Betaine treatment of cystathionine β-synthase-deficient homocystinuria; does it work and can it be improved?

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Abstract: Inactivating mutations in cystathionine β-synthase result in classical homocystinuria (HCU) and are typically accompanied by severe elevations of plasma and tissue homocysteine, methionine, S-adenosylmethionine, S-adenosylhomocysteine and significantly decreased cysteine. HCU is usually accompanied by marfanoid skeletal abnormalities, osteoporosis, ectopia lentis and/or severe myopia, cognitive impairment, and a dramatically increased incidence of atherosclerosis and thromboembolic complications of variable presentation. If untreated, HCU is a serious life-threatening disease. Betaine (N,N,N-trimethylglycine) is a zwitterionic quaternary ammonium compound that can lower homocysteine, S-adenosylmethionine, S-adenosylhomocysteine, and increase cysteine in HCU by serving as a methyl donor for the remethylation of homocysteine in a reaction catalyzed by betaine–homocysteine S-methyltransferase. This review considers the clinical efficacy and safety of betaine treatment of HCU. Possible strategies by which the efficacy of this treatment might be improved are discussed.

Keywords: homocystinuria, homocysteine, betaine, cystathionine beta-synthase, betaine–homocysteine S-methyltransferase

Introduction
In the mammalian methionine cycle, the essential amino acid methionine is transmethylated to homocysteine (Hcy), which is then either extruded into the extracellular space, transulfurated to cystathionine and subsequently converted to cysteine or remethylated back to methionine (Figure 1). Inactivating mutations in cystathionine β-synthase (CBS) result in classical homocystinuria (HCU) and are typically accompanied by severe elevations of plasma and tissue Hcy, a range of vascular and neurological sequelae and multiple connective tissue disturbances of variable presentation. If untreated, HCU is a serious life-threatening disease. This review will consider the efficacy of betaine treatment of HCU. Possible research strategies by which the efficacy of this treatment might be improved are discussed.

Betaine
Betaine (N,N,N-trimethylglycine) is a zwitterionic quaternary ammonium compound that is also known as oxyneurine, glycine-betaine, or trimethylglycine. The principal physiological functions of betaine are as an osmolyte and a methyl donor in transmethylation reactions. In this latter function, betaine supplies one-carbon units that can spare the amount of dietary methionine and choline required for nutritional purposes. In the mammalian liver and kidney, betaine serves as a methyl donor for the remethylation of Hcy in a reaction catalyzed by betaine–homocysteine S-methyltransferase (BHMT) (Figure 1) that generates methionine and dimethylglycine (DMG). In HCU,
Betaine acts to increase the plasma and tissue concentrations of methionine and reduce the concentration of Hcy. Betaine treatment has also been observed to decrease S-adenosylmethionine (AdoMet), S-adenosylhomocysteine (AdoHcy) levels, and increase plasma DMG, methylglycine (MG), also known as sarcosine and cysteine concentrations in HCU.2

Cystathionine β-synthase-deficient HCU

HCU is a recessively inherited disorder of the transsulfuration pathway of methionine metabolism that was first recognized in 1962.3,4 The defective enzyme in this condition was subsequently identified as CBS 2 years later.5 CBS is a heme protein that catalyzes the pyridoxal 5′-phosphate (PLP)-dependent condensation of serine and Hcy to form cystathionine, which is then converted to cysteine by another PLP-dependent enzyme, cystathionine γ-lyase (Figure 1). HCU is characterized biochemically by severely increased plasma and tissue Hcy, methionine, AdoMet, AdoHcy and a concomitant decrease in cysteine and frequently undetectable cystathionine.2,6 Interestingly, HCU does not exhibit elevated DMG2 and recent research has indicated that this is due to the fact that untreated HCU results in marked repression of BHMT expression.7

HCU, if untreated, is typically accompanied by marfanoid skeletal abnormalities, osteoporosis, ectopia lentis and/or severe myopia, cognitive impairment, and a dramatically increased incidence of atherosclerosis and thromboembolic disease.8 In terms of the correlation between genotype and phenotype, there is a functional trichotomy in CBS mutations. The first class of mutations completely inactivates CBS and typically results in the severest form of the disease, often referred to as classical HCU. The second class of mutations encodes CBS protein, the deficient activity of which is increased by treatment with pyridoxine.8 More recently a third functional class of CBS mutations has been observed in the C-terminal regulatory domain and result in impairment of post-translational regulation of CBS activity by AdoMet.2,9,10 To date, only a limited number of patients with these regulatory mutations have been reported, but it appears that while they incur an increased risk of thrombosis, they do not exhibit the cognitive impairment or connective tissue disturbances that are characteristic of HCU.2 There is currently little data available regarding the efficacy of betaine...
for patients with these C-terminal regulatory mutations, but it was previously noted that two patients carrying these regulatory mutations were unresponsive to therapy with either pyridoxine or betaine.²

A landmark international survey by Mudd et al in 1985 documented the natural history of a relatively large group of HCU patients by time-to-event analyses for patients before treatment.¹¹ In this study, vascular occlusions affecting both large and small arteries and veins were reported as the cardinal vascular signs. Of the 629 HCU patients studied, this study reported a total of 253 vascular events in 158 patients.¹¹ Of those vascular events, 51% involved peripheral veins (25% of which resulted in pulmonary embolism), 32% were cerebrovascular events, 11% affected peripheral arteries, while 4% led to myocardial infarction. Serious complications included severe hypertension due to renal infarcts, hemiparesis, cor pulmonale secondary to pulmonary artery occlusion, optic atrophy secondary to optic artery occlusion, and seizures or focal neurological signs due to cerebral thrombosis (sagittal sinus thrombosis). This analysis reported a mortality rate of 23% in pyridoxine-nonresponsive HCU patients and 4% in pyridoxine-responsive HCU patients by the age of 30 years. There was a 30% chance of a vascular event before the age of 20 years, which increased to 50% by the age of 30 years. This ground-breaking study effectively established baselines for subsequent studies designed to evaluate the effect of Hcy-lowering treatment upon clinical outcome.

The evolution of Hcy-lowering treatment for HCU

Biochemical efficacy of betaine treatment in pyridoxine-responsive HCU

The primary objective of HCU treatment is to reduce, and if possible normalize, the accumulation of Hcy in tissues and plasma. If this is attained during the early neonatal period and subsequently maintained, there is a good chance of preventing the development of ocular, skeletal, and thromboembolic complications and the development of normal intellectual capacity is achievable. If treatment is instigated at a later time, typically after either developmental delay or an adverse clinical event has been instrumental in initiating diagnosis, then the goal of effective biochemical control is to prevent life-threatening vascular events and to prevent further deterioration of the patient’s condition. Early efforts towards this goal focused upon dietary restriction of the Hcy precursor methionine.¹²,¹³ After the discovery of a subset of HCU patients that express a defective CBS protein, and it was found that the activity of the deficient CBS protein is increased by treatment with pharmacologic amounts of pyridoxine,¹⁴–¹⁶ methionine restriction was combined with dietary supplementation with pyridoxine. As compliance with dietary methionine restriction is particularly arduous and patient response to pyridoxine is sometimes suboptimal, additional strategies to treat HCU have been sought. To date, the most effective of these has been the use of betaine. Perry et al¹⁷ pioneered the concept of dietary supplementation with a methyl donor compound to promote the BHMT catalyzed remethylation of Hcy to methionine as a treatment for HCU. This group used dietary supplementation with the betaine precursor compound choline, in combination with a methionine-restricted diet. In addition to lowering Hcy, this strategy may have had additional benefits as choline is an essential nutrient for neurodevelopment and strict adherence to a methionine-restricted diet precludes most of the richest sources of this compound. Subsequently, Komrower and Sardharwalla reported the use of betaine in combination with methionine restriction on two patients with HCU.¹⁸ In one patient, the addition of 750 mg/day of betaine resulted in complete clearing of detectable plasma Hcy–cysteine-mixed disulfides. The other patient was treated with 6 g of betaine per day which resulted in the disappearance of both Hcy and the mixed disulfide with a concomitant marked increase in plasma methionine level. In 1985, Wilcken et al¹⁹ investigated the effects of betaine supplementation (6 g per day) upon methionine loading (4 g/m² of body area) in six treated pyridoxine-responsive patients. This group assessed the effects on plasma Hcy and methionine levels of oral methionine loads before and after adding betaine to the treatment regimen of pyridoxine and folic acid. During the 24-hour period postmethionine challenge, all patients had higher plasma methionine and Hcy and lower cysteine compared to 17 normal control subjects. After betaine, these Hcy responses were reduced to near normal. There was a direct correlation between premethionine fasting Hcy levels and mean Hcy responses during the 24-hours postmethionine load, both before (r = 0.79) and after betaine (r = 0.71). Betaine also increased plasma cysteine levels in patients with the more severe biochemical abnormalities. Collectively, these studies indicate that betaine is a useful adjuvant therapy for optimal biochemical control in pyridoxine-responsive HCU.

Biochemical efficacy of betaine treatment in pyridoxine-nonresponsive HCU

For HCU patients with mutant forms of CBS that do not respond to pyridoxine therapy, methionine restriction...
remains the cornerstone of treatment. Patients diagnosed in childhood or later find the transition from a normal diet particularly difficult and compliance is frequently poor. In this scenario, betaine is an essential component of treatment with a view towards optimal biochemical control of Hcy levels. Smolin et al have previously reported the use of betaine, 6.4 and 7 g/day, in two pyridoxine-nonresponsive patients with HCU.20 Both patients showed significant reduction of Hcy, increased methionine, and crucially, a significant improvement in clinical symptoms. Wilcken et al reported on the effects of betaine supplementation (6 g per day) in 10 patients with pyridoxine-nonresponsive HCU in conjunction with pyridoxine (100 mg/day) and folic acid (5 mg/day).21 Six of these patients were also on a protein-restricted diet. All of these patients showed a substantial reduction in total free Hcy as a consequence of betaine supplementation with the average plasma level dropping from 57 µmol/L prior to treatment to 6 µmol/L during treatment. Similarly, Wilcken and Wilcken have reported that the addition of betaine treatment to standard protein restriction in 15 pyridoxine nonresponsive patients resulted in a further 75% average decline in free plasma Hcy.22 More recently, Singh et al23 reported on the biochemical benefits of betaine treatment in five patients with pyridoxine-nonresponsive HCU who could not attain optimal metabolic control by diet alone. In these patients, the addition of betaine reduced the plasma total Hcy median level by a further 47 µmol/L. Taken together, these studies show an unequivocal benefit for betaine treatment in terms of lowering plasma Hcy levels in pyridoxine-nonresponsive HCU.

Clinical efficacy of betaine treatment in HCU
Vascular and thromboembolic complications
Since the general introduction of betaine treatment for HCU, a number of investigators have focused their attention on the question as to whether lowering Hcy levels with this compound result in significant improvement in clinical outcome. The biggest threat to the life and health of patients with HCU is vascular or thromboembolic complications, which are predominantly peripheral and cerebrovascular.24–26

Wilcken and Wilcken27 studied a group of 15 pyridoxine-nonresponsive patients who had incurred at least one vascular event previously. During the time course of this study, there were two vascular events during treatment: one fatal pulmonary embolus and one myocardial infarction. Based on previous estimates of cardiovascular events in HCU without treatment,11 21 vascular events would have been expected. Interestingly, there were no events during 258 patient-years of treatment in the 15 pyridoxine-nonresponsive patients (P < 0.005 versus expected untreated) receiving betaine. This study clearly demonstrated that treatment that lowers Hcy, markedly reduces cardiovascular risk in patients with HCU and that betaine therapy contributes importantly to this protective effect in pyridoxine-nonresponsive patients. Similar findings were detected in a subsequent study by Walter et al.27 In 2001, a multicenter observational study28 assessed the effectiveness of long-term Hcy-lowering treatment in reducing vascular risk in 158 HCU patients. Vascular outcomes were analyzed and the effectiveness of treatment in reducing vascular risk was evaluated by comparison of actual to predicted number of vascular events. The 158 patients represented 2822 patient-years of treatment, with an average of 17.9 years per patient. Plasma Hcy levels were markedly reduced from pretreatment levels, but usually remained moderately elevated. A total of 17 vascular events were observed compared to a predicted 112 vascular events which would have been expected without treatment, for a relative risk of 0.09 (95% confidence interval [CI]: 0.036–0.228; P < 0.0001). In this study, patient-years of treatment were calculated separately for pyridoxine-responders and -nonresponders. Recent work has introduced an element of caution in the interpretation of this study and others like it, because of a possible ascertainment bias due to under detection of pyridoxine-responsive HCU.29 Whilst it is true that the use of multiple treatments used simultaneously makes it impossible to unequivocally delineate the individual contribution of betaine treatment to clinical outcome in HCU, the demonstrated ability of this compound to assist in effective biochemical control strongly indicates that it exerts significant clinical benefit in this condition.

Cognitive impairment
Cognitive impairment remains the most common abnormality of the central nervous system in HCU and is frequently instrumental in the initial diagnosis, typically presenting as developmental delay in the first or second year of life.11 There is considerable variation in the presentation of cognitive impairment in HCU. A previous assessment of 629 patients by Mudd et al11 observed a relatively wide range of IQ among patients with HCU (10 to 138 with a median of 64). Pyridoxine-responsive patients had significantly higher IQs (N = 107, mean IQ = 79) compared to pyridoxine-nonresponsive patients (n = 115; mean IQ = 57; P < 0.0001). Similar levels
of variance in intellectual capacity were subsequently reported in two independent studies.\textsuperscript{30,31}

There is currently a paucity of studies that investigate the effects of treatment upon cognitive impairment in HCU and to date, none that address the effects of betaine specifically. Yap et al\textsuperscript{32} investigated the cognitive capabilities of 23 pyridoxine-nonresponsive HCU patients compared to age-matched sibling controls. Of the 23 individuals, 19 were diagnosed through newborn screening with early treatment, two were late-detected and two were untreated at the time of assessment. Thirteen of the newborn-screened group, who were compliant with treatment, had no detectable cognitive impairment, while the remaining six, who had poor compliance, developed complications. The newborn-screened, good-compliance group (n = 13) had mean full-scale IQ (FIQ) of 105.8 (range 84–120), while the poorly compliant group (n = 6) had a mean FIQ of 80.8 (range 40–103). The control group (n = 10) had a mean FIQ of 102 (range 76–116). In this study, good control was defined as plasma-free Hcy median <11 \( \mu \text{mol/L} \) and was attained solely by methionine restriction. Many patients struggle to achieve this level of control by diet alone and in this context; it is highly likely that the use of betaine in addition to methionine restriction, would almost certainly increase the number of patients able to avoid cognitive impairment as a consequence of HCU.\textsuperscript{32}

**Ocular complications**

Untreated HCU is frequently associated with ectopia lentis (dislocation of the optic lens) and myopia. Previous work by Mudd et al\textsuperscript{11} found that by the age of 10 years, 70\% of all untreated HCU patients had dislocated lenses. Subsequently, evidence has accrued indicating that early treatment delays or prevents ectopia lentis.\textsuperscript{27,33} The positive effects of optimal biochemical control upon ocular complications in HCU have recently been demonstrated.\textsuperscript{34} These investigators compared clinical outcome between 14 late-diagnosed HCU patients with lens subluxation or dislocation at diagnosis and a further 15 patients who were detected in the newborn period and remained well controlled. Of the poorly controlled patients, only 28.6\% of eyes had 20/40 vision or better and all had steadily progressive myopic astigmatism and lens subluxation. Six patients who became poorly controlled in their teens or early twenties showed significant progression of their myopia, and three developed phacomatosis or lens subluxation. All of the well-controlled patients had no evidence of lens subluxation and had 20/20 vision bilaterally. The difference in visual acuity between late-diagnosed patients and the control group was highly significant (\( P = 0.0002 \)). The differences in refractive errors between the groups were also highly significant (\( P = 0.0001 \)). Collectively these results present a strong case for a causative relationship between poor biochemical control and the risk of ocular complications in HCU.

**Skeletal abnormalities**

By the age of 15 years, approximately 50\% of HCU patients have radiological evidence of osteoporosis.\textsuperscript{24} Although no definitive study has been performed, all the available data indicates that betaine therapy is unable to completely prevent osteoporosis in HCU. In a small study of five patients with pyridoxine-nonresponsive HCU, Gahl et al found that despite significant reduction in plasma Hcy levels in all patients, there was no discernible amelioration of their osteoporosis.\textsuperscript{35} A possible explanation for the failure of betaine to improve osteoporosis in HCU is discussed below.

**Complications of betaine therapy**

Current data indicate that betaine is a relatively safe treatment. In a previous study, with a total of 825 patient-years of betaine treatment, and periods of betaine treatment up to 17 years there were no reports of significant side effects.\textsuperscript{28} Despite this favorable outcome, the use of betaine is not without risk. The primary concern with betaine treatment is the avoidance of toxic levels of methionine (>1000 \( \mu \text{mol/L} \)) formed from the remethylation of Hcy. However, evidence from patients with methionine adenosyltransferase (MAT) I/III deficiency, which incurs severely elevated methionine without any obvious adverse effects, indicates that high levels of methionine are generally tolerable.\textsuperscript{36–38} However, cerebral edema has been reported in two HCU patients exhibiting extreme hypermethioninemia induced by betaine therapy.\textsuperscript{39,40} In both cases, discontinuation of betaine resulted in reduced methionine levels and subsequent resolution of neurological symptoms and magnetic resonance imaging abnormalities. The risk of cerebral edema in betaine treatment of severely elevated Hcy is not limited to HCU and has previously been reported in a patient with MAT I/III deficiency.\textsuperscript{41}

**Optimal dosage and timing of betaine treatment in HCU**

Despite widespread usage, there is currently little consensus on optimal betaine dosage and frequency of administration in human subjects with HCU. The average decrease in plasma Hcy concentration is reported to be between 74\% and 92\% following doses of 6–20 g day\(^{-1}\) of betaine.\textsuperscript{42,43} However, some studies have found no clinical benefit using 6 g day\(^{-1}\) of betaine.\textsuperscript{44}
A number of limited trials have been performed to investigate these aspects of betaine therapy, but these have typically been limited by relatively short study durations and/or the use of normal volunteers rather than HCU patients who are likely to have different responses to the application of betaine.\textsuperscript{45–47} Some investigators have tried to model the effects of HCU for studying betaine dosing by using a methionine-loading strategy but again, the time course of this kind of analysis is not suitable for detecting possible metabolic adaption induced in long-term betaine therapy.\textsuperscript{48} In those studies that have used individuals with HCU, the data has been complicated by the need to continue a methionine-restricted diet which, given its efficacy in lowering total Hcy (tHcy), is highly likely to mask any possible diminution in the ability of betaine to lower tHcy over time.\textsuperscript{49,50} One previous study investigated the pharmacokinetics (PK) and pharmacodynamic (PD) characteristics of betaine in six patients (aged 6–17 years) with pyridoxine-nonresponsive HCU.\textsuperscript{48} Prior to commencement of the study, betaine treatment was stopped but a low-methionine diet was maintained. Betaine was then given as a single oral dose of 100 mg kg\(^{-1}\) and plasma betaine and total Hcy concentrations were measured by high-pressure liquid chromatography at frequent intervals over 24 hours. Individual plasma betaine concentration–time data were fitted by an optimal PK model, and individual concentration–time–effect data were analyzed by an indirect response PD model. Both models were then linked in an algorithm to simulate the effects of betaine dose and dosage interval on plasma Hcy concentrations. This was then applied to determine the optimal regimen of betaine to control plasma total Hcy in the HCU patients. Betaine PK was described by both mono- and biexponential disposition functions with first-order absorption and a lag time. The correlation coefficient between betaine oral clearance and body weight was 0.6. Mean betaine clearance was higher in males than in females (\(P = 0.03\)). PK–PD simulation indicated minimal benefit from exceeding a twice-daily dosing schedule and a 150 mg kg\(^{-1}\) day\(^{-1}\) dosage for betaine.\textsuperscript{49}

While this latter study is probably the best published to date regarding the optimization of the use of betaine in HCU, it does come with a number of limitations. Firstly, ethical considerations preclude the study of betaine in human subjects with HCU in the absence of the methionine-restricted diet. Secondly, betaine treatment is typically for life and the duration of the study at 24 hours was therefore unsuited to detect possible changes in the PK and PD of chronic long-term betaine treatment.

**Can the efficacy of betaine treatment in HCU be improved?**

Current knowledge indicates that betaine is essentially an adjunctive therapy that cannot effectively substitute for methionine restriction.\textsuperscript{51–53} Although current therapy is effective, adherence to the methionine-restricted diet is difficult and compliance is often poor especially with children who are diagnosed after experiencing a normal diet. As discussed above, betaine is regarded as a safe treatment for HCU, but its use is not without risk indicating that there is a need not to overdose HCU patients with betaine. Additionally, due to the relative rarity of HCU, the associated costs of manufacturing betaine to the required pharmacological grade are high. If the efficacy of betaine treatment could be increased, it is conceivable that costs could be reduced and strict adherence to the methionine-restricted diet could be relaxed thus constituting a significant improvement in quality of life for individuals with HCU. A major impediment to achieving this goal is the fact that prolonged experimentation on the PK, dosing, and frequency of long-term betaine treatment, in isolation from a methionine-restricted diet, is precluded by ethical considerations regarding patient risk. Consequently, there is a need to examine the efficacy of betaine in isolation from methionine restriction in an animal model of HCU.

**Investigating betaine treatment using transgenic and knockout mouse models of HCU**

**Cbs null mouse model of HCU**

To date, the majority of research on HCU has been performed using a CBS knockout mouse model.\textsuperscript{54} These \(Cbs^{-/-}\) animals suffer from pronounced liver injury and typically die within 2–3 weeks of birth. This neonatal semi-lethality is not mirrored in human patients and restricts the utility of the model. Recently it has been shown that betaine treatment improved the survival of \(Cbs^{-/-}\) mice and restored fertility to female \(Cbs^{-/-}\) mice, but without significantly lowering Hcy.\textsuperscript{54} Surviving \(Cbs^{-/-}\) mice failed to show any alteration in coagulation parameters compared to wild-type controls and exhibited severe liver injury, steatosis, and fibrosis that were not significantly improved by betaine treatment. The failure of betaine treatment to lower Hcy in \(Cbs\) null mice is most likely due to the influence of severe liver injury upon hepatic BHMT expression.\textsuperscript{55} The fact that betaine treatment significantly improved survival in \(Cbs\) null mice without significantly lowering tHcy indicates that this compound may
exert significant protective effects in HCU independent of its role as a substrate for BHMT.

The HO transgenic mouse model of HCU
To date, the only animal model of HCU that has been demonstrated to accurately recapitulate the biochemical response to betaine that is typically observed in human subjects with HCU, is a transgenic model in which the mouse Cbs gene is inactivated and that exhibits very low-level expression of the human CBS gene under the control of the human CBS promoter. This mouse model, designated “human only” (HO), exhibits severe elevations in both plasma and tissue levels of Hcy, methionine, AdoMet, and AdoHcy and a concomitant decrease in plasma and hepatic levels of cysteine. Betaine treatment of HO mice resulted in a highly significant lowering of average tHcy levels from 257 (standard deviation [SD] = 65) to 50 µM (SD = 16.1; P < 0.001) (Figure 2A).

![Figure 2A](https://www.dovepress.com/)

**Figure 2A** The ability of betaine treatment to decrease plasma tHcy levels in HO HCU mice diminishes with time. Plasma levels (A) of tHcy and total cysteine and (B) methionine and DMG in WT mice, untreated HO mice and HO mice on 1 and 6 weeks of betaine treatment.

**Notes:** Values shown represent the mean and SD derived from eight animals. In this figure and all subsequent graphs presented here *,**, and *** denote P values of <0.05, 0.01, and 0.001, respectively.

**Abbreviations:** HCU, classical homocystinuria; HO, human only; SD, standard deviation; tHcy, total homocysteine; WT, wild-type.

Similarly, there was a 4-, 4.5-, 5.6-, and 4.7 -fold increase in plasma methionine, DMG, MG, and cysteine respectively (P < 0.0001 for all four metabolites) (Figure 2A and B). Lowering plasma tHcy by betaine treatment also resulted in a 40% decrease in plasma AdoMet (P = 0.0039) and a fivefold decrease in AdoHcy levels (P < 0.0001). Collectively, these data indicated that the HO mouse recapitulates the biochemical response of human subjects with HCU to betaine treatment and thus constitutes a suitable model for investigating ways to optimize the therapeutic effects of this treatment in HCU.

In addition to modeling the human HCU response at the biochemical level, tail bleeding determinations indicated that HO mice clot threefold faster than wild-type controls and are in a hypercoagulative state. Betaine treatment in the absence of dietary methionine restriction significantly decreased plasma tHcy levels (P < 0.0001) and concomitantly increased the clotting time in the HO mice (P = 0.0005). Subsequent work has found that HO HCU mice exhibit a highly significant induction of the pro-inflammatory cytokines interleukin 1α (IL-1α), IL-1β, and tumor necrosis factor-α (TNF-α). Similar, constitutive inductions of multiple proinflammatory cytokines (IL-1α, IL-6, TNF-α, IL-17, and IL-12 [p70]) and chemotactic chemokines (fractalkine, macrophage inflammatory protein [MIP]-1α and MIP-1β) were observed in untreated/poorly compliant human subjects with HCU. In the human subjects, standard methionine-restriction and betaine treatment was associated with either normalization of all of the proinflammatory cytokines and chemokines investigated with the exception of TNF-α, which was reduced, but remained significantly elevated compared to the normal controls. In the HO HCU mice, betaine treatment alone was sufficient to normalize all of the observed proinflammatory cytokine expression with the exception of TNF-α, which, while significantly reduced, remained elevated compared to wild-type control mice.

TNF-α has been associated with promoting osteoporosis by activating osteoclast mediated bone resorption. This latter possibility provides a possible explanation for the fact that osteoporosis is absent from children with Marfan syndrome but is a common feature in children with HCU. In this context, it is interesting to note that reduced bone density has been found in the HO mice (Maclean, unpublished data, 2012) and in another mouse model of HCU. Although no definitive study has ever been carried out, anecdotal evidence suggests that HCU-induced osteoporosis is not completely corrected by existing treatments. These findings suggest that patients that continue to experience reduced bone density while...
receiving conventional therapy for HCU might benefit from adjuvant anti-inflammatory therapy.

Apolipoprotein apoA-I is synthesized in the liver and contributes to much of the cardioprotective effects of high-density lipoprotein. Additionally, apoA-I exerts significant neuroprotective effects that act to preserve cognition. A recent investigation of apoA-I expression in the presence and absence of betaine, in the HO mouse and betaine/methionine restriction in human subjects with this disorder reported that plasma levels of apoA-I were significantly diminished in both untreated/poorly compliant mice and humans with HCU. Betaine and methionine restriction normalized plasma levels of this apolipoprotein in humans with HCU. Interestingly, betaine treatment in the absence of methionine restriction was sufficient to normalize plasma levels of apoA-I in HO mice. Collectively this study produced data consistent with a plausible role for decreased expression of apoA-I as a contributory factor for both cardiovascular disease and cognitive impairment in HCU and that lowering Hcy with betaine may exert its protective effects at least in part by normalizing expression of this apolipoprotein. Previous work has shown that apoA-IV can mimic a number of the antiatherogenic functions of apoA-I. This study also indicated that apoA-IV expression is also significantly decreased in humans with HCU but that this effect is not significantly ameliorated by conventional treatment to lower tHcy and there remains significant scope for the improvement of therapy in HCU.

Possible metabolic adaption to long-term betaine treatment in HCU

A recent investigation of the effects of long-term betaine treatment in the absence of methionine-restriction in HO HCU mice revealed that the ability of betaine treatment to lower Hcy is significantly diminished over time. One week of betaine treatment significantly lowered the observed mean plasma tHcy level compared to the untreated HO HCU mice (50.14 ± 16.4 versus 186.1 ± 32.3; \( P < 0.0001 \)). In a 6-week betaine treatment group, there was an approximate doubling in plasma tHcy compared to the 1-week treatment group (117 ± 45.1 versus 50.14 ± 16.4; \( P = 0.0015 \)). The observed increase in plasma Hcy during prolonged betaine treatment was accompanied by a significant increase in the plasma levels of TNF-\( \alpha \) and IL-1\( \beta \) and a reversion to a hypercoagulative phenotype. The findings of this analysis are consistent with a relatively sharp threshold effect between severely elevated plasma tHcy and thrombotic risk in HCU. Plasma metabolite analysis indicated that this effect was due, at least in part, to decreased BHMT-mediated remethylation of Hcy. These findings illustrate the importance of monitoring plasma methionine and DMG levels in addition to tHcy during routine assessments of patients with HCU. If a metabolic adaption similar to that observed in the HO mice were to occur in humans, it is likely that the physician might simply assume that the patient was not taking their betaine as prescribed, but this study indicates that it is possible that such patients might be exhibiting a diminished capacity to remethylate Hcy via BHMT rather than being less compliant.

Optimization of betaine treatment will require greater understanding of the regulation of BHMT and the methionine cycle in HCU

Although much is known about the regulation of the methionine and folate cycles in normal mammals, recent work has indicated that diseases such as HCU might act to alter those processes and there is a case for specifically reevaluating this regulation in an animal model of HCU. For example, Western blotting analysis has revealed that BHMT protein levels are significantly repressed in untreated HCU HO mice, but are significantly induced in the presence of betaine treatment. The aforementioned reduction in the ability of betaine to lower plasma tHcy levels during long-term therapy with betaine poses the question as to how this metabolic adaption arises? Despite serving a fundamental metabolic role in cysteine synthesis, CBS is not ubiquitously expressed in all tissues and in the absence of transsulfuration, the methionine cycle is essentially regarded as a closed loop. Therefore, tissues that lack transsulfuration will require mechanism/s for dealing with excess methionine. It is theoretically possible that intracellular levels of both Hcy and methionine could be decreased by the transamination of methionine but previous work has shown that this is not a significant mechanism in HCU. Methionine and Hcy can be extruded into the extracellular space and ultimately excreted in the urine while relatively high levels of AdoHcy result in its excretion in the urine as a keto derivative. Additionally, AdoMet can be converted by glycine N-methyltransferase to MG which can also be excreted in the urine. The possible effects of HCU upon the regulation of these metabolic mechanisms in the presence and absence of betaine treatment are presently unknown but clearly merit further investigation.

The observation that there are significantly higher levels of BHMT protein in the HO mouse undergoing long-term...
Betaine treatment where BHMT-mediated remethylation of Hcy is diminished, raises the possibility that the BHMT protein is impaired in its function in HCU (Figure 3). Previous work has indicated that DMG or AdoMet can serve as inhibitors of BHMT function, but this seems unlikely as the plasma levels of these metabolites are also diminished in the long-term treatment group. One possible mechanism for the diminution of BHMT enzyme activity over time is oxidative stress. Research by Miller and colleagues has reported that BHMT is prone to oxidative inactivation. Subsequent research has shown that purified BHMT is inactive in the absence of a thiol-reducing agent and removal of this protective compound results in a slow irreversible loss of the BHMT catalytic zinc molecule and a concomitant loss of activity. Thus, the increased induction of BHMT expression observed in the long-term betaine treatment group could constitute an unsuccessful compensatory mechanism designed to ameliorate the effects of diminished BHMT activity. Such an interpretation would be consistent with previous work where BHMT enzyme activity was inhibited by S-(Δ-carboxybutyl)-dl-Hcy (D,L-CBHcy) in rats. In this study, rats responded to >90% inhibition of BHMT enzyme activity by inducing expression of the BHMT gene. It is therefore conceivable that coadministration of an antioxidant could facilitate maximal BHMT activity during long-term betaine treatment which would presumably result in greater clinical efficacy. The HO mouse model of HCU can serve as a useful tool for examining the optimal timing and dosing of betaine treatment with a view towards optimizing clinical outcome.

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Disclosure

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