D-Mannose is an essential monosaccharide in the structure of glycoproteins, cell-surface glycoconjugates, and glycosylated phosphatidylinositol anchors. Several studies have focused on plasma or serum mannose concentrations in patients with invasive candidiasis (1, 2), diabetes mellitus (3–8), or congenital disorders of glycosylation type 1 (9).

There are various methods to determine mannose concentrations in human plasma or serum: enzymatic methods (4, 5, 7, 10), gas-liquid chromatography (2, 11), high-resolution liquid chromatography (12), gas-liquid chromatography–mass spectrometry (6), and capillary electrophoresis (13). None of these methods is fully suitable for routine use for various reasons, e.g., the incomplete or time-consuming elimination of the ~100-fold excess of blood glucose, the use of instruments with limited availability, and the need for a large sample volume. We investigated whether a HPLC assay using an anion-exchange column would be appropriate for plasma mannose determinations. With the procedure described below, mannose could be rapidly and accurately determined in small amounts of plasma.

Boric acid, guanidine hydrochloride, sodium metaperiodate, and acetonitrile were purchased from Wako Pure Chemicals Co. Ltd. D-Mannose was obtained from Sigma Chemical Co.

We obtained blood samples from healthy individuals and from nondiabetic and diabetic patients after receiving their informed consent for this study. After collection of venous blood in tubes containing disodium EDTA (an anticoagulant) and NaF (a glycolysis inhibitor), plasma was separated by centrifugation. The plasma was mixed with an equal volume of 0.6 mol/L perchloric acid, a protein-precipitating reagent. The mixture was centrifuged at 8000 g for 10 min at 4°C, and the protein-free supernatant was used for the analysis of mannose.

We used a HPLC system (Gulliver system with a JASCO PU-980 pump) equipped with a Finepak GEL SA-121 anion-exchange column [10 cm × 6.0 mm (i.d.)]. The column was maintained at 80°C. We injected 20 µL of the supernatant prepared as described above into the HPLC system. Elution was with a gradient of 0.25 mol/L sodium borate buffer, pH 8.0 (solvent A), and 0.6 mol/L sodium borate buffer, pH 8.0 (solvent B), as follows: gradient from 80%A/20%B to 60%A/40%B for 17 min; gradient from 60%A/40%B to 0%A/100%B for 1 min; maintenance at 0%A/100%B for 17 min; gradient from 0%A/100%B to 80%A/20%B for 2 min; and maintenance at 80%A/20%B for 20 min. The eluate from the column was mixed with a reagent consisting of 50 mmol/L guanidine hydrochloride, 0.5 mmol/L sodium metaperiodate, and 0.1 mol/L boric acid in 200 mL/L acetonitrile and adjusted to pH 11.0 with 5 mol/L NaOH. This mixture was heated at 170 °C in a reaction oven. The guanidine adducts of sugars were monitored by a fluorescence detector with excitation at 310 nm and emission at 415 nm. The flow rate of both the mobile phase and the guanidine reagent was 0.5 mL/min. A diagram of the present HPLC system is shown in Fig. 1. The mannose concentration was calculated from peak areas, based on a calibration curve prepared with solutions of mannose.

References
calibrators. Plasma glucose was assayed by the glucose oxidase method with a commercial assay (Glucose B-Test; Wako).

A calibration curve generated by the present HPLC method for mannose was linear up to 400 \(\mu\text{mol/L}\). This value, corresponding to a plasma mannose concentration of 800 \(\mu\text{mol/L}\), covers the range of plasma mannose concentrations likely to be encountered clinically. As shown in Fig. 1, mannose was completely separated from glucose and other substances. The detection limit, defined as the concentration corresponding to a signal 3 SD above the mean for a calibrator free of analyte, was 5 \(\mu\text{mol/L}\).

We searched for a usable internal standard. Under the elution conditions described above except that the time for the gradient from \(80\%\) A/\(20\%\) B to \(60\%\) A/\(40\%\) B was changed from 17 min to 30 min, the plasma samples of healthy adults showed two appreciable peaks after that of mannose at 17.8 min. Retention times (in min) of the monosaccharides and their analogs detectable in human plasma were as follows: \(\text{d-}\)fructose, 21.8; \(\text{d-}\)galactose, 24.3; \(\text{d-}\)glucitol, 25.6; 1,5-anhydro-\(\text{d-}\)glucitol, 7.6; and \(\text{myo-}\)inositol, 12.5. These data suggested that the two peaks described above might correspond to those of \(\text{d-}\)fructose and \(\text{d-}\)galactose. The various monosaccharides and their analogs tested as candidates for an internal standard were found to have the following retention times (in min): \(\text{d-}\)arabinose, 21.2; \(\text{d-}\)altrose, 28.4; \(\text{d-}\)alloose, no peak; 2-deoxy-\(\text{d-}\)glucose, 12.5; \(\text{l-}\)rhamnose, 13.9; 3-\(\text{O-}\)methyl-\(\text{d-}\)glucose, 20.3; methyl-\(\text{d-}\)glucoside, 7.3; and methyl-\(\text{d-}\)glucoside, 7.4. It seems that a compound eluting between \(18\) and \(25\) min and also having a retention time clearly different from retention times of sugars detectable in human plasma would be suitable as an internal standard. However, we could not find such a compound. We therefore analyzed standard mannose samples in each set of assays performed with the present method without an internal standard.

To test the reproducibility of the method, we aliquoted three plasma samples (one from healthy individuals and two from diabetic patients) into 11 vials each and stored them at \(-20\ ^\circ\text{C}\). The within-run imprecision (CV), estimated with 10 successive assays of the three samples on the same day, was 1.4\%, 0.4\%, and 0.7\% for 19.2 \(\mu\text{mol/L}\), 79.3 \(\mu\text{mol/L}\), and 115.0 \(\mu\text{mol/L}\) mannose, respectively. Between-day variation was assessed by assaying one freshly thawed sample every day for 7 consecutive days. The mean (SD) mannose concentrations of the three samples were 18.8 \(\pm\) 0.5 \(\mu\text{mol/L}\) (CV, 2.7\%), 76.1 \(\pm\) 2.9 \(\mu\text{mol/L}\) (CV, 3.8\%), and 111.4 \(\pm\) 3.9 \(\mu\text{mol/L}\) (CV, 3.5\%), respectively. These values were similar to or better than
those reported by other researchers using enzymatic methods, e.g., a within-run CV (n = 11) of 4.4% at 80 μmol/L (10) and a between-run CV (n = 12) of 10.4% at 75 μmol/L (7).

Analytical recovery was examined using plasma specimens (160 μL each) from a reference pool containing 30.6 μmol/L mannose. Samples were supplemented with 40 μL of water, 500 μmol/L mannose, or 1 mmol/L mannose. Each supplementation was performed in triplicate and yielded recoveries of 104.0% for the lower-concentration mannose samples and 102.6% for the higher-concentration samples.

We studied potential interference from endogenous substances using a plasma pool (mannose concentration, 79 μmol/L) obtained from healthy volunteers. A one-tenth volume of the solution containing a possible interferent was added to the plasma. Glucose at 50 mmol/L, fructose at 200 μmol/L, free plus conjugated bilirubin at 340 μmol/L, hemoglobin at 5.0 g/L, uric acid at 600 μmol/L, and ascorbic acid at 20 mmol/L did not affect the measured mannose values.

The mean plasma mannose concentration of 15 samples from healthy individuals (10 from females and 5 from males), collected in the morning after fasting, was 35.6 ± 12.6 μmol/L. The mean glucose concentration of the same plasma samples was 4.64 ± 0.87 mmol/L. The observed mannose value was comparable to those reported by other investigators: e.g., 39 ± 12 μmol/L (n = 14) (6); 18.5 ± 5.5 μmol/L (n = 6) (7); 53.8 ± 9.3 μmol/L (n = 23) (9); 54.1 ± 11.9 μmol/L (n = 11) (10).

Both mannose and glucose concentrations in plasma samples (n = 21) from nondiabetic and diabetic patients were measured. A close positive correlation (r = 0.779; P < 0.01) was observed between mannose and glucose concentrations. Our data are consistent with those reported previously (6, 7). We also measured the mannose and glucose concentrations in plasma samples collected at 0, 30, 60, and 120 min after oral administration of 75 g of glucose to healthy, 14-h fasted volunteers (two females and three males). The plasma mannose concentration gradually decreased until at least 120 min after glucose ingestion, whereas the glucose concentration increased transiently, as is widely known (Table 1). To the best of our knowledge, this is the first report of the time course of changes in the plasma mannose concentration under physiologic conditions. We are now studying the mechanism of the change in the plasma mannose concentration induced by glucose load.

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In women undergoing in vitro fertilization and embryo transfer (IVF-ET), serial measurements of 17β-estradiol (E2) can be used to monitor follicular growth, and serum