Flavin-Containing Monooxygenase 1 Catalyzes the Production of Taurine from Hypotaurine [S]

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ABSTRACT

Taurine is one of the most abundant amino acids in mammalian tissues. It is obtained from the diet and by de novo synthesis from cysteic acid or hypotaurine. Despite the discovery in 1954 that the oxygenation of hypotaurine produces taurine, the identification of an enzyme catalyzing this reaction has remained elusive. In large part, this is due to the incorrect assignment, in 1962, of the enzyme as an NAD-dependent hypotaurine dehydrogenase. For more than 55 years, the literature has continued to refer to this enzyme as such. Here we show, both in vivo and in vitro, that the enzyme that oxygenates hypotaurine to produce taurine is flavin-containing monooxygenase (FMO) 1. Metabolite analysis of the urine of *Fmo1*-null mice by ^{1}H NMR spectroscopy revealed a buildup of hypotaurine and a deficit of taurine in comparison with the concentrations of these compounds in the urine of wild-type mice. In vitro assays confirmed that human FMO1 catalyzes the conversion of hypotaurine to taurine, utilizing either NADPH or NADH as cofactor. FMO1 has a wide substrate range and is best known as a xenobiotic- or drug-metabolizing enzyme. The identification that the endogenous molecule hypotaurine is a substrate for the FMO1-catalyzed production of taurine resolves a long-standing mystery. This finding should help establish the role FMO1 plays in a range of biologic processes in which taurine or its deficiency is implicated, including conjugation of bile acids, neurotransmitter, antioxidant and anti-inflammatory functions, and the pathogenesis of obesity and skeletal muscle disorders.

SIGNIFICANCE STATEMENT

The identity of the enzyme that catalyzes the biosynthesis of taurine from hypotaurine has remained elusive. Here we show, both in vivo and in vitro, that flavin-containing monooxygenase 1 catalyzes the oxygenation of hypotaurine to produce taurine.

Introduction

Taurine (2-aminoethanesulfonic acid) is one of the most abundant amino acids in mammalian tissues (Huxtable, 1992). It is an organic osmolyte involved in cell volume regulation (Huxtable, 1992) and has a variety of cytoprotective and developmental roles, particularly in neurologic and ocular tissues (Ripps and Shen, 2012). It is also involved in the formation of bile salts (Huxtable, 1992) and the modulation of intracellular calcium concentration (Lombardini, 1983). Taurine is considered an essential substance in mammals, and its deficiency has been implicated in several pathologic conditions.

Taurine is obtained from the diet and, by de novo synthesis, from cysteic acid (Edgar et al., 1994) or hypotaurine (Cavallini et al., 1954). Hypotaurine is itself an organic osmolyte and cytoprotective agent (Yancey, 2005) and acts as an antioxidant to scavenge highly reactive hydroxyl radicals (Aruoma et al., 1988). The oxygenation of hypotaurine to produce taurine was discovered in 1954 (Cavallini et al., 1954). Later, the enzyme that converts hypotaurine to taurine was reported to be a NAD-dependent hypotaurine dehydrogenase (Sumizu, 1962) but was not isolated or characterized. Subsequently, Oja and Kontro (1981) noted that the production of taurine in tissue extracts was optimal at pH 9.0 and was stimulated by oxygenation. These authors concluded that the enzyme that converts hypotaurine to taurine was not a NAD-dependent hypotaurine dehydrogenase. By overlooking the work of Oja and Kontro (1981), and giving credence to the earlier study (Sumizu, 1962), the enzyme that catalyzes the conversion of hypotaurine to taurine has continued to be reported as a hypotaurine dehydrogenase (EC 1.8.1.3), utilizing NAD as cofactor. Because an enzyme that catalyzes the reaction has not been identified or isolated, the conversion of hypotaurine to taurine has sometimes been referred to as nonenzymatic.

Here, we show in vivo through the use of a knockout-mouse line and in vitro by assays of human enzymes that oxygenation of hypotaurine to produce taurine is catalyzed by flavin-containing monooxygenase (FMO) 1.

Materials and Methods

Animals. All mice used in this study were bred at University College London. The $Fmo1^{-/-}$, $2^{-/-}$, $4^{-/-}$, and $Fmo5^{-/-}$ mouse lines were constructed as

ABBREVIATIONS: 1D, one dimensional; ca circa FMO, flavin-containing monooxygenase; HMBC heteronuclear multiple bond correlation spectroscopy HSQC, heteronuclear single quantum coherence; TSP, sodium 3-(trimethylsilyl)-2,2′,3,3′-tetradeuteropropionate.

previously described (Hernandez et al., 2006a, 2009; Gonzalez Malagon et al., 2015). Mice were genotyped as described (Hernandez et al. 2006b; Gonzalez Malagon et al., 2015). Mice were given free access to food (a standard chow diet, Teklad Global 18% ProteinRodent Diet; Harlan Laboratories, Inc., Madison, WI) and water. Animal procedures were carried out in accordance with the UK Animal Scientific Procedures Act as well as with local ethics committee approval (Animal Welfare and Ethical Review Body) and appropriate Home Office Licenses. Urine was collected between 10:00 AM and 12:00 PM (noon) from male and female mice aged 15 to 16 weeks. Urine samples were immediately frozen on solid CO_2 and stored at $-80°C$ until analyzed by ^1H NMR spectroscopy, as described below.

Enzyme Assays. All reaction mixtures (final volumes of 250 μl in a Corning Costar 96-well cell-culture plate; VWR, Lutterworth, Leicestershire, UK) were incubated in a Sunrise absorbance microplate reader (Tecan, Grödig, Austria) equipped with Magellan software, version 6.2. Reaction mixtures contained either 0.1 M potassium phosphate buffer, pH 7.4, or 0.1 M Tris-HCl, pH 8.5, 1 mM EDTA (aerated immediately before use by shaking for 5 minutes at room temperature); 2.5 mM hypotaurine (Santa Cruz Biotechnology, Heidelberg, Germany); 0.5 mM NADPH, NADH, or NAD or no cofactor; and baculosomes containing human FMO1, FMO3, or FMO5 (135 nM final concentration) (Sigma Aldrich, Gillingham, Dorset, UK) or an equivalent amount of control insect cell microsomes (Corning Life Sciences, Woburn, MA). The mixtures were incubated at 37°C for 60 minutes. Monitoring of NADPH or NADH depletion, measured at $A_{340 nm}$, revealed that reaction velocities were linear over the 60-minute period. Samples were stored at $-80°C$ until analyzed by one-dimensional (1D) ^1H NMR spectroscopy, as described below.

Methimazole S-oxygenation was measured by the method of Dixit and Roche (1984). Reaction mixtures contained final concentrations of 67 nM of human FMO1 or FMO3 in baculosomes (Sigma Aldrich), 2.5 mM methimazole, and 0.5 mM NADPH or NADH and were incubated at 37°C.

For estimation of kinetic parameters, reaction mixtures were assembled by adding the following to final concentrations in order: 0.1 M Tris-HCl (pH 8.5), 1 mM EDTA (aerated immediately before use by shaking at 37°C for 10 minutes), 0.5 mM NADPH, and baculosomes containing human FMO1 (135 nM final concentration) (Sigma Aldrich). The mixtures were equilibrated at 37°C for 3 minutes to allow formation of the active C4a-hydroperoxyflavin species of the FAD prosthetic group of FMO. Reactions were initiated by addition of hypotaurine to final concentrations of 1–10 mM, or, in the case of blank samples, an equivalent volume of buffer was added. Reaction mixtures were incubated at 37°C. The initial velocity of enzyme-catalyzed reactions was assessed by monitoring the depletion of NADPH, measured at $A_{340 nm}$. $\Delta A_{340 nm}$ was converted to Δ NADPH using a molar extinction coefficient of $6.2 \times 10^3 M^{-1}$ cm^{-1} and a light-path length of 0.73 cm. To determine substrate (hypotaurine)-dependent oxygenation of NADPH, readings from a blank sample (a reaction mixture containing no hypotaurine) were subtracted.

The ability of hypotaurine to act as a competitor substrate of FMO1 was assessed by measuring the effect of various concentrations of hypotaurine on FMO1-catalyzed S-oxygenation of methimazole. Methimazole S-oxygenation was measured by the method of Dixit and Roche (1984), as described above, in reaction mixtures containing final concentrations of 67 nM human FMO1 in baculosomes (Sigma Aldrich), 4 μM methimazole, and 2.5–100 mM hypotaurine.

Sample Preparation for NMR Spectroscopy. Urine samples (50 μl) were prepared for NMR spectroscopy as previously described (Varshavi et al., 2018). Enzyme assay samples were thawed and vortexed, and then 160 μl sample was mixed with 80 μl 0.6 M phosphate buffer, as previously described (Varshavi et al., 2018). The samples were revortexed and centrifuged at 13,000g for 5 minutes at 4°C. Supernatant (200 μl) was then pipetted into 3.0-mm outer diameter SampleJet NMR tubes (S-3.0-500-1; Norell).

NMR Spectroscopic Analysis. ^1H NMR spectra of urine and enzyme assay samples were recorded on a Bruker Avance III spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany) operating at 600.44 MHz and at a temperature of 300.0 K, using a standard 1D nuclear Overhauser spectroscopy (NOESY) presaturation pulse sequence with gradient pulses (noesygppr1d), as previously described (Varshavi et al., 2018).

NMR spectral processing was carried out in MNova (MestReNova, version 12.0.1-20560; Mestrelab Research S.L.). All concentrations or ratios of metabolites were calculated using NMR spectroscopy peak areas determined in MNova. In cases in which the signals were clear of overlap, the areas were calculated without deconvolution. In cases in which there was some peak overlap, the relevant peak areas were calculated after deconvolution, without compromise to the quantification. The following peaks did not require deconvolution: sodium 3-(trimethylsilyl)-2,2′,3,3′-tetradeuteropropionate (TSP), NMR reference standard, at 0.0; NAD at circa (ca) 9.35 and ca 9.14; and NADP at ca 9.31 and 9.10. The following peaks required deconvolution prior to area measurement: taurine at ca 3.42 and ca 3.28; hypotaurine at ca 3.35 and ca 2.67; NADPH at ca 2.85 and ca 2.75 and NADH at ca 2.81 and ca 2.69 ppm. Absolute concentrations were determined relative to the known concentration of TSP in the phosphate buffer (1.2 mg in 11.12 ml), taking into account that the area of the TSP signal is due to nine protons, whereas other signals, such as those from taurine, are each due to two protons.

The deconvolution of the peaks for metabolite quantification was done using the MNova global spectral deconvolution algorithm. The peak areas were obtained, and the residuals were manually minimized by adjusting the fitting parameters of each peak. Data were imported into Matlab (R2014b; MathWorks).

High-sensitivity analysis by ^1H NMR spectroscopy showed that the hypotaurine used as a substrate for enzyme assays contained 0.23 mol percent taurine (area measurement of taurine signal at ca 3.42 relative to the 0.55% area of the ^{13}C satellite of the hypotaurine signal at ca 3.35 ppm), and this was taken into account in our calculations.

NMR Data Deposition. Original NMR data will be deposited in MetaboLights (EBI UK) (Haug et al., 2013) after publication.

Metabolite Identification. NMR-based metabolite identification was carried out by using standard methods, as described (Dona et al., 2016), and by using information from the literature and public databases, including the Human Metabolite Database (Wishart et al., 2018) (http://www.hmdb.ca/).2018) (see Supplemental Data). Hypotaurine in $Fmo1^{-/-}$, $2^{-/-}$, and $4^{-/-}$ mouse urine showed the following features: 2.665 (t, 6.9 Hz), 58.6 (HSQC with HMBC to 36.4 and correlated spectroscopy (see Supplemental Fig. 1) to 3.365 (t, 6.9 Hz), and 36.5 (HSQC) and heteronuclear multiple bond correlation spectroscopy (HMBC) to 58.5 ppm, in agreement with literature values of 2.66 (t, 6.9 Hz), 58.5 and 3.35 (t, 6.9 Hz), and 36.2 ppm (HMDB00965, accessed from http://www.hmdb.ca/spectra/nmr_one_d/1626 on February 5, 2019). Taurine in $Fmo1^{-/-}$, $2^{-/-}$, $4^{-/-}$, and wild-type mouse urine showed the following features: 3.283 (t, 6.6 Hz), 50.6 (HSQC) with HMBC to 38.3 and correlated spectroscopy to 3.433 (t, 6.6 Hz), and 38.4 (HSQC) and HMBC to 50.5 ppm, in agreement with literature values of 3.25 (t, 6.6 Hz), 50.4 and 3.42 (t, 6.6 Hz), and 38.3 ppm (HMDB0000251, accessed from http://www.hmdb.ca/spectra/nmr_one_d/1277 on 5th February 2019). Both hypotaurine and taurine in the urine samples were unambiguously identified using the recent Metabolite Identification Carbon Efficiency (MICE) criteria (Everett, 2015).

Results

$Fmo1^{-/-}$, $2^{-/-}$, and $4^{-/-}$ **Mice Have a Deficit in Taurine Production.** Previous phenotypic analysis (Hernandez et al., 2009; Shephard and Phillips, 2010; Veeravalli et al., 2014, 2018) has identified metabolic differences between mice in which the *Fmo1, Fmo2,* and *Fmo4* genes had been deleted (*$Fmo1^{-/-}$, $2^{-/-}$,* and *$4^{-/-}$* mice) (Hernandez et al., 2009) and wild-type animals. As an extension of this work, we have used 1D ^1H NMR spectroscopy to compare the urinary metabolite profiles of the knockout-mouse line and wild-type animals. Analysis of the urine of male and female *$Fmo1^{-/-}$, $2^{-/-}$,* and *$4^{-/-}$* mice revealed signals at 2.66 and 3.37 ppm, corresponding to those of hypotaurine, which were markedly lower in the urine of wild-type mice (Fig. 1, A and B). Signals at 3.28 and 3.43 ppm, corresponding to those of taurine, were detected in the urine of both wild-type and *$Fmo1^{-/-}$*, *$2^{-/-}$,* and *$4^{-/-}$* mice, but their intensities were lower in the latter (Fig. 1, A and B). The identities of taurine and hypotaurine in urine samples were confirmed by two-dimensional NMR (Supplemental Fig. 1). The urinary ratio of taurine to hypotaurine + taurine was significantly less in *$Fmo1^{-/-}$, $2^{-/-}$,* and *$4^{-/-}$* mice than in wild-type mice ($P < 0.0001$) (Fig. 1C). In contrast, analysis of male and female *$Fmo5^{-/-}$* mice, in which the gene encoding FMO5 is disrupted (Gonzalez
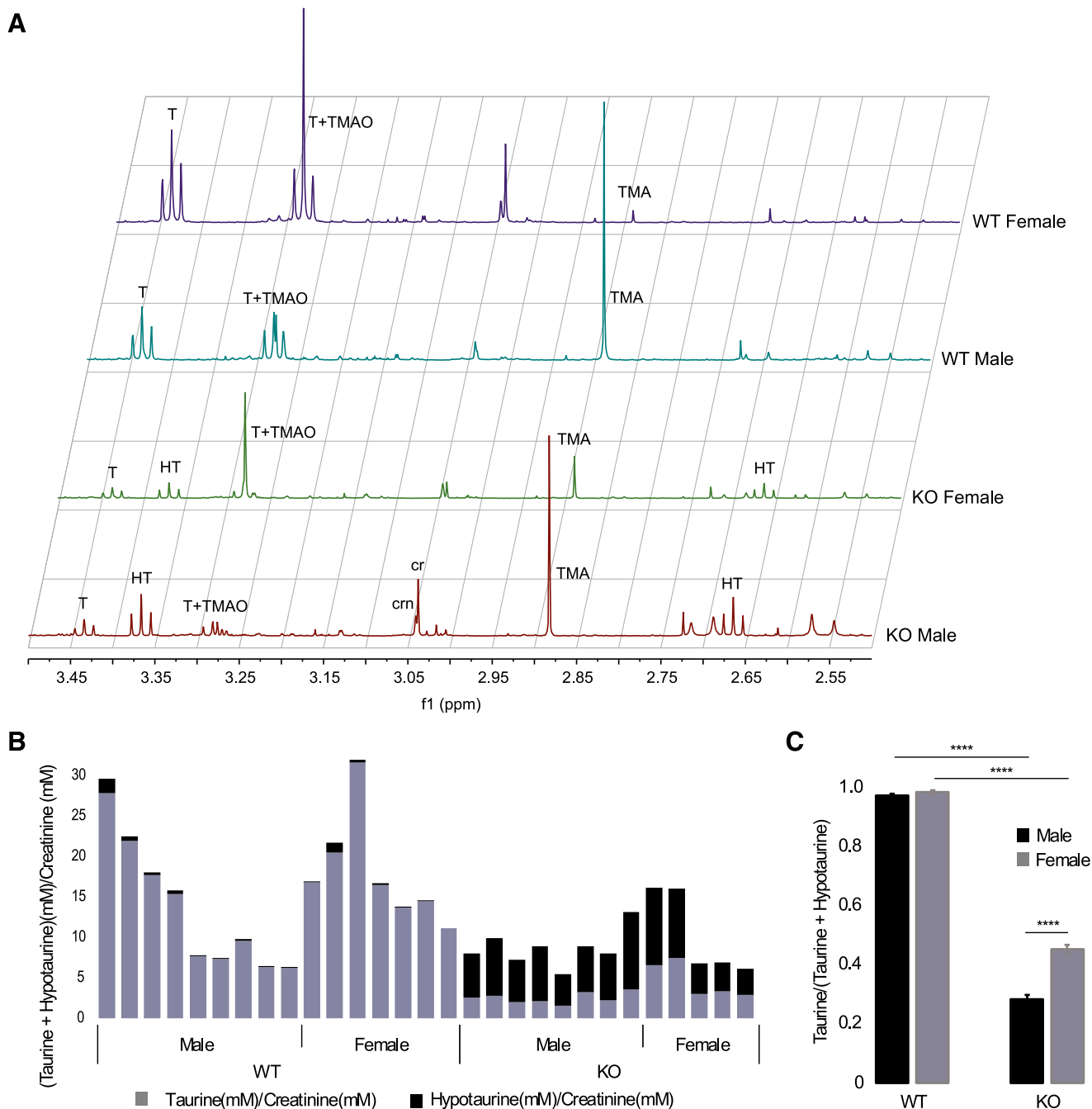
A

Fig. 1. Abundance of hypotaurine and taurine in mouse urine. (A) Representative NMR spectra of urine from individual $Fmo1^{-/-}$, $2^{-/-}$, and $4^{-/-}$ (KO) male and female mice and wild-type (WT) male and female mice. Cr, creatine; Crn, creatinine; HT, hypotaurine; T, taurine; TMA, trimethylamine; TMAO, trimethylamine N-oxide. (B) Proportion of taurine and hypotaurine (normalized to creatinine) in urine from individual male and female WT and KO mice. Taurine and hypotaurine were quantified by integration of NMR spectral peaks, as described in the *Materials and Methods* section. (C) Average ratios of taurine to taurine + hypotaurine in urine of male and female WT and KO mice. Taurine and hypotaurine were quantified by integration of NMR spectral peaks, as described in the *Materials and Methods* section. Data are expressed as means ± S.E.M. (WT male, $n = 9$; WT female, $n = 7$; KO male, $n = 8$; KO female, $n = 5$). ****$P < 0.0001$.

Malagon et al., 2015), revealed no significant difference from their wild-type counterparts in the urinary ratio of taurine to hypotaurine + taurine (Fig. 2).

Human FMO1 Catalyzes the Oxygenation of Hypotaurine In Vitro. The buildup of hypotaurine and the concomitant decrease of taurine in the urine of $Fmo1^{-/-}$, $2^{-/-}$, and $4^{-/-}$ mice (Fig. 1) suggests that the formation of taurine from hypotaurine is catalyzed by an FMO. Of the three genes deleted in the $Fmo1^{-/-}$, $2^{-/-}$, and $4^{-/-}$ knockout-mouse line, the gene encoding FMO4 is expressed at very low levels in

mice (Janmohamed et al., 2004) and humans (Dolphin et al., 1996); the gene encoding FMO2 is expressed in low amounts in mice (Janmo-hamed et al., 2004), and in most humans, the gene does not encode a functional protein because of the presence of a premature stop codon (Dolphin et al., 1998). In contrast, the gene encoding FMO1 is relatively highly expressed in a number of tissues in both mice (Janmohamed et al., 2004) and humans (Dolphin et al., 1996). Therefore, in humans, the most likely candidate for catalyzing the oxygenation of hypotaurine to produce taurine is FMO1. To investigate whether this was the case,
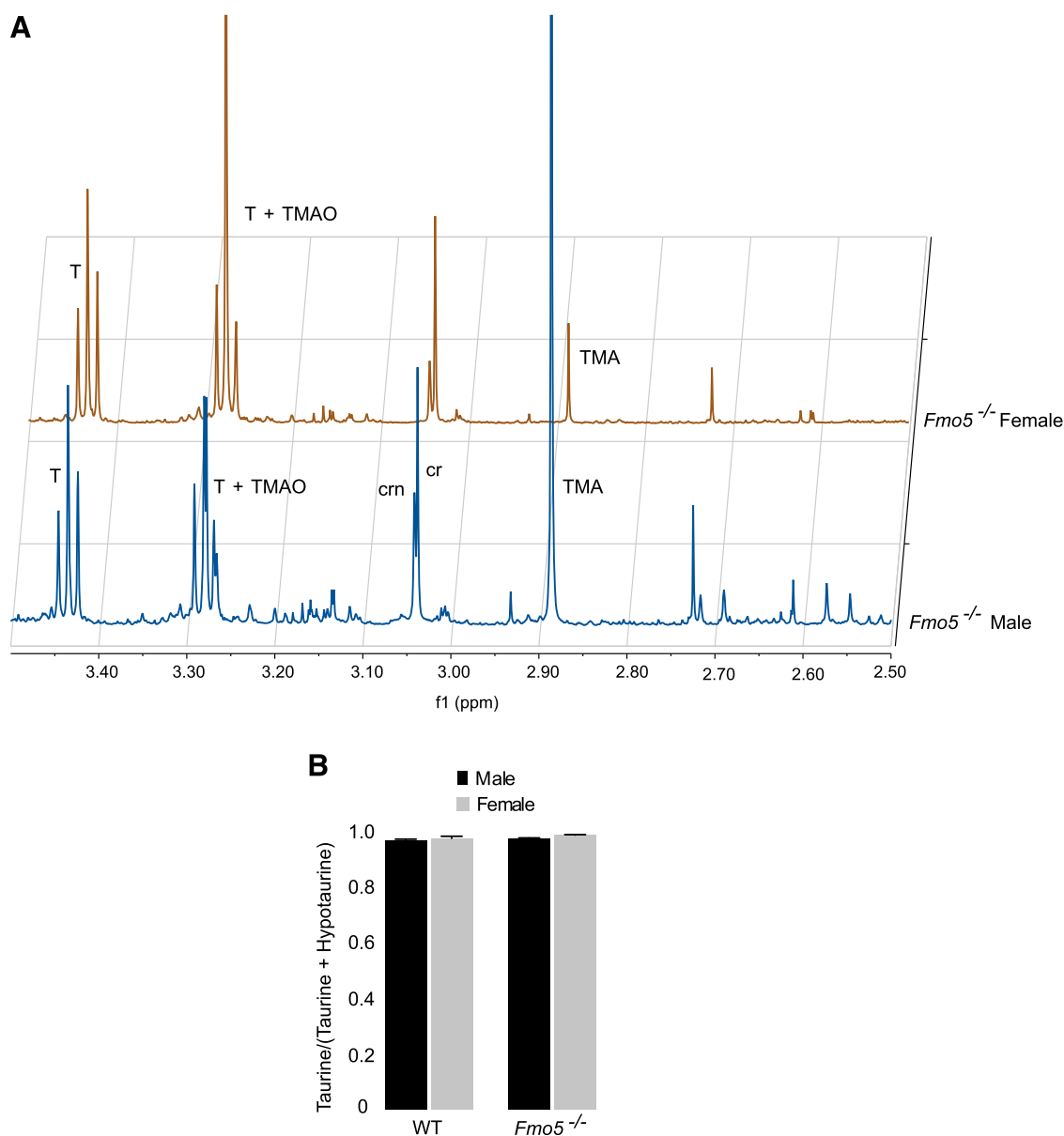
A

B

Fig. 2. Analysis of taurine and hypotaurine in the urine of $Fmo5^{-/-}$ mice. (A) Representative NMR spectra of urine from individual $Fmo5^{-/-}$ male and female mice. Cr, creatine; Crn, creatinine; T, taurine; TMA, trimethylamine; TMAO, trimethylamine N-oxide. (B) Average ratios of taurine to taurine + hypotaurine in urine of male and female wild-type (WT) and $Fmo5^{-/-}$ mice. Taurine and hypotaurine were quantified by integration of NMR spectral peaks, as described in the *Materials and Methods* section. Data are expressed as means ± S.E.M. (WT male, n = 9; WT female, n = 7; $Fmo5^{-/-}$ male, n = 14; $Fmo5^{-/-}$ female, n = 7).

baculosomes containing recombinantly expressed human FMO1 were incubated with hypotaurine and the cofactor NADPH at pH 8.5, the optimum for FMO1, and at the more physiologic pH of 7.4. Analysis of reaction products by 1D ^{1}H NMR spectroscopy identified signals at 3.276 and 3.433 ppm, corresponding to taurine, at both pH levels (Fig. 3A). As expected of an FMO1-catalyzed reaction, with NADPH as cofactor, production of taurine was greater at pH 8.5 than at pH 7.4 ($P <$ 0.01) (Fig. 3B). In comparison, very little taurine was detected in incubations of control insect cell microsomes at pH 8.5 and none at pH 7.4 (Fig. 3, A and B). The identity of taurine as a product of FMO1-catalyzed reactions was confirmed by high-resolution ultra-performance liquid chromatography electrospray mass spectrometry against an authentic reference standard (Supplemental Figs. 2 and 3) (Dona et al., 2016).

We also investigated whether the conversion of hypotaurine to taurine could be catalyzed by either of the two other major functional FMOs of humans, FMO3 and FMO5 (Overby et al., 1997). In comparison with baculosomes containing human FMO1, those containing FMO3 produced much lower amounts of taurine at pH 8.5 or 7.4, with either NADPH or NADH as cofactor ($P <$ 0.01). As expected from the analysis of the urine of $Fmo5^{-/-}$ mice (Fig. 2), production of taurine by baculosomes containing human FMO5 was similar to that produced by control insect cell microsomes (Fig. 3B). These results confirm that production of taurine from hypotaurine can be catalyzed by FMO1 and, to a much smaller extent, by FMO3 but not significantly by FMO5.

The production of taurine in the $Fmo1$-null mice was less affected in females (~55% depletion) than in males (~70% depletion) ($P <$ 0.0001) (Fig. 1C). This gender difference is likely due to the contribution in female $Fmo1$-null mice of FMO3, which is absent from the liver of adult male mice (Falls et al., 1995; Janmohamed et al., 2004). In the case of wild-type mice, the presence of FMO1, which is more effective than
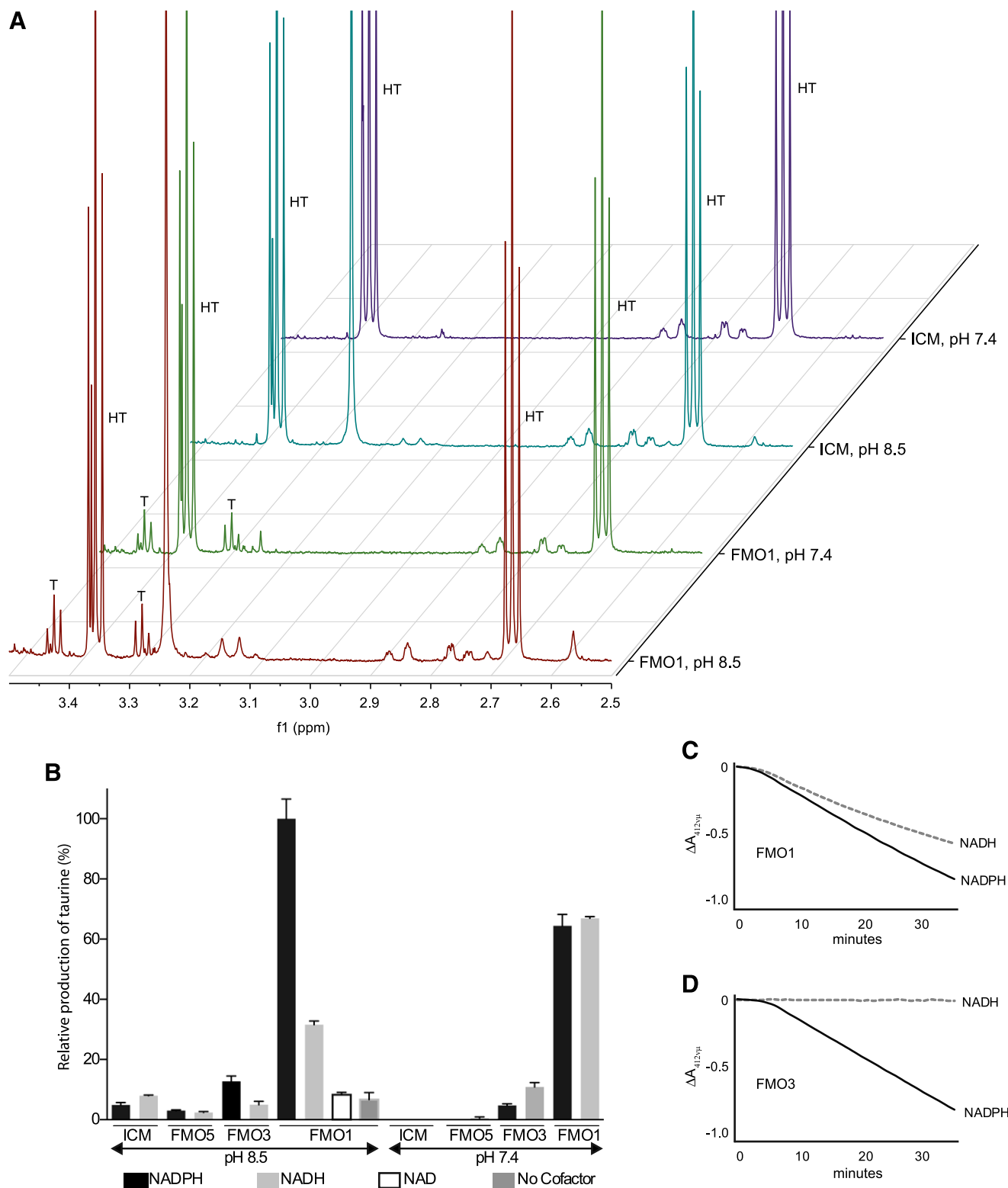
Fig. 3. Analysis of in vitro enzyme-catalyzed reactions. (A) Representative NMR spectra of products of reactions catalyzed by baculosomes containing human FMO1 or by insect cell microsomes (ICM) at pH 8.5 or 7.4. Note: the singlet signal at ca 3.364 ppm overlapping the triplet because of hypotaurine is due to methanol. HT, hypotaurine; T, taurine. (B) Production of taurine from hypotaurine in reactions catalyzed by human FMO1, FMO3, or FMO5 or by ICM at pH 8.5 or 7.4 with NADPH or NADH as cofactor. In the case of reactions catalyzed by FMO1 at pH 8.5, NAD or no cofactor were also used. Taurine production was quantified by integration of NMR spectral peaks and is plotted relative to that produced by FMO1-containing baculosomes at pH 8.5 with NADPH as cofactor (set at 100%). Data are expressed as means $\pm$ 0.5 $\times$ range (n = 2). (C and D) Representative progress curves of methimazole S-oxygenation catalyzed by human FMO1 (C) or FMO3 (D), at pH 8.5, with NADPH or NADH as cofactor. Reactions were monitored at $A_{412\ nm}$.
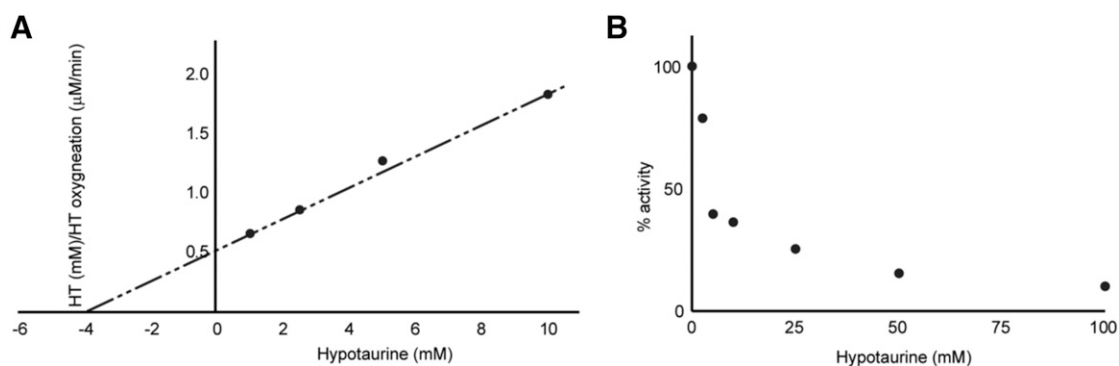
Fig. 4. Kinetic and competition analysis of FMO1-catalyzed reactions. (A) Kinetic analysis (Hanes-Woolf plot) of FMO1-catalyzed oxygenation of hypotaurine. Reaction mixtures contained human FMO1, NADPH, and various concentrations of hypotaurine. Initial velocity was measured by monitoring hypotaurine-dependent depletion of NADPH at $A_{340\ nm}$. (B) Competition of FMO1-catalyzed S-oxygenation of methimazole by hypotaurine. Reaction mixtures contained human FMO1, methimazole, NADPH, and various concentrations of hypotaurine. The concentration of methimazole was 50% of the K_M of FMO1 for this substrate. Initial velocity was measured as described in *Materials and Methods*. Methimazole S-oxygenation activity was plotted as a percentage of that in the absence of hypotaurine.

FMO3 in catalyzing the formation of taurine from hypotaurine as evidenced by analysis in vitro (Fig. 3B), greatly outweighs the effect of the presence in females of FMO3.

Human FMO1 Can Use Both NADPH and NADH as Cofactors. FMOs, despite being termed NADPH-dependent monooxygenases, have been reported to be able also to use NADH as a cofactor (Hvattum et al., 1991). We found that in catalyzing the production of taurine from hypotaurine, FMO1 could use either NADPH or NADH as cofactor (Fig. 3B). However, whereas NADPH is the more effective cofactor at pH 8.5 ($P < 0.01$), the cofactors are equally effective at pH 7.4 (Fig. 3B). When NAD was used as cofactor for FMO1, the amount of taurine produced was very low and not significantly different from that produced in the absence of cofactor (Fig. 3B). We therefore investigated whether FMO1 could use NADH as a cofactor for the S-oxygenation of other substrates. NADH was an effective cofactor for the FMO1-catalyzed S-oxygenation of the FMO model substrate methimazole (Fig. 3C). However, in the case of FMO3, NADH was ineffective as a cofactor for methimazole oxygenation, which was dependent on NADPH (Fig. 3D).

Kinetic Parameters of FMO1-Catalyzed S-Oxygenation of Hypotaurine. Kinetic parameters of FMO1-catalyzed oxygenation of hypotaurine to produce taurine were estimated under conditions at which the enzyme was most active: pH 8.5 with NADPH as cofactor (Fig. 3B). Simultaneous measurement of NADPH signal decrease and taurine signal increase in ^{1}H NMR spectra revealed that under these conditions, there was a 1:1 ratio of taurine production to NADPH oxidation. The kinetics of the enzyme-catalyzed reaction could therefore be assessed by monitoring depletion of NADPH (measured at $A_{340\ nm}$). A plot of kinetic data are shown in Fig. 4A. Direct linear plots of data (velocity vs. substrate concentration) in parameter space (Cornish-Bowden and Wharton, 1988) gave estimates for K_M of ~4.1 mM and V_{max} of ~7.5 μM minute^{-1}, giving a catalytic constant (k_{cat}) of ~55 minutes^{-1}. Further support for the ability of FMO1 to use hypotaurine as a substrate is provided by the finding that hypotaurine, at concentrations comparable with the K_M of FMO1-catalyzed hypotaurine oxygenation, acted as an effective competitor of FMO1-catalyzed S-oxygenation of the model FMO substrate methimazole (Fig. 4B).

Discussion

We have confirmed both in vivo by ^{1}H NMR metabolite profiling of the urine of *Fmo1*$^{-/-}$, *2*$^{-/-}$, and *4*$^{-/-}$ mice and in vitro by analysis of the catalytic activity of human FMOs that formation of taurine from hypotaurine is catalyzed by FMO1, a monooxygenase, and that the

enzyme can use either NADPH or NADH as cofactor for the S-oxygenation reaction. FMO1 is also able to use NADPH or NADH to catalyze the S-oxygenation of methimazole. It is not known whether the ability of the enzyme to use NADH as cofactor extends to N-oxygenation reactions.

Our results from *Fmo1*$^{-/-}$, *2*$^{-/-}$, and *4*$^{-/-}$ mice show that in the absence of FMO1, most taurine production is abolished in both males and females, suggesting that, in mice, the major source of this abundant amino acid is the FMO1-catalyzed oxygenation of hypotaurine (Fig. 5). Consistent with this, the lack of production of taurine from hypotaurine in vitro in the absence of enzyme indicates that nonenzymatic conversion does not contribute substantially to taurine production.

FMOs (EC 1.14.13.8) are best known for their role in the metabolism of xenobiotics, including therapeutic drugs (reviewed in Phillips and Shephard, 2017) and foreign chemicals such as organophosphate insecticides (reviewed in Krueger and Williams, 2005). Of the members of the FMO family, FMO1 has the broadest substrate range (reviewed in Krueger and Williams, 2005). In addition to its role in xenobiotic metabolism, FMO1 has been identified as a novel regulator of energy balance (Veeravalli et al., 2014).

As taurine can be obtained from the diet, the relative contribution of FMO1-catalyzed monooxygenation of hypotaurine to the amount of taurine in humans is not known. In human liver, *FMO1* is expressed in the fetus but is switched off after birth (Dolphin et al., 1991, 1996; Koukouritaki et al., 2002). This pattern of expression is consistent with the decline in taurine concentration in human liver after birth (Sturman and Gaull, 1975). In contrast, in adult rodent liver, the gene encoding FMO1 is highly expressed (Janmohamed et al., 2004) and taurine is abundant (Oja and Kontro, 1981). In adult humans, although *FMO1* is not expressed in liver, it is expressed in a range of extra-hepatic tissues in which the action of taurine has been implicated, such as kidney (Dolphin et al., 1996), brain (Hernandez et al., 2004), small intestine (Yeung et al., 2000), and heart (Kim et al., 2003) as well as a number of endocrine tissues, including pancreas, adrenal, and testis (Hernandez et al., 2004).

Concentrations of taurine are high in fetal brain but fall during development (Sturman and Gaull, 1975). The decline in taurine concentration in the developing brain is consistent with the decrease in expression of *Fmo1* in the mouse brain during development (Janmohamed et al., 2004). FMO1 protein is active in the mouse brain, as evidenced by its catalysis of the N-oxygenation of the tricyclic antidepressant imipramine (Hernandez et al., 2009). FMO1 would therefore be expected to contribute to the production in brain of taurine from the precursor hypotaurine.
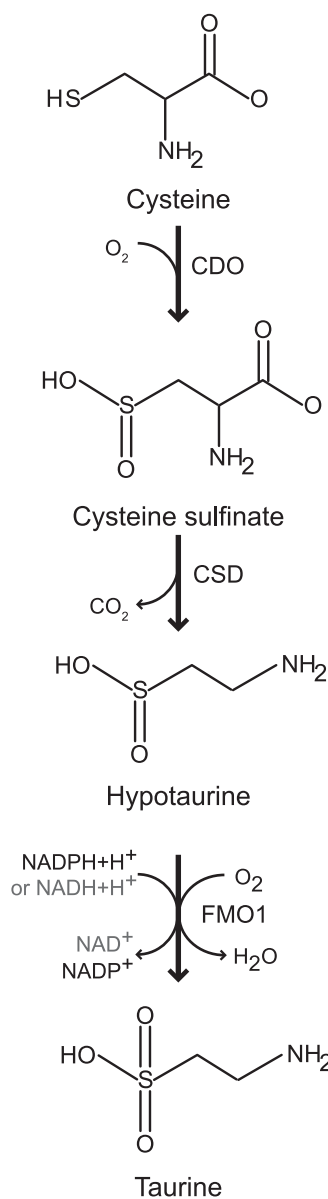
Fig. 5. Terminal steps of the biosynthetic pathway of taurine from cysteine. The final reaction in the pathway is catalyzed by FMO1, using either NADPH or NADH as cofactor. CDO, cysteine dioxygenase; CSD, cysteine sulfinate decarboxylase; FMO1, flavin-containing monooxygenase 1.

Taurine deficiency is implicated in a number of pathologic conditions, including cardiomyopathy, muscular abnormalities, and renal dysfunction (Ripps and Shen, 2012). Conversely, taurine supplementation has been reported to have positive effects on health, for instance, in lowering total plasma cholesterol (Murakami, 2015) and in overcoming insulin resistance (Ripps and Shen, 2012). Given our finding that FMO1 catalyzes the formation of taurine from hypotaurine, it is of interest that *Fmo1*-null mice exhibit some characteristics in common with those of taurine deficiency, including raised plasma concentrations of cholesterol (Veeravalli et al., 2018) and glucose (Veeravalli et al., 2014).

The K_M of FMO1 for hypotaurine is similar to that of human cysteine dioxygenase, an upstream enzyme in the taurine biosynthetic pathway (Fig. 5), for its substrate cysteine (Ye et al., 2007). Although the K_M for the FMO1-catalyzed oxygenation of hypotaurine is high, our results from the knockout-mouse line indicate that FMO1 is physiologically relevant for the production of taurine.

In addition to *Fmo1*, two other genes are deleted in the knockout-mouse line, *Fmo2* and *Fmo4*. Most humans are homozygous for a nonsense mutation of *FMO2*, c.1414C>T[p.(Gln472*)], the *FMO2*2A* allele, and do not express functional FMO2 (Dolphin et al., 1998; Whetstine et al., 2000); in the case of *FMO4*, the gene is expressed at very low levels (Dolphin et al., 1996). Thus, although we cannot eliminate the possibility that FMO2 or FMO4 are able to catalyze hypotaurine oxygenation in vitro, neither of these enzymes is likely to contribute substantially to the production of taurine in humans in vivo.

Commensurate with a role for FMO1 in endogenous metabolism, the gene contains few nonsynonymous polymorphisms (Phillips et al., 2007), each of which is present at very low frequency (Furnes et al., 2003), and only one has a significant but substrate-dependent effect on catalytic activity (Furnes and Schlenk, 2004). However, interindividual variation of up to fivefold in the expression of *FMO1* in adult human tissues such as kidney (Krause et al., 2003) and small intestine (Yeung et al., 2000) could affect taurine production and thus contribute to an intracellular deficiency of the amino acid.

Authorship Contributions

Participated in research design: Veeravalli, Phillips, Freire, Everett, Shephard.

Conducted experiments: Veeravalli, Phillips, Freire, Varshavi, Everett, Shephard.

Contributed new reagents or analytic tools: Veeravalli, Phillips, Freire, Varshavi, Everett, Shephard.

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Wrote or contributed to the writing of the manuscript: Phillips, Everett, Shephard.

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