Plasma Dependent and Independent Accumulation of Betaine in Male and Female Rat Tissues

SANDY SLOW [1,*], MICHAEL LEVER [1,2], STEPHEN T. CHAMBERS [2,3], PETER M. GEORGE [1,2]

[1]*Biochemistry Unit, Canterbury Health Laboratories,* [2]*Department of Pathology, Christchurch School of Medicine and Health Sciences, University of Otago and* [3]*Department of Infectious Diseases, Christchurch Hospital, Christchurch, New Zealand*

[*] Corresponding author: Dr Sandy Slow, Biochemistry Unit, Canterbury Health Laboratories, P.O. Box 151 Christchurch Mail Center, Christchurch, New Zealand. Tel: +64-336-40122; Fax: +64-336-40750; e-mail: sandy.slow@cdhb.govt.nz

Short title: Male and female rat tissue betaine accumulation

Summary

Tissue betaine is an intracellular osmolyte that also provides a store of labile methyl groups. Despite these important biological roles, there are few data regarding tissue betaine content. We measured the betaine concentration of plasma and various tissues (brain, heart, lungs, liver, kidney, spleen, intestine, reproductive tissues, skeletal muscle and skin) in male and female rats and assessed whether there were any gender-specific differences in betaine content or distribution and whether there was any relationship between tissue accumulation and plasma levels.

Betaine was highest in the liver and kidney with values ranging from 1.6 to 9.5 mmol/L and 2.0 to 5.4 mmol/L respectively. Plasma betaine concentrations were significantly lower than tissue levels except in the brain (≈ 25% of plasma) and skeletal muscle (similar to plasma). Regression analysis of the combined male and female data revealed a significant plasma-related accumulation of betaine in the heart, skin and skeletal muscle, while the lung, liver, kidney, spleen, and intestine showed significant plasma-related and plasma-independent accumulations of betaine. The betaine content of the skin, liver and kidney was not significantly different between males and females however in plasma and all tissues analyzed it was significantly higher in males ($P < 0.01$).

Key words: Betaine; Osmolyte; Methyl donor; Tissue content; Rats

Introduction

Betaine (glycine betaine or *N*,*N*,*N*-trimethylglycine) is a bodily requirement. It is actively accumulated in many mammalian tissues where it plays a vital role as an intracellular osmolyte, regulating cell volume by countering changes in extracellular tonicity and stabilizing macromolecules against a variety of physiological perturbations (Häussinger 1996; Schliess and Häussinger 2002; Wehner et al. 2003). In addition, betaine is an important methyl donor in one-carbon metabolism, where betaine-homocysteine methyltransferase (BHMT; E.C. 2.1.1.5), the only known enzyme which uses betaine as a substrate, mediates the transfer of a methyl group from betaine to homocysteine, forming methionine and *N*,*N*-dimethylglycine (Millian and Garrow 1998; Sunden et al.1997).

Betaine is found at high levels in certain foods (Zeisel et al. 2003; deZwart et al. 2003) and is absorbed directly from the diet via non-specific porters in the gut. In humans, depending on habitual diet, daily betaine intake varies and has previously been estimated to range between ≈300-2500 mg (Craig 2004; Olthof et al. 2003). However, recent reports suggest that normal dietary intake is much lower (Slow et al. 2005; Cho et al. 2006; Detopoulou et al. 2008) and the maximum daily betaine intake is unlikely to exceed 400-500mg. Betaine can also be endogenously produced in the liver and kidney cortex from its metabolic precursor choline (Zhang et al. 1992; Zeisel and Blusztajn 1994; Fischer et al. 2005). The concentration of betaine in human plasma and the amount excreted in the urine is under homeostatic control (Lever et al. 2004; Lever et al. 2007). It is freely filtered in the kidney and almost all (>98%) is resorbed in the proximal tubules. However, plasma levels are lower in patients with renal disease (Lever et al. 1994) and urinary excretion is elevated in some patients with diabetes mellitus (> 25%; Dellow et al. 1999) and in 10% of patients with

dyslipidaemia (Lever et al. 2005). BHMT expression and activity also has a crucial role in regulating circulating and liver betaine concentrations (Finkelstein et al. 1982) and it is likely that it is also a key determinant of extra-hepatic tissue betaine content.

Despite its two important biological functions there are few data on the tissue concentrations of betaine. It is not known if there are gender-specific differences in tissue betaine content even though it has been shown that BHMT expression and activity is influenced by various hormones with gender-specific profiles (Finkelstein et al. 1971), and there is a gender difference in plasma levels in humans (Lever et al. 1994). Similarly, whether there is any relationship between plasma and tissue betaine concentrations or any relationship between tissue betaine and plasma homocysteine concentrations remains to be investigated. Our hypothesis is that the betaine that is accumulated as a cellular osmolyte is an important metabolically available store of methyl groups. Our aim was to quantify tissue betaine concentrations, establish the variation in tissue content that is likely to be found, and determine whether there is any relationship between plasma levels and tissue accumulation or gender-specific differences. To assess this we measured the betaine content of plasma, brain, heart, lungs, liver, kidneys, spleen, intestine, reproductive tissues (testes-males; uterus, fallopian tubes and ovaries-females), skeletal muscle and skin in adult male and female rats aged between 4-12 months.

Methods

Animal protocol

The experimental protocol was approved by the Canterbury Animal Ethics Committee in compliance with the New Zealand Animal Welfare Act 1999.

Thirty adult Sprague Dawley rats (15 males; 15 females) aged between 4-12 months (≈ 400-800 g) were obtained from the Christchurch Animal Research Facility (University of Otago, Christchurch School of Medicine and Health Sciences, Christchurch, New Zealand). Animals were housed in standard caging, with a maximum of 3 animals per cage on a 12 h light cycle with free access to food and water. Animals were maintained on standard rat chow, which contained ≈ 902.1 ± 34.1 μg betaine/g diet (mean ± standard deviation of 3 measures) on analysis.

A blood sample was collected via cardiac puncture into EDTA containing tubes and stored on ice immediately following euthanasia via CO_2 inhalation. Tissue samples; heart, lung, liver, kidney, spleen, intestine, skeletal muscle, skin (with fur attached), reproductive tissues (testes-male; uterus with attached fallopian tubes and ovaries-females) and brain were rapidly dissected from each animal without exsanguination, individually wrapped in aluminium foil and flash frozen in liquid nitrogen. Prior to flash freezing the intestine, any residual food was flushed out using normal saline solution and a syringe and the skeletal muscle samples were obtained from the right hind leg for all animals. Plasma was separated within 2 hours of blood collection by centrifugation at 3000 g for 10 minutes. All tissue and plasma samples were stored frozen at -80 ºC until analysis.

Betaine extraction, derivatization and HPLC

The whole heart, brain, spleen, one kidney, the reproductive tissues (either the complete female reproductive tract or one testicle) and a portion of liver, lung, skeletal muscle, skin and intestine (≈ 1 g of each) was weighed and utilized for each animal. Tissues were immersed in liquid nitrogen and pulverized into a fine powder using a mortar and pestle. Betaine was extracted from the tissues using a modified procedure for solid food analysis, as described previously (deZwart et al. 2003). Briefly, a homogeneous paste was obtained by adding a known amount of water and further homogenizing using an Ultra-Turrax T25 homogenizer (IKA Labortechnik, Staufen, Germany). The homogenates were shaken for a minimum of 1 hour then centrifuged for 10 mins at 11,000g. The aqueous supernatant was removed and extracted with an equal volume of dichloromethane and shaken for 5 mins before centrifuging at 11 000 g for 10 mins. The aqueous phase containing the betaine was removed and stored frozen at -20 °C until analysis.

Betaine was measured in the plasma and tissue extracts by high performance liquid chromatography (HPLC) after derivatization with 2-napthacyl triflate and UV detection as previously described (Storer et al. 2006; Storer and Lever, 2006). The concentration of betaine (μmol/L tissue water) was calculated by comparing to external standards and correcting for tissue weight, water volume added and the % tissue water content as reported by Reinoso et al. (1997) and Long (1961), while plasma concentrations were corrected for water content as reported by Miller (1942).

Plasma total homocysteine

Plasma total homocysteine was measured by fluorescence polarization on an Abbott IM_X analyzer (Abbott Diagnostic Division, Abbott Laboratories, USA).

Statistics

The mean betaine concentration (± standard deviation) for plasma and tissue samples for the combined male and female data (n=30) and within each gender (n=15 each) were calculated. A normality test showed that the betaine concentrations were not normally distributed in all tissues, and therefore comparisons were carried out on log transformed data, which were acceptably normal in all cases. Two-way analysis of variance was used to detect gender differences, and for comparing the betaine content of each tissue with plasma betaine, with post-hoc Bonferroni t-test comparisons. This was corroborated by one-way repeated measures analysis of variance of the data for each gender. Linear regression analysis was used to determine the significance of plasma betaine concentration as a predictor of tissue betaine concentrations. Data were analyzed using SigmaStat version 3.1 (Systat Software Inc., San Jose, CA). Statistical significance was defined as $P < 0.05$ unless otherwise stated.

Results

Tissue betaine content

The betaine content of the male and female rat tissues varied and is shown in Table 1. Of the tissues analyzed, the liver contained the highest concentrations of betaine (1.6 to 9.5 mmol/L tissue water), followed by those of the kidney (2.0 to 5.4 mmol/L). In contrast, the brain was found to contain the lowest betaine concentrations (undetectable to 190 μmol/L).

Associations between plasma and tissue betaine concentrations

In both sexes the concentrations of betaine in all tissues, except muscle and brain, were higher than the plasma concentrations ($P < 0.001$), while the concentration in the brain was significantly lower ($P < 0.001$; Table 1). Analysis within each gender confirmed these findings and also showed that in both the female reproductive tract (uterus, fallopian tubes and ovaries) and in the testes, betaine concentrations were higher than the circulation. Although an important determinant of tissue betaine appears to be the plasma betaine concentration, the strength of this relationship varies from tissue to tissue (Table 2). Many tissues also accumulate significant amounts of betaine that are not dependent on plasma levels (the constant in Table 2). For example, the plasma betaine concentration accounts for less than 20% of the variance in liver betaine concentrations, where the accumulation is the highest. Similarly in the testes, another tissue particularly rich in betaine (2.4 to 3.4 mmol/L) most accumulation is independent of the plasma betaine (Table 2). Furthermore, the tissues that show a strong dependence on plasma betaine (combined male and female data, r^2 >0.5; heart, lung, spleen and intestine, Table 2) show a stronger correlation with each other (Pearson coefficients, $r = +0.74$ to 0.85) than with liver ($r = +0.28$ to 0.68) or

kidney (r = +0.43 to 0.53). Similar relationships are observed when each gender is analyzed separately but are less significant.

Gender differences in tissue betaine content and associations with plasma homocysteine

The betaine content in the skin, liver and kidney was not significantly different between males and females, however in plasma and in all other tissues analyzed, it was significantly higher in males (P<0.01). In the study animals the mean male plasma total homocysteine (± SD) was significantly higher than the females (4.2 ± 0.8 μmol/L vs 3.1 ± 0.5 μmol/L; $P < 0.001$). In the total animal group, plasma total homocysteine correlated with the betaine content of several tissues and with plasma betaine, but when each gender was considered separately, there was no relationship between homocysteine and plasma or tissue betaine in male rats, and the trends in the females were only marginally significant (P = 0.040 in skin, 0.058 in liver & 0.063 in spleen). Various multiple regression models with plasma betaine and homocysteine as predictor variables for tissue betaine, also failed to show any significant contribution from the homocysteine.

Discussion

Our results demonstrate that betaine is accumulated to high levels in virtually all tissues, with the highest betaine content occurring in the liver and kidney. The observed pattern of tissue betaine distribution is generally similar to the previous findings of Martin and Finkelstein (1981) in male rat tissues, but compared to that report, we found lower absolute betaine concentrations in the liver, kidney (≈ 2-fold for both tissues) and spleen (≈ 3-fold), and higher levels in the heart (≈ 2.5-fold). We also detected betaine in the lungs of all animals and in the brain in 28 of our 30 animals (Table 1). Similar to our findings in the brain, Lien (1995) detected betaine in the female rat cerebrum, cerebellum, striatum, midbrain, pons and medulla oblongata (0.4 ± 0.05 to 0.9 ± 0.02 mmol/kg water), although concentrations were higher than those we observed. The discrepancies in the betaine content between the reported studies could be the result of several factors, including genetic variation in the animals and differences in the treatment of the animals before the study. Two aspects that are likely to be particularly relevant are the betaine content of the diet and the hydration status of the animals.

Plasma betaine concentrations are under homeostatic control (Lever et al. 2004), a process that is influenced by dietary betaine or choline intake and betaine-homocysteine methyltransferase (BHMT) activity (Holm et al. 2005; Schwab et al. 2006; Collinsova et al. 2006). BHMT activity is also affected by the supply of dietary betaine, choline and methionine (Park and Garrow 1999), by hormones (Finkelstein et al. 1971; Ratnam et al. 2006) and by osmotic stress (Delgado-Reyes and Garrow 2005; Schäfer et al. 2007). Similarly, the betaine content of the liver has been shown to be controlled by dietary betaine, choline and protein intake and BHMT activity

(Finkelstein et al. 1982). Thus, it is likely that the tissue betaine content of extra-hepatic tissues is regulated in the same manner.

Tissue betaine is not necessarily derived from plasma, as (for example) liver and kidney betaine may also be derived through the two-step oxidation of choline via the choline dehydrogenase and betaine aldehyde dehydrogenase expressed in these tissues (Grossman and Hebert 1989; Huang and Lin, 2003), while the relatively high association between intestinal betaine and plasma betaine ($r^2 = 0.76$) may reflect the uptake of dietary betaine (Kettunen et al. 2001). Almost certainly, tissue betaine concentrations are also determined by the osmotic stress of the animals (Wehner et al. 2003). Indeed, most of the accumulated betaine in various tissues is likely to be because of its important role as a cellular osmolyte. The hydration state of the animals before euthanasia was not controlled in this study, thus it is likely to have contributed to at least some of the observed variation in tissue betaine concentrations. It could also explain why the correlations between various tissues are sometimes stronger than the correlations with the plasma betaine concentration. Similarly, another aspect that may contribute to some of the variation observed in the tissue betaine content in the present study is the difference in the age of the animals utilized (4-12 months). It is not known whether tissue betaine content varies with age, however, a wide range of ages was chosen for the purposes of this study so that the variability within a representative rat population could be adequately assessed.

To our knowledge this is the first time that betaine has been systematically measured in the tissues of female animals. Generally for most tissues analyzed, betaine content was higher in males compared to females, which is likely, at least in part, to be a function of BHMT activity. BHMT is the only known enzyme that utilizes betaine as a substrate and mediates the methylation of homocysteine to

methionine. Plasma homocysteine concentration may be of clinical significance in humans because elevations increase the risk of all forms of vascular disease (coronary, cerebral and peripheral; Wald et al. 2002). BHMT expression and activity plays a key role in regulating betaine concentrations and determines the fate of betaine between its two competing biological functions; stored to control cellular osmolarity or metabolized to provide a methyl group for homocysteine methylation. As well as diet, BHMT expression is influenced by various hormones, including corticosteroids, insulin, estradiol and testosterone (Finkelstein et al. 1971; Ratnam et al. 2006). This is therefore likely to cause differences in the tissue betaine content between males and females. Another possible cause is the gender difference in homocysteine concentrations, with male animals having significantly higher plasma homocysteine concentrations than females. The apparent relationships we observed between tissue betaine and plasma homocysteine concentration in the total group of animals reflects the gender differences in both betaine and homocysteine metabolism, and underlies the need for studies to be conducted on animals of each gender separately as in humans (Lever et al. 2007).

The high level of betaine observed in the testes is interesting. There is evidence to suggest that betaine, acting as a methyl donor, may have an important role in maintaining male fertility (Kelly et al. 2005). The metabolism of betaine via BHMT can ultimately lead to the production of *S*-adenosyl methionine, which is crucial in the testes for the synthesis of creatine, which is thought to be important for sperm motility and function (Lee et al. 1998). Although rat liver is the only tissue that has significant BHMT activity (Martin and Finkelstein 1981; M^{c}Keever et al. 1991), it is possible that specific cell types within a tissue produce BHMT. Indeed, a search of microarray gene chip data (Edgar et al. 2002; http://www.ncbi.nlm.nih.gov/geo/) shows that the

testes Sertoli cells express BHMT mRNA. Combined, this indicates the possibility that at least some of the accumulated betaine could be utilized as a source of methyl groups, which may contribute to testicular creatine production and thus aid in maintaining sperm motility and function.

In conclusion, the present study confirms that betaine is stored in mammalian body tissues, and these stores need to be taken into account in models of one-carbon metabolism. We provide values of the tissue betaine content in male and female rats. It functions as an important cell volume regulator or provides a source of labile methyl groups for one-carbon metabolism via the action of BHMT. Disturbances in tissue betaine concentration have the potential to have adverse consequences for health and wellbeing, and as such, it would be of particular interest to further investigate the effect of dietary betaine and choline intake on tissue betaine content.

Acknowledgements

We thank staff at the Christchurch Animal Facility for their assistance with the animals. Support from the Neurological Foundation of New Zealand, the National Heart Foundation of New Zealand, and from the Paykel Trust is acknowledged. Some of the equipment used in the analytical work was provided with assistance from the New Zealand Lottery Grants Board.

References

CHO E, ZEISEL SH, JACQUES P, SELHUB J, DOUGHERTY L, COLDITZ GA, WILLETT WC: Dietary choline and betaine assessed by food-frequency questionnaire in relation to plasma total homocysteine concentration in the Framingham Offspring Study. *Am J Clin Nutr* **83**: 905-911, 2006.

COLLINSOVA M, STRAKOVA J, JIRACEK J, GARROW TA: Inhibition of betaine-homocysteine-*S*-methyltransferase causes hyperhomocysteinemia in mice. *J Nutr* **136**: 1493-1497, 2006.

CRAIG SAS: Betaine in human nutrition. *Am J Clin Nutr* **80**: 539-549, 2004.

DE ZWART FJ, SLOW S, PAYNE RJ, LEVER M, GEORGE PM, GERRARD JA, CHAMBERS ST: Glycine betaine and glycine betaine analogues in common foods. *Food Chem* **83**: 197-204, 2003.

DELGADO-REYES CV, GARROW TA: High sodium chloride intake decreases betaine-homocysteine methyltransferase expression in guinea pig liver and kidney. *Am J Physiol Reg Inter Comp Physiol* **288**: 182-187, 2005.

DELLOW WJ, CHAMBERS ST, LEVER M, LUNT H, ROBSON RA: Elevated glycine betaine excretion in diabetes mellitus patients is associated with proximal tubular dysfunction and hyperglycemia. *Diabetes Res Clin Prac* **43**: 91-99, 1999.

DETOPOULOU P, PANAGIOTAKOS DB, ANTONOPOULOU S, PITSAVOS C, STEFANADIS C: Dietary choline and betaine intakes in relation to concentrations of inflammatory markers in healthy adults: the ATTICA study. *Am J Clin Nutr* **87**: 424-30, 2008.

EDGAR R, DOMRACHEV M, LASH AE: Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. *Nucleic Acids Res* **30**: 207-210, 2002.

FINKELSTEIN JD, KYLE WE, HARRIS BJ: Methionine metabolism in mammals. Regulation of homocysteine methyltransferases in rat tissue. *Arch Biochem Biophys* **146**: 84-92, 1971.

FINKELSTEIN JD, MARTIN JJ, HARRIS BJ, KYLE WE: Regulation of hepatic betaine-homocysteine methyltransferase by dietary betaine. *J Nutr* **113**: 519-521, 1983.

FINKELSTEIN JD, MARTIN JJ, HARRIS BJ, KYLE WE: Regulation of the betaine content of rat liver. *Arch Biochem Biophys* **218**: 169-173, 1982.

FISCHER LM, SCEARCE JA, MAR M-H, PATEL JR, BLANCHARD RT, MACINTOSH BA, BUSBY MG, ZEISEL SH: Ad libitum choline intake in healthy individuals meets or exceeds the proposed adequate intake level. *J Nutr* **135**: 826-829, 2005.

GROSSMAN EB, HEBERT, SC: Renal inner medullary choline dehydrogenase activity: characterization and modulation. Am. J. Physiol. **256**: 107-112, 1989.

HÄUSSINGER D: The role of cellular hydration in the regulation of cell function. *Biochem J* **313**: 697-710, 1996.

HOLM PI, UELAND PM, VOLLSET SE, MIDTTUN O, BLOM HJ, KEIJZER MB, DENHIJER M: Betaine and folate status as cooperative determinants of plasma homocysteine in humans. *Arterioscle Thromb Vasc Biol* **25**: 379-385, 2005.

HUANG S, LIN Q: Functional expression and processing of rat choline dehydrogenase precursor. *Biochem Biophys Res Com* **309**: 344-350, 2003.

KELLY TLJ, NEGA OR, SCHWAHN BC, ROZEN R, TRASLER JM: Infertility in 5,10-methylenetetrahydrofolate reductase (MTHFR)-deficient male mice is partially alleviated by lifetime dietary betaine supplementation. *Biol Reprod* **72**: 667-677, 2005.

KETTUNEN H, PEURANEN S, TIIHONEN K: Betaine aids in the osmoregulation of duodenal epithelium or broiler chicks, and affects the movement of water across the small intestinal epithelium in vitro. *Comp Biochem Physiol A* **129**: 595-603, 2001.

LEE H, KIM J-H, CHAE Y-J, OGAWA H, LEE M-H, GERTON GL: Creatine synthesis and transport systems in the male reproductive tract. *Biol Reprod* **58**: 1437-1444, 1998.

LEVER M, ATKINSON W, GEORGE PM, CHAMBERS ST: Sex differences in the control of plasma concentrations and urinary excretion of glycine betaine in patients attending a lipid disorders clinic. *Clin Biochem* **40**: 1225-1231, 2007.

LEVER M, GEORGE PM, DELLOW WJ, SCOTT RS, CHAMBERS ST: Homocysteine, glycine betaine, and N,N-dimethylglycine in patients attending a lipid clinic. *Metabolism* **54**: 1-14, 2005.

LEVER M, SIZELAND PC, BASON LM, HAYMAN CM, CHAMBERS ST: Glycine betaine and proline betaine in human blood and urine. *Biochim Biophys Acta* **1200**: 259-264, 1994.

LEVER M, SIZELAND PC, FRAMPTON CM, CHAMBERS ST: Short and long-term variation of plasma glycine betaine concentrations in humans. *Clin Biochem* **37**: 184-190, 2004.

LIEN Y-H H: Role of organic osmolytes in myelinolysis. *J Clin Invest* **95**: 1579-1586, 1995.

LONG C. (Ed). Biochemists Handbook. United Kingdom: E & F.N. Spon Limited, 1961, pp 796.

MARTIN JJ, FINKELSTEIN JD: Enzymatic determination of betaine in rat tissues. *Anal Biochem* **111**: 72-76, 1981.

M[C]KEEVER MP, WEIR DG, MOLLOY A, SCOTT JM: Betaine-homocysteine methyltransferase: organ distribution in man, pig, rat and sub-cellular distribution in the rat. *Clin Sci* 81: 551-556, 1991.

MILLER JR AT: Studies on tissue water I. The determination of blood water by the distillation method. *J Biol Chem* **143**: 65-73, 1942.

MILLIAN NS, GARROW TA: Human betaine-homocysteine methyltransferase is a zinc metalloenzyme. *Arch Biochem Biophys* **356**: 93-98, 1998.

OLTHOF M, VAN VLIET T, BOELSMA E, VERHOEF P: Low dose betaine supplementation leads to immediate and long term lowering of plasma homocysteine in healthy men and women. *J Nutr* **133**: 4125-4128, 2003.

PARK EI, GARROW TA: Interaction between dietary methionine and methyl donor intake on rat liver betaine-homocysteine methyltransferase gene expression and organization of the human gene. *J Biol Chem* **274**: 7818-7824, 1999.

RATNAM S, WIJEKOON EP, HALL B, GARROW TA, BROSNAN ME, BROSNAN JT: Effects of diabetes and insulin on betaine-homocysteine *S*-methyltransferase expression in rat liver. *Am J Physiol Endocrinol Metab* **290**, E933-E939, 2006.

REINOSO RF, TELFER BA, ROWLAND M: Tissue water content in rats measured by desiccation. *J. Pharmocol Toxicol Methods* **38**: 87-92, 1997.

SCHÄFER C, HOFFMANN L, HELD TK, LORNEJAD-SCHÄFER MR, BRAUERS G, GEHRMANN T, GARROW TA, HÄUSSINGER D, MAYATEPEK E, SCHWAHN BC, SCHLIESS F: Osmotic regulation of betaine homocysteine-*S*-methyltransferase expression in H4IIE rat hepatoma cells. *Am J Physiol Gastrointest Liver Physiol* **292**: 1089-1098, 2007.

SCHLIESS F, HÄUSSINGER D: The cellular hydration state: a critical determinant for cell death and survival. *Biol Chem* **383**: 577-583, 2002.

SCHWAB U, TORRONEN A, MERIRINNE E, SAARINEN M, ALFTHAN G, ARO A, UUSRTUPA M: Orally administered betaine has an acute and dose-dependent effect on serum betaine and plasma homocysteine concentrations in healthy humans. *J Nutr* **136**: 34-38, 2006.

SLOW S, DONNAGGIO M, CRESSEY P, LEVER M, GEORGE PM, CHAMBERS ST: The betaine content of New Zealand foods and the estimated intake in the New Zealand diet. *J Food Comp Anal* **18**: 473-485, 2005.

STORER MK, LEVER M: Aracyl triflates for preparing fluorescent and UV absorbing derivatives of unreactive carboxylates, amines and other metabolites. *Anal Chim Acta* **558**: 319-325, 2006.

STORER MK, MCENTYRE CJ, LEVER M: Separation of cationic Aracyl derivatives of betaines and related compounds. *J Chromatogr A* **1104**: 263-271, 2006.

SUNDEN SLF, RENDUCHINTALA MS, PARK EI, MIKLASZ SD, GARROW TA: Betaine-homocysteine methyltransferase expression in porcine and human tissues and chromosomal localization of the human gene. *Arch Biochem Biophys* **345**: 171-174, 1997.

WEHNER F, OLSEN H, TINEL H, KINNE-SAFFRAN E, KINNE RKH: Cell volume regulation: osmolytes, osmolyte transport and signal transduction. *Rev Physiol Biochem Pharmacol* **148**: 1-80, 2003.

ZEISEL SH, BLUSZTAJN JK: Choline and human nutrition. *Ann Rev Nutr* **14**: 269-296, 1994.

ZEISEL SH, MAR M-H, HOWE JC, HOLDEN JM: Concentrations of choline-containing compounds and betaine in common foods. *J Nutr* **133**: 1302-1307, 2003.

ZHANG J, BLUSZTAJN JK, ZEISEL SH: Measurement of the formation of betaine aldehyde and betaine in rat liver mitochondria by high pressure liquid chromatography-radioenzymatic assay. *Biochim Biophys Act*a **1117**: 333-339, 1992.

Table 1: The betaine content of various tissues of the male and female rat

Organ	% Tissue Water Content[1]	Male Betaine Content		Female Betaine Content	
		μmol/L	μmol/g tissue	μmol/L	μmol/g tissue
Plasma	96	186 ± 43**	0.17 ± 0.04	101 ± 37**	0.09 ± 0.03
Heart	78	372 ± 114*	0.32 ± 0.03	224 ± 77*	0.19 ± 0.02
Lung	79	500 ± 119**	0.43 ± 0.03	290 ± 96**	0.25 ± 0.02
Liver	71	4097 ± 1115	3.22 ± 0.23	4096 ± 1988	3.21 ± 0.4
Kidney	77	3785 ± 546	3.24 ± 0.12	3319 ± 1034	2.78 ± 0.22
Spleen	77	801 ± 143*	0.67 ± 0.03	519 ± 243*	0.43 ± 0.05
Skin	65	412 ± 185	0.30 ± 0.03	305 ± 153	0.22 ± 0.03
Skeletal Muscle	76	214 ± 70*	0.18 ± 0.02	124 ± 40*	0.10 ± 0.01
Brain	79	50 ± 44**	0.04 ± 0.01	26.2 ± 26.7**	0.02 ± 0.01
Intestine	75	920 ± 196**	0.75 ± 0.04	524 ± 146**	0.43 ± 0.03
Female Reproductive	76	N/A	N/A	216 ± 123	0.18 ± 0.03
Testes	86	2842 ± 292	2.63 ± 0.07	N/A	N/A

Data are means ± standard deviations. [1]Concentrations (μmol/L) are expressed as the concentration in tissue water, which were calculated from the % tissue water as reported by Reinoso et al., 1997, with the exception of plasma which was based on the report of Miller, 1941 and the female reproductive tissues, which was based on the report for rat ovary from Long (ed), 1961. Number of animals: 15 of each gender. *Significant gender difference, $P < 0.01$; **$P < 0.001$, calculated from two-way ANOVA on log-transformed data. Female reproductive tissues included the uterus, fallopian tubes and ovaries.

Table 2: Relationship between plasma and tissue betaine

Tissue	n	Slope	*P* slope	Constant	*P* const.	r^2
Heart	30	**1.7**	**<0.001**	58	0.12	0.65
Lung	30	**2.1**	**<0.001**	**94**	**0.039**	0.61
Liver	30	**11.8**	**0.015**	**2396**	**0.002**	0.19
Kidney	30	**8.1**	**<0.001**	**2388**	**0.001**	0.32
Spleen	30	**3.1**	**<0.001**	**220**	**0.012**	0.55
Skin	30	**1.5**	**0.005**	144	0.068	0.25
Muscle	30	**0.8**	**<0.001**	57	0.052	0.41
Brain	30	0.1	0.46	25	0.19	0.02
Intestine	30	**3.9**	**<0.001**	**159**	**0.019**	0.76
Female Reproductive	15	**1.7**	**0.043**	41	0.63	0.28
Testes	15	2.5	0.17	2369	**<0.001**	0.38

Predictions of tissue betaine contents (μmol/L tissue water, *y*) from plasma betaine concentrations (*x*, μmol/L). Linear regression slope and intercept (constant) parameters and coefficient of determination (r^2). Significant parameters ($P < 0.05$) are in bold. Female reproductive tissues included the uterus, fallopian tubes and ovaries.