A Novel Cysteine Sulfinic Acid Decarboxylase Knock-Out Mouse: Comparison Between Newborn and Weanling Mice

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Abbreviations

CSAD	Cysteine sulfinic acid decarboxylase
CSAD KO	Cysteine sulfinic acid decarboxylase knockout mice
CDO	Cysteine dioxygenase
CDO KO	Cysteine dioxygenase knockout mice
ADO	Cysteamine (2-aminoenthanethiol) dioxygenase
WT	Wild type (CSAD+/+)
HT	Heterozygotic mice (CSAD+/−)
HO	Homozygotic mice (CSAD−/−)
HOT	Homozygotic mice treated with 0.05 % taurine
TauT	Taurine transporter
TauT KO	Taurine transporter knockout mice
Gpx 1	Glutathione peroxidase 1
Gpx 3	Glutathione peroxidase 3
Prdx 2	Peroxireductase 2
Prdx 3	Peroxireductase 3
Prlr	Prolactin receptor
Ltf	Lactoferrin

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Upp 2	Uridine phosphorylase 2
Sds	Serine dehydratase
G1 G2, G3, G4	Generation 1, 2, 3 and 4
G1 HO	HO ($CSAD^{-/-}$) mice born from HT ($CSAD^{+/-}$) parents
G2 or G3 HO	Mice born from G1 HO or G2 HO parents

1 Introduction

The essential physiological functions of taurine in development of the brain and eye, in reproduction, endocrine regulation, kidney and cardiovascular function, membrane stabilization, osmotic regulation as well as immune function have been well documented in many laboratories (Schuller-Levis and Park 2006; Sturman 1993). Four gene products are critical to taurine homeostasis: cysteine dioxygenase (CDO; EC 1.13.11) oxidizes cysteine to cysteine sulfinic acid which is converted to hypotaurine which is then oxidized to taurine (Stipanuk 2004; Bella et al. 2000; Hosokawa et al. 1990); cysteamine dioxygenase ADO (EC1.13.11.19) which is converted cysteamine to hypotaurine (Dominy et al. 2007); the taurine transporter (TauT) (Uchida et al. 1992); and cysteine sulfinic acid decarboxylase, CSAD (EC 4.1.1.29), which is the enzyme that converts cysteine sulfinic acid to hypotaurine (Park et al. 2002).

Taurine is considered a conditionally essential amino acid in humans and primates and is required during their development. Taurine deficient animals are useful in investigations of taurine's physiological functions because it is possible to study both taurine deficiency itself and the effect of supplementation with taurine in food or drinking water. Cats and rodents have been used as animal models for taurine studies because their taurine levels could be easily manipulated (Sturman 1993; Sturman and Messing 1991, 1992). Cats have been used for taurine studies because they produce only low levels of CDO and CSAD leading to a dependence on dietary sources of taurine. However, the cat model has limitations including a long gestation period, a heterogeneous genetic background and a relatively large maintenance expense and the absence of genetic, molecular and immunological reagents. Rodents have high levels of CSAD (Schuller-Levis and Park 2006; Sturman 1993; Huxtable 2000) and taurine is not essential to their diet. Due to high concentrations of taurine in rodents, competitive inhibitors of taurine transport including guanidinoethanesulfonate (GES) or β-alanine have been used to produce taurine deficiency (Bonhaous et al. 1985; Dela Rosa and Stipanuk 1984; Jong et al. 2010). However, these chemicals have toxic side effects.

Recently genetically modified mice have been developed to model taurine deficiency using gene targeting methods. These taurine-deficient knock-out mice include taurine transporter knockout mice (TauT KO) and cysteine dioxygenase knockout mice (CDO KO). Two TauT KO mouse models show reduced levels of taurine in various tissues including heart, brain, muscle, kidney and liver (Heller-Stilb et al. 2002; Warsulat et al. 2007; Ito et al. 2008). These TauT KO models demonstrate developmental effects in various organs including the retina, liver,

brain, muscle and heart. A cysteine dioxygenase deficient (CDO KO) model was produced by deleting CDO, thereby disabling the production of cysteine sulfinic acid, a substrate for CSAD, from cysteine (Ueki et al. 2011, 2012; Roman et al. 2013). These mice have severe taurine deficiency and increased catabolism of cysteine to hydrogen sulfide, which leads to pulmonary and pancreatic toxicity.

Recently, our laboratory produced a cysteine sulfinic acid decarboxylase knockout mouse (CSAD KO) as a novel mouse model of taurine deficiency (Park et al. 2014). CSAD is an enzyme for taurine biosynthesis and a cytosolic enzyme expressed primarily in liver and kidney (Sturman 1993; Park et al. 2002). The level of CSAD activity determines the need for dietary taurine. Our laboratory demonstrated high neonatal mortality in the third and fourth generation of $CSAD^{-/-}$ homozygotes (G3 HO and G4 HO) and restoration of neonatal survival by the addition of 0.05 % taurine added to the drinking water. Compared to wild type (WT), taurine concentrations in the liver and brains of newborn pups are significantly lower except in G1 HO, which are born from $CSAD^{+/-}$ heterozygous (HT) dams which have near normal levels of serum taurine. Low taurine concentrations in the liver and brain in HOs are significantly restored by supplementation of taurine in the drinking water of the dam. Gene expression of prolactin receptor (Prlr) and lactoferrin (ltf) is decreased but gene expression of glutathione peroxidase 3 (Gpx 3) and peroxireductase (Prx 2) increased, suggesting oxidative stress may be involved in neonatal mortality. Here we compare these phenotypic changes in newborn pups and weanling mice.

2 Materials and Methods

2.1 Materials

Chemicals used in this study were purchased from Sigma Chemicals (St. Louise, MO) if not otherwise noted. Oligonucleotide primers for PCR for genotype were obtained from Eurofins MWG Operon (Huntsville, AL). Primers were designed by Primer Designer 4 (Scientific and Educational Software, Cary, NC). Taq polymerase and deoxynucleotides were purchased from New England Biolabs (Ipswich, MA). Agarose was obtained from Lonza Group LTD (Rockland, ME). Trizol and RNeasy kit for RNA extraction were obtained from Invitrogen and Qiagen (Valencia, CA), respectively. The SYBR master mix and primers used in RT^2 qPCR were purchased from Qiagen.

2.2 CSAD KO Mice

CSAD KO mice were produced previously described from our laboratory (Park et al. 2014). Briefly, chimeric CSAD KO mice were produced by injection of cells from a gene trap ES cell line (XP0392) into C57BL/6 (B6) blastocysts at the

Mouse Mutant Regional Resource Centers (MMRRC, UC Davis, CA) which were implanted into a pseudopregnant B6 mouse. Chimeric mice from MMRRC were mated with B6 (Jackson Laboratories, Bar Harbor, ME) in the animal colony at NYS Institute for Basic Research in Developmental Disabilities. Heterozygous siblings were mated to produce $CSAD^{-/-}$ homozygous pups (HO). Experimental mice were fed taurine-free chow ($LabDiet^{R}$, PMI Nutrition International, St. Louis, MO). Taurine concentrations in commercial food were confirmed by HPLC. All mice were kept under 12-h day/night with free access to food and water. Some experimental animals had exogenous taurine added to their water at 0.05 % as indicated in Results. For optimum reproductive performance, one or two females were mated to a single male. Both females and males used for mating in the taurine-treated groups were supplemented with taurine. Animals were weaned at 3 weeks of age. All mice at 1 M used in this study were separated from their dam for 1 week. All procedures involving live animals were approved by the Institutional Animal Care and Use Committee of IBR.

2.3 High Performance Liquid Chromatography (HPLC)

The liver and brain as well as plasma were obtained from all groups including WT and HT as well as G1 HO, G2 HO, G3 HO and G4 HO at birth (PD1) and 1 month of age (1M) after mice were injected *ip* with avertine (250 mg/Kg). These samples were also collected from the taurine-treated groups including G2 HOT, G3 HOT and G4 HOT. Taurine concentrations were determined using HPLC (Waters, Milford, MA) (Battaglia et al. 1999). Briefly, tissues and plasma were homogenized using 5 % TCA and centrifuged for removal of proteins. After samples were dried using a Speedvac (Savant, Holbrook, NY), they were derivatized using phenylisothiocyanate (PITC) and separated using a C18 column with a gradient of acetate buffer containing 2.5 % acetonitrile (pH 6.5) and 45 % acetonitrile solution containing 15 % methanol at 45 °C. The flow rate was 1 ml/min. Taurine concentrations were determined by comparison to a standard.

2.4 RT^2 qPCR Analysis

Total RNA was extracted using RNeasy kit (Qiagen) from the liver and was reverse-transcribed using cDNA kit according to the manufacturer's instruction (Qiagen). Quantitative real time PCR with 10 ng of cDNA were carried out in duplicate in a 7300 real-time PCR system (Eppendorff, Hauppauge, NY) using the SYBR master mix (Qiagen) and the following cycles: 2 min at 50 °C, 10 min at 95 °C and 40 cycles each at 95 °C for 15 s and 60 °C for 60 s (Park et al. 2014). RT^2 qPCR analysis was also carried out according to manufacturer's manual using β-actin as a control. All primers were purchased from Qiagen. For data analysis the Ct method

was used; for each gene fold-changes were calculated as difference in gene expression of G3 HO and G3 HOT, compared to that in WT. ΔCt was calculated by subtraction of Ct of β-actin from Ct of the interesting gene. ΔΔCt was calculated by subtraction of ΔCt of WT from ΔCt of G3 HO or H3 HOT. Fold change was determined by $2^{(-\Delta\Delta Ct)}$. More than 1 indicates gene up-regulation and less than 1 indicates gene down-regulation.

2.5 Statistical Analysis

Data are presented as mean ± SE. Statistical significance was determined using Statistica 8 (StatSoft, Tulsa, OK). Significant differences between groups were determined as $p < 0.05$ using LSD or Tukey HSD in post-hoc under one way ANOVA or t-test.

3 Results

3.1 Taurine Concentrations in the Brain, Liver and Plasma at 1 Month of Age in CSAD KO

We used HPLC to measure taurine concentrations in the liver and brain as well as plasma because the liver is a major organ for taurine production by CSAD while the brain requires taurine. Plasma taurine was measured because the vascular system supplies the taurine required by various organs.

Inactivation of one CSAD allele did affect taurine levels at 1 M: Although brain and plasma taurine concentrations in $CSAD^{+/-}$ were not decreased significantly, liver taurine concentrations were decreased to 77 % of wild type (WT). Previously, we demonstrated that G1 $CSAD^{-/-}$ born from $CSAD^{+/-}$ dams show higher taurine concentrations in the liver and brain compared to G2, G3 and G4 $CSAD^{-/-}$, presumably because of taurine transported through the placenta (Grillo et al. 2008). However, taurine concentrations in the liver, brain and plasma of G1 $CSAD^{-/-}$ at 1 M of age (1 week after weaning) fell to the same levels as in G2, G3 and G4 $CSAD^{-/-}$ (Figs. 1, 2 and 3).

While taurine concentrations in the liver from all generations of $CSAD^{-/-}$ were decreased to approximately 5 % of WT level at 1 M, taurine concentrations in the brain of $CSAD^{-/-}$ decreased to 40 % of WT. Supplementation with 0.05 % taurine in the drinking water brought liver and brain taurine concentrations to 10 and 76 % of wild type (WT), respectively. Thus, while brain taurine concentrations in $CSAD^{-/-}$ treated with taurine (HOT) were significantly increased, liver taurine concentrations were not. At PD1, G3 and G4 HOT taurine concentrations were significantly increased in both brain and liver.

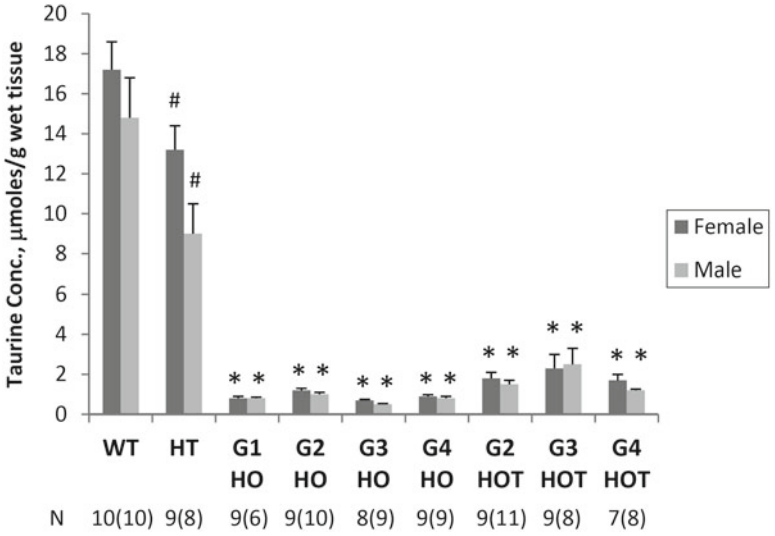

Fig. 1 Taurine concentrations in the liver at 1 M. The livers from designated number of female and male mice were homogenized with 5 % TCA and centrifuged at 10,000 g for 20 min. The supernatants were collected and dried using a Speedvac. Dried samples were derivatized using PITC. PITC-labeled taurine was determined using HPLC. Data are expressed as µmol/g wet tissue weight, mean ± SE in the liver. The number of females and males is shown in the figure. *Parentheses* are males. *$p < 0.0002$ and #$p < 0.002$ statistically significant, compared to WT

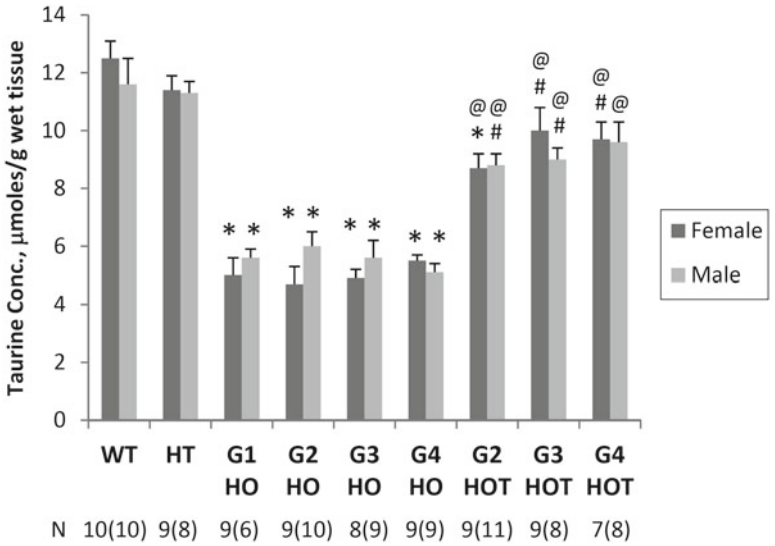

Fig. 2 Taurine concentrations in the brain at 1 M. Brain samples from 1 M mice were analyzed as in Fig. 1. Data are expressed as µmol/g wet tissue weight, mean ± SE in the brain. The number of females and males is shown in the figure with males in *parentheses*. *$p < 0.002$ and #$p < 0.05$ statistically significant, compared to WT. @$p < 0.01$ statistically significant, compared to G2, G3 and G4 HO, respectively

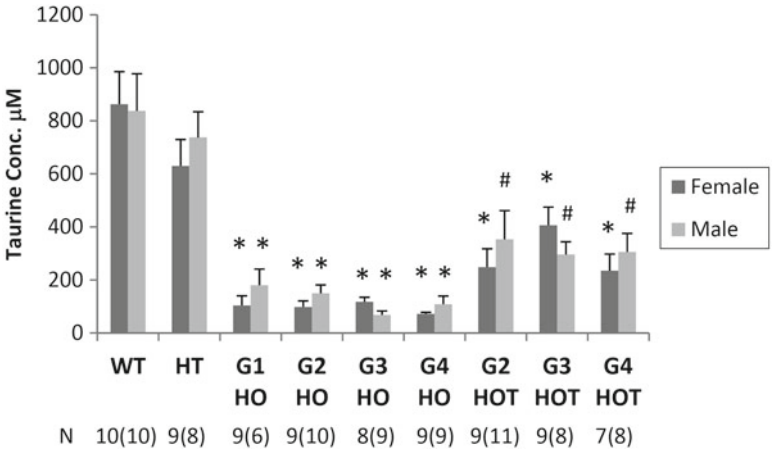

Fig. 3 Plasma taurine concentrations at 1 M. The plasma samples from 1 M mice were analyzed as in Fig. 1. Data are expressed as µM, mean ± SE. The number of females and males is shown in the figure with males in *parentheses*. *$p < 0.0002$ and #$p < 0.002$ statistically significant, compared to WT. Taurine level in treated (HOT) mice is significantly increased over the level in untreated (HO) mice ($F = 50.111$, $p < 0.001$)

Plasma concentrations in all generations of $CSAD^{-/-}$ were decreased to less than 14 % of WT and were restored to 65 % of WT with taurine supplementation in the drinking water compared to WT (Fig. 3). Females and males in all groups were not significantly different in taurine concentrations in brain, liver or plasma.

3.2 Gene Expression Measured Using RT² qPCR

We examined gene expression in CSAD KO liver using a microarray analysis. This specifically focused on genes that were candidates for influencing neonatal mortality, genes related to taurine metabolism and transport, genes for anti-oxidant enzymes, nucleotide and amino acid metabolic enzymes as well as for lactoferrin and the prolactin receptor. Taurine-related genes included CSAD, CDO, cysteamine dioxygenase (ADO) and TauT (Table 1). In contrast to gene expression at PD1, CDO was significantly decreased and TauT was significantly increased more than twofold at 1 M in G3 HO. CDO was restored in G3 HOT whereas TauT was not significantly different in G3 HOT compared to G3 HO. As expected, no CSAD expression was detected at PD1 and 1 M. ADO at both PD1 and 1 M was unaffected in both G3 HO and G3 HOT compared to WT.

Since taurine is an antioxidant we examined the expression of genes for antioxidant enzymes included glutathione peroxidase 1 and 3 (Gpx 2 and 3) as well as peroxireductase 2 (Prdx 2) (Table 2). Gpx 3 in G3 HO at 1 M was significantly increased more than twice that of WT just as it had been at PD1. However, Gpx 1

Table 1 Fold change of taurine-related genes in G3 HO and G3 HOT in the liver compared to WT

Gene	Genotype	PD1	1 month
Csad	WT	1.0±0.03[a]	1.0±0.1[b]
	G3 HO	<0.002**	<0.01**
	G3 HOT	<0.001**	<0.01**
Cdo	WT	1.0±0.05	1.0±0.06
	G3 HO	1.3±0.10*	0.7±0.03*
	G3 HOT	1.1±0.09	0.8±0.1
Ado	WT	1.0±0.03	1.0±0.05
	G3 HO	1.1±0.06	1.3±0.3
	G3 HOT	1.3±0.08*	1.1±0.2
TauT	WT	1.0±0.06	1.0±0.05
	G3 HO	1.2±0.07	2.0±0.2**
	G3 HOT	1.2±0.09	2.4±0.2**

*Significantly different compared to WT, $p<0.05$
**Significantly different compared to WT, $p<0.001$
[a]Data represent mean±SE from four WT, six G3 HO and four G3 HOT at PD1, respectively
[b]Data represent mean±SE from four mice in each group

Table 2 Fold change of antioxidant genes in G3 HO and G3 HOT in the liver compared to WT

Gene	Genotype	PD1	1 month
Gpx1	WT	1.0±0.05	1.0±0.09
	G3 HO	1.5±0.11[a]	1.4±0.1
	G3 HOT	1.2±0.09	1.6±0.2*
Gpx3	WT	1.0±0.14	1.0±0.07
	G3 HO	2.5±0.51**	2.0±0.3**
	G3 HOT	0.9±0.08	0.8±0.1
Prdx2	WT	1.0±0.05	1.0±0.07
	G3 HO	1.6±0.34	1.1±0.2
	G3 HOT	1.0±0.05	0.9±0.1
Prdx3	WT	1.0±0.10	ND
	G3 HO	1.1±0.08	
	G3 HOT	1.0±0.07	

*Significantly different, $p<0.05$. **$p<0.001$. At PD1, four mice were used in WT and G3 HOT and six mice were used in G3 HO. At 1 month, four mice were used in all groups
[a]Data are expressed as mean±SE

and Prdx 2 in G3 HO were not changed at 1 M even though Gpx 1 had been increased at PD1. Taurine supplementation restored Gpx 3 in G3 HOT to WT level at 1 M. While both uridine dephosphorylase 2 (Upp 2), a nucleotide metabolic enzyme, and serine hydratase (Sds), a amino acid metabolic enzyme, in G3 HO at PD1 were increased significantly, Upp 2 and Sds in G3 HO at 1 M were not different compared

Table 3 Gene expression of Upp2 and Sds at PD1 and 1 month in the liver

	Genotype	PD1	1 month
Upp2	WT	1.0 ± 0.02[a]	1.0 ± 0.08
	G3 HO	1.9 ± 0.51	0.8 ± 0.08
	G3 HOT	0.9 ± 0.10	0.7 ± 0.09*
Sds	WT	1.0 ± 0.22	1.0 ± 0.1
	G3 HO	2.3 ± 0.42*	0.8 ± 0.09
	G3 HOT	1.2 ± 0.10	0.6 ± 0.1*

*Significantly different, $p < 0.05$ compared to WT
[a]Data represent mean ± SE. Four mice were used in WT, G3 HO and G3 HOT at PD1 and 1 month

to WT (Table 3). Taurine treatment decreased Upp2 and Sds expression significantly in G3 HOT. Ltf and Prlr were significantly decreased in G3 HO at 1 M, as they had been a PD1. However, taurine treatment restored both at 1 M while only Prlr was restored at PD1.

4 Discussion

Weanling CSAD KO at 1 M showed a profile of taurine distribution in the liver and brain (Fig. 1, 2, and 3) that was different from the profile at PD1 (Park et al. 2014). At PD1, taurine concentrations in both the brain and liver from G2 and G3 HO were approximately 80 % lower than WT. Taurine-treated G2 and G3 HO dams had pups with PD1 liver and brain taurine concentrations that were substantially and similarly increased. However, at 1 M, taurine concentrations in the HO brain were a higher percentage of WT than in the liver (Figs. 1 and 2). After taurine treatment, CSAD**–/–** liver taurine was significantly less at 1 M than at PD1 despite the increase in liver TauT gene expression (Table 1). In contrast treatment substantially restored taurine levels in the brain at 1 M. Plasma taurine was increased significantly ($p < 0.001$) to approximately 65 % of WT level in the taurine-supplemented CSAD**–/–** mice (Fig. 3). Taurine concentrations were significantly increased in the brain of these mice but not in the liver which suggests that because the brain is not a major biosynthetic source of taurine, it may be better equipped to utilize the taurine supplied though the drinking water than the liver is. This suggests that when taurine is required for physiological function, taurine may be actively transported from the circulatory system to the organ(s) via TauT. Taurine concentrations in the splenocytes from G3 HO were decreased 56 % compared to WT, similar to the brain (data not published).

These results were similar to those in taurine-deficient knockout mouse models and in cats (Sturman 1993). Taurine concentrations in the liver from CDO KO and TauT KO are significantly decreased (Heller-Stilb et al. 2002; Ito et al. 2008; Ueki et al. 2011). However, in our study taurine concentrations in the liver and brains from females and males in all groups were not significantly different although

taurine concentrations in CDO KO females were higher than in males. Physical phenotypes observed in CDO KO (excess lacrimation and partial palpebral closure, plantigrade stance, hyperextensible toes and less-erect ears) were not observed in CSAD KO. Although neonatal mortality was detected in G3 and G4 HO, surviving CSAD KO grew normally without weight loss compared to WT. However, CDO KO weight and size is less than WT. Taurine deficiency in the cat is similar to CSAD KO with respect to taurine levels. Taurine concentrations in the liver from adult cats without taurine treatment were remarkably decreased compared to adult cats fed 0.05 % taurine. However, the developmental defects in surviving offspring from female taurine-deficient cats such as hind leg development and a peculiar gait characterized by excessive abduction and paresis, were not detected in CSAD KO (Sturman 1993).

CSAD**−/−** gene expression at 1 M is also unlike PD1. CDO in the liver from G3 HO was significantly increased due to feedback inhibition (accumulation of CSAD in CSAD KO) (Table 1). TauT expression in the liver of G3 HO and G3 HOT was also increased compared to WT at 1 M but not at PD1, indicating TauT may facilitate taurine transport to compensate for decreased taurine concentrations. CSAD gene expression was absent in both groups at both PD1 and 1 M, confirming deletion of the CSAD. The antioxidant enzyme, Gpx 3 expression was increased at 1 M although both Gpx 1 and 3 expression were increased at PD1 (Schaffer et al. 2009; Brigelius-Flohe and Maiorino 2013; Lubos et al. 2011). Alteration in these genes may contribute to neonatal death at PD1. Although G3 HO thrived, antioxidant enzymes may be needed for survival because mice are under oxidative stress due to taurine deficiency. Gpx 3 expression was restored in G3 HOT suggesting taurine may be an antioxidant. Since newborn G3 CSAD**−/−** often lacked milk spots (MS), a predictor of survival in newborn mice, we examined the gene expression of Ltf and Prlr. Ltf has innate immune function to protect newborn offspring from infection and is elevated in colostrum (Ward and conneely 2004; Legrand and Mazurier 2010; Legrand 2012). Ltf is widely present in fluids such as milk and colostrum with a high affinity for iron. Lft, an important component of the innate immune system, is important in bacteriostasis and required for optimal neutrophil function. Prolactin, a lactogenic hormone, regulates the output of insulin-like growth factor-1. Genetic ablation of Prlr results in mice which show multiple defects in reproduction leading to infertility, altered maternal behavior and reduced bone development (Brooks 2012; Binart et al. 2010; Bole-Feysot et al. 1998). Of importance, Ltf and Prlr in the liver were decreased significantly in G3 HO but only Prlr was restored by taurine-supplementation (G3 HOT) (Fig. 4) at PD1. However, at 1 M, both Prlr and Ltf were decreased significantly compared to WT and restored to WT with added taurine, suggesting these genes may be down-regulated by low taurine concentrations. Upp 2 catalyzes the cleavage of uridine to uracil and ribose and used both energy sources and nucleotide synthesis (Roosild et al. 2011; Wan et al. 2010; Johansson 2003). Serine is a substrate for Sds with production of the two products, pyruvate (precursor of glucose) and NH_3 for gluconeogenesis from amino acids (Masuda et al. 2003; Imai et al. 2003; Lopez-Flores et al. 2006) The significant increase of Upp 2 and Sds in G3 HO without MS is unclear but may show the

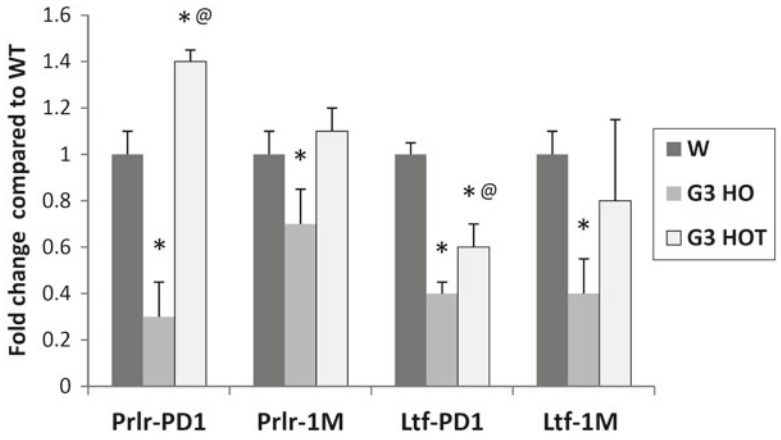

Fig. 4 Fold changes of Prlr and Ltf in G3 HO and G3 HOT compared to those in WT at PD1 and 1 M. The liver at PD 1 and 1 M were homogenized with Trizol and mRNA was extracted using RNeasy kit. After cDNA was produced by reverse transcriptase, 10 ng of cDNA was mixed with SYBR master mix and reacted with primers using PCR system. Data are expressed mean ± SE as fold change compared to WT. * is significantly ($p < 0.001$) different compared to WT, and @ is significantly ($p < 0.01$) different compared to G3 HO

critical regulation of physiological processes by uridine or may be due to starvation (Ishikawa et al. 1965). However, Upp2 and Sds in the liver from G3 HO at 1 M were not changed although expression of these two enzymes in the liver from G3 HO without MS at PD1 is dramatically increased. With taurine supplementation both genes were significantly decreased, suggesting taurine may be involved in regulation of nucleotide metabolism and gluconeogenesis.

Alteration of gene expression in CSAD KO was similar to that in other models of taurine deficiency. Gene expression in CDO KO was altered compared to WT. Expression of CSAD gene in CDO KO was increased significantly compared to WT and expression of ADO gene in CDO KO was without effect similar to CSAD KO. Although an increase of TauT in CDO KO was consistent with that in CSAD KO, TauT in taurine-treated CDO KO was restored to WT (Roman et al. 2013) but TauT in taurine-treated CSAD KO was not restored. This difference may be attributed to differences in the amount of taurine used; CSAD KO and CDO KO were treated with 0.05 and 2 % in the drinking water, respectively (Ueki et al. 2011).

5 Conclusion

Newborn G3 HO and G4 HO mice with a milk spot (MS) survive and thrive to grow well as we demonstrated previously. Surviving CSAD KO at 1 M after weaning indicated that taurine is redistributed on the basis of need, regardless of its origin, which suggests that the requirement for taurine for homeostasis and survival may

vary from organ to organ. Although CSAD KO has low reproductive performance, surviving CSAD KO mice are good models for understanding the physiological role of taurine in various disorders as well as for examining gene regulation in the absence of CSAD and/or low taurine levels. Supplementation of taurine in the food and drinking water may provide a potential prevention and treatment of various disorders caused by taurine deficiency, especially prior to or during pregnancy.

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