Evaluating taurine status: determination of plasma and whole blood taurine concentration

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Overview

The most reliable estimation of body taurine status is based on plasma and whole blood taurine concentrations.[1] Although the much greater taurine concentration in blood cells relative to plasma is remarkable (100 times higher in lymphocytes, 300 times higher in granulocytes, and 400 times higher in platelets[2-5]), the utility of the whole blood taurine concentration has only been appreciated recently.[1] The whole blood taurine pool is remarkably stable and varies only under extremes of taurine depletion or sustained supplementation, whereas the fluctuations in the plasma taurine pool reflect acute changes in taurine availability.[1] Assessing whole blood taurine also introduces less chance for technical error than assaying plasma or the taurine pools in specific blood cells.

The average plasma taurine concentration reported for healthy human adults reveals a relatively wide variation ranging from 39 to 116 μmol/L.[2-4,6-14] Such variance in the normal plasma taurine concentration suggests that some reported values are imprecise due either to analysis or sampling technique. A recurring problem when assessing plasma taurine is that spuriously elevated plasma taurine concentrations can derive from contamination by intracellular taurine, i.e., from platelets and white blood cells. This problem is directly affected by the blood-collecting procedure.[1] The choice of anticoagulant in conjunction with sample handling also influences the plasma taurine concentration. The blood-collecting and handling methods described below have proved most reliable and accurate for assessing plasma and whole blood taurine concentrations.[1]

Chromatographic techniques such as amino acid analysis based on ion-exchange chromatography,[15-17] gas-liquid chromatography[18] and high performance liquid chromatography using pre-column derivatization[19-24] have been utilized for taurine analysis. However, some of these assays have difficulty in separating taurine from other interfering amino acids. Recent procedures for removing interfering amino acids have been coupled with pre-column derivatization for rapid taurine determination by HPLC, especially in biological fluids and tissues.[12,25-27] A rapid and sensitive measure-

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ment of plasma and whole blood taurine that utilizes pre-column derivatization and HPLC after preliminary sample purification by ion-exchange column chromatography is described below.

Procedures

Blood collection and handling

Reagent: Ethylenediaminetetraacetate (EDTA) 10%. Dissolve 10 g sodium (tetra) ethylenediaminetetraacetate in water and dilute to 100 mL.

Draw venous blood from the antecubital vein into a dry, sterile disposable plastic syringe and immediately transfer into a 10% EDTA-treated (10μL/mL) plastic tube. Keep the blood samples at room temperature prior to separation of plasma. Approximately 2–3 mL of blood is needed to estimate both plasma and whole blood taurine. Transfer an aliquot of 400 μL of whole blood into a 1.5 mL microcentrifuge tube for analysis of whole blood taurine concentration. Separate plasma by centrifugation for 15 min at 1500 × g at room temperature to ensure that minimal plasma contamination occurs from the release of intracellular taurine.

Sample preparation

Deproteinization of plasma and whole blood samples

Reagent: Trichloroacetic acid (TCA) 100%. Dissolve 100g solid trichloroacetic acid in water and dilute to 100 mL.

Plasma: In a 1.5 mL microcentrifuge tube mix 400 μL plasma with 40μL 100% TCA-solution (to reach a final concentration of 9% TCA in the plasma sample). Vortex thoroughly and centrifuge for 5 min at 12000 × g in a microcentrifuge to obtain a clear protein-free supernatant.

Whole blood: Freeze the 400 μL of whole blood in the 1.5 mL microcentrifuge tube by placing on dry ice, and thaw in an ultrasonic bath. Repeat two times to achieve maximum taurine release from blood cells. Deproteinize 200 μL of the whole blood sample with 50 μL 100% TCA solution (to reach a final concentration of 20% TCA in the whole blood sample). Vortex immediately and centrifuge for 5 min at 12000 × g to obtain a clear protein supernatant.

Sample purification procedure using ion-exchange column chromatography to separate taurine from amino acids that interfere with the taurine analysis by HPLC

Reagent: Cation exchange resin AG50W-X8 (200-400 mesh) in the hydrogen form and Anion exchange resin AG 1-X (100-200 mesh) in the formate form (both from BioRad Laboratories, Richmond, CA, USA)

Prepare columns (large-volume pasteur pipettes, 0.5 cm ID x 150 mm) by layering approximately 2 cm of the cation AG–50W–X8 resin over 2 cm of the anion AG 1–X resin above a small glass-wool plug. Flush columns with 10 mL water prior to use. Filter an aliquot of 100 μL of the deproteinized whole blood or 300 μL of the deproteinized plasma supernatant through the prepared ion-exchange column. Flush the column with 3.0 mL water and collect the taurine eluant into a small disposable glass tube.

Taurine analysis by high performance liquid chromatography (HPLC)

Chromatographic solvents

Solvent 1: 0.05 M monosodium phosphate buffer, pH 5.3. Prepare by dissolving 6.9 g monobasic sodium phosphate (NaH_2PO_4) in 1000 mL double-distilled water and adjust the pH to 5.3 by adding 5 N sodium hydroxide (NaOH).

Solvent 2: 0.05 M monosodium phosphate buffer (pH 6.4) containing 75% methanol. Prepare by dissolving 6.9 g of monobasic sodium phosphate in 250 mL double-distilled water and 750 mL HPLC grade methanol.

Mobile phase: Prepare the HPLC mobile phase by combining 440 mL of Solvent 1 with 560 mL of Solvent 2 and degassing under vacuum.

Pre-column derivatization reagent

Reagents: O-Phthalaldehyde (OPA, Sigma Chemical Company, St. Louis, MO, USA)
Absolute ethanol
2-Mercaptoethanol (Sigma Chemical Company, St. Louis, MO, USA)
0.5 M borate buffer, pH 10.3. Dissolve 79.6 g sodium tetraborate in water, dilute to 500 mL and adjust pH to 10.3 by adding 5 N NaOH.

Prepare the OPA reagent by dissolving 20 mg of o-phthalaldehyde in 400 μL absolute ethanol, 10 mL 0.5 M borate buffer (pH 10.3) and 20 μL 2-mercaptoethanol. To avoid shouldering of the taurine peak on HPLC prepare this reagent fresh daily and store at room temperature in a glass tube wrapped with aluminum foil.

Taurine standard solutions

Prepare seven taurine standard solutions with concentrations ranging from 0.5 to 20 μmol/L with a 1 mmol/L taurine stock solution. The stock solution of taurine is prepared by dissolving 25.0 mg of crystalline taurine (Sigma Chemical Company, St. Louis, MO, USA) in 200 mL water. Store stock solution refrigerated in the dark. Stock solution is stable for several months. Prepare once a week the following working standards:

20 μmol/L: Dilute 2mL of the taurine stock solution in 100 mL water.
10μmol/L: Dilute 1mL of the taurine stock solution in 100 mL water.
7.5 μmol/L: Add 6 mL of the 10 μmol/L taurine solution to 2 mL water.
5 μmol/L: Add 5 mL of the 10 μmol/L taurine solution to 5 mL water.
2.5 μmol/L: Add 2 mL of the 10 μmol/L taurine solution to 6 mL water.
1 μmol/L: Dilute 1 mL of the 10 μmol/L taurine solution in 10 mL water.
0.5 μmol/L: Add 5 mL of the 1 μmol/L taurine solution to 5 mL water.

Keep all taurine working solutions refrigerated prior to use.

HPLC procedure

The HPLC method used for taurine analysis is based on that of Larsen et al.[25]

Equipment: The HPLC system included a Rainin Rabbit HP pump (Rainin Instruments, Woburn, MA, USA), a Rheodyne syringe injector with a 100 μL sample loop (Rheodyne Inc., Cotati, CA, USA), a radial-compression separation system RCA-100 with a NovaPak radial-Pak cartridge column (5NY-C18- 4 micron) and a Guard-PAK pre-column insert (μBondapak C18; Waters, Milford, MA, USA), an FD-300 dual-monochromator fluorescence detector with an 8 μL flow cell (SpectroVision, Chelmsford, MA, USA), and a Shimadzu CR3 A integrator (Shimadzu, Columbia, MD, USA). Similar HPLC systems including a similar column and a fluorescence detector can be used.

The mobile phase is pumped at a flow rate of 2mL/min. The absorbance of the OPA-taurine adduct is monitored at an excitation wavelength at 360 nm and an emisson wavelength at 455 nm. The analysis is recorded and quantitated with an integrator. The taurine concentration of the sample is calculated based on the peak area from the known concentrations of the external standard solutions. The HPLC column is flushed with methanol at a flow rate of 0.02 mL/min when not in use.

Analysis: To 1 mL of standard solution (used directly without clean-up step) or 1 mL of sample eluant, add 100 μL OPA reagent. Mix well and allow to react for exactly 1.0 min at room temperature before injecting into the HPLC. In order to obtain highest reproducibility the reaction time (1 min) must be controlled precisely.

Calculations

The taurine concentration of samples is calculated by comparing the peak area of the sample to the peak areas of the standards. Develop a standard

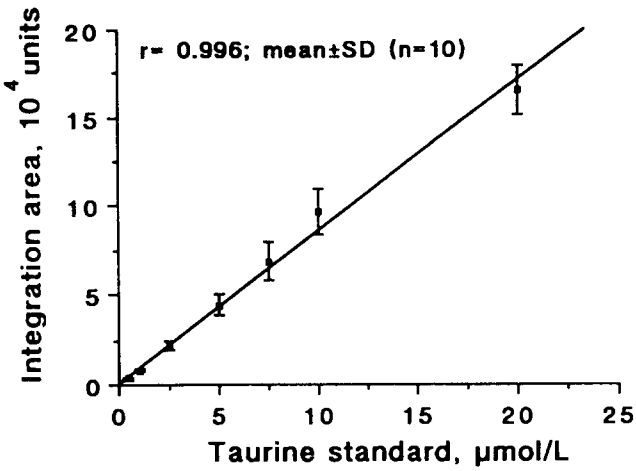


Figure 1 The taurine standard curve is generated by plotting taurine standards (μmol/L) versus integration area. The error bars indicate the SD of 10 separate determinations.

curve by plotting the integrated peak area (y-axis) versus the standard concentrations (x-axis) and calculate the slope of the linear regression (*Figure 1*). Because plasma and whole blood samples are diluted during the purification process, taurine standards ranging from 0.5 to 20 μmol/L are used to encompass the estimated range for taurine in the diluted human samples. Determine the taurine concentration in the sample from the standard curve multiplied by the appropriate dilution factor (dilution occuring during deproteinization and ion-exchange clean-up step).

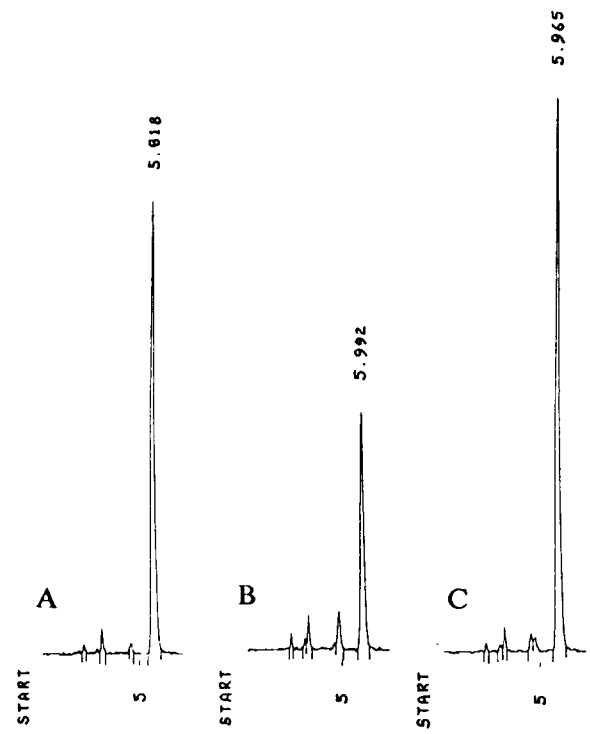
Discussion

The above method, including some modifications of earlier procedures,[12,25-27] provides a reliable, accurate and rapid determination of taurine in plasma and whole blood samples. Typical chromatograms for a taurine standard and samples of human plasma and whole blood are depicted in *Figure 2*. As seen in that figure, the sample purification procedure utilizing ion-exchange column chromatography efficiently removes amino acids such as cysteic acid, cysteine sulfinic acid, phospho-serine, phospho-threonine, phosphorylethanolamine and hypotaurine. The ability of the ion-exchange clean-up to remove compounds that interfere with taurine separation by HPLC has been described previously.[25]

Taurine recovery from the ion-exchange column, as tested by applying taurine standard solutions, was 96 ± 2.7 μmol/L (CV: 2.8%, $n=5$) for a 100 μmol/L taurine solutions and 10 ± 0.9 μmol/L (CV: 8.9%, n=5) for a 10 μmol/L taurine solution[1]. The within-day variation (CV) of 3.0% was observed for human plasma and whole blood taurine, and the day-to-day variation (CV) was 3.6% for plasma samples, and 5.7% for whole blood samples.

As indicated previously,[1] the blood-collecting and handling procedure in conjunction with the anticoagulant has an important bearing on the reliability of the taurine determination. The method described here, using plastic syringes for blood drawing, EDTA as anticoagulant, and strict handling of blood samples at room temperature, was shown to be more reliable than other sampling techniques.[1] Both icing of the blood samples or exposure to glass containers (such as commonly used Vacutainers, Becton Dickinson, Vacutainer Systems, Rutherford, NJ, USA) prior to separation of plasma can induce platelet taurine release resulting in spuriously elevated plasma taurine concentrations.[1]

The mean plasma taurine concentration in humans was 44 ± 12 μmol/L ($n=72$) with 80% of the plasma taurine values ranging between 35 and 60 μmol/L. It is presumed that this represents the normal physiological range for plasma taurine. Plasma taurine levels below 30 μmol/L may



A: Taurine standard; 5 μmol/L
B: Plasma sample with a taurine concentration of 25 μmol/L
C: Whole blood sample with a taurine concentration of 191 μmol/L

Figure 2 Typical HPLC chromatograms obtained from a taurine standard (5 μmol/L), as well as human plasma and whole blood samples.

indicate the onset of taurine depletion, although it is not known to what degree a low plasma taurine concentration concurs with depletion of whole blood or tissue stores in humans. Plasma taurine concentrations exceeding 80 μmol/L suggest spillage of intracellular taurine, although taurine concentrations of approximately 100 μmol/L have been observed after sustained dietary taurine supplementation. Normal whole blood taurine concentrations ranged between 160 and 320 μmol/L with a mean of 225 ± 38 μmol/L ($n = 30$).[1] Because plasma and whole blood taurine concentrations were not correlated under normal physiological conditions, assessment of both provides the most accurate estimate of taurine status in humans.

Although the analysis describes the preferred method for taurine determination in plasma and whole blood taurine, this method can be modified for determination of taurine in blood cells, such as platelets and leucocytes, as well as other physiologic fluids (urine) and tissues (liver).

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