healthy persons D-lactate is usually unmeasurable.
Time-Related Lactate Excretion and Serum Lactate

Urinary excretions of total lactate in two small successive samples produced during a clinical observation by CH-2 in the morning and afternoon of the same day were 40 and 240 mmol/mol creatinine, respectively. Since this difference suggested a time-related increase of lactate excretion, attention was also given to lactate excretion in 24-h urine.

During an acidic episode of CH-2 the 24-h urinary excretion of L-lactate and D-lactate was 245 and 6200 mmol/mol creatinine, respectively (Table 2). During a nonacidotic period, the total lactate excretion measured in a 24-h urine collection was nearly identical (6250 mmol/mol creatinine). The 24-h urine collections of CH-1 in a nonacidotic period consistently showed very high D-lactate and only low L-lactate excretions (Tables 2 and 3). All these data together strongly suggested that D-lactate was excreted according to a certain pattern. Therefore, L- and D-lactate excretion and concomitant pH in 6-h urine fractions of CH-1 were studied during 3 successive days in a nonacidotic period. As shown in Table 4, D- and L-lactate excretion increased during the day and cleared during the night. The pH demonstrates a corresponding pattern but decreasing during the day.

During the same days serum L- and D-lactate concentrations and pH were also studied. These data (Table 4) demonstrate increasing serum D- and L-lactate concentrations during the day, but a rather constant serum pH. The lowest pH value of 7.38 coincides with a total lactate concentration of ~3500 μmol/L.

Urinary Lactate Excretion in SB Adults

Total lactate excretions in 24-h urines of adult SB patients were relatively low (40–225 mmol/mol creatinine; median 90) (Table 2). Because of the low total lactate excretion, only in some of these urines were D- and L-lactate excretions measured. D-Lactate was excreted in all measured samples (20–110 mmol/mol creatinine; median 30), as was L-lactate (from 15 to 25 mmol/mol creatinine; median 20) (Table 2).

Discussion

D-Lactate is a very uncommon metabolite in normal individuals but is commonly detected in SB patients. Serum (both D- and L-) lactate concentrations did not essentially differ during acidic and nonacidotic periods in the SB children. Our data on serum lactate concentrations and urinary lactate excretion (a) confirm that L-lactate is metabolized and that D-lactate is accumulated and then excreted, and (b) indicate that food consumption affects D-lactate production and alters D-lactic acidemia and aciduria.

The presented data fit into the following model. In the resected intestines of SB patients, D- and L-lactate are produced (b) abundantly by fermentative activity of continuously and even dominantly present lactobacilli (manuscript in preparation), and absorbed from the intestinal lumen into blood. High serum D- and L-lactate concentrations in young SB patients during both acidic and nonacidotic episodes will result from kinetic physiological processes, which are strongly affected by the diurnal feeding pattern. Bacterial lactate production from carbohydrates will take place soon after every meal (starting after breakfast), and may result in a pulsewise influx into blood. L-Lactate will be metabolized, but influx of D-lactate will soon exceed urinary clearing and, because of accumulation, D-lactic acidemia will arise. During the night, bacterial lactate production will decrease and even stop until the next food intake in the morning, but accumulation will go on until the uptake rate equals the clearing. During the later evening and during the night the blood is cleared of D-lactate. Urinary D-lactate excretion will follow the accumulation pattern within a certain time interval. Thus, a circadian rhythm will arise for D-lactic acidemia and aciduria (Table 4).

All 24-h urines contained D-lactate excreted during the day. In accordance with the circadian rhythm, D-lactate excretions were not increased or were only slightly increased in urine samples (not the first urine passed) of both CH-1 and CH-2, which had been collected in the morning (as at the start of this study) or in the early afternoon. Therefore, SB patients should be monitored by time-related measurements in successive urine and blood samples.
The SB adults in this study were known never to have had clinical symptoms of D-lactic acidosis. The measured serum L-lactate concentrations of SB adults equaled those of SB children, but for an unknown reason their D-lactate serum concentrations were much lower than those of the SB children.

In conclusion, high metabolite concentrations are common in blood and urine of patients with a hereditary disorder, but, as far as we know, our finding high D- and L-lactate concentrations daily present in blood and (or) urine of patients with an iatrogenic disorder is unique. The high serum concentrations and urinary excretions of D-lactate are related to meals; therefore, they may be seen not only when patients are acidic but also when they are not. Thus, in an acidic SB child, the finding of a high D-lactate does not constitute strong evidence that the etiology of the acidosis is D-lactic acidemia. Since D-lactic acid is characteristic in SB patients, the practical importance of our findings is that measuring of only D-lactate in these patients will not contribute to diagnosis and is not yet useful for treatment. The conclusive finding of the circadian rhythm serves a better understanding of D-lactate metabolism in SB patients, and a better monitoring of disease states in these patients. However, for monitoring the acidic state of SB patients it is important to measure not only D-lactate, but also L-lactate in blood and urine. In non-SB patients with unexplained metabolic acidosis, intestinal bacterial overgrowth, and (or) widely fluctuating serum total lactate concentrations and (or) urinary total lactate excretions, it may be useful for diagnosis and (or) monitoring of either disease state or therapy to study the occurrence of time-related D- and L-lactate increase in serum or urine. A measured absence of a metabolite in a patient becomes more convincing after eliminating a circadian rhythm.

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References