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The betaine content of sweat from adolescent females

Shona S Craig¹, Stuart AS Craig^{2*}, Matthew S Ganio³, Carl M Maresh³, Greg Horrace¹, Kerry-Ann da Costa⁴, Steven H Zeisel⁴

Abstract

Background: This study was developed to establish whether betaine was present in the sweat of females and to determine any correlations with other sweat components.

Methods: Sweat patches were placed on eight trained adolescent Highland dancers (age = 13.6 ± 2.3 yr), who then participated in a dance class for 2 hours. Patches were removed, and the sweat recovered via centrifugation. The sweat was subsequently analyzed for betaine, choline, sodium, potassium, chloride, lactate, glucose, urea and ammonia.

Results: Betaine was present in the sweat of all subjects ($232 \pm 84 \mu\text{mol}\cdot\text{L}^{-1}$), which is higher than typically found in plasma. The concentration of several sweat components were correlated, in particular betaine with most other measured components.

Conclusion: Betaine, an osmoprotectant and methyl donor, is a component of sweat that may be lost from the body in significant amounts.

Background

Betaine is a methylamine that is widely distributed in nature where it is found in microorganisms, plants and animals. It is a significant component of many foods, including whole grains (e.g. wheat, rye), spinach, shellfish and beets [1], and low levels of dietary intake may increase disease risk [2-5]. Betaine is a trimethyl derivative of glycine that functions as an organic osmolyte to protect cells under stress (e.g. dehydration, high concentrations of electrolytes, urea and ammonia) and as a source of methyl groups for use in many key pathways via the methionine cycle [2]. Betaine accumulates in most tissues (e.g. liver, kidney, intestine, skin, muscle, etc.) [6], is non-perturbing to cellular metabolism, highly compatible with enzyme function, and stabilizes cellular metabolic function [2,7-14]. Betaine plays an important role in several aspects of human health and nutrition and recent studies show that ingestion of betaine may improve athletic performance [15-17].

Betaine concentration has been measured in many human tissues and fluids, including blood and urine, but

has not been previously studied in sweat. Sweat can be considered a filtrate of plasma, cellular and interstitial fluid that contains electrolytes (e.g. potassium, sodium, and chloride), metabolic wastes (e.g. urea, ammonia and lactic acid), and various nutrients (e.g. vitamins and choline) [18-21]. The exact composition of sweat is dependent on several factors, including absorptive mechanisms in the sweat glands that may increase or decrease the concentration of solutes. We hypothesized that since betaine is a component of plasma and skin, it is also likely to be present in sweat. In addition, the above-mentioned protective role of betaine against electrolytes and metabolic wastes may extend to the sweat gland, duct and surrounding tissue. This study was conducted to determine whether betaine is a component of sweat that may be lost from the body during exercise.

Methods

Subjects

Eight trained female Scottish Highland dancers (10-17 yr) were recruited from the Stirling Highland Dance Company, Oakdale CT. The subjects trained regularly, and were actively competing in dance competitions. Subjects attended a briefing meeting before any

* Correspondence: stuart.craig@danisco.com

²Danisco A/S, Elmsford, NY, USA

experimentation to ensure an understanding of the testing parameters and the benefits/risks of the study. The subjects and parents signed a written informed consent statement. The study was part of the Somers High School (SHS) Science Research Program and the protocol was approved by the SHS IRB.

Experimental Protocol

Sweat patches were prepared by placing two 2" × 2" gauze squares onto 4" × 4.5" adhesive film. Care was taken to minimize any cross-contamination. New disposable latex gloves were utilized for each subject. The skin on the lower back of the subjects was cleaned with gauze and distilled water, dried, and two patches were placed on both sides of the spine. The dancers then conducted a 2 hour class. The sweat patches were removed, placed in plastic 6-ml centrifuge tubes and stored on ice prior to centrifugation. The tubes were spun for 2 min at 1315 g in a benchtop centrifuge (Model 0151; Clay Adams, Parsippany, NJ). The patches were removed from the tubes, and the sweat (1-2 ml) at the bottom of the tubes was recovered. Each subject had two tubes from the two patches. The sweat from the two tubes was combined and stored frozen at -20°C prior to analysis.

Measurements

Betaine, choline, and choline metabolites were determined in duplicate by liquid chromatography/electrospray ionization-isotope dilution mass spectrometry [22]. Lactate and glucose were determined in duplicate by enzymatic techniques (YSI 2300 Stat Plus, Yellow Springs, OH). Sodium, potassium and chloride were measured in duplicate using ion selective electrodes (Medica Easy Electrolytes, Medica Corp., Bedford, MA). Urea and ammonia were measured using a COBAS Mira Plus Analyzer (Roche Diagnostics, Indianapolis, IN) and Pointe Scientific (Canton, MI) reagent sets and standards. Instruments were calibrated using NIST certified standards.

Statistics

Grubbs' test <http://graphpad.com/quickcalcs/Grubbs1.cfm> was used to determine outliers in data sets ($\alpha = 0.05$). Pearson's correlation test (SigmaPlot v11, Systat Software Inc, San Jose, CA) and Passing-Bablok regression analysis (MedCalc, Mariakerke, Belgium) were conducted to compare data sets.

Results

The measures of sweat composition are shown in Table 1. Phosphatidylcholine and sphingomyelin were also measured, but were not detected (data not shown). The mean betaine content was $232 \pm 84 \mu\text{mol}\cdot\text{L}^{-1}$. The other components of sweat were found at levels similar to that of previous studies [18,19,21]. Four data points were identified (*) as outliers via Grubb's test. Pearson's

correlations test and Passing-Bablok regression analysis were conducted for the data in Table 1. Table 2 shows the Pearson's correlation coefficients between sweat components - values that show statistical significance ($p < 0.05$) or trends ($p < 0.10$) are in bold. Betaine is correlated with all components except sodium and chloride (Fig. 1). The non-parametric regression analysis (Passing-Bablok) gave similar results (not shown). None of the Pearson's correlations for potassium remain after removal of a data point ($19.3 \text{ mmol}\cdot\text{L}^{-1}$) that is an outlier via Grubb's test (Table 1). Table 3 compares the content of sweat measured in this study with typical fasting levels published for plasma [18,23-26].

We observed that betaine levels can drop if kept at room temperature for prolonged periods; therefore, it is important when collecting sweat samples to keep them in crushed ice until frozen. We speculate that enzyme or bacterial action might reduce betaine levels, but this requires further study. Also, preliminary results (not shown) suggest that betaine levels in sweat are higher after ingestion of betaine. Future work on the relationship between plasma and sweat levels is warranted.

Discussion

This is the first study to determine the betaine content of sweat, and the average concentration in adolescent female sweat ($232 \pm 84 \mu\text{mol}\cdot\text{L}^{-1}$) was found to be about 7 times higher than that typically found in female plasma ($34 \pm 11 \mu\text{mol}\cdot\text{L}^{-1}$) [25]. The majority of constituents in sweat, such as sodium, chloride, glucose and choline, are more dilute than in the blood plasma or interstitial fluid [20]. However, some constituents are more concentrated in sweat, such as lactate, urea, ammonia, and potassium to a small extent. There are studies that support the concept of higher betaine concentrations in sweat versus plasma. Firstly, betaine is actively accumulated as an osmolyte in skin cells under osmotic and oxidative stress [12,27]. Also, there are higher betaine concentrations (expressed as $\mu\text{mol}\cdot\text{L}^{-1}$ tissue water) in rat skin (males $412 \pm 185 \mu\text{mol}\cdot\text{L}^{-1}$; females $305 \pm 153 \mu\text{mol}\cdot\text{L}^{-1}$) compared to rat plasma (males $186 \pm 43 \mu\text{mol}\cdot\text{L}^{-1}$; females $101 \pm 37 \mu\text{mol}\cdot\text{L}^{-1}$) [6].

Mean dietary intake of betaine was recently estimated to be 100-200 mg/d [28,29]. Loss via urine averages about 10 mg/d [30]. Sweat rates are variable, but daily fluid requirements for sedentary to very active persons range from 2-4 L/d in temperate climates and from 4-10 L/d in hot climates [31]. Therefore, a range of 2-10 L/d sweat loss translates to a betaine loss of approximately 50-270 mg/d from the regional sweat data. These results suggest that betaine loss through sweat is greater than that lost through urine and may even exceed dietary intake in some cases. Collection of sweat using

Table 1 Sweat composition of subjects

| Subject | Betaine (μmol·L ⁻¹) | Choline (μmol·L ⁻¹) | Lactate (mmol·L ⁻¹) | Glucose (μmol·L ⁻¹) | Sodium (mmol·L ⁻¹) | Potassium (mmol·L ⁻¹) | Chloride (mmol·L ⁻¹) | Ammonia (mmol·L ⁻¹) | Urea (mmol·L ⁻¹) |
|---------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|--------------------------------|-----------------------------------|----------------------------------|---------------------------------|------------------------------|
| 1 | 363 | 2.77 | 27.6 | 582 | 37.9 | 19.3* | 29.1 | 11.73* | 19.68 |
| 2 | 160 | 1.38 | 15.7 | 302 | 46.7 | 8.62 | 34.6 | 4.31 | 7.69 |
| 3 | 332 | 5.75* | 27.2 | 447 | 46.6 | 8.73 | 35.2 | 6.75 | 13.77 |
| 4 | 277 | 0.98 | 18.7 | 415 | 52.4 | 9.06 | 37.7 | 5.41 | 6.75 |
| 5 | 140 | 1.17 | 13.8 | 272 | 52.0 | 6.20 | 36.5 | 3.01 | 7.67 |
| 6 | 157 | 1.61 | 23.1 | 491 | 40.9 | 9.11 | 26.5 | 6.40 | 12.61 |
| 7 | 196 | 1.01 | 18.5 | 411 | 36.3 | 8.03 | 24.9 | 5.57 | 9.17 |
| 8 | 229 | 2.28 | 18.0 | 356 | 81.7* | 8.59 | 57.6* | 3.34 | 8.59 |
| Average | 232 | 2.12 | 20.4 | 410 | 49.3 | 9.7 | 35.3 | 5.81 | 10.74 |
| SD | 84 | 1.60 | 5.1 | 101 | 14.4 | 4.0 | 10.2 | 2.74 | 4.38 |

* Outlier via Grubb's Test ($p < 0.05$)

regional patches is convenient and useful for relative comparisons, but the concentration of sweat constituents tends to be higher compared to values using whole body washdown [32,33]. Therefore further work is required to accurately determine total body loss, perhaps under varied exercise conditions. In addition, it would be valuable to determine any correlation between dietary intakes, serum concentrations, sweat concentrations and level of physical activity.

The data showed several statistically significant correlations between sweat metabolites. Not surprisingly, the strongest correlation was between sodium and chloride. Betaine was correlated with all components except sodium and chloride (somewhat surprising given the known relationship between betaine accumulation and salt tolerance). The correlation between lactate and potassium agrees with the correlation found (+0.78) in a previous study [33] in males. Muscle contractions cause lactic acidosis and loss of intracellular potassium with accumulation of extracellular potassium [34]. Lactic acid acidification has been shown to counteract the effects of elevated potassium associated with muscle fatigue [35]. This may form the basis of a correlation. Betaine, lactate

and glucose were all correlated with each other. Lactate and glucose are closely related via anaerobic metabolism. Also, a study showed that ingestion of betaine led to elevated serum lactate [15]. Although the current study was not designed to determine causation, betaine may accumulate to protect the sweat gland, duct and surrounding tissue from the deleterious effects of elevated concentrations of inorganic ions, urea, ammonia and possibly lactate - which are known to perturb cellular metabolism. Betaine protects the kidney from high concentrations of electrolytes and urea [2,36,37], prevents myosin structural change due to urea [9], and protects against ammonia toxicity of neurons [14]. This may relate to the correlations between betaine, ammonia, urea, lactate and potassium found here in sweat. Further research on the significance and reproducibility of these correlations is warranted.

In conclusion, betaine is a component of sweat. Betaine is an osmoprotectant, and we speculate that it protects the sweat gland against the deleterious effects of other sweat components. Further research is warranted, such as evaluation of male and/or older athletes, sweat collection via total body washdown [38], and

Table 2 Pearson's correlations (r) for sweat components

| | Betaine | Choline | Lactate | Glucose | Sodium | Potassium | Chloride | Ammonia | Urea |
|-----------|---------|--------------------|---------|--------------------|--------|--------------------|----------|---------|--------------------|
| Betaine | x | +0.65 [#] | +0.78* | +0.69 [#] | -0.08 | +0.70 [#] | +0.03 | +0.73* | +0.67 [#] |
| Choline | | x | +0.72* | +0.36 | +0.02 | +0.21 | +0.10 | +0.36 | +0.55 |
| Lactate | | | x | +0.90* | -0.36 | +0.67* | -0.31 | +0.85* | +0.89* |
| Glucose | | | | x | -0.45 | +0.79* | -0.43 | +0.92* | +0.86* |
| Sodium | | | | | x | -0.31 | +0.99* | -0.57 | -0.43 |
| Potassium | | | | | | x | -0.23 | +0.92* | +0.85* |
| Chloride | | | | | | | x | -0.50 | -0.37 |
| Ammonia | | | | | | | | x | +0.92* |
| Urea | | | | | | | | | x |

* $p < 0.05$

[#] $p < 0.10$

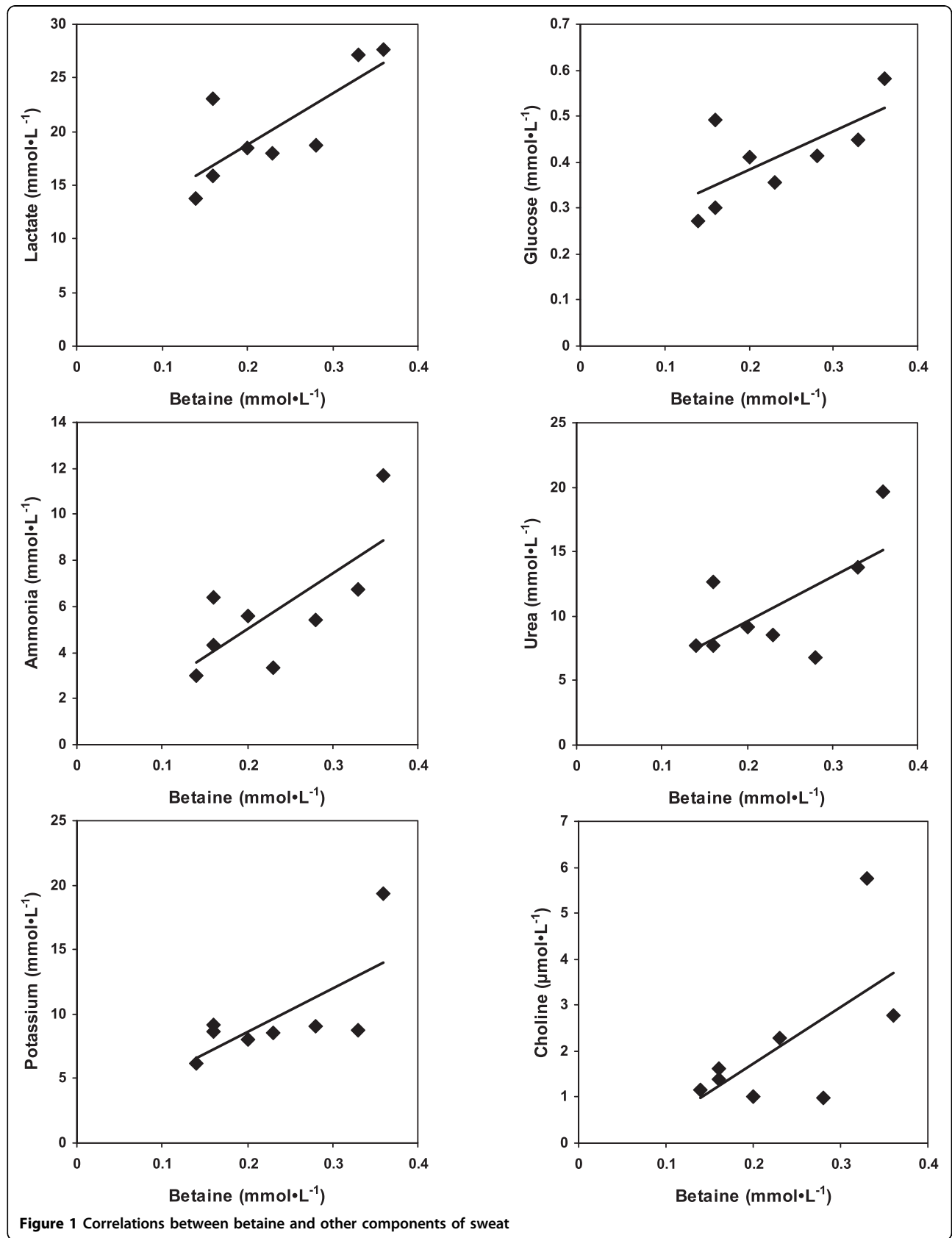


Table 3 Solute contents of sweat compared with published fasting values for plasma [18,23-26]

| | Sweat (S) | Plasma (P) |
|---|-----------|------------|
| Betaine ($\mu\text{mol}\cdot\text{L}^{-1}$) | 232 | 34.0 |
| Choline ($\mu\text{mol}\cdot\text{L}^{-1}$) | 2.1 | 14.5 |
| Lactate ($\text{mmol}\cdot\text{L}^{-1}$) | 20.4 | 0.7 |
| Glucose ($\text{mmol}\cdot\text{L}^{-1}$) | 0.41 | 4.9 |
| Sodium ($\text{mmol}\cdot\text{L}^{-1}$) | 49.3 | 141 |
| Potassium ($\text{mmol}\cdot\text{L}^{-1}$) | 9.7 | 4.1 |
| Chloride ($\text{mmol}\cdot\text{L}^{-1}$) | 35.3 | 105 |
| Ammonia ($\text{mmol}\cdot\text{L}^{-1}$) | 5.81 | 0.07 |
| Urea ($\text{mmol}\cdot\text{L}^{-1}$) | 10.74 | 5.7 |

determination of any correlation between type of exercise, plasma betaine levels, dietary intake of betaine, and sweat composition.

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Author details

¹Somers High School, Lincolndale, NY, USA. ²Danisco A/S, Elmsford, NY, USA. ³Human Performance Laboratory, Department of Kinesiology, University of Connecticut, Storrs, CT, USA. ⁴Department of Nutrition, School of Public Health and School of Medicine, University of North Carolina, Chapel Hill, NC, USA.

Authors' contributions

SSC was the primary investigator, study design, data collection/analysis and manuscript draft. SASC conceived the study, supervised, statistical analysis, manuscript preparation. MSG, KAC supervised and sweat analysis. CMM, GH, SHZ participated in concept, design, coordination and helped draft the manuscript. All authors read and approved the final manuscript.

Competing interests

Stuart Craig is employed by Danisco A/S, a manufacturer of betaine.

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