PROTOCOL #: 08-
(July 30, 2014)

(Use Protocol Manager on the COMIRB Website)

COMIRB Protocol

COLORADO MULTIPLE INSTITUTIONAL REVIEW BOARD
CAMPUS BOX F-490 TELEPHONE: 303-724-1055 Fax: 303-724-0990

Project Title: Oxidative Stress Markers In Inherited Homocystinuria And The Impact Of Taurine
Principal Investigator: Johan Van Hove, MD, PhD

1. Abstract
Cystathionine beta-synthase deficient homocystinuria (CBSDH) is an inherited disorder that is clinically silent at birth but with significant morbidity and mortality over time. Affected individuals exhibit a dramatic predisposition for thrombosis with a 50% risk of suffering a thromboembolic event by age 29. In addition, individuals incur cognitive impairment and connective tissue symptoms. Although current treatments improve outcome, limitations and adverse side effects exist, and treatment compliance is very poor. Consequently, a need for novel therapeutic strategies exists. The design of such strategies has been hampered by lack of understanding of the pathophysiology of the disease. While no single mechanism has been satisfactorily defined, data implicating oxidant stress exists. Also, CBSDH mouse model data indicate oxidative stress and inflammation play a role and that supplementation with taurine, a known antioxidant food supplement, mitigates this effect. This pilot study translates this laboratory research into clinical studies. It proposes that biomarkers of oxidative stress and inflammation are increased in human patients, that the degree of elevation is relative to the degree of elevation of homocysteine, and that short-term taurine supplementation mitigates the elevation of the biomarkers. Further, markers of oxidative stress and inflammation as well as disease-related metabolites will be related to clinically relevant platelet aggregation, vascular endothelial function and bone mineral density.

2. Hypothesis and Specific Aims

Cystathionine beta-synthase deficient homocystinuria is an inherited disorder that is clinically silent at birth but with significant morbidity and mortality over time even with best therapeutic intervention. Data from CBSDH mouse model research indicate oxidative stress and inflammation play a primary role in the pathophysiology of this disorder and that treatment with taurine, a nutritional supplement with known potent antioxidant and anti-inflammatory properties, mitigates this effect. This study translates this basic laboratory research into clinical studies, and investigates a proposed mechanism for the pathophysiology of CBSDH and the efficacy of a promising novel therapeutic intervention. The long range goal of the study is to enhance our ability to prevent adverse disease related outcomes throughout the life of individuals affected with this disorder.

Study hypothesis:

- Biomarkers of oxidative stress and inflammation are increased in CBSDH human patients
- The degree of elevation of the biomarkers of oxidative stress and inflammation is relative to the degree of elevation of homocysteine.
- Taurine supplementation mitigates the elevation of biomarkers of oxidative stress and inflammation.
- Endothelial function is abnormal in individuals with CBSDH even when receiving standard therapy, and is mitigated with taurine supplementation.
- Altered platelet aggregation is seen in CBSDH, and is mitigated with taurine supplementation.
- Decrease in bone mineral density relates to inflammatory markers, in particular TNF-α

Additional study aim:

- Pharmacokinetic analysis of taurine levels to derive C_{max}, $T_{1/2}$, and AUC .

.

3. Background and Significance

CBSDH is an inherited inborn error of metabolism caused by inactivation of cystathionine beta-synthase. This enzyme is a pyridoxal-phosphate-dependent heme protein that catalyzes the condensation of serine and homocysteine (Hcy) to form cystathionine in the first step of the cysteine biosynthetic transsulfuration pathway. The disorder is characterized by profound elevations in plasma levels of the thombogenic amino acid, Hcy, as well as elevations of methionine and S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH). Conversely, cystathionine and cysteine synthesis is effectively abolished.

Clinically, individuals with CBSDH exhibit a dramatic predisposition for thrombosis. Time-to-event graphs generated from data compiled from 629 untreated individuals show the risk of suffering a thromboembolic event is 25% by age 16 and 50% by age 29 (Mudd et al 1985). Of the events reported, 31% were cerebrovascular accidents, 51% were peripheral vein thrombosis with roughly one fourth of these involving pulmonary embolism, 4 % produced myocardial infarctions, 11% affected peripheral arteries, and 2% fell into none of these categories. These thromboembolic events constitute the major cause of morbidity and mortality in this disease (Schriver et al, 2001). In addition to the propensity toward clotting, individuals with CBSDH frequently incur cognitive impairment and exhibit a range of connective tissue disorders including markedly increased risk for ectopia lentis, marfanoid skeletal abnormalities, and osteoporosis. Incidence of CBSDH varies significantly in different populations with a recent estimate in Europe of 1 in 20,000.

There is a functional trichotomy in the nature of pathogenic mutations in CBSDH. One group of mutations is classified as “pyridoxine-responsive” as CBS enzyme function can be restored by high dose pyridoxine therapy. Such treatment effectively mitigates the biochemical and clinical findings in these individuals. The second group of functional mutations is represented by the “c-terminal CBS mutants” that are defective in their ability to respond to post-translational up-regulation by S-adenosylmethionine. Patients with this class of mutations lack mental retardation and connective tissue aspects of the phenotype and are detected after investigation of plasma Hcy levels following an idiopathic thrombotic event before the age of 40 (Maclean et al., 2004). The final group of CBSDH mutations is “classical homocystinuria” which represents the severest form of the disease.

For these latter two groups of patients, the standard treatment is a methionine (hence natural protein) restricted diet and supplementation with betaine (trimethylglycine) which serves as a methyl donor for the remethylation of Hcy to methionine catalyzed by betaine-homocysteine methyltransferase in the liver. Although methionine restriction and betaine supplementation have been successful in terms of improving clinical outcome, limitations and adverse side effects exist. Methionine restriction adversely impacts quality of life and is often associated with poor compliance. Patients diagnosed after the infantile period are very rarely able to sufficiently comply with its severe restrictions and special products. Betaine treatment in isolation often does not sufficiently lower plasma Hcy levels and can exacerbate already elevated methionine levels with a risk of cerebral edema. Illustrating the above, this clinic follows seven patients with B6 non-responsive CBSDH. All require diet therapy, as betaine therapy in isolation is not sufficient. Only one of the seven individuals is able to adhere to the dietary recommendation required to achieve a reduction in Hcy levels to a range that is therapeutic (< 50 μM total homocysteine, but still well elevated above normal which is 2-12 μM). Consequently, there is currently a need for novel therapeutic strategies for CBSDH. To date, the rational design of such strategies has been hampered by the lack of understanding of the underlying pathophysiological mechanisms involved in the disease leading to the thromboembolic events. Similarly, there is currently no knowledge about the mechanism of the skeletal and connective tissue defects in this condition. Symptoms of lens detachment, arachnoid status, and osteoporosis have persisted even in treated patients, and will require a new approach.

While no single mechanism has been satisfactorily defined in CBSDH-related thromboembolic events, a body of data has accumulated implicating oxidative stress.

General evidence:
An association between increased plasma Hcy levels and markers of oxidative stress has been reported in homocystinuria due to environmental and/or polygenic causes, as well as in CBSDH and inherited remethylation disorders. Animal studies on diet-induced homocysteinemia have shown evidence of oxidative stress, such as: (1) in rabbits elevations of thiobarbituric acid reactive substances (TBARS), a measure of reactive aldehydes formed from lipid peroxidation in rabbits (Toberek et al, 1995) and, (2) in rats elevations of monocyte chemoattractant protein-1 (MCP-1) (Hwang et al, 2008), and 3. in the aorta of rats activation of nuclear factor-Kappa-β (NF-κβ), a mediator of response to oxidative stress, with associated increased superoxide anion levels (Au-Yeung, 2004). Cultured vascular endothelial cells exposed to Hcy had induced NF-κβ activation and elevated superoxide anion levels, which was reversed by treatment of with a superoxide anion scavenger (polyethylene glycol-superoxide dismutase), supporting a causal relationship. In humans, in children and adolescents at high risk for coronary artery disease based on family history, increased plasma total Hcy (tHcy) levels and increased fasting plasma TBARS were observed, with interestingly a significant correlation between both reported (Szamosi et al, 2004).

CBSDH specific evidence:
In human CBSDH patients, reported evidence for oxidative stress includes an increase in 8-iso-prostane $F_{2\alpha}$ and an increase in extracellular superoxide dismutase. Eight-iso-$PGF_{2\alpha}$ is created by nonenzymatic lipid peroxidation by oxygen free radicals on cell membranes and LDL-particles and induces vasoconstriction and modulates the function of human platelets, hence it is particularly relevant here. Individuals with CBSDH as

compared to normal controls had increased urinary 8-iso-$PGF_{2\alpha}$ (640 ± 348 versus 321 ± 43 pg/mg creatinine; *P* = 0.0015, N = 13 patients with 3 or more samples of urine per individual), and this increase was positively correlated with plasma Hcy levels (range of tHcy levels was 17.1 umol/L – 287 umol/L; Spearman *p* = 0.398, *P* = 0.0076, N = 8 patients with 3 or more samples of urine per patient) (Davi et al, 2001). Excretion of 11-dehydro-thromboxane (TX)B_2, a marker for persistent platelet activation, in individuals with CBSDH was increased (1166 ± 415 versus 324 ± 72 pg/mg creatinine; *P*= 0.0015) and correlated with the urinary 8-iso-$PGF_{2\alpha}$ (*p*= 0.362, *P* = 0.0153). To investigate a cause and effect relationship, vitamin E supplementation (600 mg/day for two weeks) was given in 7 patients. This was associated with a decrease but not normalization in urinary 8-iso-$PGF_{2\alpha}$ (from 790 ± 159 to 559 ± 111 pg/mg creatinine, *P*=0.018) and in 11-dehydro-TXB_2 (from 1273 ± 383 to 913 ± 336 pg/mg creatinine, *P*=0.028), with an inverse correlation between urinary 8-iso-$PGF_{2\alpha}$ and plasma vitamin E levels.

A positive relationship between plasma tHcy levels and extracellular superoxide dismutase (EC-SOD) was found in a study of 62 samples in 18 individuals with CBSDH and 3 individuals with homocysteimia due to a remethylation defect over a 3.5 year period (Wilcken et al, 2000). In two newly diagnosed cases, initiation of treatment and lowering of the tHcy levels resulted in an approximate 50% reduction in plasma EC-SOD (for instance patient 1 pretreatment: tHcy 337 µmol/l and ED-SOD level 161.4 ng/ml; after treatment: tHcy 72 µmol/L and EC-SOD 71.5 ng/ml).

Finally, antioxidant therapy with oral vitamin C 1 gram per day in CBSDH showed improvement in vascular endothelial function assessed as brachial artery endothelium-dependent flow-mediated dilatation measured using high-resolution ultrasonic vessel wall-tracking (Pullin et al, 2002), but biochemical evidence of oxidative stress was not done. Endothelial dysfunction has also been reported previously in children with CBSDH (Celermajer, 1993).

Taurine is a member of the sulfur-containing amino acids and is a constituent of dietary protein being present in human milk, meat, and fish. Vegans who restrict natural protein have low plasma and urine taurine levels (Laidlaw et al, 1988) (McCarty, 2004). Taurine is also synthesized endogenously in the liver from cysteine via several enzymatic steps. Paradoxically, CBSDH blocks this natural pathway for endogenous taurine synthesis and the methionine (hence natural protein) restricted diet typically given to CBSDH patients restricts their exogenous supply of this amino acid. Low taurine levels are variable findings in patients with CBSDH followedChildren's Hospital Colorado.

Taurine's physiological properties have been extensively investigated. They involve a role in osmoregulation, an activity on calcium homeostasis, conjugation for instance with bile acids, an influence on proteins, and an overall function as a cellular protectant (Huxtable, 1992). Taurine's antioxidant properties are well recognized and its use as a protective agent in disorders resulting in oxidative stress has been investigated (Huxtable, 1992). Recent studies showing amelioration of oxidative stress include models such as rats with experimental hypothyroidism, mice with experimentally induced iron overload, and tamoxifen-induced mitochondrial oxidative damage in mice (Tas et al, 2006) (Oudit et al, 2004) (Parvez et al, 2008). Blood platelets are rich in taurine leading to the hypothesis that taurine status may impact platelet aggregation. (McCarty, 2004). To date, contrasting results of its therapeutic effect in hyperaggregation have been reported.

Typical daily taurine intake from food ranges from 40 -400 mg/day. The safety of supplementation above that was recently comprehensively reviewed by Shao and Hathcodk with the Counsel for Responsible Nutrition. (Shao and Hathcock, 2008) They looked at 30 peer-reviewed, published controlled clinical trials in humans involving administration of taurine. No significant adverse effects were seen in any trial. The highest oral dosage used in randomized controlled trials was 150 mg/kg/day or 10 grams per day over 6 months (also Durelli et al, 1983). Variable findings reported in the reviewed studies include gastric discomfort, increased as well as decreased triglycerides, and decrease in blood pressure in hypertensive individuals (but not in other patients). The lack of any consistent pattern over the 245 individuals taking the supplement provides support for its safe use. A single uncontrolled study used intravenously infused taurine 150 mg/kg 2x/day without noted side effects (Mutani et al, 1978). A single uncontrolled study noted nausea, headache, and dizziness in 2 patients (Takahashi and Nakane, 1978). Interestingly, a negative correlation was found in one uncontrolled study between plasma taurine and tHcy in normal middle-aged Korean women after taking 3 g taurine per day, reducing the tHcy level from 8.5 ±1.2 μM to 7.6 ± 1.1 μM (Ahn, 2009). Of note, taurine is available over the counter as a supplement. Suggested dosing varies and is often vague; however, Web posting comment that up to 6 grams per day as well as 12 grams per day, have been used for "treatment" with positive effect (Tolson) (Monson and Schoenstadt, 2008). In addition, taurine is found in a number of energy drinks. For instance, one can of "Monster Energy" has 1 g of taurine as does "Red Bull", "Vitamin Energy" has 2 g taurine as does "SoBe sugar free No Fear".

In humans, a 13 fold elevation in plasma taurine concentration was observed two hours after a one time dose of 1.66 grams (778 +/- 139 μM 2 hours post intake versus 64 +/- 4 μM at baseline) (Galloway et al, 2008). This decreased to a 6 fold elevation 4 hours after intake. Administration of a second 1.66 gm dose 4 hours post initial dose resulted in 16 fold elevation at five hours post initial dose (one hour post second dose). The taurine concentration then fell over the next three hours but remained significant elevated above baseline at 8 hours (161 +/- 31 μM at 8 hours versus 64 +/- 4 μM at base line). When studied and reported in other human studies, serum and urinary taurine levels increased with supplementation but effects varied widely (Sho and Hatchock, 2008). An increase in peak taurine concentration from 54.8±2.3 to 275.6±77.9 μM was reported with a dose of 6 g/day (Fujiuta et al, 1987). However, no formal pharmacokinetic data such as t½ are available for pharmacological doses of taurine. Platelets contain large quantities of taurine, and hence only plasma levels can be relied on; whereas older studies using

serum or whole blood have inconsistent and variable results due to the variable and uncontrolled degrees of release of taurine from platelets into serum.

4. Preliminary Studies; Progress Reports

To investigate in an unbiased way the mechanisms whereby CBSDH brings about its adverse effects, a microarray analysis of hepatic gene expression in a mouse model of CBSDH was done. The expression pattern was altered for multiple genes induced by `tumor necrosis factor-α (TNF-α), an inflammatory mediator, and by NF-κβ, a mediator of response to oxidative stress. Biochemical markers for oxidative stress and inflammation in the CBSDH mice were then investigated. Oxidative stress was assessed by TBARS, a measure of reactive aldehydes formed from lipid peroxidation, and was elevated in CBSDH mice ($p < 0.001$, $N = 10$) compared to control mice. Betaine therapy, decreased but did not normalize, the TBARS levels. In addition, adduction products with reactive aldehydes to proteins were increased, with fibrillin-1 being prominently sensitive to this damage. Fibrillin-1 is interesting given its role in lens fixation and marfanoid habitus. Activation of NF-κβ leads to increased cytokines such as TNF-α. Plasma levels of TNF- α and C-reactive protein (CRP) were significantly elevated in the mouse model of CBSDH ($p < 0.0001$ and $p< 0.005$ respectively). Subsequent Luminex-based cytokine profiling confirmed an increase in TNF-α in the CBSDH mice ($p < 0.0001$), and showed elevation of the inflammatory cytokines IL-1α and IL-1β ($p < 0.001$). TNF-α stimulates osteoclasts and may be important in the pathogenesis of osteoporosis. Betaine treatment lowered TNF-α and IL-1β, but did not normalize it.

Supplementation with 1% taurine in drinking water given *ad libitum* over 1 and over 2 weeks resulted in improvements of both TBARS levels and of TNF-α, IL1-α, and IL-1β without an effect on Hcy levels. For functional effects, coagulation was assessed in the mice by the bleeding time from tail cutting. CBSDH mice clot approximately 3 times faster than control mice, and taurine treatment completely abrogated this hypercoagulative phenotype. Taurine did not affect coagulation time in normal mice, indicating this effect is limited to the CBSDH pathology and is not a general effect on the components of the coagulation cascade or the natural anticoagulant/fibrinolytic system or platelet function. This implies that taurine treatment of CBSDH would have less undesirable side-effects as compared to conventional anticoagulant therapy, which currently is being taken by some adults with CBSDH.

Taken together, these observations indicate that dietary supplementation with taurine has significant therapeutic potential for treating CBSDH in humans. With its low-cost and low risk of side-effects, we propose investigation of taurine as an adjuvant therapy for betaine supplementation and methionine restriction.

Preliminary data from human CBSDH patients poorly compliant with standard therapy showed elevations of TNF-α (39.36 ± 4.85 pg/ml in un- or poorly treated and 19.33 ± 5.56 pg/ml in treated patients, versus controls 1.89 ± 0.53 pg/ml, $N = 10$, $p< 0.0001$) and TBARS (4.08 ± 0.56 nmol/ml in un- or poorly treated and 2.92 ± 0.38 treated patients, versus controls 2.23 ± 0.33 nmols/ml, $N = 10$, $p< 0.0001$ and <0.0027 respectively). Cytokine profiling confirmed significant elevations in TNF-α, and the inflammatory cytokines IL-1α and IL-1ß. Study subjects provided informed written consent for COMIRB protocol number 00-359.

5. Research Design and Methods

A. Overview - Brief Summary of Study Rationale:

Data from CBSDH mouse model research indicate that oxidative stress and inflammation play a primary role in the pathophysiology of CBSDH in its hypercoagulable state and possible in connective tissue pathology. The mouse model data also suggest that treatment with taurine, a nutritional supple ment with known antioxidant and anti-inflammatory properties, mitigates this effect. The data suggest that the effect is independent of the effect of betaine treatment, and additive to it. This basic laboratory research must be translated into clinical studies, and this study provides the first systematic study on a local scale. It investigates a proposed mechanism for the pathophysiology of CBSDH, and evaluates the efficacy of a novel therapeutic intervention. The study proposes that biomarkers of oxidative stress are elevated in individuals with CBSDH and that the elevation is relative to the elevation of Hcy. It will then treat patients with taurine (maximum 5 grams) twice a day for a total dose of 150 mg/kg/day (maximum 10 grams per day) for 4½ days. It proposes that supplementation with taurine has the potential to significantly decrease the oxidative stress and inflammation in CBSDH in humans. Biomarkers of oxidative stress and inflammation will be assessed and compared to normal values. They will also be compared to Hcy levels, levels of other disease intermediary metabolites, and markers of standard therapy for this disorder. The impact of taurine supplementation on the biomarkers of oxidative stress and inflammation will be assessed. The impact of taurine supplementation on Hcy and disease intermediary metabolites, and standard disease treatment markers will also be assessed. Further, markers of oxidative stress and inflammation as well as disease-related metabolites will be related to the clinically relevant platelet function and vascular endothelial function studies assessed via a standardized doppler method available through the UCH CCTSI. Brachial artery flow-mediated dilation (FMD) will be used as a non-invasive measure of endothelial-dependent dilatation (EDD), mediated in part by nitric oxide (Liao et al, 1991) (Dakak et al, 1998). Platelet function will be assessed by markers of chronic platelet activation and by platelet aggregation studies. The impact of taurine treatment on FMD and platelet aggregation will also be assessed.

Since inflammatory messengers such as TNF-α can stimulate osteoclasts without stimulating osteoblasts, long term elevation of TNF-α can cause osteoporosis. We will measure bone mineral density and will evaluate if the Z-scores are related to the inflammatory markers in particular to TNF-α. Such a finding would strengthen the development of a hypothesis of a possible relation, whereas its absence of such a relation would not necessarily negate this given the many parameters that can influence bone mineral density.

Finally, baseline pharmacokinetics of oral pharmacologic doses of taurine will be developed.

For safety reasons, the first two patients will be treated with a dose of 25 mg/kg/dose (maximum 1.5 g) twice daily. After review of safety parameters, the dose will then be increase to the planned dose of 75 mg/kg/dose (maximum 5 g) twice daily. The first four patients will be adults, before recruiting children in the study.

This short-term local pilot study will form the basis on which to later develop a national long-term study (orphan drug study), 4 years duration and placebo controlled, that will expand on the findings and include long-term variables such as those related to connective tissue and bone.

Primary Outcome Measure(s):

Oxidative stress and inflammation measures as follows:

- Luminex cytokine array studies to include the following cytokines
  - Interleukin 1-alpha (IL-1α)
  - Interleukin 1-beta (IL-1β)
  - Interleukin 1-ra (IL-1ra)
  - Interleukin 4 (IL-4)
  - Interleukin 8 (IL-8)
  - Interleukin 10 (IL-10)
  - TNF-alpha (TNF-α)
  - Monocyte Chemoatractant Protein-1 (MCP-1)
  - Interleukin 6 (IL-6)
  - Interleukin 12 (IL-12p70)
  - Interleukin 17 (IL-17)
  - MIP-1α
  - MIP-1β
- C-reactive protein (high sensitivity)
- Myeloperoxidase
- Transforming Growth Factor Beta (TGF-β)
- Extracelluar superoxide dismutase
- TBARS
- ELISA Assay
  - Interleukin 1-alpha (ILα)
  - Interleukin 1-beta (IL-β)
  - Interleukin 10 (IL-10)
  - TNF-alpha (TNF-α)
- 8-Isoprostane metabolites (2,3-dinor-F_2-IsoPs combined signal) of 8-iso$PGF_{2\alpha}$, urine
- Dityrosine, urine
- Glutathione reduced (GSH) and oxidized (GSSG) in whole blood

Vascular coagulability markers:

- Endothelial Function as assessed by brachial artery flow-mediated dilation (FMD): percent change induced by hypoxemia in brachial artery diameter and flow measurement (derived from diameter and flow velocity)

- Platelet aggregation
- 11-dehydro-thromboxane B2 (11-dehydro-TXB2) and 2,3-dinor-TXB2, urine

Bone mineral density by DEXA

B. Secondary Outcome Measure(s):

The relationship between the above noted oxidative stress markers and the related methionine cycle metabolites will be assessed

- Hcy
- Methionine
- Cystathionine
- Cysteine
- SAM (S-adenosylmethionine)
- SAH (S-adenosylhomocysteine)
- Dimethylglycine
- Betaine

Lipoprotein A will be assessed as confounding variable potentially modulating platelet aggregation and inflammatory response.

Molecular studies will be done given the known genotype/phenotype correlations in this disease and for verification of diagnosis.

Pharmacokinetic studies of taurine will be done for the first 12 hours following the first dose of taurine, and a steady state concentration will be obtained after 4 days of treatment.

C. Study Population Description:

Participants will be recruited from the Inherited Metabolic Diseases Clinic at Children's Hospital Colorado, The Children's Hospital of Philadelphia, and Duke University Hospital. Recruitment of patients being followed by other metabolic clinics may also be pursued if additional participants are needed. This will be done through their metabolic physician and / or through family support groups. Patients must meet all inclusion criteria. There will be no discrimination based on age gender, or ethnic heritage.

D. Total Planned Number of Subjects:

a. **For Single-Center Studies**: NA
b. **For Multi-Center Studies – Up to20**
   i. **For this location: Up to 12**
   ii. **For all locations:** Up to 20

Up to 16 patients will be exposed to taurine and analyzed. Allowing for 20 patients to sign consent is based upon the potential for patients who withdraw consent prior to drug exposure, may be potential screen failures, or fail to proceed to study after signing consent.

E. Inclusion Criteria:

Minimal inclusion criteria are:

1. A confirmed biochemical, molecular, or enzymatic diagnosis of classic homocystinuria due to cystathionine beta-synthase deficiency (OMIM 236200)

2. And not fully responsive to therapy (eg, tHcy levels above 50 µmol/L on therapy including on B6 therapy)

3. Be over 8 years old and less than 50 years. The first four patients will be adults (age 18 – 50 years).

4. Be able and willing to provide informed consent

Exclusion Criteria:

1. Age < 8 years: Individuals under 8 years of age will be excluded due to blood volume issues and concerns regarding their ability to assent

2. Age > 50 years: Individuals over 50 years will be excluded due to possible age related differences in markers of oxidative stress and inflammation

3. Pregnancy: Females who are pregnant or lactating will be excluded from the study as the influence of pregnancy on the markers is not known nor is the safety of taurine supplementation in pregnancy.

4. Continued antioxidant intake:

a. Individuals currently taking taurine, over the counter energy drinks containing taurine or other high dose antioxidants and unwilling to discontinue this for the study period (including a 2 week wash out period) will be excluded as such intake will likely impact laboratory results.

b. Individuals taking Vitamin C as a prescribed treatment for their homocystinuria will be excluded as the antioxidant therapy may impact antioxidant and inflammation markers. (As Vitamin C is not standard of care for this disease we anticipate this to have minimal impact on recruitment.)

c. Individuals currently taking platelet aggregation inhibitors such as salicylate on a self prescribed PRN basis and unwilling to discontinue this for the study period (including a washout period of at least two weeks prior to the study) will be excluded as salicylate intake will impact platelet study results. Individuals taking salicylate (or other platelet aggregation inhibitors) prescribed as a therapy for their homocystinuria or other health issues will not be asked to stop the medication. They will participate in the study, but will be excluded only from the platelet studies.

5. Medication interactions: Individuals unable or unwilling to abstain from use of cGMP phosphodiesterase 5 inhibitors (such as Viagra) during the study period will be excluded from the nitroglycerin-induced flow-mediated dilatation studies in accordance with known labeling contraindications.

6. Inflammatory status:

a. Individuals who have a significant chronic illness that has a marked inflammatory component will be excluded from the study as the illness will impact inflammatory markers.

b. Patients with an acute illness, which may impact inflammatory biomarkers, will be postponed for study entry until the acute illness is resolved. Entry into the study at a later day will be offered.

7. Cardiovascular status

a.Recent cardiovascular event. Cardiovascular events (stroke, myocardial infact, deep vein thrombosis, pulmonary embolus or thrombosis) may interfere with platelet function studies and with various mediators during the first months after the event. Patients who had such an event within the last 6 months will be excluded.

b.Patients with uncontrolled hypertension. This will remove a confounding factor from analysis of the endothelial flow studies and increase safety. 8. Informed consent: Individuals who are unwilling or unable to consent, or in the case of minors who are unwilling or unable to assent will be excluded due to lack of ability to ensure informed consent.

9. Study compliance and integrity: Individual who anticipate an inability to comply with study procedures and requirements will be excluded.

10. Hypertriglyceridemia. Individuals with a triglyceride level above 300 mg/dl will be excluded from the study. An associated increase in triglyceride levels with administration of taurine has been reported (as has a decrease in triglycerides) Exclusion of individuals who have a history of hypertriglyceridemia will decrease the likelihood that a study related increase in serum triglycerides would result in health care risks. This is a conservative approach as acute health care risk is generally seen in the setting of triglycerides > 1000 mg/dl. Historic triglyceride levels (within 5 years of the date of consent) can be used to assess eligibility. This lacking, triglycerides can be obtained as part of the study and assessed prior to administration of taurine.

F. Planned Enrollment: Enter # of Subjects in the Table Below: review the numbers for 12 and women = male (given your statement in 7A inclusion of women)

Ethnic Categories	**Gender**		
	Females	Males	Total
Hispanic or Latino	2	2	**4**
Not Hispanic or Latino	8	8	**16**
Ethnic Categories: Total of All Subjects	**10**	**10**	**20**
Racial Categories			
American Indian/Alaska Native	0	0	**0**
Asian	1	0	**1**
Native Hawaiian or Other Pacific Islander	0	0	**0**
Black or African American	1	2	**3**
White	8	8	**16**

Racial Categories: Total of All Subjects*	10	10	20

The "Ethnic Categories Total of All Subjects" **must be equal to the "Racial Categories Total of All Subjects"*

Data listed here reflects US population demographics and is an estimate..

G. Study Design Details:

First informed consent will be obtained (see section III. Informed consent plan). This section describes the active portion of the study. Patients will be evaluated after a two week washout period if the subject was taking taurine supplementation or other high dose antioxidant supplements. Prior to start of study, the PI or one of the MD co-investigators will review the list of medications and supplements used, and determine if the participant should be excluded from platelet function studies (use of platelet aggregation inhibitors) or should be excluded from FMD studies (use of cGMP phosphdiesterase 5 inhibitors) (see special considerations below in this section). Base-line observational studies will be done first. While the patient is on their standard treatment regime blood and urine samples will be obtained for biomarkers of oxidative stress, inflammatory mediators, Hcy, and other disease-specific intermediary metabolites, and for markers of treatment compliance. (See below table for laboratory study details.) The inflammatory mediators to be investigated were selected based on findings from the mouse studies. They include markers that are positively changed in the mouse model, as well as markers that have been selected to document the specificity of this inflammatory response as different from a non-specific response (e.g. IL-4 can decrease TNF-α production and we want to see that the increased TNF-α is not caused by a decrease in these factors; IL-10 would be a specific negative control not expected to be changed). The oxidative stress markers, TBARS and superoxide dismutase, were selected based on mouse model data and data from human studies in CBSDH. The oxidative stress markers, 8-isoprostane and dityrosine, are established markers of lipid and protein damage and are included in the study because they are stable and independent of dietary intake (Dalle-Donne et al, 2006). Instead of the CTSI available immunoassay for the critical oxidative stress marker 8-isoprostane, we will use the more specific and sensitive mass spectrometric assay available at Duke University Medical involving our new collaborator David Millington, PhD. Normal values for comparison will be developed by Dr. Millington. Glutathione reduced and oxidized will be measured by HPLC – tandem mass spectrometry (Steghens et al. 2003) in the laboratory of Dr. Tina Cowan, PhD at Stanford, who has used this assay in patients with metabolic diseases and has developed normal values. Processing and stabilization of samples will be done locally in the laboratory of Dr. Van Hove. Specific interleukins are repeated in the laboratory of Dr. MacLean using the same Elisa method as previous studies for comparison. Using an ELISA method to compare to the Luminex assay increases the validity of the findings, as discussed with Dr. Accurso. The above noted markers will be related to normal values as well as Hcy and other disease related metabolites. Also, a bone mineral content and density will be done using a DEXA scan. TNF-α activates osteoclasts but not osteoblasts. We will relate the DEXA results to the level of TNF-α.

The above biomarkers and disease-related metabolites will be further related to the clinically relevant vascular endothelial function studied via a standardized doppler method available through the UCH CCTSI. Brachial artery flow-mediated dilation (FMD) will be used as a non-invasive measure of endothelial dependent dilatation (EDD), mediated in part by nitric oxide (Liao et al, 1991) (Dakak et al, 1998). Ultrasound measurements of

brachial artery EDD (i.e., FMD studies) during forearm reactive hyperemia and in response to sublingual nitroglycerine (0.4mg), well-established measures of vascular endothelium–dependent and –independent vasodilatation, respectively, will be performed as previously described (Celermajer et al, 1992) (Eslirza et al, 2004). This entails ultrasound measurement of brachial arterial diameter in the resting state, followed by occlusion of the brachial artery by a blood pressure cuff for 5 minutes. The cuff is then rapidly deflated. The ensuing turbulent blood flow results in endothelial sheer stress. This activates a cascade of endothelial mediated events resulting in dilation of the vessel. The dilation is quantified by ultrasound. The maximum percent increase in vessel size after the release of the blood pressure cuff, or FMD, is a measure of endothelial-dependent vasodilatation. Following this, endothelial-independent vasodilatation is assessed. Brachial artery ultrasound measurements are again obtained in the resting state. Following this, 0.4 mg of nitroglycerin, source being Nitrostat SL tablets, is given sublingually. Nitroglycerin will not be used in patients under age 18 years. Nitric oxide is produced from the nitroglycerin without need for the endothelial cells. The nitric oxide has a direct vasodilator effect. Brachial artery diameter is again assessed via ultrasound. The maximum percent increase in vessel size after the nitroglycerin is as a measure of nitrate-mediated, endothelium-independent vasodilatation. Abnormal FMD results in the setting of normal endothelial-independent vasodilatation results suggest endothelial dysfunction. To avoid confounding variables subjects will be asked not to eat 4 hours prior to study or consume caffeine 10 hour prior to study .

The above biomarkers and disease related metabolites will be also related to clinically relevant platelet function studies. We hypothesize that the inflammatory and oxidant environment pushes normal platelets into a chronic low-grade aggregation status, and that this improves with taurine. We will measure urinary 11-dehydroTXB2 and 2,3-dinor-TXB2 to reflect aggregation status, and in vitro platelet aggregation studies to reflect the platelet function. The metabolites 11-dehydroTXB2 and 2,3-dinor-TXB2 have been reported as elevated in CBSDH patients.

Following the observational studies, participants will be treated with oral taurine 75 mg/kg (maximum5 grams) BID for 4 ½ days. The first two patients will be treated with a dose of 25 mg/kg (maximum 1.5 g) twice daily, before increasing the dose to the full study dose. USP grade taurine supplied by Solace Nutrition, lot number 6608 (Solace Nutrtition, One Research Court, Suite 450, Rockville, MD 20850) and Medisca Inc, lot number 105927 (Medisca Inc, 661 Route 3 Unit C, Plattsburgh, NY 12901) will be used. If for any reason this lot number changes, we will submit additional Certificate of Analysis to the FDA and COMIRB for the new lot numbers. Participants will be weighted on visit day one. Based on the patient weight, a prescription order will be written by the MD Co-investigator or CTRC nurse practitioner for taurine and will be given to The Children's Hospital Pharmacy Investigational Drug Services. Individual, single dose packets will be weighed and dispensed by the pharmacy to the Clinical and Translational Research Center (CTRC). Each single dose packet will be labeled with the study medication name and dose. The amount of product needed for the first 4 days of the study period will be dispensed up front in a container that is labeled with subject's name, medication name, medication dose, and directions for administration. Based on the prescription order, the Children's Hospital Pharmacy will dispense the medication to the inpatient Clinical and Translation Research Center (CTRC). The first dose of study medication will be administered orally to the study patient by a RN. The RN will mix taurine with 150mls of water or the individual's favorite juice. The RN

will review administration instructions and observe intake of the first oral dose. There after the medication will be self administered by the participant in the same manner; the patient will be instructed that once taurine is mixed with a liquid it must be consumed within thirty minutes of mixing. The last dose will be dispensed in a separate container by the Investigational Pharmacy to the outpatient CTRC with labeling and administration as that described above.

Disease metabolites and biomarkers of oxidative stress and inflammation laboratory studies will be obtained at 4 hours after the first dose, and after completion of 4 days of treatment. Comparison of results prior to treatment with results after one dose and after 4 days of treatment will be done. Functionally relevant platelet aggregation will be obtained after 4 days of treatment. Functionally relevant FMD studies will be obtained after the blood work and the last taurine dose, this being after 4 ½ days of treatment. After the blood flow studies have been completed, the patient will be discharged. Comparison of results prior to treatment and results after treatment will be done.

Pharmacokinetic studies will be done after the first dose with blood samples obtained before administration (time 0), and after 30 min, 1, 2, 4, 6, 8, and 12 hours. A steady state level will be obtained after 4 days treatment. Plasma samples will be spun down immediately to avoid platelet aggregation, and samples stored at – 70 degrees Celsius until analysis (to prevent hypotaurine from oxidation to taurine). Sample will be analyzed using an LC- MS/MS system in combination with online extraction (LC/LC-MS/MS by Dr. Uwe Christians, and pharmacokinetic results modeled using SAAM II (University of Washington) by Drs. Jeffrey Galinkin and Thomas Henthorn.

The study will usually start on a Thursday and end on a Tuesday to allow for the four day treatment period with blood and urine sample collections and ultrasound and FMD studies to be done on work-week days. Laboratory will be taken at 8:00 AM after overnight fasting or at 12 noon before lunch and after a 3 hour fast to limit the impact of dietary intake of protein . Should the taurine study be terminated early, then laboratory studies and FMD studies may be collected earlier at closing. Taurine will be dispensed by the centers investigational pharmacy, enough product given for the total length of the study. Bottles of product will be collected at the end of the study and amount of left-over product determined as a compliance check in addition to participant self-report compliance. Participants will be asked to stop use of any anti-oxidation supplement, in excess of standard multi-vitamins (1 table/day) for two weeks prior to the study. They will also be requested to avoid use of non-prescription salicylates (Aspirin) two weeks prior to the study. Potential confounding variables of (1) hormone replacement therapy, (2) other significant chronic disease (3) high physical activity, (4) smoking habits, and (5) anticoagulant use will be determined for each individual. Safety will be assessed by vital signs and a physical examination, a questionnaire on possible side effects, and basic laboratory tests of CBC, CMP, and lipid panel.

Molecular analysis of the CBSDH gene will be done in the Children's Hospital Colorado DNA Diagnostic laboratory, where this test is clinically available. We anticipate that about half the patients will already have this information clinically available, with about half the patients requiring further analysis as part of this study.

H. Laboratory Assays, Examinations and Procedures:

Study	Purpose	Laboratory
Luminex, 8 plex (IL-1α, IL-1β, IL-1ra, IL-4, IL-6, IL-10, TNF-α, MCP-1, IL,-8, IL-12p70, IL-17, MIP-1α, MIP-1β)	inflammation	CTRC
CRP	inflammations	CTRC
Myeloperoxidase	oxidative stress	CTRC
TGF-beta	inflammation	CTRC
superoxide dismutase	oxidative stress	MacLean/UCD
TBARS	oxidative stress	MacLean/UCD
Elisa assay (IL-1α,IL-1β, IL-10, TNF-α)	inflammation	MacLean/UCD
Isoprostane, urine	oxidative stress	Millington/Duke University
Dityrosine, urine	oxidative stress	Uwe Christians Anesthesiology Laboratory
Glutathione, whole blood	oxidative stress	Cowan/ Stanfod
tHcy	disease primary marker	Metabolite Laboratory, Inc
Methionine	disease intermediary	Metabolite Laboratory, Inc
Cystathionine	disease intermediary	Metabolite Laboratory, Inc
Cysteine	disease intermediary	Metabolite Laboratory, Inc
S-adenosylmethionine	disease intermediary	Uwe Christians Anesthesiology Laboratory
S-adenosylhomocystiene	disease intermediary	Uwe Christians Anesthesiology Laboratory
Dimethylglycine	compliance of standard Tx	Metabolite Laboratory, Inc
Betaine	compliance of standard Tx	MacLean/UCD
Serum amino acids (includes taurine)	compliance of experimental Tx	Denver Genetic Laboratory - Biochemical Genetic Laboratory
Taurine	Pharmacokinetics	Uwe Christians, Anesthesiology Laboratory
Platelet aggregation	Coagulation assessment	local lab*
Lipoprotein A	Atherosclerotic disease marker	Mayo
11-dehydroTXB2, 2,3-dinor-TXB2, urine	Coagulation assessment	MacLean/UCD
CBC	Safety	local lab**
CMP	Safety	local lab**
Lipid panel	Safety	local lab**
Triglyceride level	Safety	local lab**
pregnancy, urine	Safety	local lab**
Cystathionine β-synthase molecular (*CBS)*	Genotype	Denver Genetic Laboratories – Molecular Laboratory
Plasma, serum and urine freeze - 70, save		MacLean/UCD
Estradiol/Progesterone (Females	Menstrual cycle timing for	UCH CTRC

only)	FMD interpretation	

* For Colorado, this will be University of Colorado Hospital Laboratory
** For Colorado, this will be Children's Hospital Colorado

	THUR* Day 1	**FRI Day 2 8 AM**	**FRI Day 2 12 PM**	**TUE Day 6 8 AM**	**TUE Day 6 After 8 AM**
Informed consent	X				
Intake sheet (see appendix)	X				
Adverse Event/Compliance Sheet (see appendix)†		X		X	
Medical History	X				
Physical exam	X			X	
Endothelial function studies	X				X
DEXA scan	X				
Blood Luminex, CRP, myeloperoxidase, TGF-β (CTRC)	X	X	X	X	
Urine isoprostane, dityrosine		X		X	
Glutathione	X	X	X	X	
Lipoprotein A		X			
CBC, CMP, Lipid panel		X		X	
Estradiol/Progesterone (Females only)	X‡	X‡			
Urine pregnancy tests	X				
Superoxide dismutase, TBARS, Elisa, (McLean)	X	X	X	X	
Homocysteine and metabolites (Metabolite Inc)	X	X	X	X	
Betaine (MacLean)	X	X	X	X	
Serum amino acids (Biochemical Genetics)	X	X	X	X	
Plasma taurine	X	X¶	X¶	X	

Platelet aggregation studies (local lab)		X		X‡	
ThromboxaneB2 metabolites , urine		X	X	X	
Cystathionine beta-synthase molecular studies	X				
Plasma, serum and urine to freeze and save to include 1.0 ml EDTA plasma on the second day of the study	C	C	C	C	

*Day one of study will typically be on a Thursday, exceptions may exist

† The adverse event survey will also be administered over the phone one to two weeks after study completion

X = collected and analyzed

C= collected and stored for possible studies during study period

¶ = collected before administration (time 0), and after 30 min, 1, 2, 4, 6, 8, and 12 hours.

‡ = collection can occur on Day 1 or Day 2 based upon site maximum blood volume

‡= will not be collected if site maximum blood volume is reached

Practical Aspects: Samples not obtained for safety reasons will be batched to avoid inter-day variation. Analysis will be done at midway point and at study end. All samples will be stored at – 70 °C before being processed. Accurate SAM and SAH determination necessitates special handling of specimens and will be done in accordance with directions from assaying laboratory. The processing and freezing of samples for these studies will be done by the local laboratory in collaboration with the CTRC. The laboratory will be notified of anticipated samples per local standards. Accurate platelet aggregation studies necessitate that samples be analyzed within 3 - 4 hours of collection. In addition, platelet aggregation studies are sensitive to sample handling as sheer stress promotes platelet aggregation. Therefore, the samples for this study will be hand carried by study personnel (PI or assistant) to the main laboratory where they will be analyzed. Finally, collection of samples for platelet aggregation, should a heparin lock be in place, will be done in accordance to standard methods used for drawing coagulation studies, to avoid any potential confounding impact from heparin. All platelet aggregation studies will be scheduled with the local laboratory in advance in accordance with their protocol. For all draws from the heparin lock, specimens will be drawn per nursing protocol, and if not contraindicated at the time, the waste will be returned to the patient. A stopcock is used to decrease the risk of infection with returned blood. The line will be flushed with 0.9% normal saline and/or heparin as needed based on laboratory testing to be done. Flushing will use the Turbulent (push/pause) method to wash out any infusate or blood from the catheter. Samples for the 2,3-dinor-8-isoprostaglandin-F2α are collected in the morning, which reduces intraperson variation, and stored at – 70 degrees Celsius until analysis (Zhang et

al, 2009). Samples for glutathione will be placed on ice and carried to the local laboratory where they will be prepared by diluting it with the precipitating solution containing N-ethylmaleimide, sulfosalicylic acid, EDTA and methanol, and the supernatant stored at – 80 °C until shipment on dry ice to the receiving laboratory for analysis.

All specimens will be routed through either the CTRC, Children's Hospital Colorado, or MacLean laboratory for processing and distribution to appropriate laboratories as well as for freezing of samples for batching and freezing of samples for possible studies in the future.

To reduce pain with venipuncture and or peripheral catheter placement, use of EMLA cream will be offered.

Special considerations:

Patients taking salicylates or other platelet function modifying drugs (such as dipyridamole, ticlopidine, clopidogrel) will be excluded from the platelet function studies (platelet aggregration study and urine thromboxane B2 metabolites) but will otherwise participate in this study.

Patients taking cGMP phosphodiesterase 5 inhibitors (such as sildenafil, tadalafil, vardenafil) will be excluded from the FMD studies, but can participate in all other parts of the study.

These drugs will be identified during the screening interview and during the intake exam at the start of the study.

I. Documentation of a triglyceride level, obtained within the last five years, less than or equal to 300 mg/dl is required. If not available a level can be obtained any time after phone or in person consent and prior to in person re-consent at the start of the active protocol. For those living locally, they can come to Children's Hospital Colorado outpatient laboratory to have the sample collected and run as part of the study. For those living distantly, the sample can be drawn locally and sent to the Children's Hospital Colorado to be run as part of the study. Participants will be provided with a shipping container and billing code for shipping expense. The shipping cost and the cost of the lipid panel will be billed to the study. Specimen drawing fees will also be reimbursed upon receipt of bill documenting charge.
Expected Results:
It is anticipated that the biomarkers of oxidative stress and inflammation will be increased above the norm in participants and the degree of elevation will be related to Hcy levels. A decrease in the elevation of the biomarkers is anticipated following treatment with taurine. It is also anticipated that FMD studies will be abnormal in participants and that improvement will be seen following taurine supplementation. We anticipate that platelet aggregation studies will be normal, but that markers of generalized activation of platelets, thromboxane B2 metabolites, are elevated, and will reduce with treatment. Bone mineral density Z-scores will be related to baseline TNF-α levels.

J. Potential Problems:
1. Recruitment: Given the rarity of the disorder, our potential participant sample volume is low. Geographic and economic issues may make it difficult for some

potential subjects to participate in the study. Low participation in the study might impact the statistical analysis of data. Should insufficient patients be recruited locally, then advertisement through national organization will be done to increase participation. Of note, while there is one report of Vitamin C supplementation ameliorating endothelial dysfunction in individuals with CBSDH, its use has not been widely investigated and use of Vitamin C to treat homocystinuria is not standard of care. We therefore do not anticipate this excluding criteria to markedly effect recruitment.

Patients with prescribed salicylate (such as Aspirin) therapy will not have this discontinued but will be excluded from the platelet function studies. We anticipate that this will impact up to 25% of the recruited population. A recruitment of 12 patients will likely provide a minimum of 8 patients in which this clinically relevant measure can be analyzed. Should the percentage of patients taking platelet aggregation inhibitors exceed our anticipated number, then we will plan to extend the study to increase the number of patients to be enrolled. Similar considerations are for use of cGMP phosphodiesterase 5 inhibitors (such as Viagra) and exclusion of FMD studies, although we doubt that the rate of patients taking this medication in our young age group would be as high as 25%. Overall, our goal is to recruit 8 patients that participate in all aspects of the study and are treated at the target dose of 150 mg/kg/day (maximum 10 g/day).

Patients taking platelet aggregation inhibitors such as salicylate on a PRN basis that are unwilling or unable to stop taking the medication for the study period to include the two week washout period will be excluded from the study for the impact on platelet function but will participate in the remainder of the study

2. Normal values: Normal values are available for nearly all measurements. Normal values are being developed by the CTRC laboratory for the Luminex panel of inflammatory markers and for TGF-beta.. Normal values for FMD studies will be developed from patients previously analyzed in the adult CTRC using the same methodology as discussed with Kerrie Moureau.

K. Statistical Sampling, Sample Size and Analytical Plan

This study benefits from the statistical assistance provided through the CCTSI. The protocol was reviewed by biostatistician Dexiang Gao, PhD. The services of Dr. Gao will be retained through the study.

I. **The primary efficacy outcome parameters** (Aim 2) are TBARS and TNF-α. For each of these two parameters, there are two questions:

A. Is the biomarker elevated in the untreated patient compared to control?
   This question is required to validate the preliminary data.
B. Does the biomarker decrease following treatment with taurine, after 4 hours treatment (immediate response) and after 4 days of treatment (short term treatment)?
   This question evaluates the impact or treatment on the primary outcome markers

Sample size and statistical power consideration:

A. Is the biomarker (TNF-α, TBARS) elevated in CBSDH patients? Hypothesis 1 states that extracellular biomarkers of oxidative stress and inflammation in CBSDH patients will be increased compared with control patient values. The proposed study will include mostly patients who are untreated or poorly treated (non-compliant patients) for conventional

treatment, but can also have treated patients. To be conservative, we will use the preliminary data from the treated patients to power the study.
For TNF-α, preliminary data showed a mean of 19.33±5.56 pg/ml in CBSDH conventionally treated patients compared with a mean in controls of 1.89±0.53 pg/ml. When the sample size is 8, a single group t-test with a 0.05 two-sided significance level will have 99% power to detect the difference between a null hypothesis mean TNF-α of 1.89 pg/ml and an alternative mean of 19.33, assuming that the standard deviation is 5.56 pg/ml.
For TBARS, conventionally treated CBSDH patients had a mean TBARS of 2.92±0.38 nmols/ml compared with mean TBARS of 2.23±0.33 nmols/ml in controls. When the sample size is 8, a single group t-test with a 0.05 two-sided significance level will have 99% power to detect the difference between a null hypothesis mean TBARS of 2.23 nmols/ml and an alternative mean of 2.92, assuming that the standard deviation is 0.38 nmols/ml.
Analysis plan: The mean, median, standard deviation, and 95% confidence intervals for TNF-α and TBARS will be generated.

B. Does taurine decrease the primary outcome biomarkers of TNF-α and TBARS?
Sample size and statistical power consideration: We hypothesize that taurine treatment will decrease oxidative stress and inflammation in CBSDH patients, which will be evaluated by comparing the levels of TBARS and TNF-α after 4 hours and 4 days on taurine compared to the corresponding baseline levels (before treatment). The minimum number of evaluable patients on high dose taurine therapy is eight, but can be as high as 10. Eight evaluable baseline paired samples provide 80% or higher power to detect an effect size in the range of 1.2-1.5, which is the standardized (by the standard deviation of the changes) mean difference between the alternative hypothesis of reduced oxidative stress/inflammation due to taurine therapy and the null hypothesis of high oxidative stress/inflammation before taurine treatment. A paired t-test was used for the calculation with a one-sided significance level of 0.05.

Power	Effect Size
0.95	1.30
0.9	1.15
0.8	0.98

To translate the effect size into the actual detectable level of the two markers, preliminary data showed a mean baseline TNF-α of 19.33±5.56 pg/ml in treated CBSDH patients. Assume the correlation coefficient (Rho) of TNF-α between baseline and 4 days (or 4 hours) on taurine therapy is in the typical range of 0.1-0.5, and the variance of TNF-α on four days (or 4 hours) at taurine therapy time points are the same as at the baseline, then the detectable mean value of TNF-α at 4 days (or 4 hours) on therapy is 12.0 (for Rho=0.1)-13.9 (for Rho=0.5) with 80% power. Similarly, mean baseline TBARS of 2.92±0.38 nmols/ml were observed in CBSDH (treated) patients, assume that taurine can reduce TBARS level, the detectable mean value of TBARS at 4 days (or 4 hours) on therapy is 2.42 (for Rho=0.1)-2.55 (for Rho=0.5) with 80% power.

Analysis plan: The mean and standard deviation of TNF-α and TBARS at baselines and on therapy will be calculated, and the changes from baseline to therapy times will also be calculated and reported along with 95% confidence intervals. One-sample t-test will be used to test if the changes are significantly different from zero for both TNF-α and TBARS.

II. Secondary outcome measures: exploratory studies of other biomarkers of oxidative stress and inflammation, relationship to metabolites and to markers relevant of symptomatology

A. Other biomarkers of oxidative stress and inflammation: A similar analysis as outlined above for TNF-α and TBARS will be done for the exploratory biomarkers of oxidative stress and inflammation. Because of the multiple comparisons in the many biomarkers, an adjustment of p-values will be done (such a Bonferroni or given the exploratory nature, a more relaxed method such as a family wised approach (false discovery rate) may be used). The findings will be used to generate new hypotheses for further study on independent datasets.

B. Relationship to metabolites: For the relation to metabolites, the biomarkers listed in Table 1 will be correlated with total Hcy concentration using a Spearman's rank correlation coefficient, since the Hcy values are not likely to be normally distributed.

C. Relationship to pathophysiologically relevant parameters of major symptomatology: For the evaluation of endothelial function abnormalities in CBSDH patients and its mitigation with taurine supplementation, the percent change in brachial artery diameter and the percent change in brachial artery flow induced by hypoxemia, will be compared between pre-treatment and after 4 days treatment. A paired t-test will be calculated to test if the percent change is significantly different from zero. To test whether platelet aggregation is mitigated by taurine supplementation, percent aggregation will be compared pre-treatment and at day 4 post-treatment using a paired t-test. Also, urine levels of thromboxane B2 metabolites will be similarly compared. To relate the bone mineral density to TNF-α levels, the Z-score of the BMD will be related to pre-treatment TNF-α levels and the correlation assessed using a Spearman or a Pearson product moment correlation coefficient, depending on the evaluation of normality of distribution.

Normality of distribution will be assessed as needed with Shapiro-Wilk test. Statistical analysis will be done using SAS or SPSS16.0.

L. Data Management and Security Plan (contact Jeff Magouirk for assistance at 720-777-8373)**:**

The original data are paper-based, and paper copies of the data will be kept in a locked cabinet in the office of the PI. The investigators of this protocol will be working with the Data Management Services unit, of the Research Informatics department of the CCTSI to make sure the data collected in this protocol is managed in a proper fashion. The data collection for this protocol will be in a web-based application that is HIPAA compliant. It will only be access with a strong password and a user's login. The data is backed up nightly and backup tapes are sent to an offsite location. The database has a transaction log of users and an audit trail of data changes. Deidentified data will be exported for analysis in SPSS 16.0 and Excel.

M. Research Nursing Service Requirements (contact Diane Branham for assistance at 720-777-3195)**:**

Nursing assistance is sought for patients at the outpatient unit of the pediatric CTRC (see details from Section 9). Nurses will be involved in, physical exam,

obtaining data forms, placement of a venous cathether and blood collection and urine collection, medication administration.

I. Source(s) of Research Material(s):
Taurine will be obtained via The Children's Hospital Pharmacy, product having a certificate of analysis. Nitroglycerin will be supplied by The Children's Hospital Pharmacy as Nitrostat.

II. Alternative Treatments Considered:
All participants have been and will continue to be offered the opportunity to be followed by their local inherited metabolic disease clinic wherein treatment recommendations for their disease are provided in accordance with the medical staff at that clinic. There will be no recommendations for alteration of the participant's standard treatment (i.e. diet, betaine (Cystadane), vitamins B12, B6, and/or folate) during the study. Individuals prescribed Vitamin C for treatment of their homocystinuria will be excluded from the study. While there is one report of Vitamin C supplementation ameliorating endothelial dysfunction in individuals with CBSDH, its use has not been widely investigated and it is not standard of care. There will be no recommendations for changes in any anticoagulation management that the subject might have been prescribed.

Participants could choose to experiment with taking over the counter taurine. However, no data will then be available to the effects of such treatment.

Blood Draws During Study Period:

For all protocols collecting blood the following is required.

Weight of eligible subjects (in kilograms): index case an 8 year old (the lower limit of age inclusion) 30 kg child. For this index case a 3 mL/kg of body weight blood draw would be 90 mL; a 5 mL/ kg blood draw would be 150 mL.

Blood draw volumes: A maximum of 44.2mL of blood will be drawn at a given time point. The maximum volume of blood drawn in one day is 70 mL. The maximum volume of blood drawn for the entire study is 121.5mL. These quantities of blood are well within 3 mL/kg of body weight for a single blood draw and a 5 mL/kg of body weight for any 8 week period for anticipated subject population. In the unforeseen event, where in the sample volumes exceed the above noted limits; the volume drawn will be adjusted down to comply with the recommended limits.

Study Visit description	Blood drawn for research (in mL)	Blood drawn for clinical purposes (in mL)	Total mL per visit	Total mL per day	Total research in ml. per 8 week period	Total mL per 8 week period

Prior to active study	1.5	0	1.5	1.5	1.5	1.5
Study day 1, SUN 12 noon (biochemical research labs and DNA)	21.2	0	21.2	21.2	22.7	22.7.
Study day 2, FRI 8:00 AM Baseline, (biochemical research labs, safety labs, and platelet aggregation studies)	44.2	0	44.2	44.2	66.9	66.9
Study day 2, FRI 30 min taurine level	0.5	0	0.5	44.7	67.4	67.4
Study day 2, FRI 1 hr taurine level	0.5	0	0.5	45.2	67.9	67.9
Study day 2, Fri 2 hr taurine level	0.5	0	0.5	45.7	68.4	68.4
Study day 2, FRI 4 hours post initiation of therapy studies (peak value levels) 1st day of therapy (biochemical research labs)	15.2	0	15.2	60.9	83.6	83.6
Study day 2, FRI 6 hour taurine level	0.5	0	0.5	61.4	84.1	84.1
Study day 2 FRI 8 hr taurine level	0.5	0	0.5	61.9	84.6	84.6
Study day 2 FRI 12 hour taruine level	0.5	0	0.5	62.4	85.1	85.1
Study day 6, TUE 8:00 AM last day of therapy and end of study (biochemical	39.2	0	39.2	39.2	124.3	124.3

research labs, safety labs, and platelet studies)					

III. Describe Recruitment Plan. How Will You Identify and Recruit Enough Subjects:

Subjects will be primarily recruited from the Inherited Metabolic Disease Clinic patient population. All individuals identified by the PI or co-investigators as receiving care at the Inherited Metabolic Disease Clinic for CBSDH who meet the inclusion criteria will be given the opportunity to participate. Patients will be informed of the study and provided the option to participate by either (1) face to face conversation at a clinic visit, (2) by phone conversation or (3) by letter. A maximum of three phone contacts or letters will be sent to potential participants. A script for the conversation or the letter to inform of the study and to check interest will be as outlined below.

If more subjects are needed, we will also contact other physicians who provide care to patients with CBSDH in the region, informing them of our study and providing them with HIPAA-A forms to give to their patients. For the advertisement about this study, see Appendix V Supplemental Materials. It will be the duty of these non-investigator physicians and/or the patient to contact the PI regarding interest in enrollment into the study. Additional physicians specialized in metabolic diseases caring for patients with CBSDH may be informed through e-mail on the widely subscribed metab-l listing, through the SIMD website (Society for Inherited Metabolic Diseases) and through the on-line journal Perspectives in Genetic Counseling, which lists such studies for genetic counselors. This would be done via an announcement about the study by the PI. Recruitment of patients through family support groups is another option. The study will be listed on clinicaltrials.gov as required by the FDA, and may be listed with NORD (National organization for Rare Disorders). Interested individuals would then need to contact the PI regarding interest in enrollment. There will be no discrimination based on age, gender, or ethnic heritage.

Informed Consent Plan. Who/How/Where Will You Get Consents? HIPAA Authorizations?:

The consenting process consists of 5 phases:

1. Identification of potential participants
2. Screening interview (See Appendix - V Supplemental Material)
3. Consent
4. Lack of documentation of a less than or equal to 300 mg/dl triglyceride level collected within the last five years triggers a need to obtain a triglyceride level before in person re-consent process when active portion of the study starts.
5. Re-consent at start of the active phase of the study

Part 1: Identification of potential participants

Potential subjects identified from the PI's and co-investigators' patient population will be informed of the study in person, per phone, or via letter by the PI or co-investigators. This will be done without making a database as the number of potential subjects is small and eligible participants easily recalled by the PI and co-investigators. Ideally potential participants will be informed of the study face to face, most likely at a clinic visit. If they express interest in the study a screening interview will ensue (see phase 2).

Due to geographic considerations and the two week wash-out period contact of potential subjects, screening interview, and consent process may necessarily be done by phone and/or mail prior to the subject's long-distance travel to Denver. In these cases, potential subjects will be notified of the study by the PI or co-investigators by phone or mail. If the potential subject expresses interest in the study, with their consent, a screening interview will ensue.

For potential subjects recruited outside of the PI's clinical population the PI or co-investigator will respond to a HIPAA authorization (obtained by non-investigating physician) and to direct requests by the patient for information in the fashion outlined above for phone contacts.

Part 2: Screening interview.

First participants will be consented on their participation into the screening interview for eligibility into the study (See Appendix V Supplemental Materials for a script). We are asking for a waiver of the documentation of the consent of the screening interview.

Part 3: Consent:

If they meet criteria for enrollment, options to consent per phone or in person will be given. Consent process will proceed accordingly. Prior to consenting subjects/parents/guardians will be given a copy of the IRB approved consent form (Attachment A) as well as the Patient Information and Authorization (HIPAA) form. For patients not in the clinic, these forms will be mailed to them with their permission. The study will be reviewed and participants will have the opportunity to review the consents and ask questions about the study. All questions will be answered prior to signing the consent/assent. Phone consents will be done after asking the participant if they have time to do the process in a manner that will ensure they are fully informed. For a face to face consenting process, this will be documented by original signatures. For phone consents, participants will give consent to both the consenting investigator and a witness, which will be documented by the signatures of the consenting investigator and witness. Further paper-based documentation of the consent will then be done upon reconsenting (see part 4).

1. Lack of documentation of a less than or equal to 300 mg/dl triglyceride level collected within the last five years triggers a need to obtain a triglyceride level before in person re-consent process when active portion of the study starts.

Part 4. Re-consent

All patients will be re-consented when they are at the center, on the first day, prior to the active phase of the research study. Face to face consents will be done in a private room with ample time to answer any questions raised and to discuss all issues of concern to the potential subject. Consent will be documented on paper with all signatures.

Special Consent/Assent Plan (if applicable):

Assent will be obtained for children ages 8 to 17 or as per local IRB regulations. This includes phone assents when appropriate. For children ages 8 to 17 signed consent from both parents, should both parents be involved in medical care decision making, will be required. If only one parent can be reached, this will be documented.

8. Data and Safety Monitoring Plan*

A. Summarize the Data and Safety Monitoring Plan (DSMP) for this protocol:

The PI will monitor data and report on the protocol safety issues. Pregnancy test results which are part of the inclusion criteria will be reviewed the day of specimen collection, prior to 5 PM (and hence prior to start of taurine supplementation). Serum triglyceride level will also be reviewed prior to start of taurine supplementation. Those with levels greater than 300 mg/dl will be excluded from the study. Participants will be encouraged to call regarding any adverse event of concern to them during the period of supplementation. A review of systems, utilizing a form with questions targeted to capture potential adverse effects (See Intake Sheet and Adverse Event/Compliance Sheet, Appendix V – Supplemental Materials) will be administered prior to the first dose and with the last dose of taurine, and again 1 week after completion of the study (the last survey administered by telephone). This questionnaire will be administered by the PI, co-investigator, or CTRC nursing staff Participants will also be questioned directly about potential adverse effects relative to taurine intake at the time of their last dose. A physical examination including blood pressure will be done prior to taurine administration, and at the completion of the study. Blood samples for complete blood count, comprehensive metabolic panel, and a lipid panel will be obtained prior to taurine administration, and at the completion of the study.

Adverse events and monitoring lab studies data will be reviewed within 5 working days after the subject has completed the study protocol. Compiled data will be examined 1. after the first two patients have been treated at the lower dose of 50 mg/kg/day (3 g/day), 2. after the first four adults have completed the study prior to opening the study for recruitment of children, 3. at the mid-point of the next patient recruitment (after 7 subjects have completed the protocol and after 12 subjects) 4. at the end of the study by the PI, co investigators and data Safety Officers. The data Safety Officers will consist of two clinical geneticists and one alternate clinical geneticists not associated with the study but knowledgeable of the studied condition. The PI and the data Safety Officers board will be looking for repeat

adverse events (AEs) or trends in AEs. In addition, the data Safety Officers and PI and co-investigators will convene within one week of reporting of a SAE.

Current Safety Officers:

Ellen Elias, MD
Tel. 720-777-5401
Children's Hospital Colorado
Special Care Clinic
13123 E 16th Ave Box B-032
Aurora, Co 80045

Laura Pickler, MD
Tel. 720-777-7450
AOB building TCH, B032

David Manchester, MD (alternate)
Tel. 303-724-2332
Education 2 South, Room L28-4120

Unanticipated problem or events, especially problems or events that are possibly or probably related to the study or research procedures will also be looked for. This includes monitoring for serious adverse events (SAEs) since such an event is not anticipated. In an event of an SAE, the monitoring PI will discuss the event with one of the co-investigator MDs within 24 hours of knowledge of the event to allow for decisions regarding the data and the safety to all participants. All unanticipated problems and unanticipated events will also be discussed with one of the co-investigating MDs within five working days of knowledge of the problem or event. Any identified trend in AEs will also be discussed within five working days of knowledge of the trend with one of the co-investigator MDs. Of note, the minimal risk for adverse events relative to taurine intake, the low number of participants (N=16 with drug exposure) and short duration of the study (5 days) make it very unlikely that any AE trend would be detected much before the end of the study.

There are no conflicts of interest for the PI or the co-investigator MDs responsible for data safety management.

B. Describe SAEs and AEs for this protocol and the SAE/AE reporting plan:

Events that result in death, immediate hospitalization, or prolonged hospitalization to prevent death or serious disability will be considered SAEs. Any other untoward medical occurrence temporally associated with the study will be considered AE. This includes headaches, gastrointestinal discomfort, and other events captured on the adverse event/compliance sheet that are without significant health risk and/or are potentially unrelated to study. Clinically significant changes in blood pressure or safety monitoring labs will be considered an AE. Specifically, liver function results that exceed two times the upper limit of normal, triglycerides greater than 400 mg/dl, and/or hypotension, with a blood pressure <90mmHg in adults or <the 5th percentile for age in children after taurine supplementation, will

be considered an adverse event in the setting of normal subject values prior to taurine supplementation. Any intercurrent illness or injury that represents an exacerbation of pre-existing condition (e.g., increase frequency or severity) will also be considered an AE. In such cases it will be preferred to record the diagnosis as the AE rather than a series of terms relating to the diagnosis (e.g., worsening of asthma). An adverse drug reaction will be all noxious and unintended responses to taurine product. This means that a causal relationship between taurine and an AE is at least a reasonable possibility (i.e., he relationship cannot be ruled out).

The PI and one of the co-investigating MDs will determine the severity of the event and if the SAE or AE is related to the protocol using the categories noted below. Review of their determination will be sought from the Safety Officers. In the case of deferring opinions, higher grade of severity the more related rating will be accepted

Grade	**Description**
Mild	No limitation of usual activities
Moderate	Some limitation of usual activities
Severe	Inability to carry out activities

Not Related	The adverse finding was present prior to exposure to the investigational product, having the same or milder intensity OR The finding is considered likely to be related to an etiology other than the investigational product, that is, there is no evidence or arguments to suggest a causal relationship to the investigational product.
Possibly related	Exposure to investigational product and onset of the finding are reasonably related in time AND The adverse finding could be reasonably be explained by factors or causes other than exposure to investigational product.
Probably related	Exposure to investigational product and onset of the finding are reasonably related in time AND The adverse finding is more likely explained by exposure to investigational product than by other factors or causes.

SAEs will be reported to one of the co-investigator MDs as well as the Safety Officers, FDA, COMIRB, the CTRC within five working days of knowledge of the event. The participant's PCP will also be notified if deemed appropriate by the co-investigator MD. Unanticipated problems or events that result in an actual unforeseen harmful or unfavorable occurrence to the subject or others with also be reported to one of the co-investigating MDs within five working days of knowledge of the event. Information that indicates a change to the risks or benefits of the study will also be reported within five days of discovery to the Safety Officers,

FDA, COMIRB and CTRC. No new patients will be enrolled or studied until the SAE has been reviewed and appropriate action taken by the above listed bodies.

The PI will notify co-investigator MDs of abnormal safety monitoring lab results within five working days of resulted values. If deemed clinically appropriate by co-investigator MD, the subjects PCP will be notified. All other AEs will be logged and reported to COMIRB yearly or at the time of continuing review.

For full list of events to be reported to COMIRB see the Reportable Event Sheet to be used for monitoring and tracking individual events attached in Appendix V - Supplemental Materials.

A summary of data monitoring and reporting flow during the active portion of the study is noted below. A flow diagram of this information can be found in Appendix V - Supplemental Materials in the CTRC protocol application.

Pregnancy testing will be done on day 1, one day prior to the administration of taurine and reviewed by 5:00 PM that day. Positive test results will exclude the individual from participation in the study. A physical examination (to include blood pressure) and the Adverse Event Sheet Questionnaire will be completed on day 1 and just prior to taurine administration respectively. They will be repeated after administration of the last taurine dose. This data will be reviewed within 5 working days after the subject has completed the study protocol. Liver function study results that exceed two times the upper limit, triglycerides results that are greater than 400 mg/dl, and/or hypotension (blood pressure <90mmHg in adults or < the 5th percentile for age in children) as well as any clinically significant alteration in safety monitoring laboratory studies (as assessed by the co-investigating MD) that is present in two or more subjects in the setting of normal subject values before taurine supplementation will be reported to the co-investigators, FDA, COMIRB, CTRC, and Safety Officers within 5 working days of knowledge of this data. The protocol will be temporarily suspended pending review of collected data and recommendation about study continuance by Safety Officers, FDA, COMIRB and CTRC.

All unanticipated problems and unanticipated events will also be discussed with one of the co-investigating MDs within five working days of knowledge of the problem or event. Any identified trend in AEs will also be discussed within five working days of knowledge of the trend with one of the co-investigator MDs.

Compiled data will be examined for safety after 2, 4, and 8 subjects have completed the protocol and at the end of the study by the PI, co investigators and data Safety Officers The PI, co-investigators and the data Safety Officers board will be looking for repeat adverse events (AEs) or trends in AEs.

SAEs will be reported to the study project manager, the PI or one of the lead site co-investigator MDs , FDA, COMIRB, local IRB as per their requirement, and the CTRC within five working days of knowledge of the event. In addition, the data Safety Officers and PI and co-investigators will convene within one week of reporting of a SAE.

C. Describe both subject discontinuation criteria and protocol stopping criteria:

With respect to the FMD studies, if an adult systolic blood pressure is < 105 mmHg or a child's blood pressure is < 10th percentile for age, nitroglycerin will not be administered. The subject will otherwise be maintained in the study.

With respect to the study in general, subject discontinuation criteria are as follows.

- Positive pregnancy test
- Any SAE that is possibly related to the study protocol.

Protocol stopping criteria are listed below. Such events will result in the study being temporarily suspended pending review of collected data and recommendation about study continuance by Safety Officers, FDA, COMIRB and CTRC.

- Any SAE that is possibly related to the study protocol.
- Liver function study results that exceed two times the upper limit, if triglycerides are greater than 400 mg/dl, and/or if hypotension (blood pressure <90mmHg in adults or < the 5th percentile for age in children) is present in two or more subjects in the setting of normal subject values before taurine supplementation.
- Any clinically significant alteration in safety monitoring laboratory studies, as assessed by the co-investigating MD and/or the Safety Officers that is present in two or more subjects in the setting of normal subject values before taurine supplementation.

Currently there is no funding source outside of the university, thus we do not expect an outside agent to have authority to suspend this trial, other than general regulatory authorities of the institution and the FDA in its general capacity to oversee drugs and food supplements. Actions that result in suspension or termination of the protocol, whether by COMIRB or some other entity, will be reported to the Pediatric General Clinical Research Center and the IRB verbally within 24 hours and via written documentation within 2 working days.

D. Plans for assuring data accuracy and protocol compliance:

The PI will conduct protocol compliance checks. Accuracy of the laboratory data related to safety is assured via use of Clinical Laboratory Improvement Amendments (CLIA) certified laboratory.

The investigators of this protocol will be working with the Data Management Services Unit of the Research Informatics department of the CCTSI to ensure the data collected in this protocol is managed in a proper fashion. The data collection for this protocol will be in a web-based application that is HIPAA compliant. It will only be access with a strong password and a user's login. The data is backed up nightly and backup tapes are sent to an offsite location. The database has a transaction log of users and an audit trail of data changes. The paper copies of the data will be kept in a locked cabinet

10. References/Literature Cited

Au-Yeung KK, Woo CW, Sung FL, Yip JC, Siow YL, O K. Hyerhomocysteinemia activates nuclear factor-kappaB in endothelial cells via oxidative stress. *Circ Res.* 2004 Jan 9;94(1):28-36.

Ahn C S. Effect of taurine supplementation on plasma homocysteine levels of the middle-aged Korean women. *Adv Exp Med Biol* 2009;643:415-422.

Boger RH, Bode-Boger SM, Thiele W, Creutzig A, Alexander K, Frolich JC. Restoring vascular nitric oxide formation by L-arginine improves the symptoms of intermittent claudication in patients with peripheral arterial occlusive disease. *J Am Coll Cardiol.* 1998;32:1336-1344.

Celermajer DS, Sorensen K, Ryalls M, Robinson J, Thomas O, Leonard JV, Deanfield JE. Impaired endothelial function occurs in the systemic arteries of children with homozygous homocystinuria but not in their heterozygous parents. *J Am Coll Cardiol.* 1993 Sep;22(3):854-858.

Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, Deanfield J, Drexler H, Gerhard-Herman M, Herrington D, Vallance P, Vita J, Vogel R. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol.* 2002;39:257-265.

Corretti MC, Plotnick GD, Vogel RA. Technical aspects of evaluating brachial artery vasodilatation using high-frequency ultrasound. *Am J Physiol.* 1995;268:H1397-1404.

Davì G, Di Minno G, Coppola A, Andria G, Cerbone AM, Madonna P, Tufano A, Falco A, Marchesani P, Ciabattoni G, Patrono C. Oxidative stress and platelet activation in homozygous homocystinuria. *Circulation*. 2001 Sep 4;104(10):1124-1128.

Dakak N, Husain S, Mulcahy D, Andrews NP, Panza JA, Waclawiw M, Schenke W, Quyyumi AA. Contribution of nitric oxide to reactive hyperemia: impact of endothelial dysfunction. *Hypertension.* 1998;32:9-15.

Dalle-Donne I, Rossi R, Colombo R, Giustarini D, Milzani A. Biomarkers of oxidative damage in human disease. *Clin Chem* 2006;52(4):601-623.

De Curtis M, Santamaria F, Ercolini P, Vittoria L, De Ritis G, Garofalo V, Ciccimarra F. Effect of tuarine supplementation on fat and energy absorption in cystic fibrosis. *Arch Dis Child* 1992;67:1082-1085.

Durelli L, Mutani R, Fassio F. The treatment of myotonia : evaluation of chronic oral taurine threapy. *Neurology* 1983;33:599-603.

Eskurza I, Monahan KD, Robinson JA, Seals DR. Ascorbic acid does not affect large elastic artery compliance or central blood pressure in young and older men. *Am J Physiol Heart Circ Physiol.* 2004;286:H1528-1534.

Fujita T, Ando K, Noda H, Ito Y, Sato Y. Effects of increased adrenomedullary activity and taurine in young patients with borderline hypertension. *Circulation* 1987;75:525-532.

Galloway SD, Talanian JL, Shoveller AK, Heigenhauser GJ, Spriet LL. Seven days of oral taruine supplementation does not increase muscle taurine contenet or alter substrate metabolsim during prolonged exercise in humans. *J Appl Physiol* 2008:105(2):643-51

Greiner LS, Raab B, Stabler SP, Allen RH, Kraus JP, Overdier KL, Crnic LS, Maclean KN. Mouse models of CBS deficient homocystinuria: From microarrays to a novel improved treatment. Abstract C060. 5th Annual International Homocysteine Conference, Milan, Italy June 26-30,2005. Published in *Haematologica Reports*; p25, 1(3) June 2005

Haas, E. Health world on line. Staying healthy with nutrition: the complete guide to diet and nutritional medicine. Taurine [on line]. [July 27, 2009]. Available from World Wide Web: <http://www.healthy.net/scr/article.asp?ID=1971>

Huxtable R. J. Physiological actions of taurine. *Physiol Rev* 1992;72:101-163.

Hwang SY, Woo, CW, Au-Yeung KK, Siow YL, Zhu TY, OK, Homocysteine stimulates monocyte chemoattractant protein-1 expression in the kidney via nuclear factor-kappB activation. *Amer J Physiol Renal Physiol.* 2008 Jan:294(1):F236-244.

Laidlaw S, Shultz T, Cecchino J, Kopple J. Plasma and urine taurine levels in vegans. *Am J Clin Nutr.* 1988 Apr;47(4):660-663.

Liao JK, Bettmann MA, Sandor T, Tucker JI, Coleman SM, Creager MA. Differential impairment of vasodilator responsiveness of peripheral resistance and conduit vessels in humans with atherosclerosis. *Circ Res.* 1991;68:1027-1034.

Machado-Vieira R, viale CI, Kapczinski F. Mania associated with an energy drink: the possible role of caffeine, taurine, and inositol. *Can J Psychiatry* 2001;46(5):454-455.

Maclean KN, Gaustadnes M, Oliveriusová J, Janosík M, Kraus E, Kozich V, Kery V, Skovby F, Rüdiger N, Ingerslev J, Stabler SP, Allen RH, Kraus JP. High homocysteine and thrombosis without connective tissue disorders are associated with a novel class of cystathionine beta-synthase (CBS) mutations. *Hum Mutat.* 2002 Jun;19(6):641-655.

McCarty MF. Sub-optimal taurine status may promote platelet hyperaggregability in vegetarians. *Med Hypotheses.* 2004;63(3):426-33.

Monson, K, Schoenstadt, A. 2008. Med TV, Information brought to life. Congestive heart. Taurine dosage [on line]. [July 27 2008]. Available from World

Wide Web: <http://congestive-heart-failure.emedtv.com/taurine/taurine-dosage.html>

Mudd SH, Skovby F, Levy HL, Pettigrew KD, Wilcken B, Pyeritz RE, Andria G, Boers GH, Bromberg IL, Cerone R, et al.The natural history of homocystinuria due to cystathionine beta-synthase deficiency. *Am J Hum Genet.* 1985 Jan;37(1):1-31.

Mutani R, Bergamini L, Durelli L. Taurine in experimental and human epilepsy. In: Barbeau A and Huxtable R J (eds) Taurine and Neurological Disorders. Raven Press, New York, 1978, pp. 359-373.

Nakamura T, Saito Y, Kato T, Sumino H, Hoshino J, Ono Z, Sakamaki T, Nagai R. Flow-mediated vasodilation of a conduit artery in relation to downstream peripheral tissue blood flow during reactive hyperemia in humans. *Jpn Circ J.* 1997;61:772-780.

Nonaka H, Tsujino T, Watari Y, Emoto N, Yokoyama M**.** Taurine prevents the decrease in expression and secretion of extracellular superoxide dismutase induced by homocystine: Amerlioration of homocystine-induced endoplasmic reticulum stress by taurine. *Circulation* 2001;104:1165-1170

Obermann M, Schorn CF, Mummel P, Kastrup O, Maschke M. Taurine induced toxic encephalopathy? *Clin Neurol Neurosurg* 2006;108(8):812-813.

Oudit GY, Trivieri MG, Khaper N, Husain T, Wilson GJ, Liu P, Sole MJ, Backx PH. Taurine supplementation reduces oxidative stress and improves cardiovascular function in an iron-overload murine model. *Circulation*. 2004 Apr 20;109(15):1877-1885.

Passantes-Morales H, Quiroz H, Quesada O. Treatment with taurine, diltiazem, and vitamin E retards the progressive visual field reduction in retinitis pigmentosa: a 3-year follow-up study. *Metab Brain Dis* 2002;17(3): 183-197.

Parvez S, Tabassum H, Banerjee BD, Raisuddin S. Taurine prevents tamoxifen-induced mitochondrial oxidative damage in mice. *Basic Clin Pharmacol Toxicol.* 2008 Apr;102(4):382-387.

Pullin CH, Bonham JR, McDowell IF, Lee PJ, Powers HJ, Wilson JF, Lewis MJ, Moat SJ. Vitamin C therapy ameliorates vascular endothelial dysfunction in treated patients with homocystinuria. *J Inher Metab Dis*. 2002 May;25(2):107-118.

Ryan M, McInerney D, Owens D, Collins P, Johnson A, Tomkin GH. Diabetes and the Mediterranean diet: a beneficial effect of oleic acid on insulin sensitivity, adipocyte glucose transport and endothelium- dependent vasoreactivity. *Q J Med*. 2000;93:85-91.

Scriver SR, Beaudet AL, Sly WS, Valley D, Childs B, Kinzler K, Vogelstein B. The Metabolic and Molecular Bases of Inherited Disease. Eight Edition. McGraw-Hill Medical Publishing Division 2001. Disorders of Transsulfuration. P.2007-2056.

Shao A, Hathcock JN. Risk assessment for the amino acids taurine, l-glutamine and l-arginine. *Regul Toxicol Pharmacol.* 2008 Apr;50(3):376-399.

Szamosi T, Roth E, Szamosi T Jr, Tomsits E, Tordai A, Szabo T. Hyperhomocysteinemia, enzyme polymorphism and thiobarbituric acid reactive system in children with high coronary risk family history. *J Am Coll Nutr.* 2004 Oct;23(5):386-390.

Takahashi R, Nakane Y. Clinical trial of taurine in epilepsy. In: Barbeau A and Huxtable R J (eds) Taurine and Neurological Disorders. Raven Press, New York, 1978, pp. 375-385.

Taş S, Dirican M, Sarandöl E, Serdar Z.The effect of taurine supplementation on oxidative stress in experimental hypothyroidism. *Cell Biochem Funct*. 2006 Mar-Apr;24(2):153-158.

Toborek M, Kopieczna-Grzebieniak E, Drózdz M, Wieczorek M. Increased lipid peroxidation as a mechanism of methionine-induced atherosclerosis in rabbits. *Atherosclerosis*. 1995 Jun;115(2):217-24.

Wilcken DE, Wang XL, Adachi T, Hara H, Duarte N, Green K, Wilcken B. Relationship between homocysteine and superoxide dismutase in homocystinuria: possible relevance to cardiovascular risk. *Arterioscler Thromb Vasc Biol*. 2000 May;20(5):1199-1202.

Zhang M, Izumi I, Kagamimori S, Sokejima S, Yamagami T, Liu Z, Qi B. Role of taurine supplementation to prevent exercise-induced oxidative stress in healthy young men.*Amino Acids*. 2004 Mar;26(2):203-207.

Supplementary materials:

1. Intake Sheet

Oxidative Stress Markers in Inherited Homocystinuria and the Impact of Taurine Intake Sheet

Subject Name:_____ Date:_____

Characteristic	Comment
Sex	
Age	
BMI	
Salicylate intake over last two weeks	
Vitamin intake over last two weeks	
Supplement intake over last two weeks (taurine)	
Viagra intake over last two week/possible use over next 6 days	
Smoking habits	
Hypertension	
Diabetes	

Other chronic disease Infection Hormone replacement use Pregnancy Physical activity Diet prescription/compliance Medications/compliance (list all medications and dosing_	

Reviewed by PI or co-investigator on: ____
Proceed for nitroglycerin induced FMD YES/NO*
Proceed for platelet studies YES/NO*
*circle appropriate

System	Yes	No	Comments (to include any limitations of activity)
General/Constitutional			
general state of health			
sense of well being			
fever			
other			
Skin			
rash			
moisture/dryness			
other			
HEENT			
headaches			
vision			
hearing			
other			
Cardiovascular			
palpitations			
syncope			
other			
Respiratory			
difficulty breathing			
wheezing			
cough			
other			
Gastrointestinal			
appetite			
abdominal pain			
heartburn			
diarrhea			

	nausea			
	other			
Musculoskeletal				
	pain			
	cramps			
	other			
Genitourinary				
	dysuria			
	other			
Neurologic/Psychiatric				
	seizures			
	muscular coordination			
	emotional mood			
	other			
Allergic/Immunologic				
	reactions to drugs/foods			
	other			

II. Adverse Event Sheet:

Oxidative Stress Markers in Inherited Homocystinuria and the Impact of Taurine
Adverse Event/Compliance Sheet

Subject Name:_____ Date:_____ Taurine intake: _____

System	Yes	No	Comments
General/Constitutional			
general state of health			
sense of well being			
Fever			
Other			
Skin			
Rash			
moisture/dryness			
Other			
HEENT			
Headaches			
Vision			
Hearing			
Other			

Cardiovascular			
Palpitations			
Syncope			
Other			
Respiratory			
difficulty breathing			
Wheezing			
Cough			
Other			
Gastrointestinal			
Appetite			
abdominal pain			
Heartburn			
Diarrhea			
Nausea			
Other			
Musculoskeletal			
Pain			
Cramps			
Other			
Genitourinary			
Dysuria			
Other			
Neurologic/Psychiatric			
Seizures			
muscular coordination			
emotional mood			
Other			
Allergic/Immunologic			
reactions to drugs/foods			
Other			

III. Reportable event sheet

Oxidative Stress Markers in Inherited Homocystinuria and the Impact of Taurine
Reportable Event Sheet

	EVENT	Date of Knowledge of Event	Date Reported to COMIRB	Comments
Unforeseen harmful/unfavorable event, felt to possibly be related to study				
	injury			
	psychological event			
	other			

Unforeseen harmful/unfavorable event, felt to be unrelated to study			
injury			
psychological event			
other			
Unforeseen development that increases likelihood of harm			
midterm review			
new safety data on taurine supplementation			
other			
Problem involving data			
data collection			
data storage			
data privacy			
data confidentiality			
Change in subjects status			
incarceration of subject			
pregnancy of subject			
other			
Change in protocol for subject safety done prior to COMIRB review			
apparent immediate hazard			
Complaint of participant that can't be resolved or suggest risk			
complaint			
Unintentional protocol violation that harmed or may cause harm			
violation			
Noncompliance by the PI or research team			
noncompliant activity			
Other			
other			

IV. Advertisement for subject recruitment

Advertisement for subject recruitment:

You are invited to participate in a study investigating homocystiuria. The study is being conducted by (Site Specific Providers) at (Site Location). The study investigates why individuals with homocystinuria have health problems and if a new investigative treatment with taurine is beneficial. The study will not provide direct benefit to the participant but will provide generalizable information on homocystinuria.

To be eligible to participate in the study individuals must be between the ages of 8 to 50 year and have a diagnosis of homocystinuria due to cystathionine beta-synthase deficiency that is not fully responsive to therapy withtotal homocysteine (tHcy) levels greater than 50 µmol/L. Individuals cannot participate if they are (1) pregnant, (2) have hypertriglyceridemia, (3) have another significant illness, and/or (4) are being treated with Vitamin C, taurine supplementation, and/or high dose antioxidant supplementation and cannot discontinue this.. Participants who have a cold, the flu or other short term illness will be asked to delay participation until they are recovered.

The study will require the participant to be in (Site Location) for three days of the six day study period. Most commonly, participants will be in (Site Location) on Thursday and Friday and on Tuesday of the next week. Some compensation for travel and time is available.

For further information, please contact
(Insert Site Specific Information)

V. Information letter to physician for patient referral

Date

Dear

We are currently recruiting for a research study title “Oxidative Stress Markers in Inherited Homocystinuria and the Impact of Taurine”, which has been approved by the Colorado Multiple Institution Review Board. The study examines individuals with homocystinuria due to cystathionine β-synthase deficiency. It looks at biochemical markers of oxidative stress and inflammation, platelet function and endothelial function. It also examines the response to short-term taurine supplementation.

We would greatly appreciate your assistance in notifying individuals or families of individuals with homocystinuria due to cystathionine β-synthase deficiency of this study. The family can call us directly to discuss the protocol should they be interested. This is an approach which has worked well in the past. Alternatively, we have enclosed a HIPAA Research Recruitment Form. When signed, this form allows you to release contact information on the family to us. Once we have it, we will initiate the call to the family.

Please do not hesitate to call should you have questions or need more packets.

Contact Information:
(Insert Site Specific Information)

Sincerely,

(Insert Site Specific Information)

Johan L. Van Hove, MD, PhD, Lead Principal Investigator

VI. Letter and Phone Scripts for intake screening

Oxidative Stress Markers in Inherited Homocystinuria and the Impact of Taurine
Letter and Phone Recruitment Scripts

Letter script to inform of study:

Dear _____. This letter is to inform you of our research study looking at homocystinuria. The purpose of the study is to learn more about why people with homocystinuria have health problems and if a new investigational treatment with taurine is helpful. We are currently inviting participants. If you would like to learn more about the study, please let us know. You may talk to (Insert Site Specific Information – for Colorado: Cindy Freehauf, RN, CGC at 303-724-2342)).

Phone script to inform of study:

This is _____ from the Metabolic Clinic at (Site). Is this a good time to talk or would you prefer I call back.
If request is to call back later:
Thanks, I will be happy to call back., Is there a time that is better than others to call?
If request is to continue:
*Thanks. I'm calling to inform you of our research study looking at homocystinuria. [**OR** Thank you for calling to find out more about our research study looking at homocystinuria.]*
The purpose of the study is to learn more about why people with homocystinuria have health *problems and if a new investigational treatment is helpful. Specifically, we want to see if markers of oxidative stress and inflammation are increased in individuals with homocystinuria. We also want to see if taking taurine for a short period of time can decrease the markers of stress and oxidation. As part of the study, participants will be asked to come to (Site Location) for blood studies and studies that look at blood vessels and bone density. Some compensation for travel and time is available. Do you think you might be interested in participating in the study?*
If answer is no:
"Thank you for your time. Good bye."
If answer is yes:
Go to screening interview.

Phone script for screening interview:

*Before enrolling people in the study we need to determine if they are eligible. So what I would like to now is to ask you a series of question about your health and medications and supplements you might take. The questions will take about 15 minutes. The risk to take part in this interview is very small. The questions are not designed to ask you for sensitive personal information, but it is possible that some people may feel uncomfortable answering these questions. You may choose not to answer or stop participating in this interview at any time [**IF APPLICABLE** Deciding not to take part in the interview or the research study will not change any medical care you might receive from the Inherited Metabolic Diseases Clinic.]. If you qualify to take part in the study and enroll in the study, your name and the information I taking will be kept private and confidential and used only in the research study we are talking about. If you do not qualify to take part in the study*

and/or do not enroll in the study the information including your name will be discarded. Do I have your permission to continue with the interview?
If the answer is "yes"

Criteria	Description
Criteria: Diagnostic confirmation of CBSDH: *How was homocystinuria diagnosed in you? Did anyone do a mutation analysis? Are you willing to have your doctor share or send us this information for the research study?*	
Criteria: Therapeutic responsiveness *What is your treatment for homocystinuria and what is your current homocysteine level?* *Are you willing to have your doctor share or send this information with us for the research study?*	
Criteria: Age . *Are you between 8 and 50 years of age?*	
Criteria: Pregnancy If a female: *Are you, or is there a chance, that you are pregnant?*	
Criteria: Chronic illness with inflammatory component. *Do you have a chronic disease other than homocystinuria?* If the answer is yes *Could you tell me more about this, such as the name of the disorder and how severe it is?*	
Criteria: Taurine *Do you take energy drinks with taurine or take taurine as a supplement?* *Are you willing to avoid taurine or taurine containing supplements for the study period?*	
Criteria: Antioxidant intake *Is vitamin C prescribed as part of your treatment for homocystinuria?* If answer is no*:* *Do you take vitamins or other supplements with antioxidant properties outside of a typical one-a day vitamin pill?* If answer is yes*,* *Are you willing to stop the*	

supplements with the exception of a one a day vitamin for the study period?	
Criteria: Salicylate (Aspirin) intake *Do you take medications that contain Aspirin or any other medication that affects platelets?* If answer is "yes" *Is it prescribed by your physician to treat your homocystinuria or other disease state?* If answer is "no" *Are you willing to stop taking Aspirin for a three week study period?*	
Criteria: Do you take Viagra or similar products? If answer is yes: *Would you be willing or able to stop it for the study period?* If answer is no: *Would you let us know that you are using it during the study period?*	
Criteria: Intercurrent Illness *Participants who have a cold, the flu, or other short term illness will be asked to delay participation in the study until they are recovered. Would this be a problem for you? Will you let us know if you become ill when scheduled for this study?*	
Criteria: Recent cardiovascular event: *Did you have a stroke, a myocardial infarct or a deep vein thrombosis or pulmonary thrombosis or embolus in the past 6 months?*	
Criteria: Uncontrolled hypertension: *Do you have a history of high blood pressure?* If answer is yes: *Is it controlled by diet or medication?* For all individuals: *Would you be willing to share medical records showing your recent blood pressure readings?* What medications are you taking?	
Criteria: Hypertriglyceridemia: Do you have a history of elevated	

triglyceride levels? *Would you be willing to share your medical record documenting triglyceride level(s) or have a level drawn so that we can ensure your levels are not high at study start?*	

"Based on the information you gave me, it looks like you are eligible for this study. At this point you have several options. (1) I can take down you contact information, mail you the consent forms then and call you in a week or so to answer questions, go over the consent form, and consent you if you remain interested in participating in the study. (2) We can set up a time to discuss the study and consent in person, or (3) If you are not interested in the study you can let me know and the information I collected will be discarded.

_____ OK to mail information and re-contact

_____Schedule appointment

_____Not interested. **Destroy all information**

OR

"Based on the information you gave me, it looks like you are NOT eligible for this study. At this point, all information that you provided me in this screening interview will be destroyed."

_____Not eligible. **Destroy all information**

VI. Data Safety Monitoring Plan for active portion of study

DATA SAFETY MONITORING PLAN FOR ACTIVE PORTION OF STUDY