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Dietary taurine manipulations in aged male Fischer 344 rat tissue: taurine concentration, taurine biosynthesis, and oxidative markers

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Abstract

Taurine (TAU) is a ubiquitous sulfur-containing amino acid that has been proposed to be an antioxidant. The concentration of TAU decreases during aging, which may increase susceptibility to oxidative stress. Our study attempted to elucidate the mechanism for the age-dependent decrease in TAU content by examining TAU biosynthesis in aged rats. We also examined the effects of dietary TAU manipulations on TAU content and oxidative markers in aged male Fischer 344 (F344) rats. Adult (9 months) and aged (26 months) rats fed control diets, aged rats fed control diet and TAU-supplemented (1.5%) water, and aged rats fed a TAU-deficient diet were used. We observed a significant age-related decrease in TAU content in liver, kidney, and cerebellum. Dietary TAU supplementation increased tissue TAU content, whereas dietary TAU restriction had no effect. Enzyme-dependent TAU synthesis showed an age-dependent reduction in liver that was decreased further by TAU supplementation. Protein carbonyl content was elevated in the cerebral cortex and kidney of aged rats and was attenuated by TAU supplementation. A trend for a decrease in protein and acid-soluble thiol contents in hepatic tissue of aged rats was observed, and this was attenuated with dietary TAU supplementation. Our data show that a decrease in hepatic TAU biosynthesis may cause, in part, the observed decline in tissue TAU content in aged F344 rats, and TAU supplementation can restore TAU levels. Our study indicates that a decline in TAU content may exacerbate oxidative stress in aged rats, which can be reversed by dietary TAU supplementation.

Keywords: Taurine; Aging; Cysteine sulfinic acid decarboxylase; Cysteine dioxygenase; Oxidative stress

1. Introduction

Several studies have shown that TAU content declines significantly in serum [1–3] and tissues of 26- to 30-month-old F344 rats [1] as well as in several brain areas in 29-month-old rats [4,5]. Reports indicate that elderly patients (61–81 years) have significantly lower (43%) blood TAU concentration compared with younger individuals (20–38 years) [6]. This decline could exacerbate the oxidative damage that occurs during the aging process, if TAU indeed functions as an antioxidant as suggested by many studies [7–13]. The reason for such a decline of TAU remains to be fully elucidated. Possible mechanisms for this age-dependent decline in TAU levels could be increased renal excretion or decreased biosynthesis.

Previous work in our laboratory has shown that aged (26 months) F344 rats have lower urinary TAU excretion than adult rats (10 months) [2]. Corman *et al.* [14] demonstrated a similar age-related decline in urinary TAU excretion in female Wistar rats. This suggests that aged animals are actually able to conserve TAU, probably via a renal adaptive mechanism such as demonstrated in LLC-PK_1 and MDCK cells where exposure to a TAU-free medium increased Na^+-dependent TAU transport [15]. Other studies have shown an up-regulation in renal TAU transport in response to decreased dietary intake of this amino acid or disturbed TAU homeostasis [16–18]. Our previous data suggest that despite an age-related decline in renal function, the decline in TAU concentration is not due to renal dysfunction, since aged rats can renally conserve TAU [2,19].

The other possibility for the observed decline in tissue and serum TAU may be a decline in the activity of one or

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Abbreviations: CA, cysteic acid; CSA, cysteine sulfinic acid; CSD, cysteine sulfinic acid decarboxylase; CDO, cysteine dioxygenase; DNPH, 2,4-dinitrophenylhydrazin; DPPH, α,α-diphenyl-β-picrylhydrazyl; F344, Fischer 344; HPLC-ECD, high performance liquid chromatography with electrochemical detection; MDCK, Madin Darby canine kidney; PCA, perchloric acid; TAU, taurine; TBARS, thiobarbituric acid; and TCA, trichloroacetic acid.

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more biosynthetic enzymes. CSD (EC 4.1.1.29) seems to be the rate-limiting enzyme in TAU biosynthesis in the liver [20–22]. This enzyme is responsible for the enzymatic transformation of CSA to hypotaurine and CA to TAU [22] and has a high specificity for CSA and CA [23]. CSD is a pyridoxal phosphate-dependent enzyme [24], and studies have shown that its activity can be down-regulated by several factors such as thyroid hormone [25], estrogen [21], and high-protein diets [26–29]. CDO (EC 1.13.11.20) is another key enzyme involved in TAU biosynthesis [22]. This enzyme is the major route for sulfur amino acid metabolism in the liver [22], but is also found in large quantities in the brain [29,30]. CDO is important in the regulation of amino acids such as cysteine, methionine, and the peptide GSH [26,27]. CDO activity also can be regulated by dietary protein and sulfur amino acids [31,32] and hormones such as glucagon [33] and hydrocortisone [34]. Changes in TAU levels might also have an effect on GSH levels and vice versa, since TAU and GSH synthesis are tightly coupled, sharing the same precursor, cysteine [5,35,36]. Therefore, CSD and CDO are important enzymes in what is believed to be the most important TAU biosynthetic pathway in mammals, and an age-dependent down-regulation in either or both of these enzymes could lead to a decline of TAU concentration in body tissues and fluids.

The purpose of this study was to test if aging has a depressive effect on the major biosynthetic enzymes for TAU, CSD and/or CDO. These enzymes may be subject to changes, such as age-dependent post-translational changes, altering their activity and/or substrate specificity [37,38]. Tissues from male adult (9 months) and aged (26 months) F344 rats on control, TAU-supplemented, or TAU-deficient diets were used in our experiments. We tested the hypothesis that aging decreases enzyme activity, resulting in decreased tissue TAU content. We expected that dietary TAU supplementation would increase tissue TAU content but would cause a further decline in enzyme activity due to a negative feedback mechanism, whereas TAU-deficient diets would increase enzyme activity. We also measured protein oxidation and thiol levels in tissues of these animals, expecting to see an increase in protein oxidation and a decline in thiol content in aged animals. We hypothesized that TAU supplementation would decrease the levels of oxidative products and conserve thiol content, whereas dietary TAU deprivation would have the opposite effect.

2. Materials and methods

2.1. Materials

Drugs, buffer, and HPLC reagents were obtained from the Sigma Chemical Co. or Fisher Scientific. HPLC supplies were purchased from Alltech, BAS, or Varian.

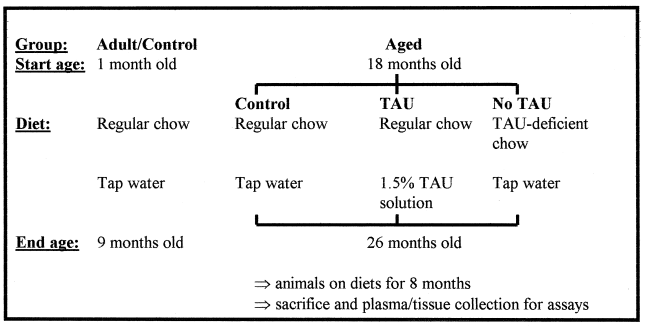

Fig. 1. Outline of the experimental design. The following assays were performed: CSD and CDO activities, protein oxidation (protein carbonyls), lipid peroxidation (TBARS), thiol content, and total serum antioxidant activity. Explanation of the measurements of each oxidation injury test can be found in section 2.

2.2. Animals and dietary treatment

Male F344 rats were purchased from Harlan-Sprague Dawley, Inc. at 1 and 18 months of age. The rats underwent dietary treatment for 8 months. Chows were purchased from Purina Mills, Inc. Animals were decapitated at the ages of 9 months (adult) and 26 months (aged). The experimental groups were as follows: adult animals fed regular rodent diet (Purina No. 5012) and tap water (adult/control), aged animals fed regular rodent diet and tap water (aged/control), aged rats fed regular rodent diet and tap water supplemented with 0.12 mol/L of TAU (1.5%; aged/TAU), and aged rats fed TAU-deficient diet (Purina No. 5729-M) and tap water (aged/no TAU).

The TAU-deficient diet was identical to the regular rodent diet except that the TAU-deficient diet contained soy-derived protein instead of fishmeal to eliminate the TAU source. The content of the precursor, methionine, was the same in the regular and TAU-deficient diets, 0.43 and 0.45%, respectively. We tested the chow for TAU content; the TAU-deficient diet was below our limit of detection ($<0.004\%$), whereas the regular diet contained 0.029% TAU. The F344 rats had free access to food and water and were kept on a 12 hr/12 hr light/dark cycle (6:00 a.m.–6:00 p.m.). The terminal weights of the adult/control, aged/control, aged/TAU, and aged/no TAU groups were (in g) 365 ± 9, 372 ± 15, 387 ± 11, and 367 ± 7, respectively, which were not statistically different. Organs, tissues, and brain areas were dissected after the rats were decapitated. Tissue samples were frozen at $-80°$ and serum at $-20°$ until further analysis. TAU content was measured in several tissues and serum, using HPLC-ECD as previously described [1]. CSD and CDO activities, protein oxidation (protein carbonyls), lipid peroxidation (TBARS), and thiol content were measured in several tissues as described below. Figure 1 shows the outline of the experimental design.

2.3. CSD activity

Tissue samples were weighed and homogenized in a 0.05 mol/L phosphate buffer, pH 6.8, with a Brinkmann tissue homogenizer (Brinkmann Instruments Co.) for about 30 sec at a setting of 4 and centrifuged at 21,000 *g* for 15 min at 4° using a Beckman Centrifuge model J2–21 (Beckman Instruments, Inc.). The supernatant was used for the enzyme assay. The assay is a modified version of that from Daniels and Stipanuk [39]. Briefly, the assay was carried out in a total volume of 0.5 mL and contained (final concentrations): 15 mmol/L of l-glutamate, 25 mmol/L of CSA, 0.8 mmol/L of pyridoxal phosphate, and 0.55 mmol/L of dithiothreitol in phosphate buffer, pH 7.0. The glutamate was added to inhibit glutamic acid decarboxylase activity on the substrate, CSA. The final assay volume contained 0.1 mL of supernatant from the above-mentioned centrifugation step. The mixture was incubated in a shaking 37° water bath for 30−60 min. Blanks contained 0.5 mL of 0.61 mol TCA/L, which was also used to stop the reaction along with incubating samples in an ice bath. The reaction mixture was centrifuged at 2000 *g* for 20 min at room temperature, and the supernatant was frozen at −20° until further purification and analysis. To analyze total CSD activity, hypotaurine was oxidized to TAU before analysis. A 0.5-mL fraction of the thawed supernatant was neutralized with NaOH/HCl as shown by the indicator solution and incubated with 0.05 mL of 30% H_2O_2 in the 37° water bath for 15 min. This mixture was then treated with 0.025 mL of a catalase solution (900 Sigma Units/0.025 mL of 1.0 mol/L potassium phosphate buffer, pH 7.1) and incubated for 10 min at room temperature. The mixture was then heated in boiling water for 3 min to denature the catalase and microcentrifuged for 3 min at room temperature to remove the denatured protein. A 0.5-mL aliquot was applied to a Dowex-50 W column (Sigma, strongly acidic cation exchanger) and eluted with 2 mL of water. A fraction of this eluent was analyzed by HPLC-ECD. The samples were considered to have enzyme activity if the TAU concentration was at least twice the concentration in the blanks. Data are expressed as nanomoles TAU per minute per milligram protein.

2.4. CDO activity

CDO activity was assayed by the method of Bagley and Stipanuk [26,31]. Tissue was weighed and homogenized in 50 mmol/L of 2-[*N*-morpholino]ethanesulfonic acid (MES) buffer, pH 6.0, using a Brinkmann tissue homogenizer. A 0.3-mL sample of the homogenate was incubated for 60 min at 37° in a shaking water bath in a final assay volume of 1 mL containing (final concentrations): 0.5 mmol/L of ferrous ammonium sulfate, 5 mmol/L of hydroxylamine-HCl, 2 mmol/L of $NAD^{+,}$ and 5 mmol/L of cysteine (20 mmol/L stock solution contained 0.05 mmol/L of bathocuproine disulfonate to prevent cysteine oxidation). The reaction was terminated by cooling the samples in an ice-cold water bath and the addition of 0.1 mL of 3 mol PCA/L. The samples were then centrifuged at 2000 *g* for 20 min at 4° and the supernatants were extracted using Dowex-50 (Sigma) columns to remove cations. The eluents were stored at −20° until further analysis by HPLC-ECD for CSA and CA since some of the CSA is oxidized to CA during storage [40]. Data are expressed as nanomoles or picomoles CSA per minute per milligram protein.

2.5. Chromatography

Samples from the above-described enzyme assays or tissue PCA extracts were assayed for amino acid content. Samples were derivatized before HPLC-ECD analysis by reacting 200 μL of the sample with 175 μL of a 9 mmol/L *o*-phthalaldehyde, 0.5% 2-mercaptoethanol solution in a 0.1 mol/L disodium borate solution (pH 10.0) for exactly 3 min. The derivatization reaction was terminated by adding 25 μL of 0.4 mol/L of iodoacetamide and 400 μL mobile phase. Iodoacetamide reacts with unreacted *o*-phthalaldehyde and diminishes electrochemical interference. Sample TAU, CSA, and CA concentrations were calculated based on external standards by a Hewlett Packard integrator.

The protocol for CSA and CA measurements was a modified version of a previously described method [41]. The mobile phase was 0.1 mol/L of sodium phosphate (dibasic), 0.13 mmol/L of Na_2EDTA, and 10% methanol, pH 6.0. The mobile phase for measuring TAU consisted of 0.08 mol/L of sodium phosphate (monobasic), 0.1 mmol/L of Na_2EDTA, 10% tetrahydrofuran, 10% acetonitrile, and 2% 1-propanol, pH 5.6, as previously described [1]. The mobile phase was vacuum filtered using Magna-R, supported nylon filters (0.45 μm, MSI) and degassed for 20 min under helium. The HPLC system consisted of a PM-11 pump, an LC-4B amperometric detector (both from Bioanalytical Systems), and a Rheodyne model 7125 injector with a 20- or 50-μL fixed loop. The columns used were Microsorb-MV, C-18, 3 μm, 4.6 mm i.d. × 10 cm columns from Rainin. Ag/AgCl reference electrodes and glassy carbon working electrodes with an applied voltage of 0.725 V were used for electrochemical detection. The detector sensitivity was between 5 and 50 nA, the flow rate was 0.7 mL/min, and the columns were at room temperature.

2.6. Protein assay

Protein content was assayed by the method of Bradford [42]. Protein values of the samples were calculated from a standard curve made with bovine serum albumin from the range of 0.063 to 1.0 mg/mL.

2.7. Protein oxidation

Oxidative damage to proteins was measured using the method of Levine *et al.* [43], which measures the carbonyl content in oxidized proteins. Introduction of carbonyl

groups into proteins is taken as presumptive evidence of oxidative modification [43]. Tissue samples from F344 rats were homogenized using a Brinkmann tissue homogenizer in a 50 mmol/L phosphate buffer, pH 7.4, containing: 1 mmol/L of Na_2EDTA, 0.5 mg/mL of aprotinin, and 40 mg/mL of phenylmethylsulfonyl fluoride. Tissue samples were divided into four 1-mL aliquots, two for blank readings and two for total reading. Nucleic acids were removed from homogenized tissue samples by adding streptomycin sulfate (1% final concentration). Samples were incubated at room temperature for 15 min and centrifuged at 6000 *g* for 10 min at room temperature. A 10 mmol/L DNPH solution (4 mL) in 2 mol/L of HCl or 2 mol/L of HCl only (4 mL) was added to the supernatants of totals and blanks, respectively. Samples were incubated for 1 hr in the dark and vortexed every 15 min. The derivatization reaction was terminated by adding 5 mL of 1.22 mol TCA/L (0.61 mol/L final concentration) and incubating the samples on ice for 10 min. Samples were centrifuged for 5 min at 1600 *g* at room temperature, and the pellets were washed three times with ethyl acetate/ethanol (1:1, v/v) to remove excess DNPH. The pellet was dissolved in 2 mL of 6 mol/L guanidine HCl in a water bath at 37° with frequent vortexing. Any insoluble material was removed by a final centrifugation step for 5 min at 1600 *g* at room temperature. The supernatants were read at 370 nm using a Beckman DU 7000 diode array spectrophotometer (Beckman Instruments, Inc.). The carbonyl content was calculated by using the absorbance at 370 nm and an absorption coefficient ϵ of 22,000 M^{-1} $cm^{-}1$. These carbonyl values were normalized with protein values of the blank samples since 10–15% of the proteins are lost in this assay. The absorption of the blanks was measured at 280 nm. Protein concentrations were calculated using a standard curve to bovine serum albumin in 6 mol/L of guanidine HCl ranging from 0.25 to 2.00 mg/mL. Data are expressed in nanomoles per milligram protein (mean ± SEM).

2.8. Lipid peroxidation

Lipid peroxidation was estimated using the TBAR test as described previously [44]. TBAR formation is a measurement of thiobarbituric acid reactive substances such as malondialdehyde, which forms during lipid peroxidation. Briefly, TBAR formation was quantitated using 1,1,3,3-tetraethoxypropane as a standard, and the absorbancy of the TBARS was read at 530 nm using a Beckman DU 700 diode array spectrophotometer. Data are expressed in nanomoles per milligram protein (mean ± SEM).

2.9. Tissue thiols

Thiol content was measured as previously described by Jocelyn [45]. Protein thiols represent the unoxidized cysteine moieties in proteins, and acid-soluble thiols are a measure of cytosolic GSH, cystine, and cysteine. Tissues or homogenates were thawed and homogenized in 20x vol. of ice-cold 0.61 mol TCA/L using a Brinkmann tissue homogenizer. The homogenates were diluted 1:1 with 1.22 mol TCA/L. The homogenates were centrifuged at 18,000 *g* for 20 min at 4°. Supernatants were used to measure soluble thiol content. Pellets were rehomogenized in the same volume of the original homogenate using 5 mmol/L of phosphate buffer with 1% SDS. A 25- to 100-μL aliquot of the sample and 475–400 μL of 1% SDS in 5 mmol/L phosphate buffer for a total of 500 μL were added to semi VIS cuvettes. Then 5,5′-dithio-bis(2-nitrobenzoic acid) (10 mmol/L, 500 μL) was added to the cuvettes, and the samples were incubated (15 min) at room temperature and read at 412 nm using a Beckman DU 700 diode array spectrophotometer. Thiol content was calculated using a standard curve to cysteine that ranged from 0 to 100 nmol. Soluble thiols were measured using the supernatants from the centrifugation step above. The supernatants were diluted 1:1 with HPLC grade H_2O, 500 μL of the diluted sample was added to 500 μL of 10 mmol 5,5′-dithio-bis(2-nitrobenzoic acid)/L, and the samples were read as described above. Samples were normalized using protein content, which was measured using the method of Bradford [42].

2.10. Total serum antioxidant capacity

Serum antioxidant capacity was measured by a method derived by Glavind [46]. Briefly, a stable free radical, DPPH, is added to serum samples and read spectrophotometrically at the absorbances of 540 and 405 nm. Antioxidants in the sample bind to DPPH causing a reduction in the absorbance at 540 nm, and the antioxidant–DPPH product causes an increase in the absorbance at 405 nm.

2.11. Statistics

Data were analyzed using a one-way ANOVA with the Tukey–Kramer multiple comparison test to compare differences among groups. If Bartlett's test for homogeneity of variance was significant, data were either transformed (log or reciprocal) or a Kruskal–Wallis nonparametric ANOVA test was used followed by Dunn's multiple comparisons test. Age-dependent differences were analyzed using unpaired *t*-tests or a non-parametric Welch's approximate *t*-test. Data with a *P* value of less than 0.05 were considered significant. The programs used for statistical analysis were InStat®, version 2.0, or Graph Pad Prism™, version 2.0 (Graph Pad Software).

3. Results

3.1. Effect of dietary TAU manipulation on enzyme activity and tissue TAU concentration in aged F344 rats

A significant age-dependent decrease in TAU concentration was observed in liver, kidney, and cerebellum of F344

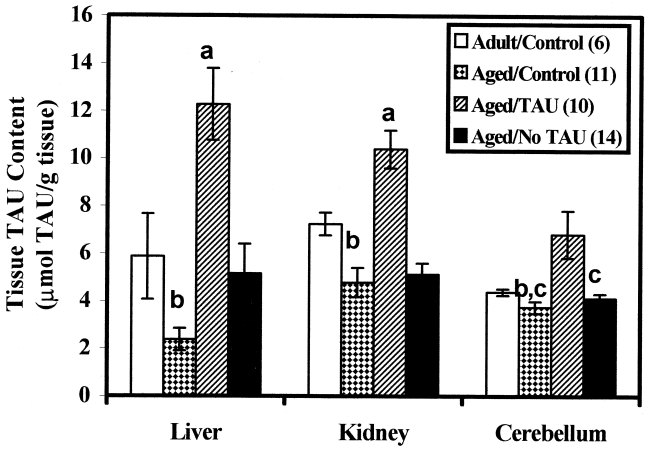

Fig. 2. Tissue TAU content in liver, kidney, and cerebellum of F344 rats on different diets. Data are presented in μmol TAU/g tissue (mean ± SEM). The number of samples is indicated in parentheses after each group. The number refers to liver and cerebellar tissues; the N for kidney tissue was 6 per group. Key: (a) $P < 0.05$ vs all other groups, (b) $P < 0.05$ vs adult/control, and (c) $P < 0.05$ vs aged/TAU.

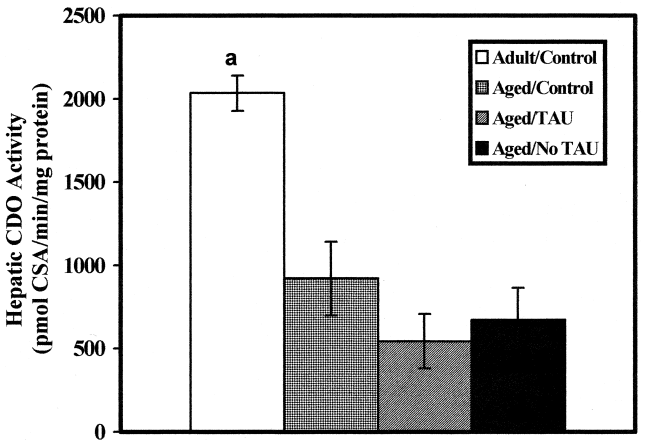

Fig. 4. Hepatic CDO activity in liver homogenates of F344 animals. Data are presented in pmol CSA/min/mg protein (mean ± SEM). The number of samples per group is the same as in Fig. 2. Key: (a) $P < 0.05$ vs all other groups.

animals (Fig. 2). The TAU concentration in these tissues was significantly higher in supplemented rats when compared with the aged/control and aged/no TAU rats (Fig. 2). TAU-deficient diets did not cause the expected decrease in TAU levels when compared with aged/control rats.

The CSD activity in the liver was significantly lower ($P < 0.05$) in the aged rats versus the adult/control rats, regardless of the diet consumed (Fig. 3). Dietary TAU supplementation depressed enzyme activity even further in the liver, showing a significantly lower ($P < 0.01$) activity versus the aged matched rats on control diets (Fig. 3). There was no significant age-dependent decrease in enzyme activity in the cerebellum (Fig. 3). CSD activity in the kidney could not be determined since total TAU production did not exceed the basal values by a sufficient (2-fold) amount to accurately estimate enzyme activity. CDO activity was measured in all four treatment groups in liver tissue, and, as seen in CSD activity, CDO activity in the liver declined in an age-dependent manner across the dietary treatment groups (Fig. 4).

3.2. Effect of aging and dietary TAU manipulation on oxidative markers in male F344 rats

Protein carbonyls were measured in the cerebral cortex, liver, and kidney. In cerebral cortex, there was a significant ($P < 0.01$) age-dependent increase in carbonyls (Fig. 5). Dietary TAU supplementation decreased carbonyl content by 19%, whereas TAU deprivation increased it by 15%

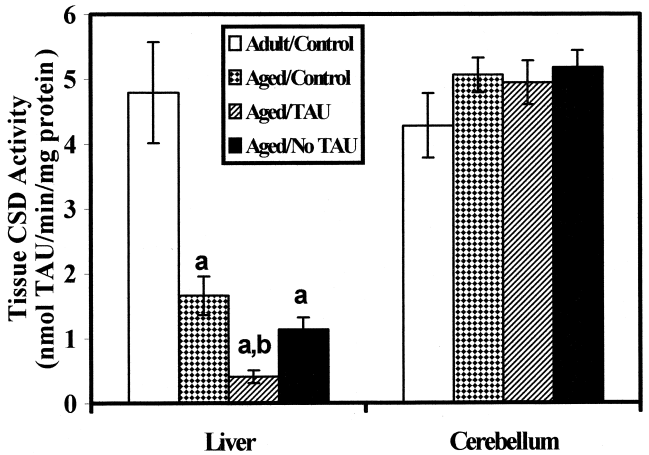

Fig. 3. CSD activity in liver and cerebellar tissue of male F344 rats. Data are presented in nmol TAU/min/mg protein (mean ± SEM). The number of samples per group is the same as in Fig. 2. Key: (a) $P < 0.05$ vs adult/control, and (b) $P < 0.01$ vs aged/control.

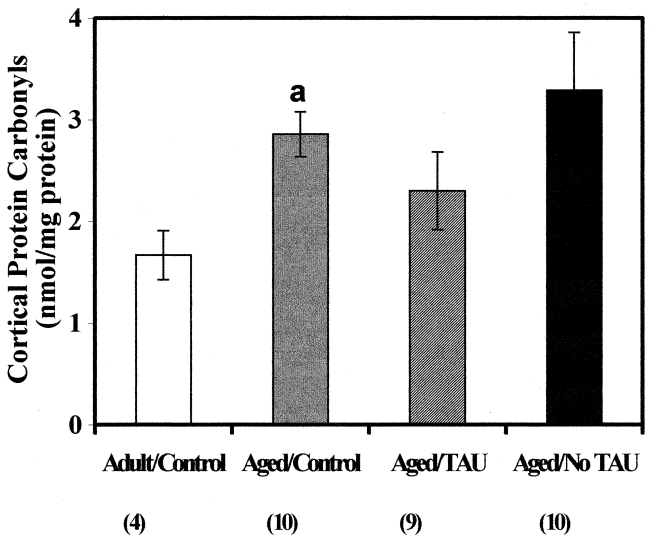

Fig. 5. Protein carbonyls (protein oxidation) in homogenates of cerebral cortex from F344 rats. Data are presented in nmol/mg protein (mean ± SEM). Numbers in parentheses designate the number of samples per group. Key: (a) $P < 0.01$ vs adult/control.

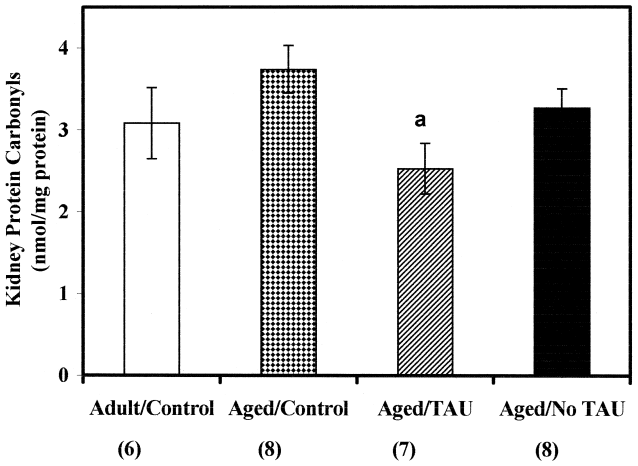

Fig. 6. Protein carbonyl measurements (protein oxidation) from kidney homogenates of F344 rats. Data are presented in nmol/mg protein (mean ± SEM). Numbers in parentheses designate the number of samples per group. Key: (a) $P < 0.05$ vs aged/control.

compared with the aged/control group (Fig. 5). TAU supplementation blunted the increase in cortical protein carbonyls. Age or dietary TAU manipulations did not have an effect on protein carbonyl content in the liver tissue of these rats (data not shown). We found an age-related increase in protein carbonyls in the kidney, and dietary TAU supplementation significantly decreased the amount of protein carbonyls in these rats (Fig. 6).

Protein thiols and acid-soluble thiols were measured in cerebral cortex and liver (Table 1). There was no significant age- or diet-dependent effect in hepatic or cortical tissue (Table 1); however, aged/control animals had a small but consistent (15%) decrease in protein thiol content in the liver. Likewise, protein thiol content was reduced about 30% in the kidney of aged rats, but this reduction was also not statistically significant (Table 2). A similar age-dependent decline (14%) in acid-soluble thiol content was observed in the liver (Table 1), which was not significant, however. TAU supplementation increased acid-soluble thiol content in liver tissue by 37% compared with the aged/control group, but this increase was not significant due to the high variability in the aged/TAU group (Table 1). Protein or acid-soluble thiols in cerebral cortex were not altered by age or dietary treatments (Table 1). TBARS in the kidney were also unaffected by age or diet (Table 2). In addition, age or dietary manipulations did not have a significant effect on the total antioxidant capacity of the serum of the F344 rats. The absorbance at 540 nm was not higher in aged animals or lower in TAU-treated animals compared with controls after the addition of the free radical DPPH to serum samples (data not shown). The other measurement, absorbance at 405 nm, also did not differ due to diet or age (data not shown).

4. Discussion

These studies show that the TAU concentration in liver, kidney, and cerebellum was decreased significantly in male aged F344 rats and that dietary TAU supplementation restored or even increased tissue TAU content when compared with adult levels (Fig. 2). Our observations of an age-related decline in tissue TAU concentration support previous reports [1,4,5]. The results from the dietary manipulation are similar to the results from another dietary TAU supplementation study [47] where male albino rats received a 17% casein diet supplemented with 1% TAU 2 weeks after weaning. This diet significantly increased TAU content in the kidney after 2 weeks and in the liver after 4 weeks. An unexpected observation in our study was that long-term dietary TAU restriction did not further exacerbate the age-related decline in tissue TAU content. In the liver, it actually seemed to blunt the age-dependent decline. Unlike humans and cats, rats are prolific TAU synthesizers [22,48], and only restricting dietary TAU does not seem to affect tissue and serum TAU concentrations. Other dietary protocols that include addition of TAU uptake inhibitors such as β-alanine or guanidinoethane sulfonate [22] or restriction of TAU precursors might be necessary to severely deplete rats of TAU. It seems that rats are able to compensate for the loss of TAU in the diet by increasing TAU production in individual tissues or in the liver, the major biosynthetic organ for *in vivo* synthesis [22,49]. Possible mechanisms of compensation could be decreasing renal TAU loss [19] or increasing enzymatic TAU production. The kidney is partly responsible for TAU homeostasis since it can control urinary TAU excretion [16–18,49,50]. Aged F344 rats show renal TAU conservation by decreasing urinary TAU excre-

Table 1
Protein thiols and acid soluble thiols in hepatic and cortical tissue of F344 rats

Treatment	Liver		Cortex	
	Protein thiols (nmol/mg protein)	Acid-soluble thiols (nmol/mg protein)	Protein thiols (nmol/mg protein)	Acid-soluble thiols (nmol/g tissue)
Adult/control	142 ± 11 (6)	5.8 ± 0.8 (3)	127 ± 22 (5)	354 ± 45 (6)
Aged/control	121 ± 10 (9)	5.0 ± 0.6 (5)	129 ± 17 (9)	407 ± 40 (8)
Aged/TAU	126 ± 10 (8)	8.0 ± 2.1 (5)	128 ± 16 (7)	453 ± 49 (10)
Aged/no TAU	119 ± 12 (9)	4.8 ± 1.1 (7)	134 ± 18 (10)	465 ± 39 (11)

Data are presented as means ± SEM. Numbers in parentheses represent the number of animals.

Table 2
Oxidative markers in renal tissue of F344 rats

Treatment	Protein thiols (μmol/g tissue)	TBARS (nmol/mg protein)
Adult/control	15.7 ± 1.95 (3)	0.66 ± 0.10 (5)
Aged/control	10.7 ± 1.01 (8)	0.63 ± 0.10 (9)
Aged/TAU	11.5 ± 1.43 (10)	0.53 ± 0.03 (7)
Aged/no TAU	11.0 ± 1.51 (11)	0.58 ± 0.04 (7)

Data are presented as means ± SEM. Numbers in parentheses represent the number of animals.

tion as observed in a metabolic study of animals at 20 and 26 months of age [2,19]. This study also showed that TAU-deficient diets significantly increased renal TAU conservation compared with control animals even further at 20 months of age. The difference due to dietary TAU restriction, however, disappears at 26 months of age since renal conservation seems to be maximized. At this age, animals must compensate for the decrease in dietary TAU intake via a *de novo* biosynthetic pathway.

We observed a significant age-dependent decrease in liver CSD activity, which is believed to be the rate-limiting step in TAU biosynthesis [20–22]. This means that the observed decline in tissue TAU content in F344 rats might be due to a decline in *de novo* hepatic TAU synthesis. In a previous study [51], however, we also observed an age-related decline in hepatic CSD activity in F344 rats but not in other strains (FBNF1, Sprague–Dawley) used in these studies, whereas we observed a similar decline in tissue and plasma TAU content across strains. Preliminary studies in our laboratory using western blot analyses did not show an age-dependent decline in CSD protein [unpublished findings]. CSD protein may be affected by post-translational processes during aging such as oxidative modifications that could render the enzyme less active or inactive [37]. We did observe a similar age-related decline in CDO activity in all strains [51], which might suggest that CDO activity is a better predictor of tissue TAU status in aging. Another important consideration in these studies is that we measured enzyme activity at high substrate concentrations, and we observed a significant decline in CSD activity presumably near its $V_{max.}$ The CSD activity present endogenously might be enough to maintain TAU levels, and the age-dependent decline in TAU levels may be due to another change in the TAU biosynthetic pathway.

Methionine and cysteine, TAU precursors, comprised 0.43 and 0.45% and 0.33 and 0.35% of the TAU-deficient and control diet, respectively. This means that the TAU-deficient diet is not depleted of TAU precursors, and TAU biosynthetic enzymes can produce TAU at the same or a higher rate under these TAU-deficient conditions. Early studies have shown that tissue and plasma TAU content fails to decline in rats fasted for up to 9 days and even increased in some tissues such as liver and muscle [52,53]. The compensation or even up-regulation of TAU content in certain tissues observed in the absence of dietary TAU might be due to an unidentified enzymatic up-regulation or the up-regulation of the tissue TAU transporter. Other observations, such as tissues with high TAU content but low or undetectable CSD activity such as skeletal muscle and heart [54], also support the concept of an alternate biosynthetic pathway for TAU or transporter-mediated redistribution of tissue TAU pools. It has been suggested that cysteamine is the main precursor for TAU synthesis in these tissues [55,56]. Other researchers also have suggested the presence of an alternate inducible biosynthetic pathway since they failed to show a decline in brain and tissue TAU concentrations in rats that were depleted of pyridoxal phosphate, the coenzyme for CSD [57,58]. This suggests a possible TAU biosynthetic pathway via an alternate CSD-independent mechanism that is stimulated under the conditions of dietary TAU deficiency. Dietary TAU deficiency also may increase TAU transporter activity as recently shown by Han *et al.* [16–18]. TAU transporter activity in the kidney seems to be manipulated at the genomic level, increasing mRNA transcription under low dietary TAU conditions and decreasing it under high dietary TAU content. This manipulation could possibly occur via a gene–nutrient interaction. Hence, low dietary TAU conditions could enhance endogenous TAU production via an alternate TAU biosynthetic pathway and increase TAU transporter activity by increasing transcription and translation of the TAU transporter in different tissues.

Our study also showed that the main biosynthetic enzyme for TAU, CSD, is subject to endproduct feedback inhibition in the liver, since dietary TAU supplementation caused a significant decline in hepatic CSD activity. Previous studies have shown that hepatic CSD and CDO activities can be modified by changing dietary intake of sulfur amino acids and/or protein [26–28,31]. In these studies, the regulation seemed to be "upstream" from TAU and to affect various enzymes responsible for cysteine metabolism such as CSD, CDO, and γ-glutamylcysteine synthase. Our studies, on the other hand, showed direct hepatic CSD regulation by TAU, one of the endproducts of cysteine metabolism. Other studies have also examined the effect of TAU supplementation on TAU biosynthetic enzymes. One study using infant Rhesus monkeys showed no change in CSD or CDO activity in brain or liver after feeding the animals a formula with a TAU supplementation of 50 μmol/100 mL for 6 or 12 months [55]. Furthermore, Sturman *et al.* [55] found that a diet free of TAU failed to increase either CDO or CSD activity. This observation is in agreement with our results. Another study using freshly weaned male albino rats and a dietary TAU supplementation of 1% only showed a decline in CSD activity in kidney, but not in liver or brain after 2 or 4 weeks on the diet [47]. A direct comparison of the results obtained by Sturman *et al.* [55] and Loriette *et al.* [47] with our data is difficult due to differences in experimental design. However, all of these studies agree with our

observation that dietary TAU manipulation had no effect on CDO activity.

CSD activity in the cerebellum was not affected by aging in F344 rats. Even though some researchers show colocalization of TAU and CSD in neuronal cells in the brain [59,60], recent studies have localized CSD protein in glial cells in the CNS [61,62] by double immunofluorescence staining. Therefore, the loss of TAU in the cerebellum could be a reflection of neuronal loss, and CSD would not be lost due to its localization in astrocytes. Another explanation for the decline in brain TAU content in aging might be a decline in transport capacity at the blood–brain barrier or a down-regulation of another "upstream" enzyme responsible for the availability of precursors, such as the biosynthesis of cysteine from methionine or an enzymatic step of an alternate TAU-producing biosynthetic pathway.

Our study also replicated previous studies that found a significant increase in protein carbonyls in brain tissue during aging [63–66]. There was a significant increase in protein carbonyls in cortical tissue of aged F344 rats when compared with adult animals. We also observed a subtle non-significant age-dependent increase in oxidized proteins in kidney tissue, whereas there was no age- or diet-related change in the liver. Since TAU may function as an antioxidant, dietary TAU supplementation should attenuate this age-related increase in oxidative protein damage. We observed a blunting of the age-related increase of oxidative products in the cerebral cortex and a significant attenuation in the kidney in aged rats given oral TAU supplementation compared with age-matched controls. The results observed in cortical and renal tissue match the expectations, which were that an age- and/or diet-dependent change in protein carbonyls correlated with changes in TAU content. There was a significant age-related decline in TAU concentration in the kidney and a non-significant decline in the cerebral cortex. TAU supplementation can significantly increase TAU content in both of these tissues ([19]; Fig. 2). These data support previous observations that TAU acts as an antioxidant *in vivo* [11–13]. Removing TAU from the diet did not exacerbate markers of oxidative stress in aged rats. The reason for this might be the fact that we were unable to actually deplete these tissues of TAU with the TAU-deficient diet due to a possible compensatory mechanism active under these conditions in rodents. In contrast, humans do not have the biosynthetic capacity for TAU that rodents exhibit [67].

Thiol groups are reducing agents and an indirect measure of oxidative damage. Lower amounts of thiols are indicative of an increase in oxidative stress and damage [68]. Protein thiols did not show a significant age- or diet-dependent change in renal, hepatic, or cortical tissues of male F344 rats. Thus, increased dietary TAU did not appear to result in a significant shunting of cysteine into glutathione synthesis. Dietary TAU deficiency also did not have an effect on thiol content. Dietary TAU has been shown to improve antioxidant defense in rats, since it lessens the decline in lung GSH in response to a tumor necrosis factor α injection [69], and a recent study has also found that TAU could blunt an increase in oxidized GSH seen in diabetes without altering total GSH levels [70]. Our observation in aged rats does not support this; however, a trend for an increase in soluble thiol in livers might suggest that dietary TAU may improve antioxidant defenses. Measurements for total serum antioxidant capacity failed to show a significant age- or diet-related effect, even though serum TAU levels in these F344 rats were decreased with aging and increased in TAU-supplemented rats [2]. This supports a previous suggestion by Aruoma *et al.* [71] that TAU is not a good direct free radical scavenger. Future studies, however, should measure oxidized and reduced GSH levels directly in response to chronic dietary TAU manipulation to better understand the antioxidant status under these dietary conditions. The observation that dietary TAU supplementation significantly decreased carbonyl levels in kidney and cortex indicates that this treatment can improve weakened antioxidant defenses during aging in certain tissues.

TAU and GSH biosynthesis are closely related because they have the precursor cysteine in common [5,36]. This fact could lead to an increase in acid-soluble thiols, which mainly represent GSH, in response to an increase in dietary TAU and TAU feedback inhibition on CSD and CDO. Strolin Benedetti *et al.* [5] have argued previously that the age-dependent decline in brain TAU might be due, in part, to an increased need for GSH. GSH levels decline during aging [72,73], possibly because GSH detoxifies hydrogen peroxide produced by increased monoamine oxidase [5] and accumulating reactive oxygen species. This could increase the flow of cysteine down the enzymatic pathway, resulting in GSH production. Indeed, Strolin Benedetti *et al.* [74] showed that lifelong treatment with monoamine oxidase inhibitors eliminates the age-dependent decline of TAU in rat brain. Further studies need to be completed to elucidate the cause-and-effect relationship between TAU and GSH during aging and dietary TAU manipulations.

In conclusion, we showed that simply elevating dietary TAU intake can reverse the decline in tissue and serum TAU content observed during aging in F344 rats. This might have therapeutic implications since many beneficial functions, such as Ca^{2+} flux modulation, osmoregulation, and antioxidation [22], have been proposed for TAU. Indeed, we did show that oxidative markers are increased during aging and that elevation of TAU content via dietary supplementation attenuated some age-dependent markers of oxidative damage. This might be due to a combination of direct and indirect effects: (a) the direct antioxidant effects of TAU on protein or DNA [44,75], (b) the direct inhibitory effect of TAU on cytotoxic quinones and ROS formation [44], and (c) the indirect effect of TAU on the increase in GSH, a crucial endogenous antioxidant. A decline of TAU during aging and a loss of these functions might exacerbate the aging process. The mechanism responsible for this observed decline is still unclear, but might be due, in part, to

a decline in CSD and CDO-dependent biosynthesis in F344 rats. Since dietary TAU supplementation significantly elevated tissue TAU content in aged rats, it is unlikely that an age-related deficit in intestinal absorption of TAU is a major contributor to TAU deficiency in aging. Alternative explanations for the age-dependent decline could be a selective TAU sequestration in muscle tissue and/or an increase of GSH production decreasing TAU precursor availability that could lead to a decline of TAU in other tissues. TAU utilization in detoxification and/or conjugation reactions might also increase in aging and contribute to a decline in tissue content. We further showed that dietary TAU restriction in rodents does not seem to be detrimental since it did not induce a further decrease in tissue TAU content compared with age-matched controls. It seems, however, that this dietary condition might induce an alternative enzymatic pathway and/or the TAU transporter activity to maintain levels seen in control animals. Our studies suggest a possible beneficial effect of oral TAU supplementation, especially for pathologies that might be induced by free radical mechanisms during aging.

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References

[1] Dawson R Jr, Wallace DR. Taurine content in tissues from aged Fischer 344 rats. Age 1992;15:73–81.
[2] Dawson R Jr, Eppler B, Patterson TA, Shih D, Liu S. The effects of taurine in a rodent model of aging. Adv Exp Med Biol 1996;403:37–50.
[3] Wallace DR, Dawson R Jr. Decreased plasma taurine in aged rats. Gerontology 1990;36:19–27.
[4] Palkovits M, Banay-Schwartz M, Lajtha A. Taurine levels in brain nuclei of young adult and aging rats. In: Pasantes-Morales H, Martin DL, Shain W, Del Río RM, editors. Taurine: functional neurochemistry, physiology, and cardiology. New York: Wiley-Liss, 1990:45–51.
[5] Strolin Benedetti M, Russo A, Marrari P, Dostert P. Effects of ageing on the content in sulfur-containing amino acids in rat brain. J Neural Transm 1991;86:191–203.
[6] Jeevanandam K, Young DH, Ramias L, Schiller WR. Effect of major trauma on plasma free amino acid concentrations in geriatric patients. Am J Clin Nutr 1990;51:1040–5.
[7] Alvarez JG, Storey BT. Taurine, hypotaurine, epinephrine, and albumin inhibit lipid peroxidation in rabbit spermatozoa, and protect against loss of motility. Biol Reprod 1983;29:548–55.
[8] Banks MA, Porter DW, Martin WG, Castranova V. Ozone-induced lipid peroxidation and membrane leakage in isolated rat alveolar macrophages: protective effects of taurine. J Nutr Biochem 1991;2:308–13.
[9] Dawson R Jr, Tang E, Shih D, Hern H, Hu M, Baker D, Eppler B. Taurine inhibition of iron-stimulated catecholamine oxidation. Adv Exp Med Biol 1998;442:155–62.
[10] Nakamori K, Koyama I, Nakamura T, Nemoto M, Yoshida T, Umeda M, Inoue K. Quantitative evaluation of the effectiveness of taurine in protecting the ocular surface against oxidant. Chem Pharm Bull (Tokyo) 1993;41:335–8.
[11] Schuller-Levis GB, Gordon RE, Park E, Pendino KJ, Laskin DL. Taurine protects rat bronchioles from acute ozone-induced lung inflammation and hyperplasia. Exp Lung Res 1995;21:877–88.
[12] Timbrell JA, Seabra V, Waterfield CJ. The *in vivo*, and *in vitro* protective properties of taurine. Gen Pharmacol 1995;26:453–62.
[13] Trachtman H, Futterweit S, Bienkowski RS. Taurine prevents glucose-induced lipid peroxidation and increased collagen production in cultured rat mesangial cells. Biochem Biophys Res Commun 1993;191:759–65.
[14] Corman B, Pratz J, Poujeol P. Changes in anatomy, glomerular filtration, and solute excretion in aging rat kidney. Am J Physiol 1985;248:R282–7.
[15] Jones DP, Miller LA, Chesney RW. Adaptive regulation of taurine transport in two continuous renal epithelial cell lines. Kidney Int 1990;38:219–26.
[16] Han X, Budreau AM, Chesney RW. Molecular cloning and functional expression of an LLC-PK1 cell taurine transporter that is adaptively regulated by taurine. Adv Exp Med Biol 1998;442:261–8.
[17] Han X, Budreau AM, Chesney RW. Functional expression of rat renal cortex taurine transporter in *Xenopus laevis* oocytes: adaptive regulation by dietary manipulation. Pediatr Res 1997;41:624–31.
[18] Han X, Budreau MA, Chesney RW. Adaptive regulation of MDCK cell taurine transporter (pNCT) mRNA: transcription of pNCT gene is regulated by external taurine concentration. Biochim Biophys Acta 1997;1351:296–304.
[19] Dawson R Jr, Liu S, Eppler B, Patterson T. Effects of dietary taurine supplementation or deprivation in aged male Fischer 344 rats. Mech Ageing Dev 1999;107:73–91.
[20] de la Rosa J, Stipanuk MH. Evidence for a rate-limiting role of cysteinesulfinate decarboxylase activity in taurine biosynthesis *in vivo.* Comp Biochem Physiol 1985;81B:565–71.
[21] Kaisaki PJ, Jerkins AA, Goodspeed DC, Steele RD. Cloning and characterization of rat cysteine sulfinic acid decarboxylase. Biochim Biophys Acta 1995;1262:79–82.
[22] Huxtable RJ. Taurine in the central nervous system and the mammalian actions of taurine. Prog Neurobiol 1989;32:4471–533.
[23] Do KQ, Tappaz ML. Specificity of cysteine sulfinate decarboxylase (CSD) for sulfur-containing amino-acids. Neurochem Int 1996;28:363–71.
[24] Tang XW, Hsu C-C, Schloss JV, Faiman MD, Wu E, Yang C-Y, Wu J-Y. Protein phosphorylation and taurine biosynthesis *in vivo* and *in vitro.* J Neurosci 1997;17:6947–51.
[25] Jerkins AA, Steele RD. Cysteine sulfinic acid decarboxylase activity in response to thyroid hormone administration in rats. Arch Biochem Biophys 1991;286:428–32.
[26] Bagley PJ, Stipanuk MH. The activities of rat hepatic cysteine dioxygenase and cysteinesulfinate decarboxylase are regulated in a reciprocal manner in response to dietary casein levels in rats. J Nutr 1994;124:2410–21.
[27] Bella DL, Stipanuk MH. High levels of dietary proteins or methionine have different effects on cysteine metabolism in rat hepatocytes. Adv Exp Med Biol 1996;403:73–84.
[28] Jerkins AA, Steele RD. Dietary sulfur amino acid modulation of cysteine sulfinic acid decarboxylase. Am J Physiol 1991;261:E551–5.
[29] Ida S, Ohkuma S, Kimori K, Kuryama K, Morimoto N, Ibata Y. Regulatory role of cysteine dioxygenase in cerebral biosynthesis of taurine. Analysis using cerebellum from 3-acetylpyridine-treated rats. Brain Res 1985;344:62–9.

[30] Misra CH, Olney JW. Cysteine oxidase in brain. Brain Res 1975;97: 117–26.
[31] Bagley PJ, Stipanuk MH. Rats fed a low protein diet supplemented with sulfur amino acids have increased cysteine dioxygenase activity and increased taurine production in hepatocytes. J Nutr 1995;125: 933–40.
[32] Kohashi N, Yamaguchi K, Hosokawa Y, Kori Y, Fujii O, Ueda I. Dietary control of cysteine dioxygenase in rat liver. J Biochem (Tokyo) 1978;84:159–68.
[33] Hosokawa Y, Yamaguchi K, Kohashi N, Kori Y, Ueda I. Decrease of rat liver cysteine dioxygenase (cysteine oxidase) activity mediated by glucagon. J Biochem (Tokyo) 1978;84:419–24.
[34] Hosokawa Y, Yamaguchi K, Kohashi N, Kori Y, Fujii O, Ueda I. Immaturity of the enzyme activity and the response to inducers of rat cysteine dioxygenase during development. J Biochem (Tokyo) 1980; 88:389–94.
[35] Chen TS, Richie JP Jr, Lang CA. The effect of aging on glutathione and cysteine levels in different regions of the mouse brain. Proc Soc Exp Biol Med 1989;190:399–402.
[36] Stipanuk MH, Coloso RM, Garcia RAG, Banks MF. Cysteine concentration regulates cysteine metabolism to glutathione, sulfate and taurine in rat hepatocytes. J Nutr 1992;122:420–7.
[37] Stadtman ER. Protein oxidation and aging. Science 1992;257: 1220–4.
[38] Rothstein M. Altered proteins, errors and aging. In: Segal HL, Rothstein M, Bergamini E, editors. Protein metabolism in aging. New York: Wiley-Liss, 1990:3–14.
[39] Daniels KM, Stipanuk MH. The effects of dietary cysteine level on cysteine metabolism in rats. J Nutr 1982;112:2130–41.
[40] Bagley PJ, Hirschberger LL, Stipanuk MH. Evaluation and modification of an assay procedure for cysteine dioxygenase activity: high-performance liquid chromatography method for measurement of cysteine sulfinate and demonstration of physiological relevance of cysteine dioxygenase activity in cysteine catabolism. Anal Biochem 1995;227:40–8.
[41] Dawson R Jr, Wallace DR. Kainic acid-induced seizures in aged rats: neurochemical correlates. Brain Res Bull 1992;29:459–68.
[42] Bradford MM. A rapid, and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248–54.
[43] Levine RL, Williams JA, Stadtman ER, Shacter E. Carbonyl assays for determination of oxidatively modified proteins. Methods Enzymol 1992;233:346–71.
[44] Dawson R Jr, Baker D, Eppler B, Tang E, Shih D, Hern H, Hu M. Taurine inhibition of metal-stimulated catecholamine oxidation. Neurotox Res 2000;2:1–15.
[45] Jocelyn PC. Spectrophotometric assay of thiols. Methods Enzymol 1987;143:44–67.
[46] Glavind J. Antioxidants in animal tissue. Acta Chem Scand 1963;17: 1635–40.
[47] Loriette C, Pasantes-Morales H, Portemer C, Chatagner F. Dietary casein and taurine supplementation: effects on cysteine dioxygenase and cysteine decarboxylase activities and taurine concentration in brain, liver and kidney of the rat. Nutr Metab 1979;23:467–75.
[48] Gaull GE. Taurine in pediatric nutrition: review and update. Pediatrics 1989;83:433–42.
[49] Garcia RAG, Stipanuk MH. The splanchnic organs, liver and kidney have unique roles in the metabolism of sulfur amino acids and their metabolites in rats. J Nutr 1992;122:1693–701.
[50] Friedman A, Albright PW, Chesney RW. Dietary adaptation of taurine transport by rat renal epithelium. Life Sci 1981;29:2415–9.
[51] Eppler B, Dawson R Jr. Cysteine sulfinate decarboxylase and cysteine dioxygenase activities do not correlate with strain-specific changes in hepatic and cerebellar taurine content in aged rats. Mech Ageing Dev 1999;110:57–72.
[52] Awapara J. The taurine concentration of organs from fed and fasted rats. J Biol Chem 1955;218:571–7.
[53] Wu C. Metabolism of free amino acids in fasted and zein-fed rats. J Biol Chem 1953;207:775–87.
[54] Weinstein L, Griffith OW. Multiple forms of rat liver cysteinesulfinate decarboxylase. J Biol Chem 1987;262:7254–63.
[55] Sturman JA, Messing JM, Rossi SS, Hofmann AF, Neuringer M. Tissue taurine content, activity of taurine synthesis enzymes and conjugated bile acid composition of taurine-deprived and taurine-supplemented rhesus monkey infants at 6 and 12 mo of age. J Nutr 1991;121:854–62.
[56] Tappaz M, Legay F, Almarghini K, Henry S, Rιmy A. Cysteine sulfinic acid decarboxylase (CSD) in the brain. In: Pasantes-Morales H, Martin DL, Shain W, Del Río RM, editors. Taurine: functional neurochemistry, physiology, and cardiology. New York: Wiley-Liss, 1990:53–68.
[57] Hope DB. The persistence of taurine in the brains of pyridoxine-deficient rats. J Neurochem 1957;1:364–9.
[58] Nyffenegger E, Lauber K, Aebi H. Die Taurineausscheidung normaler und B_6-avitaminotischer Ratten nach Ganzkφrperbestrahlung. Biochem Z 1960;333:226–35.
[59] Chan-Palay V, Lin CT, Palay S, Yamamoto M, Wu JY. Taurine in the mammalian cerebellum: demonstration by autoradiography with [^{3}H]taurine and immunocytochemistry with antibodies against the taurine-synthesizing enzyme, cysteine-sulfinic acid decarboxylase. Proc Natl Acad Sci USA 1982;79:2695–9.
[60] Magnusson KR, Clements JR, Wu JY, Beitz AJ. Colocalization of taurine- and cysteine sulfinic acid decarboxylase-like immunoreactivity in the hippocampus of the rat. Synapse 1989;4:55–69.
[61] Reymond I, Almarghini K, Tappaz M. Immunocytochemical localization of cysteine sulfinate decarboxylase in astrocytes in the cerebellum and hippocampus: a quantitative double immunofluorescence study with glial fibrillary acidic protein and S-100 protein. Neuroscience 1996;75:619–33.
[62] Tappaz M, Almarghini K, Do K. Cysteine sulfinate decarboxylase in the brain: identification, characterization, and immunocytochemical location in astrocytes. In: Huxtable R, Michalk DV, editors. Taurine in health and disease. New York: Plenum Press, 1994:257–68.
[63] Carney JM, Carney AM. Role of protein oxidation in aging and in age-associated neurodegenerative diseases. Life Sci 1994;55:2097–103.
[64] Oliver CN, Ahn B, Moerman EJ, Goldstein S, Stadtman ER. Age-related changes in oxidized proteins. J Biol Chem 1987;262:5488–91.
[65] Beckman KB, Ames BN. The free radical theory of aging matures. Physiol Rev 1998;78:547–81.
[66] Dean R, Gebicki J, Gieseg S, Grant AJ, Simpson JA. Hypothesis: a damaging role in aging for reactive protein oxidation products? Mutat Res 1992;275:387–93.
[67] Gaull GE. Taurine as a conditionally essential nutrient in man. J Am Coll Nutr 1986;5:121–5.
[68] Soszynski M, Bartosz G. Decrease in accessible thiols as an index of oxidative damage to membrane proteins. Free Radic Biol Med 1997; 23:463–9.
[69] Pathirana C, Grimble RF. Taurine and serine supplementation modulates the metabolic response to tumor necrosis factor α in rats fed a low protein diet. J Nutr 1992;122:1369–75.
[70] Obrosova IG, Stevens MJ. Effect of dietary taurine supplementation on GSH and NAD(P)-redox status, lipid peroxidation, and energy metabolism in diabetic precataractious lens. Invest Ophthalmol Vis Sci 1999;40:680–8.
[71] Aruoma OI, Halliwell B, Hoey BM, Butler J. The antioxidant action of taurine, hypotaurine and their metabolic precursors. Biochem J 1988;256:251–5.
[72] Farooqui MYH, Day WW, Zamorano DM. Glutathione and lipid peroxidation in the aging rat. Comp Biochem Physiol 1987;88B:177–80.
[73] Nakata K, Kawase M, Ogino S, Kinoshita C, Murata H, Sakaue T, Ogata K, Ohmori S. Effects of age on levels of cysteine, glutathione and related enzyme activities in livers of mice and rats and an attempt

to replenish hepatic glutathione level of mouse with cysteine derivatives. Mech Ageing Dev 1996;90:195–207.

[74] Strolin Benedetti M, Kettler R, Marrari P, Cini M, Da Prada M, Dostert P. The effects of lifelong treatment with MAO inhibitors on amino acid levels in rat brain. J Neural Transm Park Dis Dement Sect 1990;2:239–48.

[75] Messina S, Dawson R Jr. Attenuation of oxidative damage to DNA by taurine and taurine analogs. Adv Exp Med Biol 2000;483:355–67.