Chapter 7

The Role of Cholesterol in the Pathogenesis of Hypertension-Associated Nonalcoholic Steatohepatitis

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Additional information is available at the end of the chapter

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Abstract

Dietary cholesterol is a crucial risk factor for nonalcoholic steatohepatitis (NASH). Our recent studies indicated that high cholesterol intake was associated with the pathogenesis of hypertension-associated NASH. We developed a novel hypertensive rat model of NASH by feeding stroke-prone spontaneously hypertensive rats (SHRSP5/Dmcr) a high fat and cholesterol (HFC) diet. Histological features resembling human NASH were observed in this model. Furthermore, we investigated the kinetics of cholesterol in the rats fed an HFC diet and determined that suppression of bile acid (BA) detoxification led by HFC feeding results in cytotoxic BA accumulation in hepatocytes, which induces inflammatory response and liver damage. Sex differences in fibrogenesis were also observed in this model, and we found this was associated with a different ability in BA detoxification. Since SHRSP5/Dmcr rats are hypertensive, we investigated the role of hypertension in NASH progression by comparing NASH development among SHRSP5/Dmcr rats, spontaneously hypertensive rats and their original strain, Wistar Kyoto, with normal blood pressure. HFC diet induced more severe hepatic fibrosis in the hypertensive strains compared with the normotensive one. In conclusion, dietary cholesterol plays an essential role in the pathogenesis of NASH, and the combined action of cholesterol and hypertension further aggravates its progression.

Keywords: cholesterol, hypertension, nonalcoholic steatohepatitis, spontaneously hypertensive rat, Wistar Kyoto rat, CYP7A1, kinetics of bile acids, gender differences in fibrogenesis

1. Introduction

High dietary cholesterol intake may lead to increased risk of diseases such as cardiovascular disease and diabetes [1, 2]. Although the recommendation to restrict daily dietary cholesterol

intake (300 mg) was removed from the 2015–2020 Dietary Guidelines for Americans [3], it is still recommended that individuals minimize cholesterol consumption. Animal foods such as egg yolk, meats, dairy products, fish, and poultry are major sources of dietary cholesterol. Meanwhile, dietary cholesterol is not found in plant foods. Instead, many plants contain phytosterols, which are chemically similar to cholesterol, and can therefore compete with it and decrease its absorption in the intestinal tract [4]. The effect of dietary cholesterol on plasma cholesterol levels remains undetermined, since the body may suppress endogenous cholesterol synthesis in response to additional cholesterol ingestion [1]. Some studies have suggested that dietary cholesterol increases serum total cholesterol (TC), low-density lipoprotein (LDL) cholesterol, as well as the ratio of LDL to high-density lipoprotein cholesterol [5–8], which are considered to be associated with risk of vascular diseases.

Dietary cholesterol is also linked to the pathogenesis of nonalcoholic fatty liver disease (NAFLD)/nonalcoholic steatohepatitis (NASH) [9, 10]. NAFLD is one of the most common chronic liver diseases worldwide and comprises a spectrum of liver damage, from simple steatosis (a benign non-progressive condition) to NASH, the advanced form that may progress to hepatic cirrhosis or hepatocellular carcinoma [11]. The pathological characteristics of NASH include steatosis, hepatocellular ballooning, lobular inflammation, and hepatic fibrosis. Cholesterol may contribute to NASH development by being catabolized in the liver into bile acids (BAs), which are hepatotoxic and cause liver damage [12]. Li et al. demonstrated that dietary cholesterol exacerbates liver damage and hepatic inflammation in mice fed a high-fat diet [13]. Subramanian et al. reported that an LDL receptor-deficient mouse fed a high-fat, high-carbohydrate diet was a good animal model of NAFLD/NASH, and showed that dietary cholesterol worsened hepatic steatosis and inflammation in this model [9].

In addition, NAFLD/NASH was described as a hepatic manifestation of metabolic syndrome, and its development was associated with hypertension, obesity, diabetes, and hyperlipidemia [14, 15]. Some studies have shown an increased prevalence of NAFLD/NASH among hypertensive patients [16–18]. Using spontaneously hypertensive (SHR) rats fed a choline-deficient diet as a hypertensive animal model of NASH, and its normotensive control, the Wistar Kyoto (WKY) rat [19], Ikuta et al. revealed that hypertension enhances the progression of NASH. We previously developed a novel animal model of hypertension-associated NASH by feeding stroke-prone spontaneously hypertensive5/Dmcr (SHRSP5/Dmcr) rats a high fat and cholesterol (HFC) diet [20]. Further studies from our group suggested that dietary cholesterol may have a potential effect on the development of hypertension-associated NASH (unpublished).

In this chapter, we will discuss the crucial role of dietary cholesterol in the progression of hypertension-associated NASH.

2. The development of a novel animal model of NASH and the mechanism underlying the progression of NASH in this model

The mechanisms of the pathogenesis of NASH are not completely understood, partly due to a lack of ideal animal models with histological patterns that resemble human NASH. Matsuzawa et al. showed that an HFC diet (an atherogenic, high-fat diet containing 1.25%

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cholesterol and 60% fat) induced steatohepatitis, cellular ballooning, and fibrosis in the livers of male C57Bl/6J mice [21]. We previously established an HFC diet-induced NASH model using hypertensive SHRSP5/Dmcr rats [20].

SHRSP5/Dmcr rats are the fifth substrain of the stroke-prone spontaneously hypertensive (SHRSP) rat [20, 22], which is derived from the SHR strain [23]. To establish this strain, SHRSP rats were fed an HFC diet for 1 week, then those with high serum cholesterol levels (600–900 mg/dL in females and 300–600 mg/dL in males) were selected for brother–sister inbreeding. Selective inbreeding was repeated and offspring with increased hypercholesterolemic responses were obtained. Although the SHRSP5/Dmcr rats, formally known as arteriolipidosis-prone rats, were developed as an animal model of arteriosclerosis, marked enlargement and an abnormal whitish color of the liver were noted in the 47th generation. These findings prompted our studies on HFC diet-induced liver damage in this strain.

In order to determine whether the HFC diet-fed SHRSP5/Dmcr strain was a suitable model of NASH, we investigated hepatic histopathological changes following HFC feeding [20]. Male SHRSP5/Dmcr rats at 10 weeks of age were fed either an HFC (35.3% crude lipid and 5% cholesterol) or control diet (4.8% crude lipid and no additional cholesterol) for 2, 8, and 14 weeks. We found that the HFC diet induced microvesicular steatosis and lymphocyte infiltration at 2 weeks. Macrovesicular steatosis, ballooned hepatocytes with eosinophilic Mallory-Denk bodies, and multilobular necrosis were observed in the livers of rats fed an HFC diet at 8 weeks. The severity of steatosis and hepatocyte ballooning was further increased at 14 weeks. Meanwhile, a progressive deterioration of hepatic fibrosis occurred during HFC feeding. Slight pericellular and perivenular fibrotic changes, bridging fibrosis, and end-stage honeycomb fibrosis were observed at 2, 8, and 14 weeks, respectively. In addition, the HFC diet induced a progressive increase in indicators of liver damage, including serum levels of alanine transaminase (AST), aspartate transaminase (ALT), and γ-glutamyltranspeptidase (γ-GTP). Matteoni et al. classified human NAFLD into four types according to histological analysis of liver biopsy specimens: type 1, fatty liver alone; type 2, fat accumulation and lobular inflammation; type 3, fat accumulation and ballooning degeneration; and type 4, fat accumulation, ballooning degeneration, and hepatic fibrosis [24]. The histological characteristics observed in the liver of the SHRSP5/Dmcr strain at 2, 8, and 14 weeks of HFC feedings were very similar to those in type 2, type 3 or 4, and type 4 human NAFLD, respectively. Therefore, all pathological stages of NAFLD can be observed in the SHRSP5/Dmcr strain during HFC feeding. In addition, obesity, insulin resistance, and diabetes were not observed in this model. Therefore, it represents an excellent model of NAFLD/NASH without obesity and diabetes, and is useful for studying the pathogenesis and therapeutics of this disease.

We further investigated the molecular mechanisms underlying the progression of HFC-induced NASH in the SHRSP5/Dmcr strain [25]. Rats were fed either an HFC or control diet for 2, 8, and 16 weeks, and expression of genes involved in inflammation and hepatic fibrosis was evaluated. Tumor necrosis factor α (TNF-α), a proinflammatory cytokine, was reported to be upregulated in the livers of NASH patients [26]. We showed that the HFC diet increased the hepatic expression of TNF-α in SHRSP5/Dmcr rats at all time points. Nuclear factor κB (NF-κB; p50/p65) and inhibitor of κBα, the proteins involved in NF-κB signaling, which is regulated by TNF-α and plays an important role in inflammatory response, were also upregulated by the HFC diet. Hepatocyte injury and inflammation led to hepatic fibrosis via hepatic stellate

cell (HSC) activation, which results in the production and deposition of extracellular matrix (ECM) [27]. The HFC diet induced the upregulation of transforming growth factor-β1 (TGF-β1), a profibrotic cytokine that promotes HSC activation, prior to the appearance of obvious hepatic fibrosis (at 2 weeks). Its upregulation was also observed at subsequent stages (at 8 and 16 weeks). Expression of alpha smooth muscle actin (α-SMA) and platelet-derived growth factor-B, involved in hepatic fibrosis, were elevated at 8 weeks of HFC feeding, indicating extensive activation of HSC at this time point. Alpha-1 type I collagen, the major component of ECM, was produced by activated HSC (myofibroblast) and was markedly elevated at 8 and 16 weeks, corresponding to the appearance of extensive liver fibrosis observed at the same time points.

In order to investigate the role of dietary cholesterol in the pathogenesis of HFC diet-induced NASH in SHRSP5/Dmcr rats, we compared hepatic histological changes induced by a high fat (HF) diet and those by an HFC diet (unpublished). As described above, the HFC diet induced severe steatosis, lymphocyte infiltration, ballooned hepatocytes, and fibrosis in the livers of the rats. In contrast, HF feeding only led to mild hepatic steatosis and lymphocyte infiltration, while liver fibrosis was not observed. It was suggested that dietary cholesterol may play a key role in the transition from simple steatosis to fibrotic steatohepatitis, the progressive stage, during the progression of NAFLD/NASH.

3. The role of hypertension in the progression of NASH

SHRSP5/Dmcr rats are hypertensive, making this strain an ideal model in which to study the correlation between hypertension and NASH. In our previous study, we investigated the mechanism underlying the development of hypertension-associated NASH using three strains of a rat: normotensive WKY, hypertensive SHR and SHRSP5/Dmcr [28]. As mentioned previously, SHRSP5/Dmcr was established from the SHRSP strain, which was derived from SHR strain that was developed from normotensive WKY rats by selective inbreeding of the rats with spontaneously high systolic blood pressure in normal conditions [29]. Male rats with a blood pressure of 150–175 mmHg persisting for more than 1 month, and females with a blood pressure of 130–140 mmHg were mated, and the offspring with high blood pressure (over 150 mmHg persisting for more than 1 month) were selected for further inbreeding. The severity of hypertension was elevated from generation to generation, and all the rats from the third to sixth generation developed spontaneous hypertension by 15 weeks of age. Since the SHR and WKY originated from the same parental outbred Wistar rats, the WKY strain was used as the normotensive control for the SHR and SHRSP5/Dmcr strains. The blood pressure in the adult male rats of the three strains, WKY, SHR, and SHRSP5/Dmcr, were 130, 235, and 180 mmHg, respectively [28].

In our study, the normotensive WKY strain, and two hypertensive SHR and SHRSP5/Dmcr strains were fed either the HFC or control diet for 8 weeks. Changes to liver pathology and expression of proteins associated with inflammation and oxidative stress were determined [28]. We evaluated serum levels of AST, ALT, and γ-GTP, and confirmed that mild liver damage occurred in the hypertensive strains in the absence of HFC feeding, suggesting that hypertension may be a risk factor for chronic liver disease. The HFC diet induced more severe lobular inflammation and hepatic fibrosis in the hypertensive strains compared with the normotensive

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strain. The severity of the hepatic fibrosis observed in the SHRSP5/Dmcr strain was even higher compared with that of the SHR strain. The HFC diet induced elevation of serum inflammatory cytokines, TNF-α and TGF-β1, in the hypertensive strains, whereas an increase in TGF-β1 was not observed in the normotensive rats. The combination of TNF-α and TGF-β1 may trigger a more severe inflammatory response in the hypertensive rats by regulating the activation of downstream inflammatory signaling such as NF-κB and mitogen-activated protein kinase (MAPK) pathways. Increased activation of NF-κB and MAPK (p38 and JNK) signaling occurred in the hypertensive strains, which may have contributed to the more severe lobular inflammation observed in these rats. In addition, oxidative stress, defined as an imbalance between the production of reactive oxygen species (ROS) and their elimination by antioxidant defenses, may lead to cellular injury and chronic inflammation [30]. An increase in oxidative stress in NASH patients was previously reported [31, 32]. We measured serum thiobarbituric acid reactive substances levels and found that oxidative stress was significantly elevated in hypertensive strains fed an HFC diet but not in normotensive rats (unpublished data). Meanwhile, in hypertensive rats, the HFC diet suppressed the nuclear factor erythroid 2-related factor 2 (Nrf2)/Kelch-like ECH-associated protein 1 (Keap1) pathway, involved in antioxidative defenses [33]. We also found that hepatic levels of superoxide dismutase-1 (SOD-1) [25] and SOD-2 [28], that contribute to antioxidant defense by catalyzing the dismutation of superoxide anions [34], were decreased in hypertensive SHRSP5/Dmcr rats fed the HFC diet. The decrease in SOD-2 expression induced by HFC feeding was not observed in normotensive WKY and hypertensive SHR strains [28]. This could suggest that an increase in oxidative stress and a lower antioxidative capacity may trigger a more severe inflammatory response and liver damage in hypertensive rat strains following HFC feeding, compared with normotensive strains.

4. The role of cholesterol in the development of hypertension-associated NASH

As previously stated, dietary cholesterol intake is considered a risk factor for NAFLD/NASH. The liver is a crucial organ implicated in the regulation of cholesterol metabolism, including the synthesis and secretion of cholesterol, as well as the synthesis of BAs from cholesterol (a major pathway for hepatic cholesterol catabolism) and BA detoxification [35]. Disturbed cholesterol homeostasis in the liver is thought to be associated with the pathogenesis of NAFLD/NASH [35]. Our study showed that the HFC diet increased serum and hepatic levels of TC in the hypertensive SHR and SHRSP5/Dmcr strains, as well as the normotensive WKY strain [28]. It is worth noting that the increase in hepatic TC levels in the hypertensive rats was significantly lower than those in the normotensive WKY strain. Therefore, we postulated that more cholesterol was consumed for the synthesis of BAs in the livers of the hypertensive rats. In addition, serum TC levels in the hypertensive strains fed the control diet were markedly lower compared with those of the normotensive WKY strain, suggesting that the dysregulation of cholesterol metabolism may play an important role in the progression of hypertension-associated NASH.

In order to investigate the kinetics of cholesterol during the development of HFC-induced NASH in our hypertensive SHRSP5/Dmcr rat model, we evaluated the expression of proteins

involved in de novo cholesterol synthesis, cholesterol uptake from bloodstream in the form of LDL, cholesterol secretion into blood in the form of very-low-density lipoprotein, and BA synthesis and detoxification [36].

4.1. De novo cholesterol synthesis and its uptake from blood

Excessive intake of cholesterol may suppress de novo cholesterol synthesis via a feedback mechanism dependent on the transcriptional factor sterol regulator element-binding protein 2 (SREBP-2) [35]. SREBP-2 resides in the endoplasmic reticulum and remains there when cholesterol is abundant in hepatocytes; however, SREBP-2 is activated in response to low levels of cholesterol and translocated to the nucleus, where it triggers the expression of various genes, including low-density lipoprotein receptor (LDLR) and 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR). HMGCR is the rate-limiting enzyme for cholesterol biosynthesis. Our study showed that HMGCR was downregulated in the livers of SHRSP5/Dmcr rats during consumption of the HFC diet (2, 8, and 14 weeks), although SREBP-2 expression remained unchanged [36]. It was proposed that additional signaling, except SREBP-2, may be required for cholesterol synthesis in our rat model. The HFC diet decreased the expression of LDLR and LDLR-related protein 1, which are required for clearing cholesterol-contained lipoproteins from the blood by the liver [37]. Therefore, excessive intake of dietary cholesterol led to accumulation in the liver and consequently resulted in suppression of cholesterol synthesis and uptake.

4.2. BA synthesis and excretion

There are two major pathways of BA synthesis. The classic pathway is initiated by cholesterol 7 alpha-hydroxylase (CYP7A1), the rate-limiting enzyme, followed by the catalytic action of sterol 12 alpha-hydroxylase (CYP8B1) [38]. On the other hand, the initial step in the alternative (acidic) pathway is catalyzed by sterol 27-hydroxylase (CYP27A1), followed by oxysterol 7alpha-hydroxylase (CYP7B1). The major primary BAs, cholic acid (CA) and chenodeoxycholic acid (CDCA), are produced from cholesterol in the liver, while the secondary BAs, lithocholic acid (LCA) and deoxycholic acid (DCA), are generated from CDCA and CA in the intestines, respectively. After synthesis, conjugation of Bas is required for effective transport and detoxification [39]. BAs are conjugated with amino acids (taurine or glycine) or sulfate, mediated by BA coenzyme A synthase and BA amino acid transferase, and sulfotransferase (SULT2A1), respectively. Some BAs are glucuronidated by UDP-glucuronosyl *N*-transferases (UGT1A1, 2B4, and 2B7). Amino acid-conjugated BAs are excreted from the liver into the bile canaliculi via the bile salt export pump (BSEP), an ATP-binding cassette (ABC) transporter protein located in the canalicular membrane of hepatocytes [40]. Multidrug-resistant protein 2 (MRP2) is another ABC transporter implicated in the transport of sulfated or glucuronidated BAs to bile, while MRP3, located in the basolateral membrane of hepatocyte, is responsible for the transport of BAs from the liver to the blood. In addition, bile acid-activated nuclear receptors (a group of transcriptional factors), such as farnesoid X receptor (FXR), pregnane X receptor (PXR), and constitutive androstane receptor (CAR), are implicated in the regulation of BA metabolism, including synthesis, transport, and detoxification [39]. Several studies have reported that activation of FXR, PXR, and CAR inhibits transcription of the CYP7A1 gene in hepatocytes, and therefore suppress BA synthesis [41–43]. Activation of FXR and PXR also induces expression of the BA transporter proteins, BSEP and MRP2 [44–46].

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Increased levels of hepatic BA were observed in NASH patients and were correlated with inflammation and fibrosis in the liver [47]. In our SHRSP5/Dmcr model, the HFC diet increased hepatic levels of CYP7A1 but decreased levels of CYP8B1, while CYP27A1 was downregulated and CYP7B1 was upregulated [36]. We used ultra-performance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS) to further determine the hepatic levels of 21 types of BA in rats fed the HFC or control diet [48]. The HFC diet significantly increased total BA levels in the liver at 2 weeks, but decreased it at 8 weeks. We also investigated the composition of the total BA in the rats' livers. In the total BA pool, the relative proportions of CDCA species, which are hydrophobic and show high cytotoxicity [49], were markedly elevated at 8 and 14 weeks, whereas hydrophilic CA species, with lower toxicity, were significantly decreased at 14 weeks. The ratio of total CA to CDCA was prominently reduced by HFC feeding at 8 and 14 weeks. Most BAs (about 90% of the total) in the livers of rats fed the control diet were taurine-conjugated. In contrast, glycine-conjugated BAs were predominant in HFC-fed rats. In addition, canalicular transporters, BSEP and MRP2, were reduced in the livers of the rats during HFC feeding (2, 8, and 14 weeks), whereas MRP3, the basolateral transporter, was significantly increased at 8 and 14 weeks [36]. Therefore, the accumulation of total BAs in the rats' liver at 2 weeks of HFC feeding may have resulted from suppressed BA excretion to the bile duct, mediated by BSEP and MRP2 transporter proteins. Meanwhile, the decrease in total BA levels in the liver at 8 weeks may have been triggered by an increase in MRP3-mediated BA excretion to the blood. Furthermore, we demonstrated that the ratio of CA to CDCA was negatively correlated with liver injury (macrovesicular steatosis, serum ALT levels, and fibrotic area), whereas total glycol-BA/total tauro-BA was positively correlated. Therefore, the accumulation of BAs at 2 weeks of HFC-feeding, led by dysregulated BA synthesis and excretion, may trigger liver damage during the initial stages of NAFLD/NASH. Furthermore, a decrease in nuclear FXR, PXR, and CAR was observed in the livers of rats following HFC feeding. The downregulation of these nuclear receptors may be responsible for the increase in CYP7A1, as well as the decrease in BSEP and MRP2.

4.3. BA detoxification

Toxic BA accumulation in the liver induces hepatocyte injury, and BA hydrophobicity is correlated with cytotoxicity [12]. The order of BA hydrophobicity was reported to be CA < CDCA < DCA < LCA [12]. Hydrophobic BAs are potent inflammatory agents, whereas the hydrophilic BAs are anti-inflammatory [38]. Hydrophobic BAs stimulate ROS generation in hepatic mitochondria and lead to oxidative stress, hepatocyte apoptosis, and subsequent liver damage [50, 51]. BAs with detergent properties may also induce damage in hepatocyte membranes by binding to membrane components and disrupting the integrity of the plasma membrane [12, 52].

BA metabolism is tightly regulated to prevent the retention of excessive BAs in the liver [12]. Sulfation and glucuronidation of BAs, catalyzed by SULT2A1 and UGT, respectively, are major detoxification pathways of Bas [53, 54]. These reactions increase the solubility of BAs, enhance their fecal and urinary excretion, and reduce their toxicity. In addition, the nuclear receptors, PXR and CAR, protect hepatocytes from BA toxicity by regulating the transcription of genes involved in BA detoxification, including SULT and UGT [55, 56]. Our study showed that the HFC diet impaired BA detoxification by inducing the downregulation of PXR and CAR and further suppressing SULT2A1-catalyzed sulfation and UGT-catalyzed glucuronidation in the hypertensive SHRSP5/Dmcr rats [36].

5. CYP7A1

Our previous study showed that dysregulated expression of enzymes involved in BA synthesis led to the accumulation of BA in the livers of SHRSP5/Dmcr rats fed an HFC diet [36]. We further investigate the role of CYP7A1 in the pathogenesis of hypertension-associated NASH, and evaluated its hepatic levels in hypertensive SHR and SHRSP5/Dmcr rats, and the normotensive WKY strain [28]. Constitutive CYP7A1 levels were markedly higher (over 300-fold) in the hypertensive strains compared with those in the normotensive WKY strain. Upregulation of CYP7A1 may result in an excessive accumulation of toxic BAs, such as hydrophobic BAs, which may lead to oxidative stress and liver damage. In addition, Kamisako et al. showed that the Nrf2 pathway may regulate the expression of genes associated with BA synthesis and fatty acid metabolism, including CYP7A1 [57]. Our study showed increased activation of Nrf2 signaling in the livers of hypertensive rats fed a control diet compared with the normotensive WKY, which might be the responsible for the overexpression of CYP7A1 in the hypertensive strains [28].

6. Gender differences in NASH development

The prevalence and severity of human NAFLD/NASH varies with gender and age [58]. Yatsuji et al. studied 193 Japanese patients with NASH (86 women and 107 men) and showed a predominance of the disease in women over 50 years old, yet a greater prevalence in men aged 30–40 years [59]. Williams et al. reported that NAFLD patients were more likely to be male, older, and hypertensive [60]. The incidence of NAFLD/NASH is higher in men than premenopausal women (less than 50 years of age), while this immediately increases in women after menopause. Therefore, sex hormones such as estradiol may influence gender differences in NASH. In our study, we regarded female SHRSP5/Dmcr rats aged 12–24 weeks to correspond to the menopausal age in women. We also found female rats were less susceptible to HFC diet-induced liver damage compared with males [61]. Hence, our rat model may be useful for studies into gender differences in HFC-induced NASH. In order to investigate the related mechanisms, mature female and male SHRSP5/Dmcr rats (10 weeks old) were fed either an HFC or control diet for 2, 8, and 14 weeks. The severity of hepatic fibrosis was markedly lower in the female rats compared with the males. Although HFC feeding significantly reduced serum estradiol levels in female rats at 2 weeks, these levels were still much higher in females compared with males during HFC feeding, suggesting that this female hormone may contribute to the gender difference in NASH. In addition, only minor gender differences were noted in the expression of CYP7A1, CYP8B1, CYP27A1, and CYP7B1, the enzymes involved in BA synthesis, as well as MRP3 and BSEP, the proteins associated with BA transport. On the other hand, the enzymes implicated in BA detoxification, UGT and SULT2A1, as well as the nuclear receptors, CAR and PXR, were significantly suppressed in the male rats fed the HFC diet, whereas expression of these proteins was only slightly changed in females following HFC feeding. Since estradiol, which markedly decreases in women after menopause, may stabilize CAR and PXR proteins [61, 62], these results suggested a stronger capacity of BA detoxification associated with higher estradiol levels may be responsible for the resistance to HFC-induced liver damage and hepatic fibrosis in female rats compared with males.

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7. Treatment of NAFLD/NASH

NAFLD/NASH is related to poor lifestyle, including unhealthy diet habit and lack of exercise, which may, in turn, lead to excessive weight gain. Therefore, dietary intervention and exercise, targeted at weight loss, are the primary therapies for obesity-related NAFLD/NASH [63]. Vilar-Gomezet al. evaluated the effect of weight loss through lifestyle modifications on the improvement of NASH-related histologic features [64]. The study included 293 patients with NASH who followed a recommended lifestyle over 52 weeks to reduce body weight, including a low-fat, hypocaloric diet (750 kcal per day) and walking (200 min per week). Among these patients, 30% lost ≥5% of their weight at 52 weeks, 25% showed resolution of steatohepatitis, and 47% showed reduced nonalcoholic fatty liver disease activity score (NAS). This study also reported that the extent of weight loss was associated with histologic improvement. A higher proportion of patients with ≥5% weight loss had NASH resolution compared with those with ≤5% weight loss. Furthermore, 45% of patients with ≥10% weight loss showed regression of hepatic fibrosis.

Although NAFLD/NASH is closely linked with obesity and diabetes, it may also occur in the absence of these diseases [65]. As described before, the hypertensive SHRSP5/Dmcr rat represents a good model of NAFLD/NASH without obesity and diabetes [20]. We used this model to investigate the efficacy of dietary intervention for improving HFC-induced NASH [66]. Rats were fed an HFC diet for 2 weeks (before the appearance of hepatic fibrosis) or 8 weeks (after the appearance of fibrosis), then subsequently fed a control diet for 6 or 12 weeks. We found that dietary intervention prior to the appearance of fibrosis markedly improved steatosis and suppressed the HFC-induced increase in serum AST, ALT, and TC. On the other hand, dietary intervention after the appearance of fibrosis was unable to suppress the increase in serum ALT and hepatic TC. Although the dietary intervention (in both cases) reset the increased expression of fibrosis-relative proteins, TGF-β1 and α-SMA, it only slightly reduced the fibrotic area compared with continuous HFC feeding. Taken together, dietary intervention was able to completely or partially improve steatosis, inflammation, and cholesterol accumulation in the livers of rats fed an HFC diet, although this was not enough to improve hepatic fibrosis.

In addition, several pharmacological agents use in the treatment of NASH, including vitamin E and pioglitazone, have been tested [67, 68]. Oxidative stress and insulin resistance are considered as key factors implicated in the progression of NASH, and are, therefore, attractive targets for the treatment of NASH [69]. Sanyal et al. tested the efficacy of vitamin E, a lipid-soluble antioxidant, and pioglitazone, an insulin sensitizer, in NASH patients without diabetes [69]. The 247 patients included in this study received 800 IU vitamin E (84 subjects), 30 mg pioglitazone (80 subjects), or placebo (83 subjects) daily for 96 weeks. Both vitamin E and pioglitazone were associated with improvements in hepatic steatosis and lobular inflammation, as well as a reduction of serum AST and ALT, compared with the placebo. However, neither drug had a significant effect on hepatic fibrosis. In conclusion, lifestyle intervention (controlled dietary intake as well as exercise) may be the first choice for NAFLD/NASH treatment and should be optimized, while pharmacological management can be used as an auxiliary method, and should be further tested in large studies with long-term outcomes.

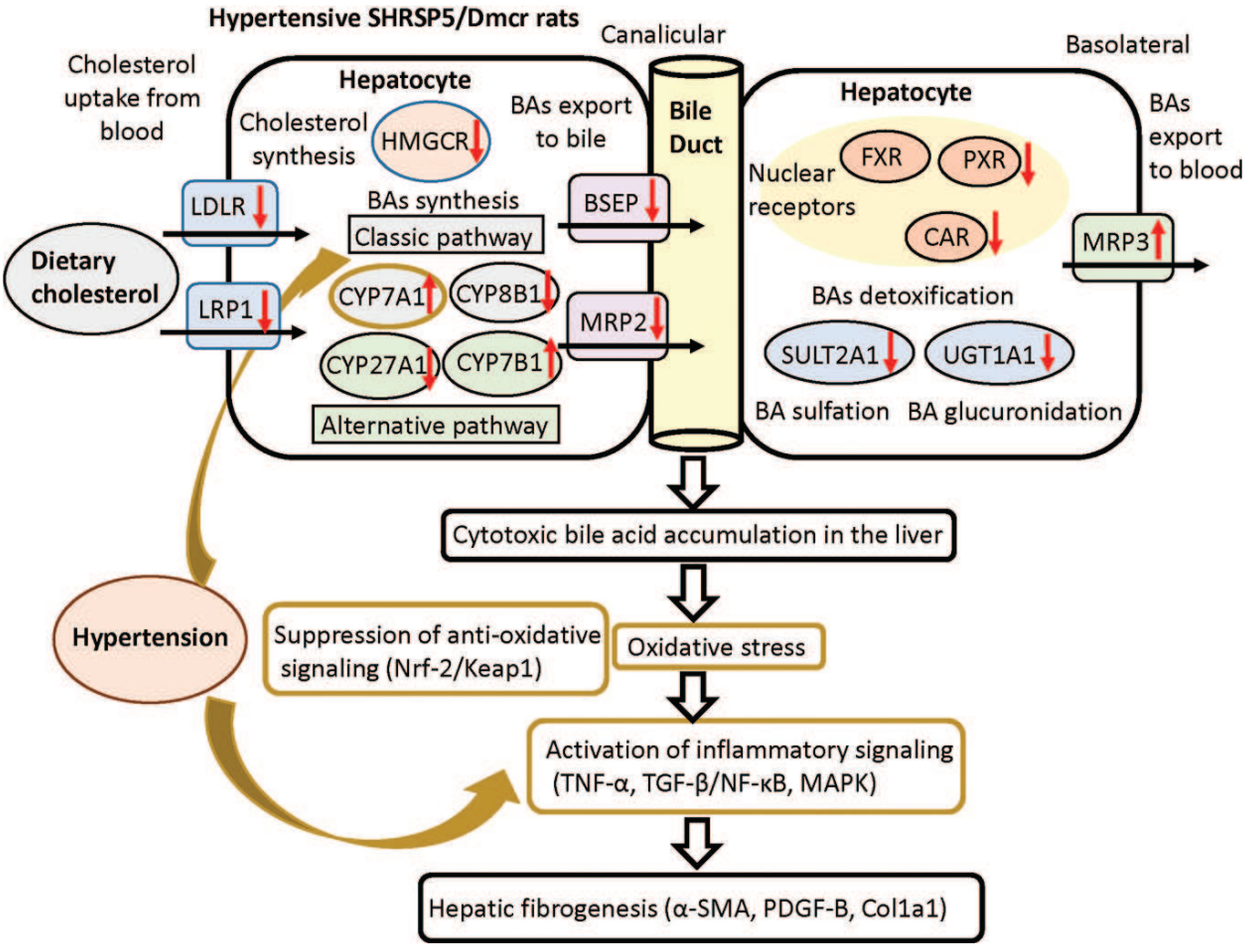

Figure 1. Possible mechanism underlying pathogenesis of HFC diet-induced fibrotic steatohepatitis in hypertensive SHRSP5/Dmcr rats [25, 35, 47]. In response to cholesterol accumulation in the liver triggered by HFC feeding, de novo cholesterol synthesis and its uptake were suppressed, indicated by a reduction in HMGCR, as well as LDLR and LPR1. The HFC diet induced dysregulated BA synthesis (upregulated CYP7A1 and CYP7B1, as well as downregulated CYP8B1 and CYP27A1) and export (downregulated BSEP and MRP2, as well as upregulated MRP3), and led to BA accumulation in hepatocytes. In addition, the HFC diet suppressed BA detoxification by decreasing the expression of nuclear receptors (PXR and CAR), and further downregulating SULT2A1 and UGT1A1, BA detoxification enzymes. Furthermore, cytotoxic BA accumulation in hepatocytes-induced oxidative stress, which activated inflammatory signaling (TNF-α, TGF-β/NF-κB, MAPK) and resulted in hepatitis. Hepatic inflammation-induced upregulation of fibrosis-related genes (α-SMA, PDGF-β, Col1a1) and led to hepatic fibrosis. Additionally, hypertension enhanced the deterioration of HFC-induced fibrotic steatohepatitis by upregulating CYP7A1, further leading to BA accumulation in hepatocytes and increased oxidative stress. On the other hand, hypertension induced the suppression of anti-oxidative signaling (Nrf-2/Keap1) following HFC feeding. Therefore, elevated oxidative stress and suppressed anti-oxidative capacity triggered a more severe inflammatory response in the hypertensive rats fed an HFC diet, as indicated by increased activation of inflammatory signaling (TNF-α, TGF-β/NF-κB, MAPK). BA, bile acid; HMGCR, 3-hydroxy 3-methyl-glutaryl-coenzyme A reductase; LDLR, low density lipoprotein receptor; LRP1, LDLR-related protein 1; CYP7A1, cholesterol 7α-hydroxylase; CYP8B1, sterol 12α-hydroxylase; CYP27A1, sterol 27-hydroxylase; CYP7B1, oxysterol 7α-hydroxylase; BSEP, bile salt export pump; MRP2, multidrug resistance-associated protein 2; MRP3, multidrug resistance-associated protein 3; FXR, farnesoid X receptor; PXR, pregnane X receptor; CAR, constitutive adrostane receptor; SULT2A1, sulfotransferase 2A1; UGT1A1, UDP-glucoronosyltransferase 1A1; TNF-α, tumor necrosis factor-α; TGF-β, transforming growth factor; NF-κB, nuclear factor kappa B; MAPK, mitogen-activated protein kinase; α-SMA, α-smooth muscle actin; PDGF-B, platelet-derived growth factor subunit B; Col1a1, alpha 1 type 1 collagen; Nrf-2, nuclear factor erythroid 2-related factor 2; Keap1, Kelch-like ECH-associated protein 1.

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8. Conclusions

In our previous study, we established a novel model of fibrotic steatohepatitis by feeding hypertensive SHRSP5/Dmcr rats an HFC diet. Histological features resembling human NASH were observed in the rats, suggesting that this model is useful for studying hypertension-associated NASH. We compared NASH development among hypertensive strains (SHRSP5/Dmcr and SHR) and the normotensive WKY strain, and showed that hypertension accelerates progression of HFC-induced NASH by elevating BA synthesis (CYP7A1), inducing increased activation of inflammatory signaling (MAPK and NF-κB), and suppressing signaling associated with antioxidant defense (Nrf2/Keap1). To elucidate the role of cholesterol in NASH development, we investigated the kinetics of cholesterol in this model, and found that the HFC diet induced dysregulation of BA synthesis and suppression of BA detoxification, therefore resulting in cytotoxic BA accumulation in hepatocytes, which further induced oxidative stress, followed by activation of signaling involved in hepatic inflammation and fibrosis (**Figure 1**). Sex differences in fibrogenesis were also observed in this model and were associated with a different sensitivity to BA toxicity. More sustained expression of nuclear receptors, CAR and PXR, and the enzymes involved in BA detoxification, UGT and SULT, contributed to the stronger resistance to HFC-induced liver damage in female rats compared with males. In conclusion, our studies demonstrate that dietary cholesterol may play a crucial role in the progression of NASH-associated hypertension and provide a basis for NAFLD/NASH treatment involving restriction of cholesterol intake.

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