A Novel Cysteine Sulfinic Acid Decarboxylase Knock-Out Mouse: Taurine Distribution in Various Tissues With and Without Taurine Supplementation

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Abstract Taurine, a sulfur containing amino acid, has various physiological functions including development of the eye and brain, immune function, reproduction, osmo-regulatory function as well as anti-oxidant and anti-inflammatory activities. In order to understand the physiological role, we developed taurine deficient mice deleting a rate-liming enzyme, cysteine sulfinic acid decarboxylase (CSAD) for biosynthesis of taurine. Taurine was measured in various tissues including the liver, brain, lung, spleen, thymus, pancreas, heart, muscle and kidney as well as plasma from CSAD knock-out mice (CSAD KO) with and without treatment of taurine in the drinking water at the age of 2 months (2 M). Taurine was determined using HPLC as a phenylisothiocyanate derivative of taurine at 254 nm. Taurine concentrations in the liver and kidney from homozygotes of CSAD KO (HO), in which CSAD level is high, were 90% and 70% lower than WT, respectively. Taurine concentrations in the brain, spleen and lung, where CSAD level is low, were 21%, 20% and 28% lower than WT, respectively. At 2 M, 1% taurine treatment of HO restored taurine concentrations in all tissues compared to that of WT. To select an appropriate taurine treatment, HO were treated with various concentrations (0.05, 0.2, 1%) of taurine for 4 months (4 M). Restoration of taurine in all tissues except the liver, kidney and lung requires 0.05% taurine to be restored to that of WT. The liver and kidney restore taurine back to WT with 0.2% taurine. To examine which enzymes influence taurine concentrations in various tissues from

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WT and HO at 2 M, expression of five taurine-related enzymes, two antioxidant enzymes as well as lactoferrin (Lft) and prolactin receptor (Prlr) was determined using RT^2 qPCR. The expression of taurine transporter in the liver, brain, muscle and kidney from HO was increased except in the lung. Our data showed expression of glutamate decarboxylase-like 1(Gadl-1) was increased in the brain and muscle in HO, compared to WT, indicating taurine in the brain and muscle from HO was replenished through taurine transporter and increased biosynthesis of taurine by up-regulated Gadl-1. The expression of glutathione peroxidase 3 was increased in the brain and peroxireductase 2 was increased in the liver and lung, suggesting taurine has anti-oxidant activity. In contrast to newborn and 1 month CSAD KO, Ltf and Prlr in the liver from CSAD KO at 2 M were increased more than two times and 52%, respectively, indicating these two proteins may be required for pregnancy of CSAD KO. Ltf in HOT1.0 was restored to WT, while Prlr in HOT1.0 was increased more than HO, explaining improvement of neonatal survival with taurine supplementation.

These data are essential for investigating the role of taurine in development of the brain and eye, immune function, reproduction and glucose tolerance.

Keywords CSAD KO • Taurine distribution of various tissues • Gene expression • CSAD KO with taurine treatment

Abbreviations

2 M and 4 M	The age of 2 months and 4 months
ADO	Cysteamine (2-aminoenthanethiol) dioxygenase
CDO KO	Cyteine dioxygenase knockout mice
CSAD KO	Cysteine sulfinic acid decarboxylase knockout mice
CDO	Cysteine dioxygenase
CSAD	Cysteine sulfinic acid decarboxylase
Gpx 3	Glutathione peroxidase 3
HO	Homozygotic mice ($CSAD^{-/-}$)
HOT	Homozygotic mice treated with 1% taurine
HOT0.05, HOT0.2 and HOT1.0	Homozygotic mice treated with 0.05%, 0.2% or 1% taurine, respectively
Ltf	Lactoferrin
Prdx 2	Peroxireductase 2
Prlr	Prolactin receptor
TauT KO	Taurine transporter knockout mice
TauT	Taurine transporter
WT	Wild type ($CSAD^{+/+}$)

1 Introduction

Taurine is considered a conditionally essential amino acid in humans and primates and is required during their development. 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 (Schuller-Levis and Park 2006; Sturman 1993). 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 the food and 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 cycteine dioxygenase (CDO) and cysteine sulfinic acid decarboxylase (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.

Five 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 converts 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); Glutamate decarboxylase-like 1 (GADL-1) which produces taurine from CSAD and aspartate (Liu et al. 2012; Winge et al. 2015). Recently four genetically modified mice have been developed to model taurine deficiency using gene targeting methods. These taurine-deficient knock-out mice include two taurine transporter knockout mice (TauT KO), cysteine dioxygenase knockout mice (CDO KO) and cysteine sulfinic acid decarboxylase (CSAD 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. Our laboratory produced a cysteine sulfinic acid decarboxylase knockout mouse (CSAD KO) as a novel mouse model of taurine deficiency (Park et al. 2014). We 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, 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. Subsequently newborn pups and mice at 1 M after weanling were compared for changes in taurine distribution in the brain and liver and gene expression (Park et al. 2015). Data demonstrated that a decrease in taurine concentrations in the liver from weanling mice are more profound than those in the brain. Surviving CSAD KO 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.

In order to confirm this finding, we examined nine tissues including the liver, brain, kidney, pancreas, thymus, spleen, heart, lung and muscle as wells as blood at 2 M with and without 1% taurine in the drinking water. Taurine concentrations at 2 and 4 M were compared in nine tissues and plasma. Various taurine amounts including 0.05, 0.2 and 1.0% at 4 M were examined for appropriate amount of taurine supplementation for restoring the taurine concentrations in various tissues to WT. Gene expression correlated to different taurine concentrations in various tissues were also examined in this study.

2 Materials and Methods

2.1 Materials

Chemicals used in this study were purchased from Sigma Chemicals (St. Louis, 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 as previously described from our laboratory (Park et al. 2014). 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. 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 various concentrations of taurine including 0.05, 0.2 and 1.0% in the drinking water as indicated in Results and Discussion and offspring used in this study were treated with same taurine concentrations after weaning. All mice at the age of 2 and 4 months (2 M and 4 M) used in this study were separated from their dam 3–4 week after birth. All procedures involving live animals were approved by the Institutional Animal Care and Use Committee of IBR.

2.3 High Performance Liquid Chromatography (HPLC)

All tissues including the liver, brain, kidney, spleen, thymus, heart, lung, muscle and pancreas as well as plasma were obtained from all groups including WT, HO and HO treated with various taurine (HOT) after mice were sacrificed with *ip* injection of avertin (250 mg/Kg). 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% acetonitril (pH 6.5) and 45% acetonitril 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 Ananlysis

Total RNA was extracted using RNeasy kit (Qiagen) from various tissues including the liver, brain, kidney, muscle and lung from WT, HO and HO treated with 1% taurine (HOT1.0) 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 (Effendorff, 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 (19). 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 HO and HOT1.0, 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 HO or HOT. Fold change was determined by $2^{\wedge\Delta\Delta Ct}$. More than one indicates gene up-regulation and less than one indicates gene down-regulation.

2.5 Statistical Analysis

Data are presented as mean ± SE. Statistical significance was determined using Statistica 13 (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.

3 Results

3.1 Taurine Concentrations in Various Tissues

Since the decrease of taurine concentrations in the liver and brain from HO were remarkably different compared to WT at the age of 1 M, we determined taurine concentrations in 9 tissues including the liver, brain, kidney, pancreas, spleen, thymus, lung, heart and muscle as well as plasma from WT, HO and HO treated with 1% taurine in the drinking water (HOT1.0) at 2 M. Taurine concentrations in various tissues are variable (Figs. 1 and 2).

The liver and kidney in HO showed the lowest taurine concentrations. Both the liver and kidney produce taurine due to high levels of CSAD in WT and decreased taurine concentrations 90% and 70% in CSAD KO, respectively (Fig. 2). Meanwhile,

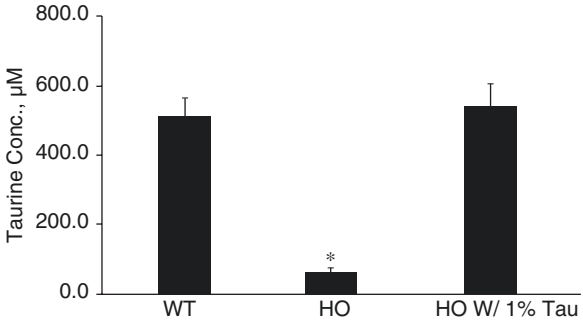

Fig. 1 Plasma taurine concentrations in WT, HO and HO with 1% taurine treatment at the age of 2 months. Eight mice were used in each group. Data are expressed mean ± SE. *$p < 0.05$ statistically significant, compared to WT

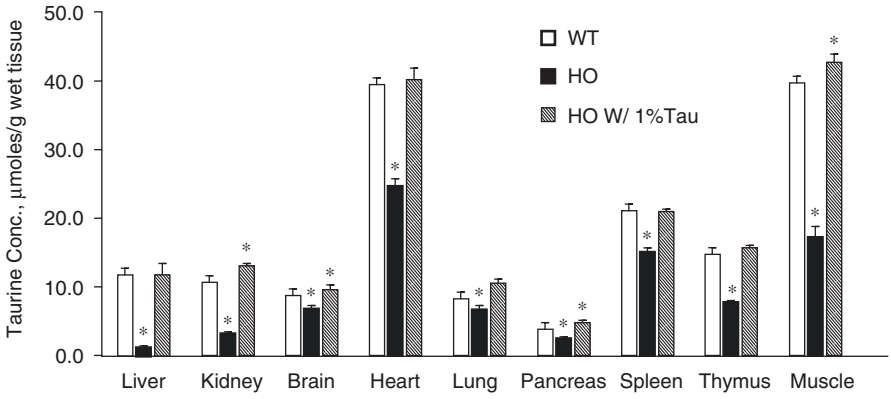

Fig. 2 Taurine concentrations in various tissues from WT, HO and HO with 1% taurine treatment at the age of 2 months. Eight mice were used in each group. Data are expressed mean ± SE. *p < 0.05 statistically significant, compared to WT

the brain, spleen and lung, which have low CSAD levels and transport taurine through TauT, were decreased 21%, 21% and 28% lower than WT, respectively. At 2 M, HOT1.0 restored taurine concentrations in all tissues to those of WT (Fig. 2). Taurine concentraions in some tissues including the kidney, brain, pancreas and muscle from HOT1.0 are significantly increased. Plasma taurine concentrations in HO CSAD KO were 88% lower than WT and restored completely to WT when HO were treated with 1% taurine (Fig. 1). Previously we demonstrated that 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 (Park et al. 2015; Ueki et al. 2011). Taurine concentrations in this study were obtained from both females and males.

3.2 Taurine Treatment of Various Taurine Treatment Including 0.05, 0.2 and 0.1% in the Drinking Water

Four month old mice to 2 M old mice were compared to determine how much taurine concentrations is changed in WT and HO (Table 1). All tissues at 4 M except the heart, muscle and lung from HO were not significantly different, compared to 2 M. However, taurine concentrations in the heart, muscle and lung from HO at 4 M were significantly increased although the heart and muscle were in WT of CSAD KO not significantly different at both ages. Interestingly, taurine concentrations in the lung from WT and HO at 4 M was increased 3.2 times and 2.5 times compared to in both WT and HO at 2 M, respectively. Lower taurine treatment including 0.05 and 0.2% were compared to 1% taurine treatment in the drinking water at 4 M. 0.05% taurine treatment in HO restored taurine concentrations in all tissues except the liver and kidney. Taurine concentrations in the liver and kidney were restored to

Table 1 Taurine concentrations in various tissues from WT and HO at the age of 2 and 4 months

	WT		HO	
	2 M (8)[c]	4 M (8)	2 M (8)	4 M (8)
Plasma[a]	512 ± 53.3	319.7 ± 29.7	65.0 ± 11.1	77.7 ± 10.2
Liver[b]	11.8 ± 0.9	9.0 ± 0.9	1.2 ± 0.1	1.8 ± 0.2
Kidney	10.7 ± 0.6	12.6 ± 0.2	3.2 ± 0.2	5.5 ± 0.4
Brain	8.8 ± 0.6	10.5 ± 1.0	6.9 ± 0.5	6.8 ± 0.6
Heart	39.4 ± 3.6	38.6 ± 0.6	24.7 ± 1.0	32.4 ± 0.7[d]
Lung	8.3 ± 0.3	26.2 ± 4.4[d]	6.7 ± 0.6	17.2 ± 2.9[d]
Pancreas	3.9 ± 0.2	4.5 ± 0.4	2.6 ± 0.2	3.2 ± 0.3
Spleen	21.0 ± 0.2	20.7 ± 0.5	15.1 ± 0.6	15.8 ± 0.6
Thymus	14.7 ± 1.0	15.7 ± 1.1	7.7 ± 0.3	11.2 ± 0.5
Muscle	39.6 ± 1.0	44.2 ± 1.2	17.4 ± 1.4	31.5 ± 1.2[d]

[a]Data from plasma are expressed as mean ± SE (μM)
[b]Data from various tissues are expressed as mean ± SE (μmoles/g wet tissue)
[c]Number in parentheses is number of mice used in each group
[d]Significant difference statistically between the age of 2 and 4 months in WT and HO, respectively, $p < 0.05$. All HO in both ages are significantly different, compared to WT, <0.05

Table 2 Taurine concentrations in various tissues from WT, HO, HO with 0.05, 0.2 and 1.0% taurine

	WT[c]	HO	HOT0.05	HOT0.2	HOT1.0
Plasma	319.7 ± 29.7[a]	77.7 ± 10.2[d]	422.8 ± 90.1	385.8 ± 82.6	540.4 ± 65.2[d]
Liver	9.0 ± 0.9[b]	1.8 ± 0.2[d]	5.9 ± 1.1	7.1 ± 1.2	10.7 ± 0.7
Kidney	12.6 ± 0.2	5.5 ± 0.4[d]	8.6 ± 0.5	12 ± 0.9	15.2 ± 1.0[d]
Brain	10.5 ± 1.0	6.8 ± 0.6[d]	10.7 ± 0.5	10.6 ± 0.8	13.7 ± 0.9[d]
Spleen	20.7 ± 0.5	15.8 ± 0.6[d]	20.8 ± 1.0	22.3 ± 0.6	21.7 ± 0.4
Thymus	15.7 ± 1.1	11.2 ± 0.5[d]	14.8 ± 1.1	17.1 ± 0.4	18.7 ± 0.8
Pancreas	4.5 ± 0.4	3.2 ± 0.3[d]	4.8 ± 0.6	5.3 ± 0.2	5.0 ± 0.2
Heart	38.6 ± 0.6	32.4 ± 0.7[d]	37.6 ± 0.9	39.2 ± 1.3	43.2 ± 1.8[d]
Muscle	44.2 ± 1.2	31.5 ± 1.2[d]	45.5 ± 1.5	44.4 ± 2.3	49.0 ± 1.4[d]
Lung	26.2 ± 4.4	17.2 ± 2.9	18.7 ± 3.3	18.3 ± 4.3	21.7 ± 3.9

[a]Data from plasma are expressed as mean ± SE (μM)
[b]Data from various tissues are expressed as mean ± SE (μmoles/g wet tissue)
[c]Eight mice were used in each group
[d]Significant difference statistically between WT to HO, HOT0.05, HOT0.2 and HOT1.0, $p < 0.05$

WT when HO were treated with 0.2% taurine solution (Table 2). Taurine concentrations in four tissues including the kidney, brain, heart and muscle as well as plasma were increased significantly when HO were treated with 1% taurine at 4 M. The taurine concentration in the lung from WT and HO as well as HO treated with various taurine in the drinking water were not significantly different at 4 M, not consistent with results from 2 M (Fig. 2). Taurine concentrations in the lung from WT, HO and HOT were not statistically different because the data from the lung are remarkably variable in individual mice.

3.3 Gene Expression Measured Using RT² qPCR

Taurine tissue distribution was remarkably different in various tissues (Fig. 2 and Table 1). In order to examine correlation of taurine tissue concentrations as well as various taurine biosynthetic enzymes and taurine transporter, gene expression of four enzymes including Csad, Cdo, Ado and Gadl-1 as well as TauT was determined using five tissues from females of WT, HO and HOT1.0 at 2 M (Table 3). Gene expression of five proteins in five tissues including the liver, brain, lung, muscle and kidney from HO was compared to WT. The taurine transporter was increased more than 1.5 times in four tissues including the liver, brain, kidney and muscle from HO compared to WT. TauT in the lung from HO were not significantly different compared to WT. TauT in all tissues from HOT1.0 was restored to WT. CSAD KO in all tissues from HO and HOT1.0 was extremely low, confirming the absence of CSAD. Cdo was increased in the brain from HO and Ado was increased in the brain and muscle compared to WT, while Gadl-1 was increased in the brain and muscle compared to WT. Cdo in the brain from HOT1.0 was even increased compared to HO. Meanwhile, Ado in both tissues was restored to WT. Gadl-1 in the brain from HOT was recovered to WT but that in the muscle was even increased compared to HO. Gadl-1 was not detected in the liver and kidney which is expressed previously described by Liu et al. (Liu et al. 2012). In addition to taurine-related genes, antioxidant-related genes including Gpx3 and Prdx2 were determined as measured

Table 3 Fold changes[a] in various tissues from WT, HO and HO with 1% taurine treatment

Tissue	Genotype	Csad	Cdo	Ado	Gadl-1	TauT
Liver	WT	1.00[b]	1.00	1.00	ND	1.00
	HO	<0.01	0.80	1.20	ND	1.70
	HOT1.0	<0.01	1.20	1.30	ND	1.40
Brain	WT	1.00	1.00	1.00	1.00	1.00
	HO	0.05	1.70	1.87	3.10	2.30
	HOT1.0	0.05	2.20	1.32	1.10	1.40
Lung	WT	1.00	1.00	1.00	ND[c]	1.00
	HO	0.01	0.88	0.72	ND	1.05
	HOT1.0	0.02	1.04	1.05	ND	0.08
Muscle	WT	1.00	1.00	1.00	1.00	1.00
	HO	0.02	0.56	1.91	1.98	1.77
	HOT1.0	0.02	1.06	1.39	2.68	1.23
Kidney	WT	1.00	1.00	1.00	ND	1.00
	HO	0.01	1.23	1.23	ND	1.62
	HOT1.0	0.02	0.86	0.86	ND	0.91

[a]Fold change was determined by $2^{\wedge\Delta\Delta Ct}$

[b]Data are expressed as average of two mice. Similar results were obtained from one additional experiment

[c]ND means "not detected"

Table 4 Fold change[a] in various tissues from WT, HO and HO with 1% taurine treatment

Tissue	Genotype	Gpx3	Prdx2
Liver	WT	1.00[b]	1.00
	HO	1.26	1.52
	HOT1.0	1.07	1.42
Brain	WT	1.00	1.00
	HO	1.98	1.10
	HOT1.0	1.90	0.74
Lung	WT	1.00	1.00
	HO	1.14	1.58
	HOT1.0	1.45	1.41
Muscle	WT	1.00	NT[c]
	HO	0.97	NT
	HOT1.0	0.82	NT
Kidney	WT	1.00	1.00
	HO	1.38	1.38
	HOT1.0	1.06	1.06

[a]Fold change was determined by $2^{\wedge\Delta\Delta Ct}$

[b]Data are expressed as average of two mice. Similar results were obtained from one additional experiment

[c]NT means "not tested"

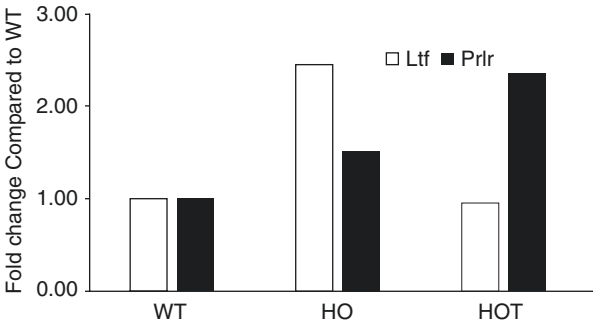

Fig. 3 Fold changes of Prlr and Ltf in HO and HOT compared to those in WT at the age of 2 months. HOT mice were treated with 1% taurine in the drinking water. Data are expressed as mean of two mice. Similar results were obtained from one additional experiment

previously in newborn pubs and 1 month old mice (1 M) (Table 4). Gpx3 was increased in the brain from HO and HOT1.0, while Prdx2 was increased in the liver and lung. Prolactin receptor (Prlr) and lactoferrin (Ltf), which were significantly decreased at newborn pups and weanling mice in previous studies, were included in this study to compare 2 M to newborn pups and 1 M (Fig. 3) (Park et al. 2015). In contrast to newborn pubs and weanling mice, both genes were increased in HO compared to WT at 2 M. However, Lft in HOT were recovered to WT, while Prlr in HOT1.0 was increased compared to HO.

4 Discussion

Taurine tissue distribution in WT, HO and HOT was examined because taurine concentrations in the liver and brain are remarkably different as previously described (Park et al. 2015). Taurine concentration in the liver from HO at the age of 2 months was decreased much more than those in the brain from HO which has low CSAD levels although taurine concentrations in both of the liver and brain from pups of HO were low. (Park et al. 2014, 2015). Taurine concentrations in nine tissues including the liver, kidney, brain, lung, heart, spleen, thymus, pancreas, and muscle as well as plasma from HO and HOT1.0 were compared to WT. Data demonstrated that the liver and kidney from HO, which has high CSAD levels, showed the greatest decrease, compared to WT (Fig. 2). The spleen, brain and lung were the least decrease, compared to WT. The heart and muscle were less decrease than the liver and kidney. These data indicated that taurine concentration was replenished through TauT and/or by biosynthesis of taurine *in situ* using up-regulated Ado and/or Gadl-1 depending on tissue (Table 3). All three proteins were up-regulated in the brain to maintain relatively high taurine HO compared to WT. Even Cdo in the brain from HO was up-regulated to produce more CSA which is a substrate of Gadl-1. Since TauT in the brain in HOT1.0 was restored to WT, Cdo in HOT1.0 in the brain was increased more than HO to produce CSA for restoring taurine to WT. Due to lack of Gadl-1 and CSAD in the liver and kidney in HO, taurine is low in the liver and kidney from HO although TauT was up-regulated by taurine deficiency (Fig. 2 and Table 3). These results demonstrated that taurine deficiency by deleting the CSAD was involved in the dynamic regulation of taurine-related genes to fulfill requirement of taurine for homeostasis. (Figs. 1 and 2, Table 3). The lung is a unique tissue in which any genes examined are not regulated in HO and HOT1.0 compared to WT.

Taurine concentrations in the lung from WT and HO at 4 M were remarkably increase compared to 2 M (Table 1). Taurine concentrations in the lung from WT, HO and three HOTs including HO0.05, HO0.2 and HO1.0 were not significantly different with various amounts of taurine (Table 2). These data indicated that the lung may not be regulated expression of taurine-related genes and TauT in both WT and HO by taurine-deficiency and/or deletion of CSAD gene. Up-regulation of Gpx3 in the lung from HO may partially explain anti-oxidant activity of taurine (Table 4). Our results with Gadl-1 enzyme, which produce taurine from CSA and aspartate, was not consistent from previous results of Liu et al. and Winge et al. (Liu et al. 2012; Winge et al. 2015). Although expression of Gadl-1 was detected in the kidney, muscle and brain previously, Gadl-1 was expressed in the brain and muscle but not in the kidney in our study (Table 3). It is of interest that taurine in the lung, muscle and heart were remarkably increased in HO at 4 M, compared to 2 M, indiciating taurine may be required more for maintaining their physiological functions. In contrast to CSAD KO, TauT KO shows low taurine concentrations in all tissues including the heart, muscle, eye, brain, kidney and plasma (Heller-Stilb et al. 2002; Ito et al. 2008) due to the absence of Tau T. Taurine concentrations in the liver from CDO KO are significantly low, similarly to CSAD KO (Ueki et al. 2011). CSAD gene expression was absent in both HO and HOT in this study, confirming deletion of CSAD (Table 3).

The antioxidant enzyme, Gpx 3 expression in the liver was not changed at 2 M although Gpx 3 expression were increased in both newborn pups and weanling mice (Table 4) (Park et al. 2014, 2015; Schaffer et al. 2009; Brigelius-Flohe and Maiorino 2013; Lubos et al. 2011). However, Gpx3 expression in the brain was increased. Prdx2 expression in the liver and lung was increased at 2 M. Gpx 3 expression in the brain was restored in HOT1.0. Alteration of these genes may indicate taurine deficiency may induce oxidative stress. Since Ltf and Prlr in the liver from newborn pups and weanling mice were decreased in HO, we also examined these gene expression at 2 M. 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 with a high affinity for iron in fluids such as milk and colostrum. Ltf, an indispensable component of the innate immune system, has 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). While Ltf and Prlr are decreased significantly in liver from HO at newborn and 1 M, both genes were increased at 2 M (Park et al. 2014, 2015). In contrast to newborn and weanling mice, Prlr in HOT1.0 at 2 M was increased even more than HO. These data suggest that mature mice may require taurine to maintain a stable pregnancy. Antibacterial lactoferrin is high in colostrum same as taurine and Ig A (Sturman 1993; Sanchez et al. 1992; Weaver et al. 1991). An increase in lactoferrin in HO may compensate for taurine deficiency because lactoferrin expression is recovered to WT in HOT0.1. In contrast to lactoferrin, an increase of prolactin receptor expression in HO and HOT1.0 may be required for increased survival of offspring as demonstrated previously in our laboratory. The survival rate in HOT0.05 was remarkably high, 92%, compared to HO, 13%. These results supported improvement of neonatal survival with taurine supplementation. Alteration of gene expression was observed in CDO KO as in CSAD KO. Expression of Csad gene in the liver from CDO KO was increased significantly compared to WT and expression of Ado gene in CDO KO was without effect. However, Ado in the brain in CSAD KO was increased at 2 M but not in the liver. Regulation of TauT in CSAD KO is consistent to that in CDO KO. TauT expression in CDO KO is increased and restored to WT in taurine-treated CDO KO (Roman et al. 2013). These data indicated that taurine deficiency as well as deletion of taurine biosynthetic enzymes may be involved in gene regulation of various genes.

5 Conclusion

Taurine concentrations in the liver and kidney which have high levels of CSAD were decreased more severely than the other tissues, compared to WT. Taurine centrations in the brain in CSAD KO were decreased much less by increasing Cdo,

Ado, Gadl-1and TauT, compared to the liver. These data indicated that taurine concentrations in HO of CSAD KO was replenished through an increase of TauT and/or taurine-bioxsynthetic enzymes according to necessity of taurine in various tissues. Redistribution of taurine and regulation of gene expression in various tissues from HO of CSAD KO are important for understanding the role of taurine in development of the brain, immune function, reproduction and glucose tolerance.

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References

Battaglia A, Bortoluzza A, Galbucci F, Eusebi V, Giorgianni P, Ricci R, Tosi R, Tugnoli V (1999) High performance liquid chromatographic analysis of physiological amino acids in human brain tumor by pre-column derivatization with phenylisothiocyanate. J Chromatogr B 730:81–93

Bella DL, Kwon YH, Hirschberger LL, Stipanuk MH (2000) Post-transcriptional regulation of cysteine dioxygenase in rat liver. Adv Exp Med Biol 483:71–85

Binart N, Bachelot A, Bouilly J (2010) Impact of prolactin receptor isoforms on reproduction. Trends Endocrinol Metab 21:362–368

Bole-Feysot C, Goffin V, Edery M, Binart N, Kelly PA (1998) Prolactin (PRL) and its receptor: actions, signal transduction pathways and phenotypes observed in PRL receptor knockout mice. Endocr Rev 19(3):225–268

Bonhaous DW, Pasantes-Morales H, Huxtable RJ (1985) Actions of guanidinoethane sulfonate on taurine concentration, retinal morphology and seizure threshold in the neonatal rat. Neurochem Int 7:263–270

Brigelius-Flohe R, Maiorino M (2013) Glutathione peroxidases. Biochim Biophys Acta 1830(5):3289–3303

Brooks CL (2012) Molecular mechanisms of prolactin and its receptor. Endocr Rev 33(4):1–22

Dela Rosa J, Stipanuk MH (1984) Effect of guanidinoethanesulfonate administration on taurine levels in rat dams and their pups. Nutr Res Int 30:1121–1125

Dominy JE, Simmons CR, Hirshberger LL, Hwang J, Coloso RM, Stipanuk MH (2007) Discovery and characterization of a second mammalian thiol dioxygenase, cysteamine dioxygenase. J Biol Chem 282:25189–25251

Heller-Stilb B, van Royen C, Rascher K, Hartwig HG, Huth A, Seeliger MW, Warskulat U, Haeussinger D (2002) Disruption of the taurine transporter gene (*taut)* leads to retinal degeneration in mice. FASEB J 16:231–233

Hosokawa Y, Matumoto A, Oka J, Itakura H, Yamaguchi K (1990) Isolation and characterization of a complemantary DNA for rat liver cysteine dioxygenase. Biochem Biophys Res Commun 168:473–478

Huxtable RJ (2000) Expanding the circle 1975–1999: sulfur biochemistry and insights on the biological functions of taurine. Adv Exp Med Biol 483:1–25

Ito T, Kimura Y, Uozumi Y, Takai M, Muraoka S, Matsuda T, Ueki K, Yoshiyama M, Ikawa M, Okabe M, Schaffer SW, Fujio Y, Azuma J (2008) Taurine depletion caused by knocking out the taurine transporter gene leads to cardiomyopathy with cardiac atrophy. J Mol Cell Cardiol 44:927–937

Jong CJ, Ito T, Mozaffari M, Azuma J, Schaffer S (2010) Effect of beta-alanine treatment on mitochondrial taurine level and 5-taurinomethyluridine content. J Biomed Sci 17(Suppl 1):S25

Legrand D (2012) Lactoferrin, a key molecule in immune and inflammatory processes. Biochem Cell Biol 90(3):252–268

Legrand D, Mazurier J (2010) A critical review of the roles of host lactoferrin in immunity. Biometals 23(3):365–376

Liu P, Ge X, Ding H, Jiang H, Christensen BM, Li J (2012) Role of glutamate decarboxylase-like 1 (GADL-1) in taurine biosynthesis. J Biol Chem 287(49):40898–40906

Lubos E, Loscalzo J, Handy DE (2011) Glutathione peroxidase-1 in health and disease: from molecular mechanism to therapeutic opportunities. Antioxid Redox Signal 15(7):1957–1997

Park E, Park SY, Wang C, Xu J, LaFauci G, Schuller-Levis G (2002) Cloning of murine cysteine sulfinic acid decarboxylase and its mRNA expression in murine tissues. Biochim Biophys Acta 1574:403–406

Park E, Park YS, Dobkin C, Schuller-Levis G (2014) Development of a novel cysteine sulfinic acid decarboxylase knockout mouse: dietary taurine reduces neonatal mortality. J Amino Acids 2014:346809. 1–11

Park E, Park SY, Dobkin C, Schuller-Levis G (2015) A novel cysteine sulfinic acid decarboxylase knock-out mouse: comparison between newborn and weanling mice. Adv Exp Med Biol 803:3–16

Roman HB, Hirschberger LL, Krijt J, Valli A, Kozich V, Stipanuk MH (2013) The cysteine dioxygenase knockout mouse: altered cyteine metabolism in nonhepatic tissues leads to excess H_2S/HS^- production and evidence of pancreatic and lung toxicity. Antioxid Redox Signal 19(12):1321–1336

Sanchez L, Calvo M, Brock JH (1992) Biological role of lactoferrin. Arch Dis Child 67(5):657–661

Schaffer SW, Azuma J, Mozaffari M (2009) Role of antioxidant activity of taurine in diabetes. Can J Physiol Pharmacol 87(2):91–99

Schuller-Levis G, Park E (2006) Is taurine a biomarker? Adv Clin Chem 41:1–21

Stipanuk MH (2004) Role of the liver in regulation of body cysteine and taurine levels: a brief review. Neurochem Res 29:105–110

Sturman JA (1993) Taurine in development. Physiol Rev 73:119–146

Sturman JA, Messing JM (1991) Dietary taurine content and feline production and outcome. J Nutr 121:1195–1203

Sturman JA, Messing JM (1992) High dietary taurine effects on feline tissue taurine concentrations and reproductive performance. J Nutr 122:82–88

Uchida S, Kwon HM, Yamauchi A, Preston AS, Marumo F, Handler JS (1992) Molecular cloning of the cDNA for an MDCK cell Na(+)- and Cl(−)-dependent taurine transporter that is regulated by hypertonicity. Proc Natl Acad Sci U S A 89:8230–8234

Ueki I, Roman HB, Valli A, Fieselmann K, Lam J, Peters R, Hirschberger LL, Stipanuk MH (2011) Knockout of the murine cysteine dioxygenase gene results in severe impairment in ability to synthesize taurine and an increased catabolism of cysteine to hydrogen sulfide. Am J Physiol Endocrinol Metab 301:E668–E684

Ueki I, Roman HB, Hirschberger LL, Junior C, Stipanuk MH (2012) Extrahepatic tissues compensate for loss of hepatic taurine synthesis in mice with liver-specific knockout of cysteine dioxygenase. Am J Physiol Endocrinol Metab 302:E1292–E1299

Ward PP, Conneely OM (2004) Lactoferrin: role in iron homeostasis and host defense against microbial infection. Biometals 17(4):204–208

Warsulat U, Heller-Stilb B, Oermann E, Zilles K, Haas H, Lang F, Haessinger D (2007) Phenotype of the taurine transporter knockout mouse. Methods Enzymol 428:439–458

Weaver LT, Wadd N, Taylor CE, Greenwell J, Toms GL (1991) The ontogeny of serum IgA in the newborn. Pediatr Allergy Immunol 2(2):72–75

Winge I, Teigen K, Fossbakk A, Mahootchi E, Kieppe R, SKoelberg F, Kaempe O, Haavik J (2015) Mammalian CSAD and GADL-1 have distinct biochemical properites and patterns of brain expression. Neurochem Int 90:173–184