Taurine attenuates hepatic steatosis in a genetic model of fatty liver disease

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ABSTRACT — Mice lacking the farnesoid X receptor (FXR) are used as a genetic model for nonalcoholic fatty liver disease because their livers exhibit hepatic steatosis and inflammation. The influence of taurine drinking on disrupted hepatic function was investigated using female Fxr-null mice. Significant decreases in the levels of hepatic damage-associated diagnostic markers, hepatic triglycerides, non-esterified fatty acids, and total bile acids were found in Fxr-null mice that had drunk water containing 0.5% taurine for four weeks. Hepatic but not serum taurine concentrations were significantly increased in these mice. The expression levels of oxidative stress-related genes (Hmox1 and Gsta1) and fatty acid synthetic genes (Acc1 and Scd1) were significantly decreased in these mice. These results suggest that drinking taurine improves hepatic steatosis and dysfunction caused by a lack of FXR.

Key words: Taurine, FXR, Steatosis, NAFLD

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is currently considered the most common type of liver disease and is characterized by excessive fat accumulation in the liver. It ranges from simple steatosis to a more aggressive form, nonalcoholic steatohepatitis, which may progress into hepatic fibrosis, cirrhosis, or hepatocellular carcinoma (Loguercio et al., 2001; Matteoni et al., 1999; Mulhall et al., 2002; Neuschwander-Tetri and Caldwell, 2003). Oxidative stress, lipotoxicity, and inflammation play key roles in NAFLD progression (Arteel, 2012; Bugianesi, 2008; Ibrahim et al., 2011).

Mice lacking the farnesoid X receptor (FXR, Fxr-null mice) develop hepatic steatosis, and their livers show elevated levels of triglycerides (TGs), non-esterified fatty acids (NEFAs), total cholesterol (TC), and bile acids (Sinal et al., 2000). Furthermore, elevated hepatic damage-associated diagnostic markers, such as serum alanine aminotransferase (ALT) and alkaline phosphatase (ALP), were found in Fxr-null mice (Kitada et al., 2003). Hepatic inflammation and fibrogenesis were observed in three-month-old Fxr-null mice (Nomoto et al., 2009; Liu et al., 2012). Fxr-null mice had a high incidence of hepatocarcinoma at 12 months of age (Kim et al., 2007; Yang et al., 2007). Based on these perspectives, Fxr-null mice are considered an ideal genetic NAFLD model.

Taurine (2-aminoethanesulfonic acid) is one of the most abundant amino acids in mammalian tissues. Taurine is supplied to the body by dietary ingestion from healthy drinks and marine products and via de novo synthesis from cysteine. Taurine has many biological and physiological properties such as antioxidation, osmoregulation, and bile acid conjugation; furthermore, it plays an important role in maintaining normal lipid metabolism (Chen et al., 2016; Huxtable, 1992). However, there are several contradicting reports on the influence of taurine supplementation on hepatic steatosis in mice fed a high-fat diet. Some studies suggest that long-term taurine supplementation leads to enhanced hepatic steatosis in mice fed a high-fat diet (Branco et al., 2015) whereas others report that taurine attenuates the development of hepatic steatosis in mice fed a high-fat diet (Chen et al., 2006). Conversely, several reports suggest that taurine exhibits a cholesterol-lowering effect in a hypercholesterolemia model (Chen et al., 2012). These studies were performed using a diet (high-fat and/or high cholesterol)-induced disease model. In diet-induced disease models, protection against the disease can be evaluated for the simultaneous administration of taurine.

In the present study, the influence of taurine supplementation was evaluated using a genetic model for fatty
liver disease (Fxr-null mice). Because the genetic disease model has already established the pathological condition before taurine treatment, the improvement in disease status but not protection against the disease can be evaluated. We utilized a genetic model for fatty liver disease, Fxr-null mice, and evaluated taurine-mediated improvement of this disease.

MATERIALS AND METHODS

Animal treatment, sample collection, and histological analysis

Fxr-null mice were kindly provided by Dr. Frank J. Gonzalez (National Institute of Health, Bethesda, MD) (Sinal et al., 2000). Eight-week-old C57BL/6N mice were purchased from Charles River Laboratories Japan, Inc. (Kanagawa, Japan) for use as the wild-type mice. The Fxr-null and wild-type mice were housed under a standard 12-hr light-dark cycle (7 a.m.-7 p.m.). Age-matched groups of eight- to nine-week-old female mice were used for all experiments because our previous study demonstrated that female Fxr-null mice were more sensitive to lipid metabolism than male Fxr-null mice (Miyata et al., 2016, 2011, 2017). The mice were fed standard rodent chow (NMF; Oriental Yeast Co. Ltd., Tokyo, Japan). Several studies were performed with 1% (w/v) taurine containing water (Kim et al., 2017; Li et al., 2019; Park et al., 2017). In the present study, mice were given 0.5% (w/v) or 2% (w/v) taurine in their drinking water for four weeks. After four weeks of drinking this water, the mice were killed between 9 a.m. and 11 a.m. For histological analysis, some of the liver sections were stained with hematoxylin and eosin. All experiments were performed in accordance with the guidelines for animal experiments of the National Fisheries University (Yamaguchi, Japan). The protocol was approved by the Institutional Animal Care and Use Committee at the National Fisheries University (Permission No. 2018-18-2).

Determination of hepatic damage-associated diagnostic markers and hepatic lipid levels

Serum ALT and ALP activities were determined using the commercial kits, Transaminase CII-B-test Wako and LabAssay™ ALP (Wako Pure Chemicals, Osaka, Japan), respectively. The hepatic samples were prepared as described previously (Miyata et al., 2010). Hepatic TGs, NEFAs, TC, and bile acids were determined using the Triglyceride E-test Wako, NEFA E-test Wako, and Cholesterol E-test Wako kits and the total bile acid-test Wako (Wako Pure Chemicals), respectively.

Determination of mRNA levels

Hepatic total RNA was isolated by the acid guanidine–phenol–chloroform method. Single-strand cDNA was synthesized using an oligo (dT) primer and the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). The cDNA underwent real-time quantitative polymerase chain reaction (qPCR) using SYBR Premix Ex Taq™ II (Tli RNaseH Plus) (Takara Bio, Shiga, Japan) with the TP870 Thermal Cycler Dice Real-Time System (Takara Bio). The relative mRNA levels were calculated by the comparative threshold cycle method. The specific forward and reverse primers used in real-time qPCR are described in a previous report (Miyata et al., 2017).

Determination of bile acid composition

The hepatic bile acid composition was analyzed by high-performance liquid chromatography (HPLC) as described previously (Kitada et al., 2003). The contents of β-muricholic acid (βMCA), tauro-β-muricholic acid (TβMCA), urusodeoxycholic acid (UDCA), tauroursodeoxycholic acid (TUDCA), cholic acid (CA), taurocholic acid (TCA), chenodeoxycholic acid (CDCA), taurochenodeoxycholic acid (TCDCa), deoxycholic acid (DCA), taurodeoxycholic acid (TDCA), lithocholic acid (LCA), and taurolithocholic acid (TLCA) were measured.

Determination of taurine levels

Taurine concentrations were determined using HPLC. The livers and serum were homogenized using 12% trichloroacetic acid and centrifuged to remove proteins. Trichloroacetic acids were removed by the extraction of diethyl ether. The samples were derivatized with o-phthalaldehyde (OPA) and separated with an L-column2 ODS (2.1 × 150 mm) (Chemicals Evaluation and Research Institute, Japan) by gradient elution using 50 mM potassium phosphate buffer (pH 7.0) without or with 40% acetonitrile. OPA-derivatized compounds were measured by fluorescence using an excitation wavelength of 360 nm and an emission wavelength of 455 nm.

Statistical analysis

The data are presented as means ± S.D. In the animal experiments, statistical significance was analyzed using a one-way ANOVA followed by Dunnett’s test or the Student’s t tests using the software Excel Statistics 2015 (Social Survey Research Information Co. Ltd., Tokyo, Japan).
Taurine improves hepatic dysfunction caused by a lack of FXR

RESULTS

Hepatic and serum taurine concentration

Fxr-null mice and the wild-type mice were given 0.5% (w/v) or 2% (w/v) taurine in their drinking water for four weeks. No significant alterations in amount of drinking were found among all of the groups. The hepatic taurine concentrations in the Fxr-null mice given 0.5% taurine or 2% taurine in their drinking water (0.5% taurine group or 2% taurine group) were significantly higher than those given 0% taurine in their drinking water (control group) (Fig. 1). The hepatic taurine concentrations in the wild-type mice in the 2% taurine group but not in the 0.5% taurine group were significantly higher than those in the control group. In both Fxr-null mice and wild-type mice, the serum taurine concentrations in the 2% taurine group but not in the 0.5% taurine group were significantly higher than those in the control group.

Influence of drinking taurine on hepatic biochemical parameters

The ratio of liver weight to body weight was increased in the wild-type and Fxr-null mice in the 2% taurine group, although the increase was not statistically significant (Table 1). Hepatic diagnostic damage marker, ALT, and ALP activities were markedly decreased in Fxr-null mice in the 0.5% taurine group compared to those in the control group (Fig. 2A, B). However, these activities were not further decreased in Fxr-null mice in the 2% taurine group. These activities were low in the wild-type mice compared to those in the Fxr-null mice. No significant differences in these activities were observed among the control, 0.5% taurine, and 2% taurine groups in the wild-type mice. Increases in hepatic taurine concentration might affect hepatic bile acid metabolism. Thus, we measured hepatic bile acid levels and bile acid composition. Hepatic total bile acid concentrations were significantly decreased in Fxr-null mice in the 0.5% taurine group but not in the 2% taurine group (Fig. 2C). TCA and TβMCA were the major hepatic bile acids observed in the Fxr-null mice (Fig. 2D). Hepatic TCA and TβMCA levels were significantly decreased in Fxr-null mice in the 0.5% taurine group. Hepatic βMCA, UDCA, CDCA, TCDCA, DCA, TDCA, LCA, and TLCA were not detected in all of

Fig. 1. Influence of drinking taurine on hepatic and serum taurine levels in Fxr-null and wild-type mice. A) Hepatic taurine levels. B) Serum taurine levels. The mice were fed water containing 0.5% or 2% taurine for four weeks. The values are presented as the mean ± S.D. (n = 6). Significant differences were assessed by Dunnett’s test (*p < 0.05; **p < 0.01 vs. 0% group).

Table 1. Body and hepatic weights in Fxr-null and wild-type mice.

|                | Wild-type          | Fxr-null          |
|----------------|--------------------|-------------------|
|                | 0% | 0.5% | 2%  | 0% | 0.5% | 2.0% |
| Body weight (g)| 21.8 ± 0.9 | 22.4 ± 0.9 | 23.0 ± 0.4 | 20.5 ± 1.1 | 20.8 ± 1.3 | 20.0 ± 1.5 |
| Liver (g)      | 1.17 ± 0.06 | 1.14 ± 0.12 | 1.32 ± 0.06 | 1.60 ± 0.45 | 1.42 ± 0.47 | 1.83 ± 0.28 |
| Liver/body weight ratio (%) | 5.38 ± 0.18 | 5.11 ± 0.60 | 5.74 ± 0.24 | 7.30 ± 1.81 | 6.89 ± 2.53 | 9.54 ± 0.78 |

Mice were fed a 0.5% or 2% taurine containing water for four weeks. Values are presented as the mean ± S.D. (n = 6).
Influence of drinking taurine on lipid levels

Hematoxylin and eosin (H&E) staining of liver sections showed several vacuoles due to lipid depositions in Fxr-null mice of the control group but not in Fxr-null mice of the taurine groups (Fig. 3). None were found in the wild-type mice of all groups. Consistent with the results of H&E staining, hepatic TG levels were markedly decreased in Fxr-null mice in the 0.5% and 2% taurine groups compared to those in the control group (Fig. 4A). The levels in the Fxr-null mice in the 0.5% and 2% taurine groups decreased compared to those in the wild-type mice. The alterations in hepatic NEFA levels were similar to those of hepatic TG levels. Hepatic NEFA levels were significantly decreased in Fxr-null mice in the 0.5% and 2% taurine groups. Conversely, no significant alterations of hepatic TC levels were observed among all groups of Fxr-null mice. In the wild-type mice, no significant decreases in hepatic TG, TC, or NEFA levels were observed with taurine supplementation. Serum TG levels were markedly decreased in the Fxr-null mice in the 0.5% and 2% taurine groups compared to those in the control group (Fig. 4B). The levels in the Fxr-null mice in the 0.5% and 2% taurine groups decreased compared to those in the wild-type mice. No significant decreases in serum NEFA or TC levels were observed in Fxr-null mice supplemented with taurine. Serum TG, TC, and NEFA levels were not decreased in wild-type mice supplemented with taurine.
Influence of drinking taurine on gene expression

To explore the mechanisms involved in the taurine-mediated attenuation of hepatotoxicity in Fxr-null mice, changes in the hepatic mRNA levels of oxidative stress-related genes (Hmox1, Nqo1, and Gsta1) were analyzed in Fxr-null mice. The expression levels of these genes were significantly increased in Fxr-null mice (Liu et al., 2012; Miyata et al., 2017). The expression levels of Hmox1 and Gsta1 were significantly decreased in Fxr-null mice in the 0.5% taurine group but not in the 2% taurine group compared to those in the control group (Table 2). To explore the mechanisms involved in the taurine-mediated reversion to hyperlipidemia in the Fxr-null mice, changes in the mRNA levels of lipid and drug metabolizing enzymes (Fasn, Scd1, Acc1, Cyp3a11, and Cyp7a1) were analyzed in Fxr-null mice. The expression levels of Acc1 and Scd1 were significantly decreased in Fxr-null mice in the 0.5% and 2% taurine groups.

DISCUSSION

The present study demonstrated that taurine supplementation ameliorated hepatic steatosis in Fxr-null mice. Serum TG levels were also decreased in taurine drinking Fxr-null mice. The taurine-mediated amelioration of hepatic steatosis might be at least in part due to decreased hepatic lipogenesis in Acc1 and Scd1. Murakami et al. reported that taurine attenuated the development of hepatic steatosis by inhibiting oxidative stress in mice fed a high-fat diet (Murakami et al., 2018). In the genetic model of fatty liver disease in this study, Fxr-null mice, taurine attenuated hepatic steatosis and reversed the elevated expression of the oxidative stress genes Hmox1 and Gsta1. Taurine-mediated attenuation of hepatic steatosis is likely related to the suppression of oxidative stress. Oxidative stress is spontaneously enhanced in Fxr-null mice, which may be attributable to a continuously high level of hepatic bile acids (Nomoto et al., 2009). Taurine-mediated reduction of hepatic bile acid levels is likely involved in the amelioration of hepatic steatosis.

Decreases in ALT and ALP activities and hepatic bile acid levels were alleviated in the 2% taurine group in Fxr-null mice, compared to those in the 0.5% taurine group. Oxidative stress-related genes, Hmox1 and Gsta1 expression levels were also correlated with these levels. It seems that 2% taurine drinking had, at least in part, negative effects on the improvement of hepatic functions in Fxr-null mice.
Several reports suggest that taurine exhibits a cholesterol-lowering effect due to the induction of CYP7A1, which is a rate-limiting enzyme for bile acid synthesis (Chen et al., 2012). Treatment with taurine decreased serum cholesterol levels in rats and mice fed a high cholesterol diet (Murakami et al., 2000). Taurine stimulates cholesterol catabolism by elevating CYP7A-mediated bile acid production, resulting in the reduction of hepatic and serum cholesterol.

Fig. 4. Influence of drinking taurine on hepatic and serum lipid levels in Fxr-null and wild-type mice. A) Hepatic total cholesterol (TC), triglyceride (TG), and non-esterified fatty acid (NEFA) levels. B) Serum total cholesterol (TC), triglyceride (TG), and non-esterified fatty acid (NEFA) levels. The mice were fed water containing 0.5% or 2% taurine for four weeks. Values are presented as the mean ± S.D. (n = 6). Significant differences were assessed by Dunnett's test (*p < 0.05; **p < 0.01 vs. 0% group) or the Student’s t-test (**p < 0.05; ***p < 0.01 vs. 0% wild-type group).

Table 2. Influence of taurine drinking on hepatic mRNA levels in Fxr-null mice.

| Gene     | Fxr-null 0% | Fxr-null 0.5% | Fxr-null 2% |
|----------|-------------|--------------|-------------|
| Fasn     | 1.00 ± 0.83 | 1.02 ± 0.70  | 1.12 ± 0.64 |
| Scd1     | 1.00 ± 0.50 | 0.56 ± 0.38* | 0.53 ± 0.48*|
| Acc1     | 1.00 ± 0.48 | 0.45 ± 0.31* | 0.45 ± 0.40*|
| Cyp3a11  | 1.00 ± 0.59 | 0.85 ± 0.56  | 0.84 ± 0.30 |
| Cyp7a1   | 1.00 ± 0.69 | 1.65 ± 1.27  | 1.61 ± 0.30 |
| Hmox1    | 1.00 ± 0.46 | 0.44 ± 0.27* | 0.72 ± 0.35 |
| Gst1     | 1.00 ± 0.52 | 0.44 ± 0.30* | 0.75 ± 0.83 |
| Nqo1     | 1.00 ± 0.30 | 1.14 ± 0.81  | 1.46 ± 0.65 |

Mice were fed a 0.5% or 2% taurine containing water for four weeks. Values are presented as the mean ± S.D. (n = 6). Significant differences (*p < 0.05) were assessed by Dunnett’s test. Acc1, acetyl-CoA carboxylase 1; Cyp7a1, cytochrome P450 7a1; Cyp3a11, cytochrome P450 3a11; Fasn, fatty acid synthase; Gst1, glutathione S-transferase alpha 1; Hmox1, heme oxygenase 1; Nqo1, (NAD(P)H quinone oxidoreductase 1; Scd1, stearoyl CoA desaturase 1.
serum cholesterol levels in rats or mice fed a high cholesterol diet (Chen et al., 2005). In Fxr-null mice, drinking taurine did not markedly reverse the elevated hepatic and serum cholesterol levels. Taurine might be able to suppress the elevation of cholesterol levels in hypercholesterolemia models fed a high cholesterol diet, but it did not reverse the elevated cholesterol levels in the genetic disease model (Fxr-null mice). Increased bile acid pool size, hepatic bile acid synthesis, and bile acid reabsorption in the intestine have been found in Fxr-null mice (Kok et al., 2003). Although drinking taurine did not decrease the Cyp7a1 mRNA levels in the Fxr-null mice, drinking taurine reduced hepatic bile acid levels. Drinking taurine might increase biliary bile acid output and decrease intestinal bile acid reabsorption in an FXR-independent manner. Chen et al. reported that the fecal bile acid levels of hypercholesterolemia rats fed a high cholesterol diet increased after taurine supplementation (Chen et al., 2003). Taurine feeding inhibits ileum bile acid absorption in rats fed a high cholesterol/fat diet (Nishimura et al., 2009). Taurine might be able to alter bile acid metabolism under unusual conditions such as the lack of FXR and the administration of high levels of cholesterol.

In the present study, drinking taurine reduced elevated hepatic TG levels in Fxr-null mice. To our knowledge, this is the first demonstration that drinking taurine attenuates hepatic steatosis in a genetic NAFLD model. Further studies are necessary to understand the taurine-mediated regulation of fat and cholesterol metabolism.

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Conflict of interest---- The authors declare that there is no conflict of interest.

REFERENCES

Arteel, G.E. (2012): Beyond reasonable doubt: who is the culprit in lipotoxicity in NAFLD/NASH? Hepatology, 55, 2030-2032.

Branco, R.C., Batista, T.M., Camargo, R.L., Borck, P.C., Ribeiro, R.A., Zoppi, C.C., Lollo, P.C., Morato, P.N., Boschero, A.C. and Carneiro, E.M. (2015): Long-term taurine supplementation leads to enhanced hepatic steatosis, renal dysfunction and hyperglycemia in mice fed on a high-fat diet. Adv. Exp. Med. Biol., 803, 339-351.

Bugianesi, E. (2008): Nonalcoholic fatty liver disease (NAFLD) and cardiac lipotoxicity: another piece of the puzzle. Hepatology, 47, 2-4.

Chen, S.W., Chen, Y.X., Shi, J., Lin, Y. and Xie, W.F. (2006): The restorative effect of taurine on experimental nonalcoholic steatohepatitis. Dig. Dis. Sci., 51, 2225-2234.

Chen, W., Guo, J., Zhang, Y. and Zhang, J. (2016): The beneficial effects of taurine in preventing metabolic syndrome. Food Funct., 7, 1849-1863.

Chen, W., Guo, J.X. and Chang, P. (2012): The effect of taurine on cholesterol metabolism. Mol. Nutr. Food Res., 56, 681-690.

Chen, W., Nishimura, N., Oda, H. and Yokogoshi, H. (2003): Effect of taurine on cholesterol degradation and bile acid pool in rats fed a high-cholesterol diet. Adv. Exp. Med. Biol., 526, 261-267.

Chen, W., Suruga, K., Nishimura, N., Gouda, T., Lam, V.N. and Yokogoshi, H. (2005): Comparative regulation of major enzymes in the bile acid biosynthesis pathway by cholesterol, cholate and taurine in mice and rats. Life Sci., 77, 746-757.

Huxtable, R.J. (1992): Physiological actions of taurine. Physiol. Rev., 72, 101-163.

Ibrahim, S.H., Kohli, R. and Gores, G.J. (2011): Mechanisms of lipotoxicity in NAFLD and clinical implications. J. Pediatr. Gastroenterol. Nutr., 53, 131-140.

Kim, H.W., Blomkalns, A.L., Oghi, M., Thomas, M., Gavrilova, D., Nolte, B.S., Cassis, L.A., Thompson, R.W., Weiss, R.M., Lindower, P.D., Blanco, V.M., McCormick, M.L., Daugherty, A., Fu, X., Hazen, S.L., Stansfield, B.K., Hsu, Y., Fulton, D.J., Chatterjee, T. and Weintrob, N.L. (2017): Role of myeloperoxidase in abdominal aortic aneurysm formation: mitigation by taurine. Am. J. Physiol. Heart Circ. Physiol., 313, H1168-H1179.

Kim, I., Morimura, K., Shah, Y., Yang, Q., Ward, J.M. and Gonzalez, F.J. (2007): Spontaneous hepatocarcinogenesis in farnesoid X receptor-null mice. Carcinogenesis, 28, 940-946.

Kitada, H., Miyata, M., Nakamura, T., Tozawa, A., Homma, W., Shimada, M., Nagata, K., Sinal, C.J., Guo, G.L., Gonzalez, F.J. and Yamazoe, Y. (2003): Protective role of hydroxysteroid sulfotransferase in lithocholic acid-induced liver toxicity. J. Biol. Chem., 278, 17838-17844.

Kok, T., Hulzebos, C.V., Wolters, H., Havinga, R., Agellon, L.B., Stellard, F., Shan, B., Schwarz, M. and Kuipers, F. (2003): Enterohepatic circulation of bile salts in farnesoid X receptor-deficient mice: efficient intestinal bile salt absorption in the absence of ileal bile acid-binding protein. J. Biol. Chem., 278, 41930-41937.

Li, K., Shi, X., Luo, M., Inam-U-Llah, Wu, P., Zhang, M., Zhang, C., Li, Q., Wang, Y. and Piao, F. (2019): Taurine protects against myelin damage of sciatic nerve in diabetic peripheral neuropathy rats by controlling apoptosis of schwann cells via NGF/Akt/GSK3β pathway. Exp. Cell Res., 383, 111557.

Liu, N., Meng, Z., Lou, G., Zhou, W., Wang, X., Zhang, Y., Zhang, L., Liu, X., Yan, Y., Lai, L., Forman, B.M., Xu, Z., Xu, R. and Huang, W. (2012): Hepatocarcinogenesis in FXR-/- mice mimics human HCC progression that operates through HNF4α regulation of FXR expression. Mol. Endocrinol., 26, 775-785.

Loguerocio, C., De Girolamo, V., De Sio, I., Tuccillo, C., Ascionne, A., Baldi, F., Budillon, G., Cimino, L., Di Carlo, A., Di Marino, M.P., Morisco, F., Picciotto, F., Terraccione, L., Vecchione, R., Verde, V. and Del Vecchio Blanco, C. (2001): Non-alcoholic fatty liver disease in an area of southern Italy: main clinical, histological, and pathophysiological aspects. J. Hepatol., 35, 568-574.

Matteoni, C.A., Younossi, Z.M., Gramlich, T., Boparai, N., Liu, Y.C. and McCullough, A.J. (1999): Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. Gastroenterology, 116, 1413-1419.

Miyata, M., Kinoshita, Y., Shinno, K., Sugiura, Y. and Harada, K.
(2016): Hepatic n-3/n-6 polyunsaturated fatty acid shift improves hepatic steatosis in farnesoid X receptor-null mice. Fish. Sci., 82, 529-536.

Miyata, M., Nomoto, M., Sotodate, F., Mizuki, T., Hori, W., Nagayasu, M., Yokokawa, S., Ninomiya, S. and Yamazoe, Y. (2010): Possible protective role of pregnenolone-16 alpha-carbonitrile in lithocholic acid-induced hepatotoxicity through enhanced hepatic lipogenesis. Eur. J. Pharmacol., 636, 145-154.

Miyata, M., Sakaida, Y., Matsuzawa, H., Yoshinari, K. and Yamazoe, Y. (2011): Amelioration of disrupted hepatic lipogenesis in Fxr-null mice by human FGF19 treatment. Biol. Pharm. Bull., 34, 1885-1889.

Miyata, M., Shinno, K., Kinoshita, T., Kinoshita, Y. and Sugiu, Y. (2017): Fish oil feeding reverses hepatomegaly and disrupted hepatic function due to the lack of FXR signaling. J. Toxicol. Sci., 42, 671-681.

Mulhall, B.P., Ong, J.P. and Younossi, Z.M. (2002): Non-alcoholic fatty liver disease: an overview. J. Gastroenterol. Hepatol., 17, 1136-1143.

Murakami, S., Kondo, Y. and Nagate, T. (2000): Effects of long-term treatment with taurine in mice fed a high-fat diet: improvement in cholesterol metabolism and vascular lipid accumulation by taurine. Adv. Exp. Med. Biol., 483, 177-186.

Murakami, S., Ono, A., Kawasaki, A., Takenaga, T. and Ito, T. (2018): Taurine attenuates the development of hepatic steatosis through the inhibition of oxidative stress in a model of nonalcoholic fatty liver disease in vivo and in vitro. Amino Acids, 50, 1279-1288.

Neuschwander-Tetri, B.A. and Caldwell, S.H. (2003): Nonalcoholic steatohepatitis: summary of an AASLD Single Topic Conference. Hepatology, 37, 1202-1219.

Nishimura, N., Yamamoto, T. and Ota, T. (2009): Taurine feeding inhibits bile acid absorption from the ileum in rats fed a high cholesterol and high fat diet. Adv. Exp. Med. Biol., 643, 285-291.

Nomoto, M., Miyata, M., Yin, S., Kurata, Y., Shimada, M., Yoshinari, K., Gonzalez, F.J., Suzuki, K., Shibasaki, S., Kurosawa, T. and Yamazoe, Y. (2009): Bile acid-induced elevated oxidative stress in the absence of farnesoid X receptor. Biol. Pharm. Bull., 32, 172-178.

Park, E., Park, S.Y., Cho, I.S., Kim, B.S. and Schuller-Levis, G. (2017): A novel cysteine sulfinic acid decarboxylase knock-out mouse: taurine distribution in various tissues with and without taurine supplementation. Adv. Exp. Med. Biol., 975, 461-474.

Sinal, C.J., Tohkin, M., Miyata, M., Ward, J.M., Lambert, G. and Gonzalez, F.J. (2000): Targeted disruption of the nuclear receptor FXR/BAR impairs bile acid and lipid homeostasis. Cell, 102, 731-744.

Yang, F., Huang, X., Yi, T., Yen, Y., Moore, D.D. and Huang, W. (2007): Spontaneous development of liver tumors in the absence of the bile acid receptor farnesoid X receptor. Cancer Res., 67, 863-867.