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ScienceDirect
Clinical Biochemistry 42 (2009) 706–712
CLINICAL BIOCHEMISTRY

Plasma and urine betaine and dimethylglycine variation in healthy young male subjects

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Received 12 November 2008; received in revised form 3 February 2009; accepted 3 February 2009
Available online 13 February 2009

Abstract

Objectives: We aimed to compare the individuality (within subject consistency) of plasma and urine betaine and *N*,*N*-dimethylglycine.

Design and methods: In two separate groups of 8 males (ages 19 to 40), plasma (10) and urine (6) samples were collected either over a single day or over an 8 week period. The individuality of the betaine and *N*,*N*-dimethylglycine plasma concentrations and excretions were estimated by one-way repeated measures analysis of variance. The reliability coefficients and indices of individuality were calculated. The between-subject variation in the study population was compared with that in a normal population ($n=192$ for plasma, 205 for urine).

Results: Plasma betaine concentrations were significantly different between subjects over 24 h and 8 weeks ($p<0.00001$). Plasma dimethylglycine concentrations were different over 24 h. Urine betaine and dimethylglycine excretions were different in both ($p<0.0001$). Betaine was more individual than dimethylglycine in both plasma and urine. Compared with a normal healthy population, the between-subject variation in plasma betaine was less ($p<0.001$) in the study group, but similar for dimethylglycine and for urine betaine.

Conclusions: Plasma betaine and urinary betaine excretions are more individual than dimethylglycine. Plasma and urine betaine are highly individual in the general population.

Keywords: Glycine betaine; Dimethylglycine; Biological variation; Urine betaine; Individuality

Introduction

Betaine (glycine betaine or *N*,*N*,*N*-trimethylglycine), and its metabolite *N*,*N*-dimethylglycine, are emerging as markers of pathological processes in vascular disease, renal disease, diabetes and possibly other chronic diseases [1–3]. Betaine has two important metabolic functions. It is a major osmolyte, responsible for cell volume regulation in many tissues [4] and countering urea cytotoxicity in kidneys [5]. Several tissues store millimolar or higher concentrations of betaine [6]. Betaine is also a source of methyl groups for the regeneration of *S*-adenosylmethionine, and tissue betaine is the major store of metabolically available methyl groups in mammals. Thus a disruption of betaine homeostasis is likely to have complex metabolic consequences.

Plasma betaine concentrations are usually much lower than tissue concentrations [6], are little affected by osmotic stress, and have been reported to remain stable for years [2]. On the other hand, they are affected by the dietary intake of betaine [7–9]. Minimal betaine is normally excreted in the urine, and the urinary excretion does not correlate with plasma concentrations. When normal subjects receive a betaine supplement little of the additional betaine appears in the urine, despite the increase in plasma betaine [7–9], and urine betaine is also not significantly affected by osmotic stress [10]. Instead, individual subjects appear to excrete an approximately constant amount of betaine for weeks and possibly years. Less is known about the control of dimethylglycine. The fractional clearance of dimethylglycine is not as low as that of betaine [3] but the predominant route for its clearance is probably by mitochondrial oxidative demethylation by dimethylglycine dehydrogenase rather than urinary clearance. Plasma and urinary dimethylglycine do increase after a betaine load, though this

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doi:10.1016/j.clinbiochem.2009.02.001

only accounts for a small proportion of the betaine supplied and appears to be transitory [9].

During the course of studies on the effects of betaine supplementation we collected repeated plasma and urine samples, over a single day and over an eight-week period, on healthy young adult male subjects. Here we report some measures of the variation in plasma betaine and dimethylglycine concentrations, and of their urinary excretions, between these young men. In particular, we focus on the degree to which individual subjects tend to return consistent results; when this trend is strong there is said to be high individuality and there is low within-subject variability compared with the between-subject variability. We also extrapolate these results from the selected study groups to the general population.

Methods

Subjects

Healthy Caucasian male volunteers aged 19–40 years were recruited by local advertisement. All subjects provided written informed consent following ethical approval of the study by the Canterbury Ethics Committee (Christchurch, New Zealand). Subjects had no current or previous history of vascular or renal disease, were free from acute or chronic illness requiring prescription medication, did not take dietary or vitamin supplements, were non-smokers and in good health based on medical history and physical examination. Subjects were excluded if they displayed any of the following; abnormal hemoglobin concentration (<130 g/L), abnormal plasma homocysteine concentration (<5 or >15 μmol/L), low vitamin B_{12} (<120 pmol/L), B_6 (<35 nmol/L) or red cell folate (<445 nmol/L), or the MTHFR TT genotype. Eight subjects were enrolled for a short-term study (repeat tests over 24 h), and a separate 8 subjects for a longer study (8 weeks duration).

Normal control subjects were recruited in a series of studies, each approved by the Canterbury Ethics Committee, and all gave written informed consent. The data used here is based on plasma samples from 105 male subjects and 87 females, and urine samples from 104 males, 93 females and 8 infants for whom the sex was not recorded.

Study designs

The full study designs are described elsewhere. The data analysed here is from the control arm (no treatment) of the short-term study [8] and from the baseline results of the longer study [9]. In the short-term study, subjects were asked to abstain from foods known to be high in betaine or choline [11–13], caffeine and alcohol for 2 days, and fast for 12 h, prior to treatment. Water and food intake was closely controlled throughout the day, with subjects receiving 100 mL water hourly and, immediately following the 3 h blood sampling time-point, and a standard lunch which was estimated to be low in betaine and choline from food composition databases [11–13]. Fasting venous blood samples were collected at baseline ($t=0$ h), via an indwelling cannula in the antecubital fossa, into EDTA tubes and immediately placed on ice. Blood samples were collected hourly at 1, 2, 3, 4, 5, 6, 7, 8 h and 24 h. A fasting urine sample was obtained at baseline, with subsequent sampling every 2 h at 2, 4, 6, 8 h and an overnight urine collection from 8 h to 24 h. Plasma was separated by centrifugation at 2000 ×g for 10 min within 2 h of blood collection. Urine sample volumes were recorded and a 10 mL aliquot removed. All samples were stored at −20 °C before analysis. Thus there were 10 plasma and 6 urine samples over a 24-hour period. Plasma concentrations of betaine and *N*, *N*-dimethylglycine, and urine concentrations of betaine, dimethylglycine and creatinine were measured.

The longer study was a randomized crossover study with 2 treatments: (A) a betaine rich-diet (supplying on average 845 mg betaine/day) (B) oral betaine supplementation (0.5 g trimethylglycine taken twice daily, Life Extension, Florida, USA, providing 1040 mg betaine/day by analysis) which was similar to that supplied in the betaine-rich diet. The study was conducted over 8 consecutive weeks, with a 5 day pre-treatment period (sample collections on day-5 and -1) immediately prior to the 14 day treatment period (designated day 0–13) in which subjects were randomly assigned to receive either treatment A or B, followed by a 5 day post-treatment period (sample collections on day 14 and 18) and a 14 day wash-out period between treatments. The pre-treatment samples, and the five-day post-treatment samples from both arms were used in this study. There were 10 plasma samples and 6 urine samples in this study also.

No subjects participated in both studies.

Biochemical analyses

Betaine and dimethylglycine were measured in plasma and urine by high performance liquid chromatography (HPLC) after derivatization with 2-naphthacyl triflate and UV detection as previously described [14,15]. Urine creatinine was measured using the Jaffé reaction with an Abbott Aeroset Analyzer (Abbott Laboratories, Abbott Park, IL 60064, USA), and urine betaine and dimethylglycine excretion calculated as a ratio to creatinine and (in the longer term study) as mg betaine/24 h.

Comparison with general population

Data on plasma and urine betaine in normal subjects had been collected on control groups over 15 years and this pooled data was used to compare the variance of the estimates in the young male study group with that in the general population.

Statistical analyses

The presence of individuality was primarily assessed by one-way repeated measures analysis of variance, which is equivalent to a two-way analysis of variance with the subjects and sample times as the factors. The reliability coefficient was calculated as the ratio of the between-subject variance to total variance in these two-way analyses [16,17]. The index of individuality [18] was also estimated, using the simplified relationship CV_I/CV_G of Fraser [19] where CV_I is the within-subject coefficient of