Proton Nuclear Magnetic Resonance Spectral Profiles of Urine from Children and Adolescents with Type 1 Diabetes, Cecilia Zuppi,1,2 Irene Messana,1,3 Päivi Tapaniainen,4 Mikael Knip,5 Federica Vincenzoni,1 Bruno Giardina,1,2 and Matti Nuutinen4* (1 Institute of Biochemistry and Clinical Biochemistry, Catholic University Rome, 00168 Rome, Italy; 2 Center for the Chemistry of Biologically Active Molecules, National Council of Research, 00168 Rome, Italy; 3 Department of Sciences Applied to Biosystems, University of Cagliari, 09100 Cagliari, Italy; 4 Department of Pediatrics, University of Oulu, FIN-90014 Oulu, Finland; 5 Hospital for Children and Adolescents, University of Helsinki, FIN-00029 Helsinki, Finland; * address correspondence to this author at: Department of Pediatrics, University of Oulu, PO Box 5000, FIN-90014 Oulu, Finland; fax 358-8-315-5559, e-mail matti.nuutinen@oulu.fi)

The assessment of renal function is crucial in type 1 diabetes because nephropathy is one of the major complications. The measurement of urinary albumin excretion is the most widely used method for monitoring kidney involvement (1), although the use of other markers, such as enzymes or proteins derived from tubular cells (2), has also been proposed. 1H nuclear magnetic resonance (NMR) spectroscopic analysis of urine may provide useful information because it allows the analysis, in a single image, of several metabolites that reflect diverse renal functions, including intermediary metabolism as well as tubular and medullary cell function.

In this study, we compared the 1H NMR spectra of urine samples from children and adolescents with type 1 diabetes, but without microalbuminuria or any other renal complication, with spectra of samples from healthy individuals matched for sex and age. The aim of this comparison was to obtain basic knowledge of possible differences in the urinary excretion or concentrations of a series of metabolites between patients with type 1 diabetes and nondiabetic individuals, with the prospect of evaluating the utility of diabetes-associated differences in the assessment of the patients’ metabolic control.

1H NMR spectra were registered at 25 °C, following a standardized protocol (3) and using a Gemini 300 apparatus (Varian) operating at 300 MHz. Metabolite quantification was performed using peak heights normalized in relation to the signal of creatinine. Thus, the values obtained for each metabolite are expressed as millimoles per mole of creatinine present in the sample.

Spot urine samples were obtained from 25 patients with type 1 diabetes. Patients (15 girls and 10 boys; mean age, 13.9 years; range, 6.5–17.8 years) with normal serum and urinary creatinine and without any signs of microvascular complications were recruited into the study haphazardly among patients older than 6 years visiting the Outpatient Diabetes Clinic, Department of Pediatrics, University of Oulu. Seven patients were younger than 10.0 years, 11 were 10.0–14.0 years, and 7 were older than 14.0 years. The mean duration of diabetes was 5.3 years (range, 0.0–12.0 years). Urine samples were also obtained from 25 healthy school children matched for age and sex.

Written informed consent was obtained from the parents of both patients and controls. All urine samples were frozen at −80 °C for 1H NMR analysis, which was performed within 1 month after the sampling. The storage of urine has been shown to cause no loss of available biochemical information (3).

The patients’ glycated hemoglobin A1c (HbA1c) was assayed with the DCA 2000 analyzer (Bayer Diagnostics) at the visit when the urine sample was collected. On the basis of the HbA1c values, the patients were divided into groups with HbA1c <8.0% (n = 10), HbA1c 8.0–10.0% (n = 9), and HbA1c >10.0% (n = 6). The duration of diabetes was >6 years in 7 patients, 3–6 years in 8 patients, and <3 years in 10 patients.

Data are given as mean ± SE. P <0.05 was considered statistically significant.

Typical 1H NMR spectra for urine samples from a patient affected by type 1 diabetes and a healthy individual are shown in Fig. 1, A and B, respectively. The most prominent peaks present in both samples, although at different concentrations, were lactate (1.33 ppm), alanine (1.49 ppm), acetate (1.94 ppm), citrate (2.57 and 2.72 ppm), dimethylamine (2.73 ppm), creatinine (3.06 ppm; reference for quantification), trimethylamine N-oxide (3.29 ppm), and hippurate (7.81 ppm).

Spectral analyses also showed the presence of acetone in the urine of two patients (2.24 ppm; 87.0 and 948.0 mmol/mol of creatinine, respectively) and the presence of acetocacete (2.29 ppm; 55.0 mmol/mol of creatinine) in one, which were not revealed by dipsticks used for
routine analysis. Moreover, the high glucose concentrations observed in 11 patients made it impossible to quantify trimethylamine N-oxide and some other metabolites, including glycine (3.57 ppm) and betaine (3.27 ppm), consistently present in the controls, because of the overlap of their respective peaks with the glucose signals.

The ratios of metabolites to creatinine in the nondiabetic children and adolescents were generally comparable to those reported earlier in healthy adults (3), but that of acetate was significantly higher in the Finnish children than in the Italian adults (37.1 ± 5.5 vs 8.3 ± 1.3 mmol/mol of creatinine; $P < 0.001$). The metabolites were also analyzed as a function of age based on the three age groups established. No significant differences were observed among the age groups, except for citrate, which decreased significantly with age ($P = 0.003$), being $254 \pm 34$ mmol/mol of creatinine in the youngest (<10 years) age group, 209 ± 29 mmol/mol of creatinine in the children 10–14 years of age, and 113 ± 15 mmol/mol of creatinine in children >14 years. In contrast, there were no differences in urinary citrate in relation to age. Significant differences were observed in several metabolites between the patients and the age- and sex-matched controls. Highly significant differences were seen for citrate, alanine, lactate, and hippurate, which were all higher in diabetic children than in the unaffected individuals (Table 1). Urinary lactate ($P < 0.01$) and acetate ($P < 0.03$) were increased in the patients with poor metabolic control ($\text{HbA}_{1c} > 8.0\%$) in comparison with patients with good metabolic control ($\text{HbA}_{1c} < 8.0\%$). No differences were observed for the other metabolites in relation to $\text{HbA}_{1c}$, although a wider range was characteristic of the patients with impaired metabolic control.

Alanine, lactate, acetate, and citrate were significantly

The vertical expansion of the region corresponding to the glucose resonances has been decreased fivefold. For experimental details, see the text.
(P <0.04) higher in the patients with glucosuria than in those without glucosuria. No correlation was found between the duration of type 1 diabetes and the urinary excretion of the different metabolites (as estimated by the ratio to creatinine).

The mechanism of the increase of citrate, alanine, lactate, and hippurate in type 1 diabetes is not known, but these metabolites are affected by metabolic factors, the glomerular filtration rate, and tubular function. The importance of the hyperexcretion of these metabolites for the enhanced glomerular filtration rate characteristic of type 1 diabetes is unknown, but lactate and acetate were higher in those patients with HbA1c >8.0%.

We hypothesize that the consistently higher citrate, alanine, and hippurate might reflect the increased glomerular filtration rate characteristic of type 1 diabetes (4) and/or a modification of the transport mechanisms at the tubular level. The latter may be related either to altered cellular function or to the presence of high glucose concentrations in the tubular lumen.

In particular, in our patients, the citrate concentration was weakly (P = 0.04) correlated with glucosuria, but not correlated with HbA1c disease duration, and age, suggesting that the increase of citrate may be mainly related to the enhanced glomerular filtration rate and partially to the effect of glucosuria on the citrate transport carrier. Furthermore, citrate reabsorption is mainly sensitive to the tubular cell pH (5). In this respect, the absence of citrate in the two patients with good glycemic control (HbA1c <7.5%) may be attributed to an alteration of the reabsorption mechanisms in proximal cells.

The increased alanine and lactate excretions (as estimated by the ratio to creatinine) may be related to increased gluconeogenic (6) and glycolytic activities, respectively. We observed, however, a further increase in lactate excretion in patients with HbA1c >10.0% and found a significant relationship between glucosuria and both alanine and lactate concentrations. High glucose concentrations in the tubular lumen have been shown to alter tubular uptake of these metabolites (7).

The observed increase in the hippurate concentration may be attributable to increased hepatic availability of its precursors, mainly acetyl-CoA, in patients with type 1 diabetes. High hippurate excretion may reflect an adequate acid excretion rate (8), and a reduced concentration of hippurate indicates a reduced ability of the kidney to eliminate acids and may hence be considered an early marker of impaired renal function.

The ability of NMR spectroscopy to detect all three ketone bodies provides simultaneous information on their specific relationships. Further information on metabolic status was obtained by assessing acetate concentration, which was higher in patients with HbA1c >10.0% and with glucosuria >180 mmol/L, i.e., in patients with inadequate metabolic control.

In conclusion, NMR spectroscopy may provide a relevant method for the study of metabolites that could be useful markers in monitoring metabolic status and renal function in patients with type 1 diabetes. Such metabolites are not readily measurable with traditional techniques.

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References

Table 1. NMR results for urine samples from children with type 1 diabetes (n = 25) and age- and sex-matched controls (n = 25).

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Controls*</th>
<th>Type 1 diabetes*</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
</tr>
<tr>
<td>Citrate</td>
<td>217.4</td>
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</tr>
<tr>
<td>TMAO o</td>
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<td>10.4</td>
</tr>
<tr>
<td>Alanine</td>
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<td>4.7</td>
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<tr>
<td>Lactate</td>
<td>39.6</td>
<td>6.6</td>
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<tr>
<td>Hippurate</td>
<td>311.3</td>
<td>42.2</td>
</tr>
<tr>
<td>Acetate</td>
<td>37.1</td>
<td>5.5</td>
</tr>
</tbody>
</table>

*Results are expressed as mmol/L of creatinine.

o TMAO, trimethylamine oxide; ND, not determined; NS, not significant.

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**Nanotechnology and Applications: An All-Language Literature Survey Including Books and Patents, Larry J. Kricka**

1. Department of Pathology and Laboratory Medicine, University of Pennsylvania Medical Center, 3400 Spruce St., Philadelphia, PA 19104.
2. The Children’s Hospital of Philadelphia, University of Pennsylvania School of Medicine, 310-C Abramson Pediatric Research Center, 34th St. and Civic Center Blvd., Philadelphia, PA 19104; *author for correspondence: fax 215-662-7529, e-mail kricka@mail.med.upenn.edu

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