thromboplastin time reagents are used when PT (ISI, 1.0; Quick method) and activated partial thromboplastin times are longer than the corresponding times for patient samples containing citrate samples, the times for PT coagulation and activated partial thromboplastin times in the reaction mixture. The Ca$^{2+}$ concentration in the reaction mixture was 1.3 mmol/L. When 0.109 mol/L citrate samples were used in the Quick method, the lowest Ca$^{2+}$ concentration in the reaction mixture was 1.3 mmol/L. The Ca$^{2+}$ concentrations in the coagulation mixtures are clearly different between two sample citrate concentrations with the Quick method.

When a sample is diluted (1 + 9) with citrate solution, the dilution occurs in plasma, not in whole blood, and depending on the hematocrit, the plasma volume will differ from sample to sample. The situation is similar if too little blood is drawn into the collection tube and the tube is underfilled. The Ca$^{2+}$ concentration in patient plasma can sometimes be low. The citrate concentration is markedly variable in plasma, as well as in the coagulation reaction mixture in the Quick method.

Because the Ca$^{2+}$ concentration is lower in 0.129 mol/L citrate samples, the times for PT coagulation and activated partial thromboplastin time measurements are longer than the corresponding times for patient groups designated as "no anticoagulant therapy" and "anticoagulant therapy" when PT (ISI, 1.0; Quick method) and activated partial thromboplastin time reagents are used. Duncan et al. (8) showed that the concentration of citrate in the sample affects the results and ISI calibration (Quick methods). The citrate concentration exerts an ~10% effect on the ISI of reagents. The above-mentioned results of two studies agree well with the PT and calcium results in our study.

With Owren’s PT, the mean difference in the Ca$^{2+}$ concentration in the reaction mixture was 0.01 mmol/L (0.4%) and the CVs were 4.9% for 0.129 mol/L citrate and 3.0% for 0.109 mol/L. The sample citrate concentration has no effect on the Owren method, and this method is also less sensitive to other preanalytical variables by reason of the small sample volume (5%) in the reaction mixture. Because the Owren and Quick methods depend differently on sample citrate concentrations (Ca$^{2+}$ in the reaction mixture), the correlation is not so good.

We subscribe to the demand for unification of recommendations of a sample citrate concentration of 0.109 mol/L to improve results for PT and activated partial thromboplastin times (8, 9). For results obtained by the Owren method, ISI correction should be used to standardize patient INR results and anticoagulant therapy to be the same as the results obtained by the Quick method because the recommendations for care are made according to the Quick method. The patients whose samples were analyzed by the Owren method were receiving too much medication and were in greater danger of bleeding.

I thank the staff of the Laboratory at the Valkeakoski District Hospital for their assistance.

References

Assay for Hexosamine Pathway Intermediates (Uridine Diphosphate-N-Acetyl Amino Sugars) in Small Samples of Human Muscle Tissue, Paul N. Span,1,2 Marie-José J.M. Pouwels,2 André J. Ollilhaar,1 Renko R. Bosch,3,2 Ad R.M.M. Hermus,2 and C.G.J. (Fred) Sweepe1 (Departments of 1 Chemical Endocrinology and 2 Internal Medicine, Division of Endocrinology, University Medical Centre Nijmegen, 6500 HB Nijmegen, The Netherlands; * address correspondence to this author at: Department of Chemical Endocrinology 530, University Medical Centre Nijmegen, PO Box 9101, 6500 HB Nijmegen, The Netherlands; fax 31-24-3541484, e-mail p.span@ace.azn.nl)

It has been suggested that the hexosamine biosynthetic pathway is involved in the pathogenesis of insulin resistance in patients with type 2 diabetes mellitus because the
activity of the rate-limiting enzyme of this pathway, glutamine:fructose-6-phosphate amidotransferase (EC 2.6.1.16), is increased in skeletal muscle of patients with type 2 diabetes mellitus (1). In rats, the skeletal muscle concentrations of the major end products of this pathway, i.e., UDP-N-acetylgalactosamine (UDP-GalNAc) and UDP-N-acetylglucosamine (UDP-GlcNAc), are highly correlated with the degree of insulin resistance (2). HPLC-based method have been applied to measure UDP-hexosamines in rat or murine tissues (2–7). In contrast to capillary zone electrophoresis (8, 9), these methods are reportedly not sensitive enough to measure hexosamines in small samples of tissue, and most are not able to separate the glucose-galactose epimers of UDP-hexosamines and UDP-epoxides (9).

For our studies into the role of the hexosamine pathway in type 2 diabetes mellitus, we were interested in a method to assess UDP-galactose (UDP-Gal), UDP-glucose (UDP-Glc), UDP-GalNAc, and UDP-GlcNAc in small samples of human muscle tissue, e.g., percutaneous muscle biopsies. To this end, we optimized and characterized an HPLC-based assay (5). The assay was suitable for application in small samples of human muscle tissue (>30 mg) and separated all of the above-mentioned analytes of interest.

Human muscle tissue (musculus gluteus maximus) was obtained from 17 patients during hip replacement surgery, after approval by the institutional human research committee and after informed consent. Twelve patients (6 females and 6 males) were normoglycemic with hemoglobin A1c values of 4.4–5.9%. Five patients (four females and one male) were diabetic, with hemoglobin A1c values of 7.2–9.6%. Tissues were homogenized with homogenization buffer (100 mmol/L KCl, 1 mmol/L EDTA, 50 mmol/L KH2PO4, pH 7.5; 5 mL of buffer/g of tissue) in a dismembrator (45 s at highest setting; Braun) using liquid nitrogen. For optimization and characterization of the assay, a pool of eight muscle tissues from normoglycemic patients was used. After centrifugation at 60 000 g for 15 min at 4 °C, 1600 pmol of UDP-mannose and 25 pmol of UDP-xylose were added to the supernatant as internal standards. A typical HPLC elution profile of UDP-sugars is shown in Fig. 1. The assay was linear over the entire range measured. Linear regression analysis of measured amounts (y; corrected for endogenous concentrations) vs added amounts (x) gave the following coefficients (standard errors in parentheses): UDP-Gal, y = 0.92 (± 0.001)x + 0.006 (± 0.002); UDP-Glc, y = 0.98 (± 0.004)x – 0.02 (± 0.011); UDP-GalNAc, y = 0.97 (± 0.001)x + 0.005 (± 0.002); and UDP-GlcNAc, y = 1.02 (± 0.001)x – 0.002 (± 0.002); R² >0.999 for all.

Interassay CVs, based on five replicate assays of 15- and 30-mg equivalents of a pooled muscle homogenate and at six added concentrations, are shown in Table 1. Even in a muscle tissue equivalent as low as 15 mg (wet weight), CVs were ≤13% for all UDP-sugars. Intraassay CVs at endogenous concentrations in a 30-mg tissue sample are shown in Table 1.

The limit of detection, defined as 3 times the SD of the background signal, and the limit of quantification, defined as 10 times the SD, were 0.04 and 0.125 nmol/mL of homogenate, respectively. This limit of quantification was equivalent to 0.625 mmol/g of tissue (wet weight). Considering the amounts of UDP-sugars found in the pooled

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**Fig. 1. HPLC elution profile of UDP-sugars.**

UDP-mannose and UDP-xylose were added as internal standards. A typical HPLC profile of endogenous UDP-Gal, UDP-Glc, UDP-GalNAc, and UDP-GlcNAc in a 30-mg (wet weight) equivalent human muscle tissue pool is shown.
In muscle tissues, a minimum of 37, 13, 32, and 20 mg of tissue (wet weight) would be sufficient to accurately quantify UDP-Gal, UDP-Glc, UDP-GalNAc, and UDP-GlcNAc, respectively.

Aliquots of muscle homogenate could be kept at −80 °C for at least 3 months, and freeze-thawing of the muscle homogenate pool did not lead to diminished concentrations of UDP-sugars. The fractions obtained after strong ion-exchange chromatography could be kept at room temperature overnight without degradation.

In 12 normoglycemic subjects the following concentrations, mean (SD), of UDP-sugars were found: UDP-Gal, 1.19 (0.35) nmol/g of tissue; UDP-Glc, 3.99 (1.1) nmol/g of tissue; and UDP-GlcNAc, 6.61 (3.4) nmol/g of tissue. These amounts did not differ between male and female subjects and exhibited gaussian distributions. In muscle tissue from 5 diabetics, we found similar (1.96) nmol/g of tissue; UDP-Gal, 4.40 (0.61) nmol/g of tissue; UDP-GlcNAc, 8.20 (1.96) nmol/g of tissue; and UDP-GalNAc, 6.1 (3.9) nmol/g of tissue; UDP-Glc, 13.9 (5.4) nmol/g of tissue. These amounts for at least 3 months, and freeze-thawing of the muscle homogenate pool did not lead to diminished concentrations of UDP-sugars. The fractions obtained after strong ion-exchange chromatography could be kept at room temperature overnight without degradation.

### Table 1. Interassay CVs for UDP-sugars.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Endogenous</th>
<th>Addition (% of endogenous)</th>
<th>Intraassay</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 mg</td>
<td>30 mg</td>
<td>25</td>
</tr>
<tr>
<td>UDP-Gal</td>
<td>5.9</td>
<td>6.6</td>
<td>5.5</td>
</tr>
<tr>
<td>UDP-Glc</td>
<td>3.3</td>
<td>6.9</td>
<td>4.7</td>
</tr>
<tr>
<td>UDP-GalNAc</td>
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<td>12</td>
<td>9.2</td>
</tr>
<tr>
<td>UDP-GlcNAc</td>
<td>6.1</td>
<td>3.9</td>
<td>2.8</td>
</tr>
</tbody>
</table>

*CVs for five separate determinations in a 15- or 30-mg wet weight muscle tissue pool and six added concentrations.

Liver-Type Arginase Is a Highly Sensitive Marker for Hepatocellular Damage in Rats, Masaki Ikemoto,† Shoji Tsunekawa,2 Yoshinobu Toda,3 and Masayuki Totani4 (1 College of Medical Technology, Kyoto University, Kyoto 606-8507, Japan; 2 Department of Surgery, NTT West Kyoto Hospital, Kyoto 601-8441, Japan; 3 Study Center for Human Remains, Faculty of Medicine, Kyoto University, Kyoto 606-8507, Japan; 4 The National Institute of Health and Nutrition, Shinjuku-ku, Tokyo 162-8636, Japan; † author for correspondence: fax 81-75-751-3945, e-mail mmas@kuhp.kyoto-u.ac.jp)

Hepatic enzymes in serum, such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT), are routinely measured in serum for the diagnosis of hepatic disease; however, these enzymes are not liver specific because they are widely distributed in nonhepatic tissues. In contrast, urea cycle enzymes, i.e., liver-type arginase (ARG), ornithine carbamoyltransferase (OCT), and argininosuccinate synthase (AS), exist almost exclusively in the liver (1–3) and may serve as more specific markers of liver damage.