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Sex differences in the control of plasma concentrations and urinary excretion of glycine betaine in patients attending a lipid disorders clinic

Michael Lever [a,b,*], Wendy Atkinson [a], Peter M. George [a,b], Stephen T. Chambers [b]

[a] *Clinical Biochemistry Unit, Canterbury Health Laboratories, Christchurch, New Zealand*
[b] *Pathology Department, Christchurch School of Medicine and Health Sciences, Christchurch, New Zealand*

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Abstract

Objectives: To find whether the control of betaine metabolism differs between male and female patients and identify the effects of insulin and other hormones.

Design and methods: Data from non-diabetic lipid clinic patients (82♀ and 76♂) were re-analyzed by sex. Data on insulin, thyroid hormones and leptin were included in models to identify factors affecting the circulation and excretion of betaine and its metabolites.

Results: Different factors influenced plasma concentrations and urinary excretion of betaine, dimethylglycine and homocysteine in males and females. In males, apolipoprotein B (negative), thyroid stimulating hormone (positive) and insulin (negative) predicted circulating betaine, consistent with betaine-homocysteine methyltransferase mediated control. In females, insulin positively predicted plasma dimethylglycine. Urinary betaine excretion positively predicted circulating homocysteine in males ($p<0.001$), whereas dimethylglycine excretion (also indicating betaine loss) was a stronger positive predictor ($p<0.001$) in females. Carnitine affected betaine homeostasis.

Conclusions: Betaine metabolism is under endocrine control, and studies should use sex stratified groups.

Keywords: Betaine; Dimethylglycine; Betaine-homocysteine methyltransferase; Urine betaine; Homocysteine; Insulin; Carnitine; Thyroid hormones

Introduction

Glycine betaine (also known as "betaine" or "trimethylglycine") is a cell-volume regulator in mammalian tissues [1–3] that is also a reserve of methyl groups for methylation reactions, and its metabolism is a major determinant of circulating homocysteine in mice [4]. This is mediated by betaine-homocysteine methyltransferase which is known to be regulated both by the supply of methyl groups [5] and by osmotic stress [6]. The circulating concentrations of glycine betaine are lower than tissue concentrations and are tightly controlled around individual set-points [7]. Urine excretion of glycine betaine is normally minimal (>95% resorbed: [8]), but some patients excrete large amounts, including many (>25%) diabetic patients and about 10% of patients with established vascular disease [9–11].

Glycine betaine is obtained from the diet [12–14] and also by the metabolism of choline [15–17] and its deficiency may have direct clinical consequences [1]. In particular, betaine is accumulated by arterial endothelial cells in response to osmotic changes [18–20]. The relevance of its potential effect on homocysteine concentrations can be questioned since doubt has been recently thrown on the role of homocysteine as a risk factor for vascular disease [21,22]. These studies show that, in patients who have had a first vascular event, supplementing the whole group with folate and other B vitamins does not decrease the incidence of second events, even though the median circulating homocysteine is lowered significantly. Nevertheless there is still strong evidence for an association between homocysteine and vascular disease, recently reviewed [23], and there is a need to look at other factors that affect, or may be affected by, homocysteine [24]. Betaine loss may be one such factor since if a subset of the population has elevated homocysteine as a consequence of betaine deficiency, then treating this group with folate will not correct that deficiency. Specifically inhibiting

* Corresponding author. Biochemistry Unit, Canterbury Health Laboratories, PO Box, 151, Christchurch 8140, New Zealand.
E-mail address: michael.lever@chmeds.ac.nz (M. Lever).

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betaine-mediated homocysteine methylation in mice causes up to a seven-fold increase in circulating homocysteine [4], and the results of betaine supplementation in humans are consistent with this being general in mammals [25]. Betaine loss may be pathogenic through causing an increase in homocysteine, or the elevation of homocysteine may be a marker of a pathogenic loss of betaine.

Previously we reported that, among patients attending a lipid disorders clinic, abnormal glycine betaine excretion was more frequent than expected [11]. There was an increased incidence of vascular disease in patients with elevated (>30 mmol/mol creatinine) excretion, and they also had higher circulating homocysteine than matched patients attending the same clinic. Since our earlier report there has been further evidence of hormonal control of glycine betaine metabolism therefore we have included thyroid hormone, insulin and leptin measurements on the patients and confirmed that these are factors in controlling betaine metabolism in this population. During this reevaluation, we observed that the pattern of factors affecting the male and female subjects was different. We report these differences here and suggest that studies on populations including both sexes should be interpreted with caution.

Methods

Patients

One hundred and seventy consecutive patients attending the adult lipid disorders clinic at Christchurch Hospital, New Zealand were enrolled into the study. The hospital ethics committee approved the study protocol, and all patients gave informed consent. A summary of the cohort and protocols has been previously published [11], and Table 1 shows the differences between male and female patients for the more important features. In summary, we recorded the date of birth, sex, known disease, weight, body mass index (BMI), body fat percent, and current medication. The body fat ratio was measured by bioelectrical impedance analysis, using a "Body Fat Analyzer TBF 501", Tanita Corporation, Tokyo, Japan. Established cardiovascular disease was defined as previously diagnosed angina, stroke, vascular lesions (identified by ultra sound or angiography), angioplasty or coronary artery bypass graft. Paired blood and urine samples were collected in the morning after 12–14 h overnight fast.

Twelve of the patients with established diabetes were excluded. The remaining 158 patients were confirmed to be normoglycemic. The group was made up of 82 female subjects (mean age 55.0 with standard deviation 11.5 years) and 76 males (mean age 50.5±12.1 years). Fasting plasma insulin, thyroid function tests and leptin were measured on most of the patients when originally seen, but these were not included in the original data analysis; there are 4 to 8 missing values for each of these. The actual numbers of patients in the statistical analyses are noted in Tables 2–6 in the Results section.

Laboratory methods

Glycine betaine, *N*,*N*-dimethylglycine and carnitine were measured in plasma and urine by high performance liquid chromatography (HPLC) after derivatization with 2-naphthacyl triflate [26,27].

Creatinine was measured in plasma and urine using the Jaffé reaction on the Abbott Aeroset Analyzer (Abbott Laboratories,

Table 1
Properties of study population by sex

	Females	Males	*p*
Number of patients	82	76	
Age (years)	56.0 (48.0–63.0)	51.5 (43.0–59.0)	0.024
BMI	27.2 (24.5–30.9)	28.4 (25.7–31.4)	0.151
Percent body fat	43 (34–50)	28 (23–33)	<0.001
Plasma glycine betaine (μmol/L)	20.0 (15.0–25.6)	24.9 (19.2–31.9)	<0.001
Urine betaine excretion	8.1 (4.8–15.6)	7.4 (5.0–13.9)	0.430
Plasma dimethylglycine (μmol/L)	1.8 (1.4–2.2)	2.1 (1.5–2.7)	0.032
Urine dimethylglycine excretion	3.2 (1.8–6.6)	3.8 (1.7–7.1)	0.963
Plasma total homocysteine (μmol/L)	9.6 (7.7–12.2)	9.8 (8.5–12.2)	0.526

The medians and interquartile ranges given for each sex, with the probability of a sex difference (rank sum test). Excretions quoted as mmol metabolite per mol creatinine.

Table 2
Hormone characteristics of the study population

	Units	Insulin pmol/L	Leptin μg/L	TSH mU/L	FTI	HOMA-IR
Lower quartile	Female	31.8	13.9	1.04	87	1.1
	Male	35.3	3.9	1.03	84	1.2
Median	Female	48.0	19.4	1.46	96	1.6
	Male	51.0	5.6	1.42	96	1.7
Upper quartile	Female	70.0	28.4	2.50	109	2.3
	Male	73.0	8.2	2.40	103	2.4
Patient numbers	Female	79	78	80	77	79
	Male	71	72	74	74	71
Reference ranges	Female		7–35			
	Male	10–80	2–10	0.4–4	55–160	<2.5

Quartiles for hormone concentrations in the study population, with the locally derived reference ranges at the bottom.

Table 3
Predictors of plasma glycine betaine concentrations (μmol/L)

Predictor variable (in group)	Coefficient
All female patients ($n=82$; $r^2=0.19$)	
Plasma carnitine (μmol/L)	+0.28±0.08
Carnitine excretion (log(urine carnitine/creatinine))	−4.3±1.5
All male patients ($n=71$; $r^2=0.22$)	
Plasma apolipoprotein B (g/L)	−8.8±3.3
Plasma acetylcarnitine (:mol/L)	+0.58±0.21
TSH (mU/L)	+2.23±1.05
Insulin (pmol/L)	−0.051±0.021

Final multiple linear regression model using independent variables that are significant in best subsets and forwards stepwise regression models. The number (*n*) is the number of patients included in the final model. Coefficients are quoted with standard errors, and for a model with only the tabulated variables.