

Short Communication

Temporal reproducibility of taurine measurements in frozen serum of healthy postmenopausal women

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Animal studies and small clinical trials have shown that taurine (2-aminoethanesulphonic acid), a sulphur-containing molecule mainly obtained from the diet in human subjects, has a variety of biological actions that are related to atherosclerosis and cardiovascular functions. However, epidemiological studies of taurine and CHD risk are lacking. We evaluated whether a single measurement of serum taurine could serve as an estimate for long-term serum levels. Serum taurine was measured using HPLC in three annual samples from thirty postmenopausal women selected from the New York University Women's Health Study. Overall, serum taurine values ranged from 62.8 to 245.3 nmol/ml, with a mean of 140 nmol/ml. The intraclass correlation coefficient of a single measurement of serum taurine was 0.48 (95% CI 0.26, 0.68), which can be improved to 0.65 by using the mean of two annual measurements. The CV was 7%. These results indicate that the mean of two or more annual measurements of serum taurine is a sufficiently reliable measure of long-term serum levels that can be used in epidemiological studies.

Taurine: HPLC: Reproducibility: Epidemiology: Biomarkers

Taurine (2-aminoethanesulphonic acid), a sulphur-containing molecule, is synthesised in the liver from methionine and cysteine by coenzyme pyridoxal-5'-phosphate, the active form of vitamin B₆⁽¹⁾. However, human subjects have a low level of cysteine sulphinic acid decarboxylase, which is required for the biosynthesis of taurine⁽²⁾; therefore, most taurine comes from the diet, especially from seafoods and the dark meats of poultry. Mean daily intake of taurine in adult human subjects has been estimated to be between 40 and 400 mg⁽³⁾, typically falling closer to the lower end of the range. Several small clinical trials and ecological studies have suggested that taurine can lower cholesterol and blood pressure. However, prospective case-control or cohort studies examining the possible health effects of taurine are lacking.

In epidemiological studies, usual dietary intake can be measured using a FFQ with information on quantity and frequency of food consumption. However, the content of taurine in seafoods differs appreciably by type, and in meat, it depends on the cut of the meat⁽¹⁾, which is the information that is not often collected in FFQ. For instance, raw turkey dark meat contains 24.4 µmol/g of taurine, while raw turkey white meat contains only 2.3 µmol/g of taurine⁽¹⁾. Therefore,

commonly used FFQ that are not designed to measure taurine intake are likely to have a limited validity as a result of omitted food items and lack of detailed information about specific cuts. In addition, FFQ cannot measure the small amount of taurine which is endogenously synthesised. Biological measurements are more objective than FFQ, which may be affected by recall bias, and may better reflect the 'internal dose', measuring taurine from both exogenous and endogenous sources.

Temporal reproducibility refers to the consistency of measurements from the same person at different times⁽⁴⁾. The reproducibility of a biomarker can provide useful information about its performance in measuring the usual level of exposure, which is of key interest in epidemiological studies. Serum or whole blood taurine measurements are considered markers of long-term intracellular taurine concentration because they are not rapidly influenced by recent taurine intake, unlike measurements from plasma or urine^(5,6). In many prospective epidemiological studies, serum samples instead of whole blood samples were collected, and therefore, it is useful to assess the reproducibility of serum taurine to judge its utility in epidemiological studies.

Abbreviation: ICC, intraclass correlation coefficient.

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The aim of the present study is to evaluate the temporal reproducibility of a single measurement of serum taurine using repeated measurements over time.

Methods and materials

New York University Women's Health Study

The New York University Women's Health Study (NYUWHS) enrolled 14 274 women, 34–65 years of age, between 1985 and 1991 at a breast cancer screening centre in the New York City. At the time of enrolment and at annual screening visits, participants were asked to donate blood and complete self-administered questionnaires. Blood was kept in covered tubes at room temperature (21–26°C) for 1 h, and was then refrigerated at 4°C for 30 min to allow clot retraction. Tubes were then centrifuged for 15 min at 3500 rpm. Supernatant was pooled into a 15 ml transfer polystyrene tube, and was partitioned into 1 ml capped plastic vials. Serum samples were stored at –80°C until use⁽⁷⁾. The present study was approved by the Institutional Review Board of New York University School of Medicine. Written informed consent was obtained from all the subjects at the time of enrolment.

Temporal reproducibility study design and subject selection

We selected thirty postmenopausal women at random from the New York University Women's Health Study participants who (1) donated blood annually five or more times during follow-up visits; (2) had not been diagnosed with CVD or cancer; and (3) were not selected as controls in any ongoing case–control studies nested within the cohort. Serum samples donated at the second, third and fourth visits were measured for the thirty participants. Samples from later visits were selected to conserve baseline blood donations for nested case–control studies. For quality control purposes, duplicate aliquots from ten samples, selected at random from the ninety reproducibility samples, were measured to assess the CV. Laboratory personnel was blinded to the identity of each of the donors.

Chromatographic instrumentation

All HPLC measurements were made using the following Waters equipment (Millford, MA, USA): Multi λ Fluorescence Detector 2475; Autosampler 717 Plus; Binary Pump 1525; Inline Degasser AF; Bus SAT/IN Module. The excitation wavelength was set at 333 nm, the emission wavelength at 450 nm, the gain at 1 and the emission/sample energy units full scale (EUFS) at 10 000. Peak areas were calculated using Waters Breeze software. A YMC Hydrosphere (150 mm \times 4.6 mm; S-5 μ m, 12 mm) C18 column (Waters) was protected by a Spherisorb ODS Supelco (1 cm \times 4.6 mm; 5 μ m) guard column (Sigma-Aldrich, St Louis, MO, USA).

The mobile phase⁽⁸⁾ consisted of 1.0 mmol/l potassium phosphate buffer, 85% HPLC-grade water (Fisher Scientific, Pittsburgh, PA, USA) and 15% acetonitrile (Sigma-Aldrich Chromasolv Plus for HPLC \geq 99.9%), pH 6.8. The 0.5 mmol/l potassium phosphate solutions were made by dissolving 17.0 g potassium phosphate monobasic for HPLC (Fluka, Buchs, Switzerland) in 250 ml HPLC-grade water,

and 21.8 g potassium phosphate dibasic anhydrous for HPLC (Fluka) in 250 ml HPLC-grade water. The phosphate solutions were combined until the pH reached 6.8, and the solution was filtered using a cellulose acetate 0.22 bottle top filter (Corning, Lowell, MA, USA). Mobile phase flow was set at 1 ml/min.

Standards and reagents

Taurine standards (Sigma) were prepared (50, 40, 30, 20, 10, 5 and 2.5 μ g/ml) using HPLC-grade water, and were stored at –80°C for up to 3 months, at which point, they were discarded, new standards were made and a new calibration curve was generated (described below).

Reagents used were based on, but slightly modified from, the reagents used in a previously published protocol⁽⁹⁾. *o*-Phthalaldehyde pre-column derivatisation was used because it is simpler, less time consuming, less expensive, more reproducible and more sensitive when compared with an ion exchange HPLC with post-column ninhydrin reaction⁽¹⁰⁾. The working solution reagents were made as follows: (1) 40 μ l of 3-mercaptopropionic acid (Biochemika grade; Fluka) were added to 1 ml methanol (HPLC grade; Fisher Scientific); (2) 27 mg of *o*-phthalaldehyde (Sigma-Aldrich HPLC) were added to 1 ml methanol; (3) 0.1 mmol/l solution of sodium borate was prepared by dissolving 38.1 g sodium tetraborate (Fisher Scientific) in 1 litre HPLC-grade water by adjusting the pH to 9.65 and filtering. 3-Mercaptopropionic acid was made daily, while *o*-phthalaldehyde was made once a week and was stored at –20°C. A working solution was made daily by adding together 20 μ l *o*-phthalaldehyde, 40 μ l 3-mercaptopropionic acid and 940 μ l of 0.1 mm-sodium borate.

Sample preparation and derivatisation

New York University Women's Health Study serum samples were thawed for approximately 15 min before 100 μ l of serum were added to 400 μ l of 100% methanol in a 1.5 ml microcentrifuge tube (Fisher Scientific). After 6 min of incubation, the solution was centrifuged (HERMLE Z180M Labnet) in a cold room for 5 min at 8000 g. The supernatant was removed and centrifuged for 1 min at 8000 g through a filter tube (Costar Spin-X LC Centrifuge Tube 0.22 μ m Nylon Propylene). One hundred microlitres of the filtered sample (or 20 μ l taurine standard and 80 μ l methanol) were added to 150 μ l of working solution and 750 μ l of 0.1 mm-sodium borate. After exactly 7 min, 20 μ l of the sample (or standard) were injected into the HPLC machine, and measured with the HPLC column held at ambient temperature. The standard was run for 22 min, while the sample was run for 60 min.

Calibration curve

Various concentrations of taurine standard were run to create a calibration curve. The standard was identified as the only peak that changed area and height when run at different concentrations. The retention time of taurine was approximately 6 min. Peak area for each of the concentrations of taurine standard was plotted against concentration. A calibration

curve was fitted to the data, and was used to estimate taurine concentration for each sample.

Statistical analysis

Taurine serum values were log base 2 transformed to reduce the skewness of distribution. The intraclass correlation coefficient (ICC)⁽¹¹⁾, the proportion of total variability (within- and between-subject) due to variability between the subjects, was estimated using repeated biological samples from the same individuals⁽¹²⁾ using PROC VARCOMP in Statistical Analysis Systems (version 9.1; SAS Institute, Inc., Cary, NC, USA), and the exact 95% CI was calculated⁽¹³⁾. The closer the ICC is to 1, indicating little within-subject variation relative to the between-subject variation, the better a single measurement of a biomarker is at differentiating the relative ordering of the level among the individuals⁽⁴⁾.

The overall CV was estimated by dividing the SD of each pair of duplicates by the mean of that pair, and averaging the pair-specific CV. The overall CV served as the index of laboratory method error.

The temporal reproducibility of a biomarker can be improved by averaging repeated measurements. The temporal reproducibility of the mean of multiple measurements, R_m , can be estimated as $R_m = (m \times R)/(1 + (m - 1)R)$ ⁽¹²⁾. In this formula, m is the number of independent replicate measurements from a subject, and R is the reproducibility (ICC) of a single measurement.

Results

Serum samples were stored for an average of 19.1 years. At the time of the second blood donation, the mean age of the study participants was 57 years. Ninety-two percentage of the subjects in the reproducibility study were white, 57% were never smokers and 62% had higher education, and the mean BMI was 25.3 kg/m² at baseline. This distribution was similar to that of postmenopausal women who gave more than three blood samples in the overall New York University Women's Health Study cohort (i.e. 80% white, 49% never smokers and 60% with higher education, and mean BMI of 25.6 kg/m²).

Taurine was detected in 100% of the samples. Table 1 shows the mean values and standard deviations of taurine measurements for each of the three annual samples. The range of serum taurine did not differ greatly by year of collection, with an overall range from 63 to 245 nmol/ml.

Mean taurine varied by year of collection from 129 to 149 nmol/ml, but the SD was similar across the years.

The ICC for a single measurement of taurine was 0.48 (95% CI 0.26, 0.68). The CV was 7%, indicating that the laboratory measurement error was small. There was no significant correlation between the mean of the three annual measurements of serum taurine and age, BMI or smoking status of the participants (data not shown). Using the formula discussed earlier, we calculated how much the ICC would increase with an increasing number of repeated measurements for each individual. Two samples from each individual would increase the ICC to 0.65, while three samples would improve the ICC to 0.73.

Discussion

Using three annual samples from thirty women, we estimated the temporal reproducibility of a single measurement of serum taurine as 0.48, which is on the lower side of the typical reproducibility values (0.5–0.7) of commonly used exposure variables in epidemiological studies⁽⁴⁾, indicating that a single measurement would be insufficient to differentiate individuals with regard to their long-term serum taurine concentrations in our study population. The average of at least two measurements, though, would provide a fairly good measure of exposure (ICC = 0.65).

Low reproducibility of biomarkers of exposure can result in a reduction of the statistical power of a study. Although statistical techniques are available to correct for exposure measurement errors in epidemiological studies, such methods can only be applied to exposures with a good reproducibility^(14,15). The present results emphasise that when multiple samples are available for the study subjects, it is possible to evaluate health effects of serum taurine levels with a satisfactory reproducibility.

Taurine concentrations measured in this reproducibility study ranged from 63 to 245 nmol/ml, which are values similar in magnitude to those reported previously^(16,17). Taurine has been shown to be very stable at high temperatures⁽¹⁸⁾, and it is not affected by freeze–thaw cycles⁽¹⁹⁾. Consistent with these observations, our data indicate that although the samples used in our study were frozen for almost 20 years, levels of serum taurine were similar to the values reported in the literature using samples stored for a shorter time. However, additional research is needed to assess taurine stability over greater lengths of storage time.

The study subjects were all healthy at the time of blood donation, ensuring that serum taurine levels were not

Table 1. Annual mean, standard deviation, and the overall mean and 10th and 90th percentiles of thirty New York University Women's Health Study serum taurine measurements (Mean values and standard deviations; median and ranges)

Samples	Taurine value (nmol/ml)				
	Mean	SD	Median	Range	10th and 90th percentiles
Second year	129	39	126	65–201	79, 190
Third year	149	41	157	63–241	97, 188
Fourth year	141	40	132	72–245	99, 196
All	140	40	138	63–245	92, 191

influenced by disease or a change in diet due to disease. The standardisation of sample collection, storage and handling are also major strengths of the present study⁽⁷⁾, as a delay between sample donation and preparation can result in variations in concentration of taurine⁽¹⁰⁾. Furthermore, the study assessed relatively long-term reproducibility using samples collected annually. However, it should be noted that our study population included only postmenopausal, mostly Caucasian women, and the results may not be generalisable to other populations. Our samples were collected during the time when taurine was not used frequently as a supplement or as an ingredient in 'energy' drinks, such as Red Bull. Given the popularity of including taurine in these products, information on their use should be collected in current epidemiological studies.

These results suggest that the average of two annual measurements would lead to a fairly good assessment of a woman's long-term average level of taurine. This finding should be considered when planning the use of serum taurine measurement as a biomarker in epidemiological studies.

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