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Vitamin D Attenuates Non-Alcoholic Fatty Liver Disease in High-Fat Diet-Induced Obesity Murine Model

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Purpose: Obesity and metabolic syndrome are acknowledged as key factors contributing to the development of non-alcoholic fatty liver disease (NAFLD). Vitamin D (VitD) is a multifaceted secosteroid hormone known for its anti-fibrotic and anti-inflammatory properties, with its deficiency often linked to obesity. Our study aimed to investigate whether VitD supplementation could mitigate the liver pathology associated with NAFLD.

Materials and Methods: The NAFLD model was developed by subjecting male C57BL/6 mice to a high-fat diet (HFD) for 14 weeks. These mice were supplemented with VitD through intraperitoneal injection at a dosage of 7 μg/kg, administered three times per week for 7 weeks.

Results: HFD resulted in VitD deficiency, insulin resistance, and increased liver weight. It elevated serum levels of liver aminotransferases and triglyceride, ultimately leading to steatohepatitis with fibrosis. This model exhibited increased levels of transforming growth factor (TGF)-β1, pro-inflammatory cytokines, HNF4α transcription factors, reactive oxygen species (ROS), renin-angiotensin system activity, and epithelial-mesenchymal transitions (EMT) within the liver. Supplementation with VitD resulted in the recovery of liver weight, improvement in histologic features associated with steatohepatitis, and reduction in alanine aminotransferases and triglyceride levels induced by the HFD. Additionally, it mitigated the HFD-induced over-expressions of TGF-β1 and fibrosis-related genes, along with pro-inflammatory cytokines and ROS. Notably, no adverse effect was found due to VitD supplementation in this model.

Conclusion: VitD ameliorates steatohepatitis within obesity-induced NAFLD through its multifaceted pathways. VitD supplementation emerges as a potentially safe, cost-effective, and direct treatment approach for NAFLD patients dealing with obesity or metabolic dysfunction.

Key Words: High-fat diet, non-alcoholic fatty liver disease, NAFLD, vitamin D

INTRODUCTION

Obesity and metabolic dysfunction stand as the primary pathogenic contributors to non-alcoholic fatty liver disease (NAFLD), intertwined with heightened insulin resistance, elevated serum triglycerides, intracellular lipid metabolites, and a multitude of systemic pro-inflammatory mediators, such as C-reactive protein, tumor necrosis factor-α (TNF-α), transforming growth factor-β (TGF-β), leptin, interleukin (IL)-6, and reactive oxygen species (ROS). These factors collectively underscore the critical mechanisms driving the progression of NAFLD.[1] As obesity and metabolic syndrome prevalence escalates, NAFLD's incidence surges, making it the foremost cause of liver cirrhosis in developed nations.[2,3]

Currently, the absence of direct pharmacotherapy for NAFLD necessitates a reliance on diet control and exercise for its management.[4] Intriguingly, epidemiological studies consistently

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report reduced vitamin D (VitD) level in obesity, metabolic syndrome,[5-7] and NAFLD patients,[8,9] hinting at the potential of VitD supplementation as a direct therapeutic avenue for these conditions, NAFLD included. While traditionally recognized for its role in calcium and bone metabolism, VitD serves as a multifaceted secosteroid hormone capable of modulating over 250 genes, exerting both up-regulatory and down-regulatory effects. It has demonstrated a capacity to mitigate pro-inflammatory and pro-fibrotic gene expressions.[10] Moreover, VitD deficiency heightens renin-angiotensin system (RAS) activity, implicated in inducing fibrosis across various organs, including the liver.[11] Given the multifaceted nature of NAFLD's pathogenesis, VitD has emerged as a potentially safe and cost-effective treatment approach for this disease. However, existing randomized clinical trials (RCTs) investigating VitD supplements for NAFLD have shown inconclusive results.[8,12,13]

This study aimed to assess the protective role of VitD supplementation in mitigating NAFLD liver pathology within a high-fat diet (HFD)-induced obesity mouse model.

MATERIALS AND METHODS

Mice model and experimental design

Male C57BL/6 mice, purchased from Japan-SLC (Hamamatsushi, Shizuoka, Japan), were used for this study. The study scheme was approved by the Institutional Animal Care and Use Committee (IACUC) of Yonsei University College of Medicine (IACUC number: 2017-0349). Each group consisted of six mice maintained in conventional animal facilities under standard conditions (room temperature of 21–24°C, relative humidity 45%–70%, 12-hour light/dark cycle), which have been approved by the Association for Assessment and Accreditation of Laboratory Animal Care International. All mice were randomly allocated to each group, fed a normal diet (ND) or HFD for 14 weeks, and sacrificed. The ND (D12450B; Research Diets Inc., New Brunswick, NJ, USA) contained 10% kcal from fat and 10 gm of vitamin mixture in 773.85 gm of diet. HFD (D12492; Research Diets Inc.) contained 60% kcal from fat and 10 gm of vitamin mixture in 1055.05 gm of diet. Mice were weighed weekly. VitD (Kwangdong Pharma, Seoul, Korea; 7 μg/kg, approximately 280 IU/kg) was injected three times per week for 7 weeks (Supplementary Fig. 1, only online). All measurement were done by researchers who were blinded to the groups.

Oral glucose tolerance test and insulin tolerance test

Oral glucose tolerance test (OGTT) and insulin tolerance test (ITT) were performed at the 14th week after HFD or ND feeding. Food was withheld overnight for OGTT and for 4 hours for ITT. OGTT was performed by oral administration of glucose (2 g/kg body weight; Sigma Aldrich, St. Louis, MO, USA). For ITT, mice were administered insulin (0.75 IU/kg; Sigma Aldrich) by intraperitoneally injection. Blood glucose from the tail vein was measured at 0, 30, 60, 90, and 120 minutes after glucose or insulin injection using blood glucose strips and the Accu-Check glucometer (Roche, Mannheim, Germany).

Liver harvest and serum biochemical assays

Mice were deeply anesthetized by intraperitoneal injection of pentobarbital (50 mg/kg; Hanlim Pharm, Seoul, Korea). A midline laparotomy incision was performed, and the maximum amount of blood possible was collected from the heart. The liver was then harvested, and pieces of extracted liver were immersed in 10% neutral-buffered formalin. The remainder of the liver was snap-frozen in liquid nitrogen. Serum was harvested following centrifugation of clotted blood samples and examined for biochemical parameters: serum triglyceride, calcium, alanine aminotransferase (ALT), and alkaline phosphatase (ALP) levels using an automated clinical chemistry analyzer (Dri-Chem 4000i; Fujifilm, Tokyo, Japan).

Histopathologic examination

Liver specimens embedded in paraffin blocks were sectioned into 4-μm thickness slices. Each slice was treated with xylene and dehydrated by immersion in a graded alcohol series. Slices were then stained with hematoxylin & eosin (H&E) and Masson's trichrome. Liver specimens embedded in OCT compound blocks were cryo-sectioned into 5-μm thickness slices. Slices were stained with Oil Red O solution. Tissue sections were examined using an Olympus BX40 microscope in conjunction with an Olympus U-TV0.63XC digital camera (Olympus BX53F, Center Valley, PA, USA).

Liver homogenate and enzyme-linked immunosorbent assay

To analyze the protein levels, 100 mg of liver tissues were lysed with RIPA buffer (Thermo Fisher Scientific Inc., Rockford, IL, USA) using a TissueLyser II (Qiagen, Hilden, Germany). After centrifugation, supernatants from liver homogenate were collected to measure TGF-β1, IL-1β, TNF-α, renin, angiotensin-2, angiotensin-2 receptor type 1 (AGTR1), and angiotensin-2 receptor type 2 (AGTR2) levels. Concentrations of each protein in liver homogenate were measured using ELISA (TGF-β1, IL-1β, TNF-α, renin, angiotensin-2: R&D Systems, San Diego, CA, USA; AGTR1, AGTR2: MyBioSource, San Diego, CA, USA) according to the manufacturer's instructions. To evaluate the degree of oxidative stress, TBARS assay for malondialdehyde (MDA) in liver homogenate was performed (DoGenBio, Seoul, Korea). The procedure was performed following the recommended protocols of the manufacturers.

RNA purification, reverse transcription, and real-time PCR amplification

Total RNA was isolated from extracted livers using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). cDNA synthesis was carried out with RNA to cDNA EcoDry premix kit (Takara Bio,

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Kusatsu, Japan). The procedure was performed following the recommended protocols of the manufacturers. A polymerase chain reaction (PCR) master mix (Power SYBR Green PCR Master Mix: Applied Biosystems, Warrington, UK) was used, and quantitative PCR performed on the StepOnePlus™ PCR System (Applied Biosystems). The relative expression levels of target genes were normalized to GAPDH levels.

Statistical analysis

All results are expressed as mean±standard error of mean. Data were analyzed using one-way analysis of variance (ANOVA) followed by Bonferroni post-hoc test. Body weight, ITT and OGTT were analyzed using repeated-measures ANOVA. Statistical analyses were performed using SPSS statistical software version 12.0 (SPSS Inc., Chicago, IL, USA), with p value <0.05 considered statistically significant.

RESULTS

HFD triggers alternation in body and liver weights, OGTT, ITT, and serum 25-OH-VitD levels

In the HFD groups, body weight exhibited a consistent increase starting from the first week of feeding. By the 14th week, the body weight had doubled in the HFD group compared to the ND group (p=0.006) (Fig. 1A). Furthermore, the liver weight in the HFD group was 2.4 times heavier than that in the ND group (3.18±0.53 g vs. 1.33±0.30 g, p<0.001). While VitD supplementation did not impact the body weight of the HFD group, it notably reduced the liver weight compared to the untreated HFD group (2.48±0.42 g vs. 3.18±0.53 g, p=0.015) (Fig. 1B).

By the 14th week, the HFD group displayed impaired OGTT (p<0.001) and ITT (p=0.018) compared to the ND group (Fig. 1C and D). Notably, VitD supplementation did not influence these tolerance levels (OGTT: p=0.637, ITT: p=0.356). Intriguingly, serum 25-OH VitD levels, measured at week 14, were notably lower in the HFD group in contrast to the ND group (p=0.002), and VitD supplementation successfully increased these levels as anticipated (p<0.001) (Fig. 1E).

Additionally, the mRNA expression of the VitD receptor (VDR) was diminished in the HFD group (p<0.001), but was restored by VitD supplementation (p<0.001) (Fig. 1F). Moreover, the mRNA expression of CYP2R1, a crucial 25-hydroxylase of VitD in the liver, was reduced in the HFD group (p=0.008), and this decline was reversed by VitD (p= 0.018) (Fig. 1G). However, there were no discernible differences in the levels of CYP27B1 (HFD: p=0.439, VitD: p=0.799), the VitD 1α-hydroxylase responsible for converting 25-OH VitD3 to calcitriol (Fig. 1H).

Alterations in serum biochemical markers and liver pathology induced by HFD and VitD supplementation

Comparison of various serum biochemical parameters among the groups revealed significant elevations in ALT (p=0.003), ALP (p=0.016), and serum triglyceride (p=0.003) levels due to HFD, with subsequent improvements observed upon VitD supplementation (ALT: p=0.027, ALP: p=0.019, triglyceride: p=0.031) (Fig. 2A–C). However, no alterations were observed in serum calcium levels following either HFD or VitD supplementation (Fig. 2D).

Histopathological examination unveiled distinct differences between the groups. HFD induced notable steatosis, confirmed through H&E staining and Oil Red O staining, which was markedly ameliorated by VitD supplementation. Additionally, liver fibrosis, identified via Masson's trichrome staining, displayed more conspicuous collagen bands in hepatic sinusoids of the HFD group. These fibrotic features notably improved after VitD supplementation (Fig. 3).

The assessment of epithelial-mesenchymal transitions (EMT) markers in the liver revealed HFD-induced enhancements in mRNA levels of collagen 1, collagen 3 (Fig. 4A and B), and α-SMA (Fig. 4C). VitD supplementation attenuated collagen 1 (p=0.002) and collagen 3 (p=0.026) and showed a trend toward attenuation in α-SMA (p=0.095). E-cadherin was down-regulated (p=0.039), and N-cadherin (p=0.036) displayed a tendency to increase due to HFD, but VitD supplementation did not fully reverse these changes. Other EMT markers, namely fibronectin (p<0.001) (Fig. 4D), vimentin (p=0.006) (Fig. 4G), and Twist (p<0.001) (Fig. 4H), were upregulated by HFD and down-regulated by VitD supplementation. Conversely, mRNA expression of Snail (p=0.150) and Slug (p=0.309) remained unchanged due to HFD (Fig. 4I and J).

Alternation in gene expressions associated with steatosis within the liver

In our investigation utilizing liver tissues and quantitative RT-PCR, we aimed to assess the impact of VitD supplementation on the expression of steatosis-related genes. As anticipated, mRNA levels of various steatosis-related genes-ChREBP (p=0.005) (Fig. 5A), SREBP-1 (p=0.006) (Fig. 5C), LPIN1 (p=0.004) (Fig. 5D), and FASN (p=0.032) (Fig. 5E)-exhibited enhancement due to HFD, whereas PEPCK (p=0.880) (Fig. 5B) and leptin (p=0.113) (Fig. 5F) remained unaffected. Notably, the elevations, particularly in ChREBP (p=0.040) and LPIN1 (p=0.029), were significantly mitigated by VitD supplementation.

Furthermore, our investigation encompassed the examination of HNF4α expression in the liver using quantitative RT-PCR analyses. The mRNA levels of HNF4α (p<0.001) (Fig. 5G) were diminished by HFD and subsequently restored with VitD supplementation (p=0.007).

Alterations in pro-fibrotic, pro-inflammatory cytokines, and transcription factors within the liver due to HFD and VitD supplementation

The impact of HFD and VitD supplementation on various pro-inflammatory and pro-fibrotic cytokines in the liver was ob-

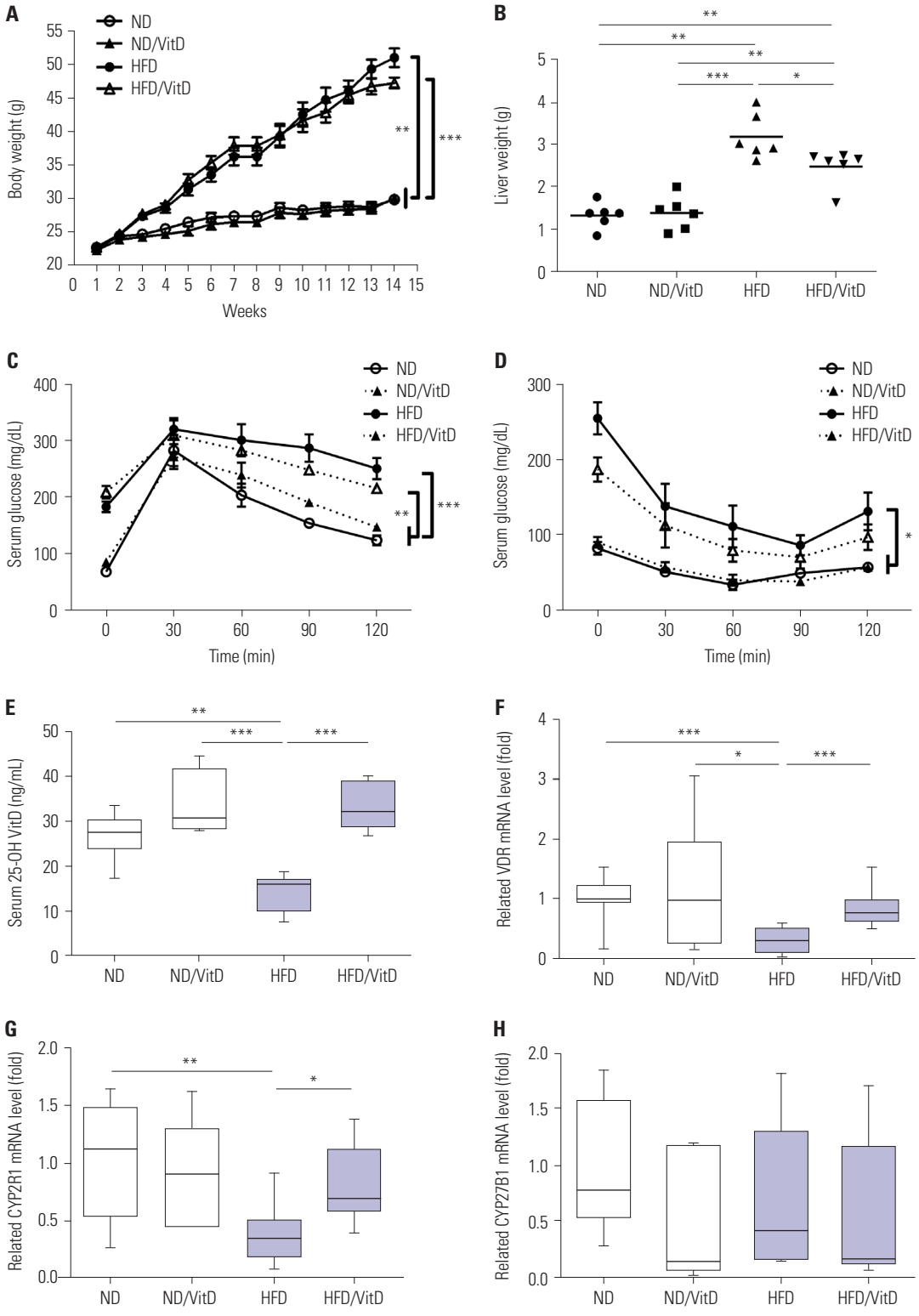

Fig. 1. Changes of metabolic and VitD-related parameters in HFD-induced NAFLD model. (A) Body weight change, (B) liver weight at 14th week, (C) oral glucose tolerance test at 14th week, (D) insulin tolerance test, (E) serum 25-OH VitD concentration, (F) mRNA expression of VitD receptor in liver, (G) mRNA expression of CYP2R1, 25-OH hydroxylase of VitD, and (H) CYP27B1 mRNA expression in liver, 1-OH hydroxylase of VitD in liver. (A, C, and D) were analyzed with repeated ANOVA test. (E–H) were analyzed with one-way ANOVA with Bonferroni correction. Each group consisted of six mice, and data was from the representative experiment. *p<0.05; **p<0.01; ***p<0.001. ND, normal diet; VitD, vitamin D; HFD, high-fat diet; VDR, VitD receptor; NAFLD, non-alcoholic fatty liver disease; ANOVA, analysis of variance.

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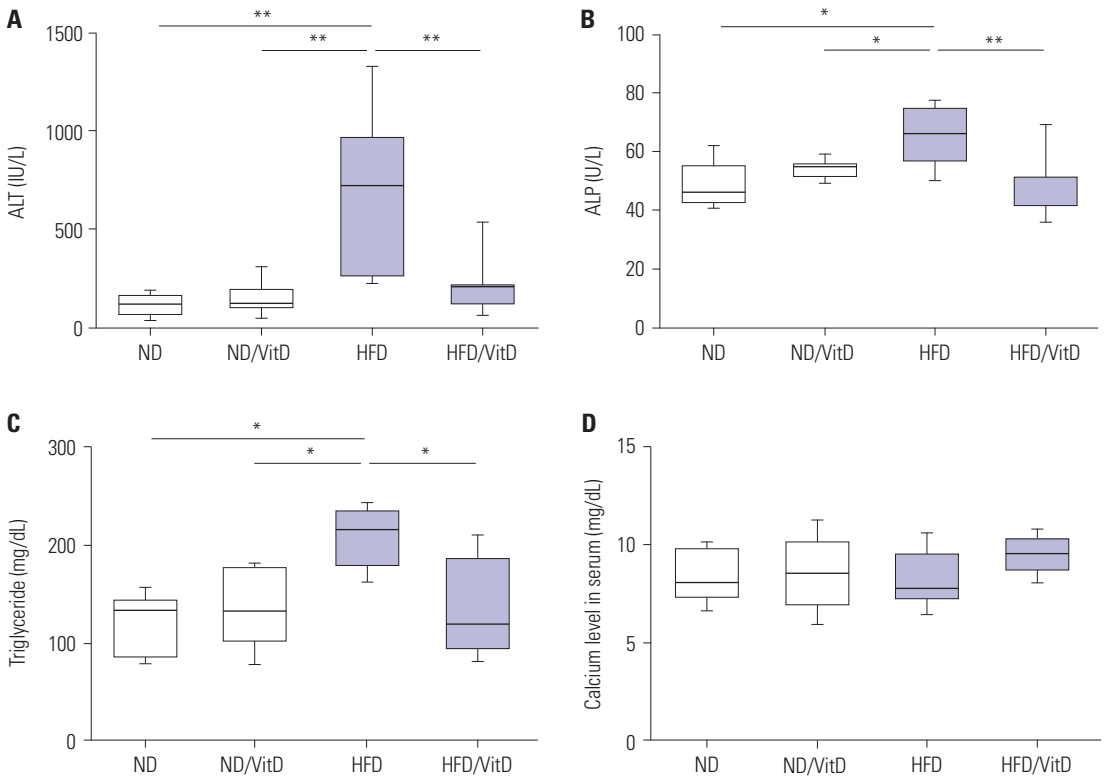

Fig. 2. Change of liver function status, triglyceride, and calcium concentrations in serum by HFD and administration of VitD. (A) Alanine aminotransferase (ALT) concentration. (B) Alkaline phosphatase (ALP) concentration. (C) Triglyceride. (D) Calcium concentration. All figures were analyzed using one-way ANOVA with Bonferroni correction. *p<0.05; **p<0.01. ND, normal diet; VitD, vitamin D; HFD, high-fat diet; ANOVA, analysis of variance.

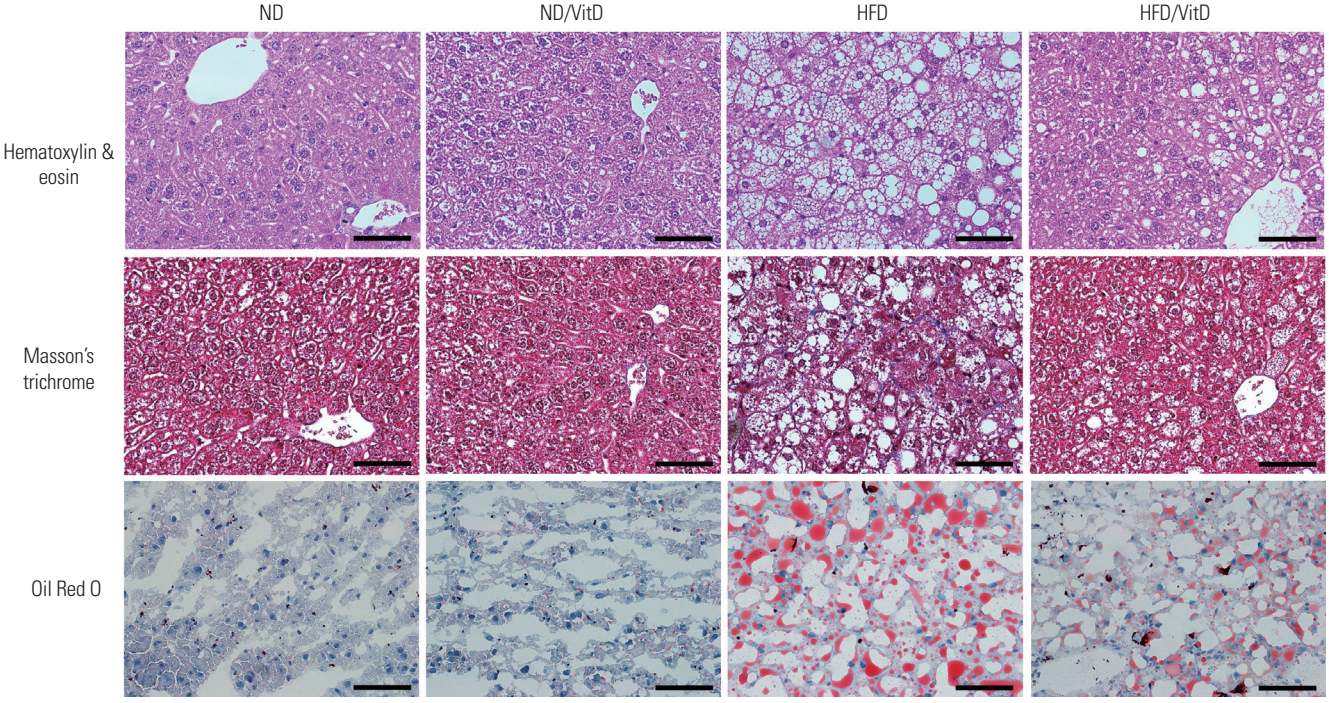

Fig. 3. Liver histology features of HFD-induced NAFLD model with VitD supplementation. Liver tissues were stained with hematoxylin & eosin, Masson's trichrome, and Oil Red O stains (Scale bars, 50 μm). ND, normal diet; VitD, vitamin D; HFD, high-fat diet; NAFLD, non-alcoholic fatty liver disease.

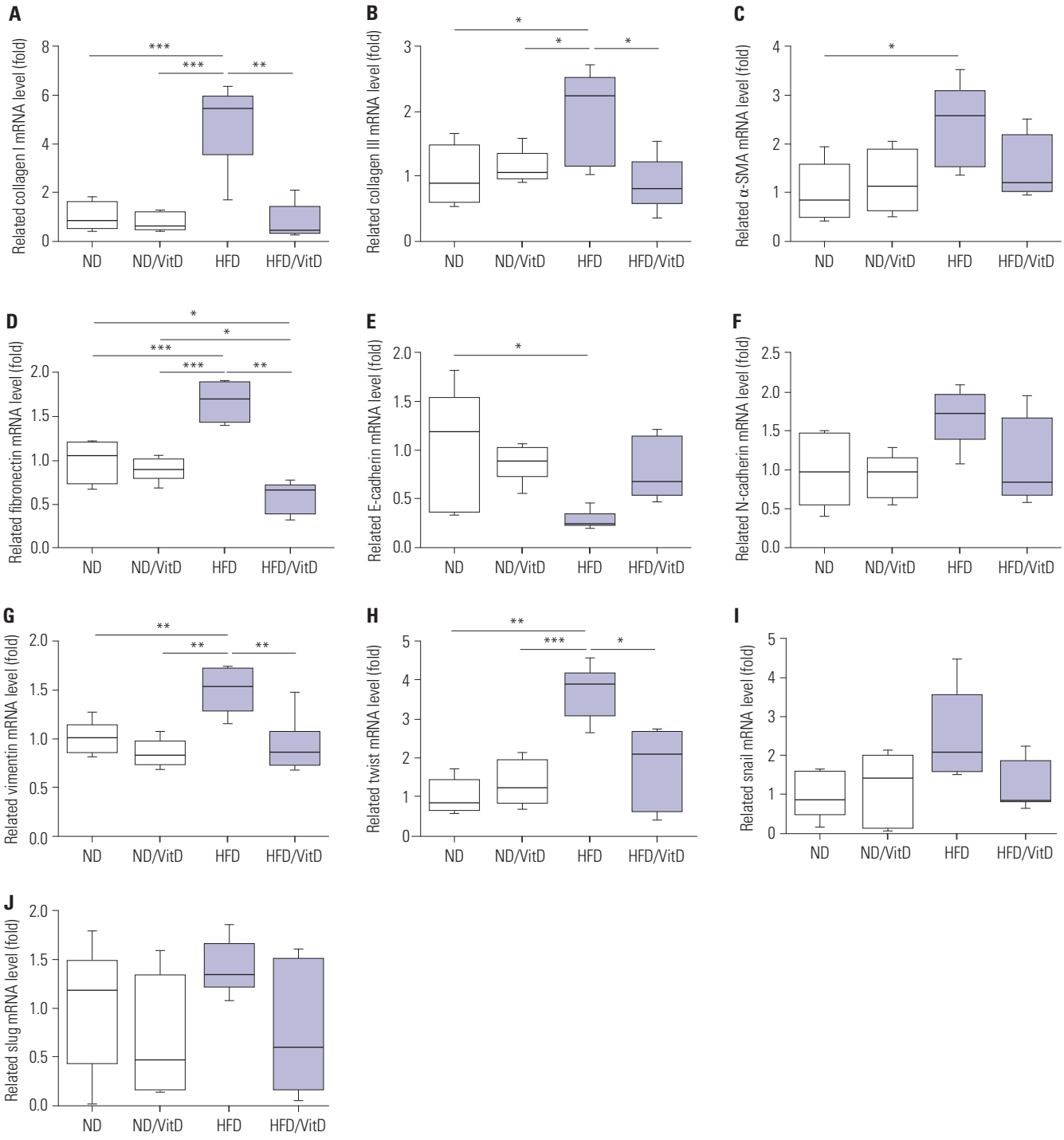

Fig. 4. Change of mRNA expressions of fibrosis and EMT-related genes in liver. (A) Collagen 1. (B) Collagen III. (C) α-SMA. (D) Fibronectin. (E) E-cadherin. (F) N-cadherin. (G) Vimentin. (H) Twist. (I) Snail. (J) Slug. *p<0.05; **p<0.01; ***p<0.001. ND, normal diet; VitD, vitamin D; HFD, high-fat diet; EMT, epithelial-mesenchymal transitions.

served in our study. Notably, TGF-β1, a fibrogenic cytokine, displayed significant upregulation at both protein (p=0.021) and mRNA and levels (p=0.017) due to HFD (Fig. 6A and E). Other cytokines involved in mediating inflammation, such as IL-1β (Fig. 6B and F) and TNF-α (Fig. 6C and G), exhibited increased mRNA and protein levels in the HFD liver, which were subsequently decreased by VitD. Additionally, the expression of NF-κB, a crucial transcription factor, showed enhancement due to HFD (p=0.012) and displayed a tendency toward reduction with VitD supplementation (p=0.166) (Fig. 6D).

To explore whether VitD supplementation influences oxidative stress, we assessed the liver's MDA level. We observed an increase in MDA levels induced by HFD (p=0.033), which significantly decreased upon VitD supplementation (p=

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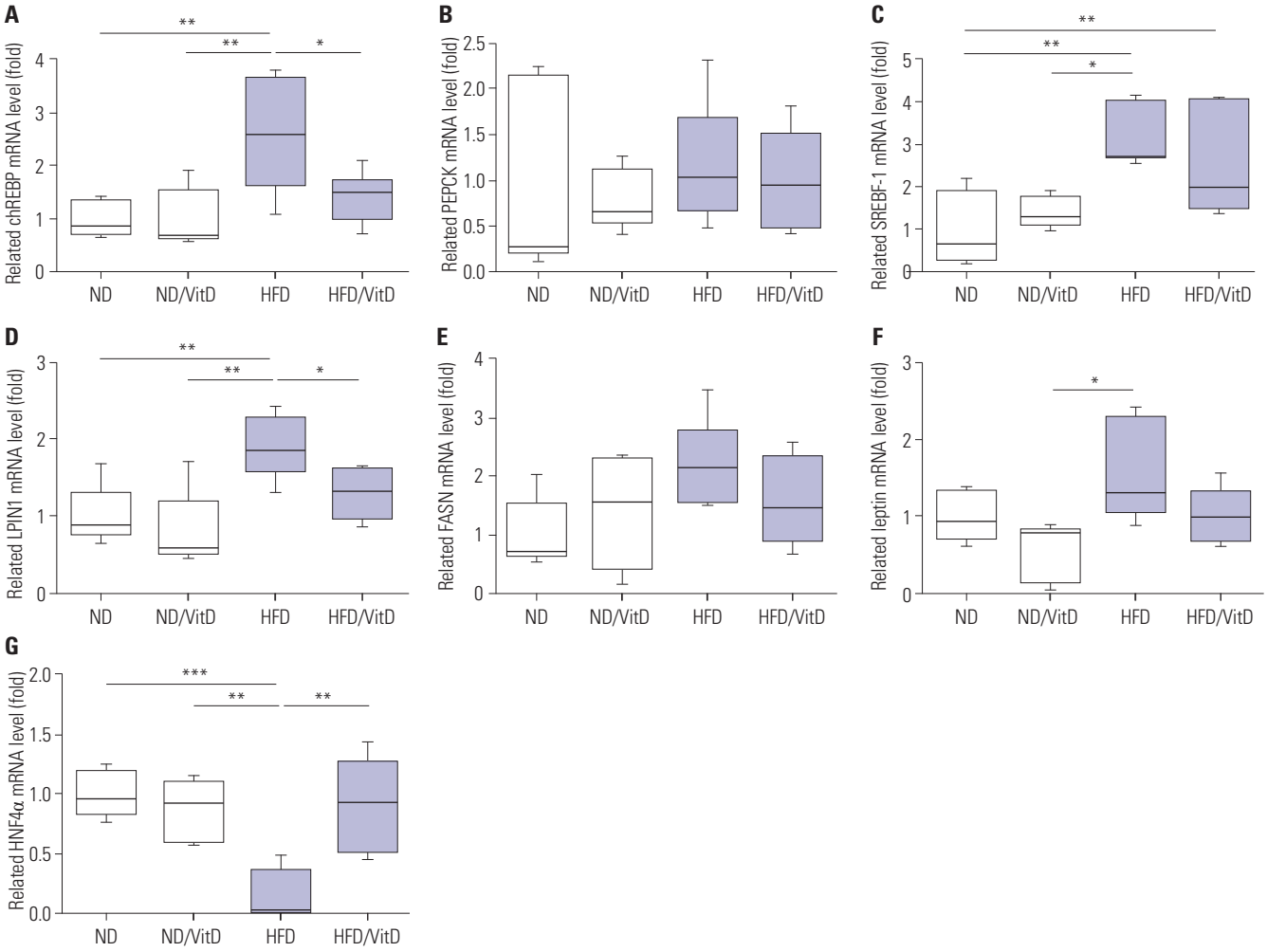

Fig. 5. Change of mRNA expressions of steatosis-related genes in liver. (A) ChREBP, (B) PEPCK, (C) SREBP-1, (D) LPIN1, (E) FASN, fatty acid synthase, (F) leptin, and (G) HNF4α were measured by qPCR. All figures were analyzed with one-way ANOVA with Bonferroni correction. *p<0.05; **p<0.01; ***p<0.001. ND, normal diet; VitD, vitamin D; HFD, high-fat diet; ANOVA, analysis of variance.

0.048) (Fig. 6H).

Impact of VitD supplementation on RAS in both serum and liver

The serum levels of renin (p=0.024) and angiotensin-2 (p=0.005) exhibited an increase due to HFD, and these levels notably decreased upon VitD supplementation (renin: p=0.010, angiotensin-2: p=0.016) (Fig. 7A and D). Similarly, in liver tissue, ELISA measurements revealed elevated renin (p=0.005) (Fig. 7B) and angiotensin-2 (p=0.019) (Fig. 7E) levels following HFD, which consistently decreased with VitD supplementation (renin: p=0.022, angiotensin-2: p=0.003) (Fig. 7B and E). Furthermore, the heightened mRNA expression of renin and angiotensinogen observed in the liver due to HFD was also notably attenuated by VitD (renin: p=0.002, angiotensinogen: p=0.018) (Fig. 7C and F). Angiotensin-2 plays a pivotal role in liver fibrogenesis, acting via two primary receptors, AGTR1 and AGTR2. To explore this, we measured the expression levels of AGTR1 and AGTR2 in the liver using ELISA and quantitative RT-PCR. Intriguingly, both the protein (p=0.022) and mRNA (p=0.049) expression levels of AGTR1 were significantly elevated by HFD, and these levels were normalized with VitD supplementation (protein: p=0.020, mRNA: p=0.056) (Fig. 7G and I). Conversely, the protein (HFD: p=0.321, VitD: p=0.902) and mRNA (HFD: p=0.169, VitD: p=0.895) levels of AGTR2 remained unaltered by both HFD and VitD (Fig. 7H and J).

DISCUSSION

The pathogenesis of NAFLD remains multifaceted and complex, with multiple factors potentially contributing to its development. The "multiple parallel hits" theory offers insight into this intricate process. It suggests that several simultaneous factors play a role in the onset and progression of NAFLD. Factors such as fat accumulation within hepatocytes, activation of hepatic macrophages and stellate cells, and the release of pro-fibrotic and pro-inflammatory cytokines from visceral adipose tissue all contribute to the development of NAFLD.[1] Moreover, increased intracellular lipid metabolites might induce cell inju-

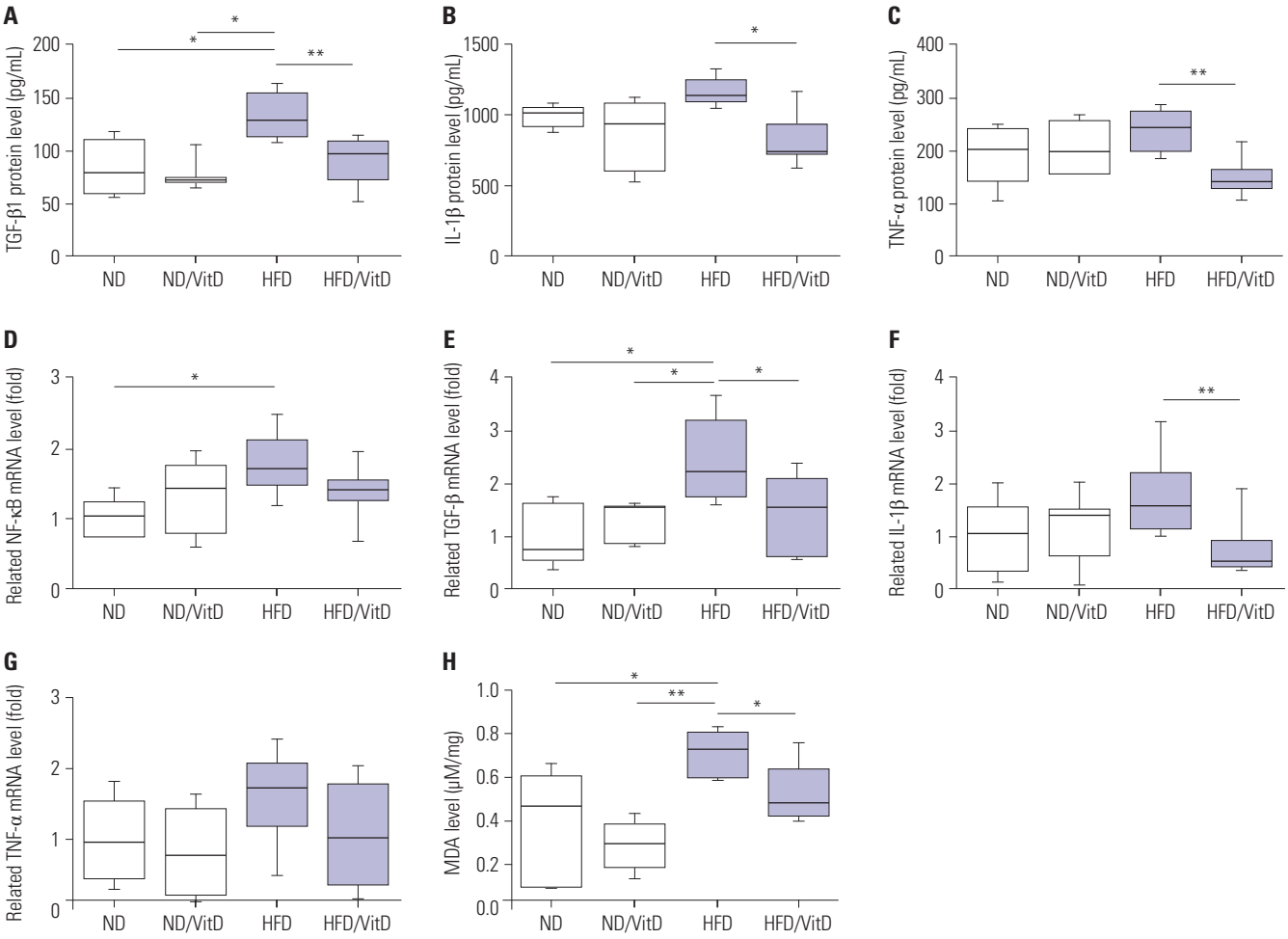

Fig. 6. Change of pro-fibrotic and pro-inflammatory cytokines levels in liver by HFD and VitD supplement. TGF-β1 (A and E), IL-1β (B and F), and TNF-α (C and G) were measured by ELISA and qPCR. NF-κB (D) was measured by qPCR, and MDA as ROS marker (H) was measured by TBARS assay. All figures were analyzed with one-way ANOVA with Bonferroni correction. *p<0.05; **p<0.01. ND, normal diet; VitD, vitamin D; HFD, high-fat diet; TNF, tumor necrosis factor; TGF, transforming growth factor; IL, interleukin; MDA, malondialdehyde; ROS, reactive oxygen species; ANOVA, analysis of variance.

ry and insulin resistance, rendering hepatocytes more susceptible to inflammation. Our data aligned with this theory, demonstrating how obesity leads to heightened levels of pro-fibrotic and pro-inflammatory cytokines, increased ROS, elevated RAS activity, upregulated transcription factors associated with lipogenesis in liver tissues, heightened serum triglycerides, and insulin resistance. Together, these factors may collectively contribute to the development and progression of NAFLD, reflecting the multifaceted nature of this condition and the interplay among various pathways and mechanisms.

The restorative effects of VitD supplementation on HFD-induced metabolic alterations, steatosis, and liver fibrosis in mice indeed come as a surprise, yet they align with the diverse hormonal actions associated with VitD. The pleiotropic mechanisms of VitD suggest its potential in reversing these changes.

Notably, VitD deficiency is a well-documented phenomenon in obesity. Several proposed mechanisms attempt to explain the decreased levels of VitD in individuals dealing with obesity. These mechanisms span various aspects, including altered distribution and sequestration of VitD in adipose tissue[14,15] and reduced sunlight exposure due to lifestyle changes or decreased outdoor activity. However, decreased expression of CYP2R1, hepatic 25-hydroxylase in liver, was recently reported as a possible mechanism for 25-OH VitD deficiency in obese mice,[16] and our study confirmed the decreased expression of hepatic CYP2R1 in this HFD-induced obesity model. This feature was consistent with many epidemiology studies which showed inverse correlation of VitD concentration and body mass index.[17-19]

The association of VitD deficiency with fibrosis in major organs, including the lungs, heart, and kidneys, has been well-documented. In our previous study, we demonstrated how obesity-induced VitD deficiency contributes to lung fibrosis and airway hyperresponsiveness.[20] This association between VitD levels and fibrotic conditions across various vital organs underscores the broader impact of VitD deficiency on multiple physiological systems and its potential implications for various health conditions.

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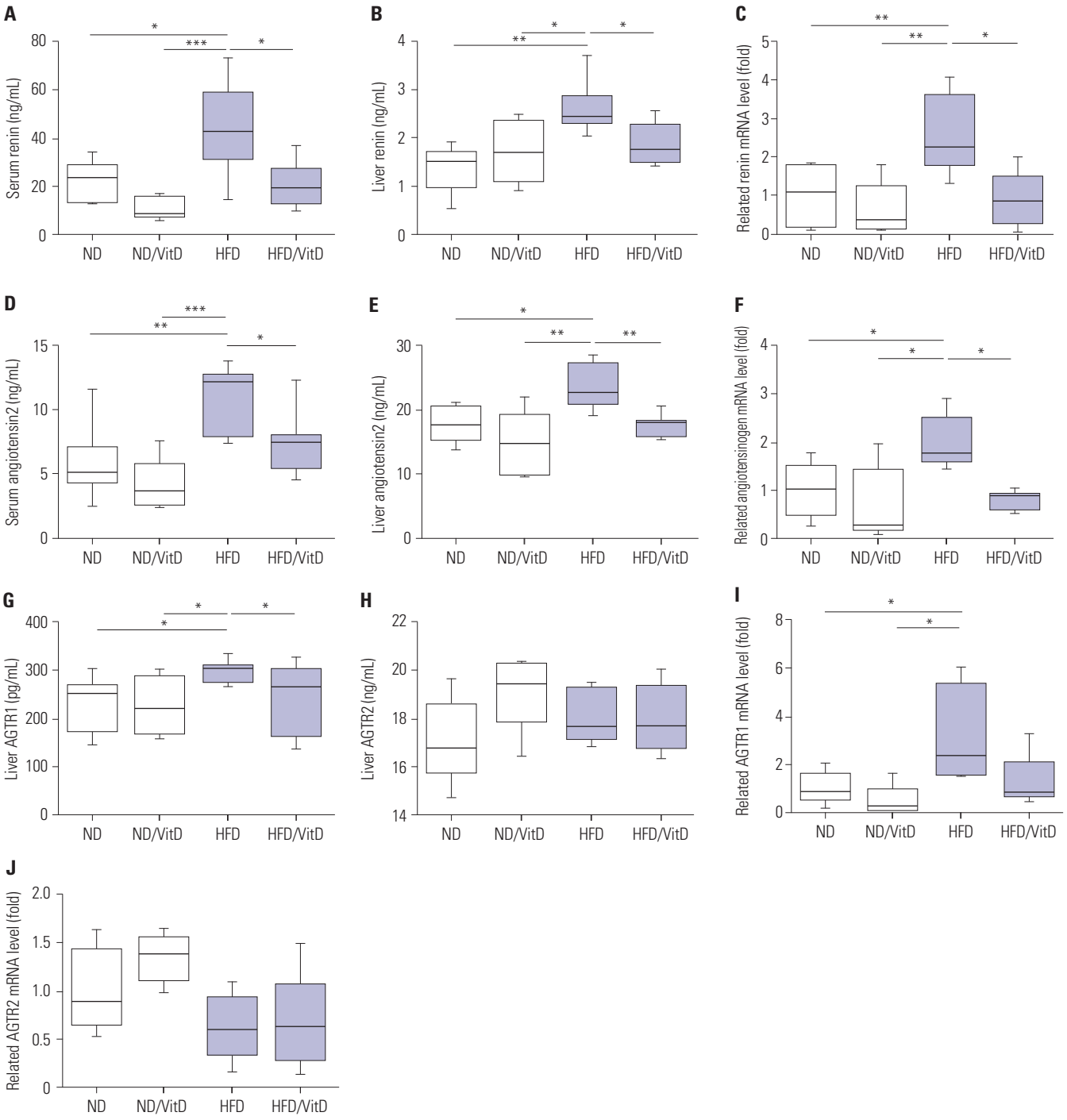

Fig. 7. Effect of VitD supplement on RAS in liver. Renin (A and B), angiotensin2 (D and E), AGTR1 (G), and AGTR2 (H) were measured by ELISA. The mRNA expression levels of renin (C), angiotensinogen (F), AGTR1 (I) and AGTR2 (J) were measured by qPCR. All figures were analyzed using one-way ANOVA with Bonferroni correction. *p<0.05; **p<0.01; ***p<0.001. ND, normal diet; VitD, vitamin D; HFD, high-fat diet; RAS, renin-angiotensin system; ANOVA, analysis of variance.

The consistent findings across multiple rodent experiments investigating NAFLD have indeed reported the attenuation of both steatosis and liver fibrosis through VitD supplementation.[20-24] This robust body of evidence further underscores the potential therapeutic role of VitD in mitigating the pathological changes associated with NAFLD.

The widespread expression of VDR across various cell types involved in liver function, including adipocyte,[25] stellate cells,[21,23] hepatocyte,[22] and Kupffer cells,[24] highlights the potential for VitD supplementation to exert its effects through multiple pathways. By targeting these diverse cell types, VitD supplementation could indeed play a role in suppressing the

expression of adipokines, pro-inflammatory cytokines, pro-fibrotic mediators, and transcription factors associated with hepatic lipogenesis. Hepatic stellate cells are widely acknowledged as a pivotal source of pro-fibrotic factors, particularly TGF-β1, in various models of liver fibrosis, including thioacetamide-induced liver fibrosis models and in vitro studies involving human stellate cells.[26] Numerous studies have highlighted the strong association between VitD deficiency and increased translational expression of TGF-β1.[20,21,27] These investigations have consistently shown that VitD supplementation effectively attenuates the expression of TGF-β1 in liver cells.[21,23] Ding, et al.'s[28] study demonstrating the spontaneous development of liver fibrosis in VDR knockout mice offered crucial insights into the role of VDR in liver health. Their research unveiled an intriguing mechanism where the VDR binding cistron coincides with the SMAD3 binding site on DNA, leading to co-occupancy. They found that the addition of VDR ligands effectively inhibited the binding of SMAD to these co-regulatory gene sites. This study underscores the intricate interplay between VDR signaling and the regulation of fibrotic pathways, shedding light on potential strategies for preventing or managing liver fibrosis through VDR modulation.

The high expression of VDR in hepatic macrophages observed in the HFD-induced steatohepatitis model suggests its potential involvement in the inflammatory processes. Additionally, in vitro studies have indicated that these macrophages, characterized by their elevated VDR expression, also produce pro-inflammatory cytokines, such as IL-1β, IL-6, and TNF-α.[24] Chronic exposure to pro-inflammatory cytokines and ROS plays a significant role in inducing hepatic injury, setting the stage for conditions like steatohepatitis and fibrosis.[1] VitD has been demonstrated to exert anti-inflammatory effects by suppressing the production of pro-inflammatory cytokines. One of the mechanisms through which VitD achieves anti-inflammatory properties is by inhibiting the NF-κB and MAPK signaling pathways.[25,26] NF-κB and MAPK pathways are key regulators of inflammatory responses, orchestrating the expression of various pro-inflammatory genes, including cytokines such as IL-1β, IL-6, and TNF-α.

The RAS plays a significant role in the development and progression of liver fibrosis.[29,30] Close relationship between RAS and TGF-β1 pathway has been well-documented. There exists a cross-talk between RAS and TGF-β1 signaling, often operating through an autocrine mechanism.[31,32] Hirose, et al.[33] showed that AGTR1 blocker inhibited fibrosis in a rat NASH model. AGTR1 blocker reduced the activation of hepatic stellate cells, oxidative stress, expression of TGF-β1, and expression of fibrosis-related genes, such as collagen.

Angiotensin-2, the primary effector of RAS, exerts its fibrotic effects mainly through the action of AGTR1, which is expressed on activated hepatic stellate cells—the key players in fibrosis progression. Animal studies have highlighted the efficacy of angiotensin-2 receptor blockers in attenuating hepatic fibrosis, emphasizing the therapeutic potential of targeting the RAS pathway to mitigate fibrotic processes within the liver.[34,35] Indeed, Powell, et al.'s[36] findings were compelling, demonstrating a significant association between high TGF-β1 and angiotensinogen-producing genotypes. This association correlated with a higher risk of hepatic fibrosis in a chronic hepatitis C model. The genetic predisposition linked to increased production of both TGF-β1 and angiotensinogen underscores the intricate interplay between these factors and their impact on fibrotic processes within the liver. Our findings indicating increased levels of renin and angiotensin-2 in the HFD-induced obesity model, subsequently decreased with VitD supplementation, aligned with the broader understanding of the RAS in metabolic disorders, such as obesity, and its potential modulation by VitD. This interplay between RAS, AGTR1, and TGF-β1 signaling pathways represents a significant area of interest in understanding and managing liver fibrosis.

The liver-specific transcription factor, HNF4α, plays pivotal roles in regulating various metabolic pathways within hepatocytes, encompassing glycolysis, lipogenesis, fatty acid oxidation, and gluconeogenesis.[37,38] Notably, in individuals with NAFLD, hepatic HNF4α protein levels are markedly diminished.[39] Recent studies, such as the one conducted by Yang, et al.,[40] have demonstrated that administering HNF4α mRNA transfection can potentially mitigate liver fibrosis, as evidenced in both in vivo and in vitro experiments. Interestingly, our study revealed reduced levels of HNF4α mRNA in the HFD-induced NAFLD model, with an intriguing twist—VitD supplementation elevated the HNF4α mRNA level. Further supporting this finding, Zhang, et al.[22] reported on the beneficial effects of VitD in NAFLD pathology, suggesting a mechanism involving the interaction between the VDR and HNF4α.

The data from RCTs assessing the impact of VitD supplementation on NAFLD presented inconsistent findings.[13,41-43] While some trials conducted liver biopsies[43] or MRI studies[41] to evaluate liver histology or fat content before and after VitD supplementation, they failed to show significant differences in NASH or NAFLD patients, contrasting with the outcomes observed in preclinical studies.

However, there have been some studies, like the one conducted by Geier, et al.,[42] that demonstrated a beneficial effect of VitD supplementation on biochemical liver damage markers in NASH. Moreover, a recent meta-analysis of RCTs focusing on NAFLD suggested that VitD supplements might have modest beneficial effects on ITT and ALT levels, although no significant impact was observed on AST and triglyceride levels.[13] These findings come with certain limitations, including variations in disease severity among enrolled patients, short-term interventions, and relatively small sample sizes. To validate and ascertain the precise role of VitD supplementation in NAFLD, there is a consensus that well-designed, large-scale RCTs are necessary. Such trials need to carefully consider factors such as disease severity, duration of intervention, and the

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number of enrolled patients to provide more conclusive evidence regarding the effectiveness of VitD supplementation in managing NAFLD.

In conclusion, our study highlights that the significant metabolic changes induced by HFD contribute to the development of steatohepatitis and liver fibrosis. Moreover, this study underscores the potential of VitD to mitigate obesity-associated steatohepatitis and liver fibrosis through diverse pathways, as depicted in the graphic abstract. VitD suppresses pro-fibrotic and pro-inflammatory cytokines, reduces ROS production, modulates RAS activities, and influences the HNF4α transcript factor associated with steatosis in the liver. By addressing these multifaceted factors, VitD supplementation emerges as a potentially safe, cost-effective, and direct treatment approach for individuals with NAFLD linked to obesity or metabolic dysfunction.

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REFERENCES

1. Cimini FA, Barchetta I, Carotti S, Bertoccini L, Baroni MG, Vespasiani-Gentilucci U, et al. Relationship between adipose tissue dysfunction, vitamin D deficiency and the pathogenesis of non-alcoholic fatty liver disease. World J Gastroenterol 2017;23:3407-17.
2. Younossi Z, Anstee QM, Marietti M, Hardy T, Henry L, Eslam M, et al. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. Nat Rev Gastroenterol Hepatol 2018;15:11-20.
3. Song K, Yang J, Lee HS, Kim SJ, Lee M, Suh J, et al. Changes in the prevalences of obesity, abdominal obesity, and non-alcoholic fatty liver disease among Korean children during the COVID-19 outbreak. Yonsei Med J 2023;64:269-77.
4. Moore JB. From sugar to liver fat and public health: systems biology driven studies in understanding non-alcoholic fatty liver disease pathogenesis. Proc Nutr Soc 2019;78:290-304.
5. Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF. Decreased bioavailability of vitamin D in obesity. Am J Clin Nutr 2000;72:690-3.
6. Ford ES, Ajani UA, McGuire LC, Liu S. Concentrations of serum vitamin D and the metabolic syndrome among U.S. adults. Diabetes Care 2005;28:1228-30.
7. Botella-Carretero JI, Alvarez-Blasco F, Villafruela JJ, Balsa JA, Vázquez C, Escobar-Morreale HF. Vitamin D deficiency is associated with the metabolic syndrome in morbid obesity. Clin Nutr 2007;26:573-80.
8. Zhang Z, Thorne JL, Moore JB. Vitamin D and nonalcoholic fatty liver disease. Curr Opin Clin Nutr Metab Care 2019;22:449-58.
9. Cho YH, Kim JW, Shim JO, Yang HR, Chang JY, Moon JS, et al. Association between vitamin D deficiency and suspected nonalcoholic fatty liver disease in an adolescent population. Pediatr Gastroenterol Hepatol Nutr 2019;22:233-41.
10. Sassi F, Tamone C, D'Amelio P. Vitamin D: nutrient, hormone, and immunomodulator. Nutrients 2018;10:1656.
11. Li YC, Qiao G, Uskokovic M, Xiang W, Zheng W, Kong J. Vitamin D: a negative endocrine regulator of the renin-angiotensin system and blood pressure. J Steroid Biochem Mol Biol 2004;89-90:387-92.
12. Barchetta I, Cimini FA, Cavallo MG. Vitamin D supplementation and non-alcoholic fatty liver disease: present and future. Nutrients 2017;9:1015.
13. Guo XF, Wang C, Yang T, Li S, Li KL, Li D. Vitamin D and non-alcoholic fatty liver disease: a meta-analysis of randomized controlled trials. Food Funct 2020;11:7389-99.
14. Pramyothin P, Biancuzzo RM, Lu Z, Hess DT, Apovian CM, Holick MF. Vitamin D in adipose tissue and serum 25-hydroxyvitamin D after roux-en-Y gastric bypass. Obesity (Silver Spring) 2011;19:2228-34.
15. Drincic A, Fuller E, Heaney RP, Armas LA. 25-hydroxyvitamin D response to graded vitamin D_3 supplementation among obese adults. J Clin Endocrinol Metab 2013;98:4845-51.
16. Roizen JD, Long C, Casella A, O'Lear L, Caplan I, Lai M, et al. Obesity decreases hepatic 25-hydroxylase activity causing low serum 25-hydroxyvitamin D. J Bone Miner Res 2019;34:1068-73.
17. Barchetta I, Carotti S, Labbadia G, Gentilucci UV, Muda AO, Angelico F, et al. Liver vitamin D receptor, CYP2R1, and CYP27A1 expression: relationship with liver histology and vitamin D3 levels in patients with nonalcoholic steatohepatitis or hepatitis C virus. Hepatology 2012;56:2180-7.
18. Targher G, Bertolini L, Scala L, Cigolini M, Zenari L, Falezza G, et al. Associations between serum 25-hydroxyvitamin D3 concentrations and liver histology in patients with non-alcoholic fatty liver disease. Nutr Metab Cardiovasc Dis 2007;17:517-24.
19. Bozic M, Guzmán C, Benet M, Sánchez-Campos S, García-Monzón C, Gari E, et al. Hepatocyte vitamin D receptor regulates lipid metabolism and mediates experimental diet-induced steatosis. J Hepatol 2016;65:748-57.
20. Han H, Chung SI, Park HJ, Oh EY, Kim SR, Park KH, et al. Obesity-induced vitamin D deficiency contributes to lung fibrosis and airway hyperresponsiveness. Am J Respir Cell Mol Biol 2021;64:357-67.
21. Beilfuss A, Sowa JP, Sydor S, Beste M, Bechmann LP, Schlattjan M, et al. Vitamin D counteracts fibrogenic TGF-β signalling in human

hepatic stellate cells both receptor-dependently and independently. Gut 2015;64:791-9.
22. Zhang H, Shen Z, Lin Y, Zhang J, Zhang Y, Liu P, et al. Vitamin D receptor targets hepatocyte nuclear factor 4α and mediates protective effects of vitamin D in nonalcoholic fatty liver disease. J Biol Chem 2020;295:3891-905.
23. Abramovitch S, Dahan-Bachar L, Sharvit E, Weisman Y, Ben Tov A, Brazowski E, et al. Vitamin D inhibits proliferation and profibrotic marker expression in hepatic stellate cells and decreases thioacetamide-induced liver fibrosis in rats. Gut 2011;60:1728-37.
24. Dong B, Zhou Y, Wang W, Scott J, Kim K, Sun Z, et al. Vitamin D receptor activation in liver macrophages ameliorates hepatic inflammation, steatosis, and insulin resistance in mice. Hepatology 2020;71:1559-74.
25. Abbas MA. Physiological functions of vitamin D in adipose tissue. J Steroid Biochem Mol Biol 2017;165(Pt B):369-81.
26. Ding C, Wilding JP, Bing C. 1,25-dihydroxyvitamin D3 protects against macrophage-induced activation of NFκB and MAPK signalling and chemokine release in human adipocytes. PLoS One 2013;8:e61707.
27. Li SR, Tan ZX, Chen YH, Hu B, Zhang C, Wang H, et al. Vitamin D deficiency exacerbates bleomycin-induced pulmonary fibrosis partially through aggravating TGF-β/Smad2/3-mediated epithelial-mesenchymal transition. Respir Res 2019;20:266.
28. Ding N, Yu RT, Subramaniam N, Sherman MH, Wilson C, Rao R, et al. A vitamin D receptor/SMAD genomic circuit gates hepatic fibrotic response. Cell 2013;153:601-13.
29. Okamoto K, Tajima H, Ohta T, Nakanuma S, Hayashi H, Nakagawara H, et al. Angiotensin II induces tumor progression and fibrosis in intrahepatic cholangiocarcinoma through an interaction with hepatic stellate cells. Int J Oncol 2010;37:1251-9.
30. Yoshiji H, Kuriyama S, Yoshii J, Ikenaka Y, Noguchi R, Nakatani T, et al. Angiotensin-II type 1 receptor interaction is a major regulator for liver fibrosis development in rats. Hepatology 2001;34(4 Pt 1):745-50.
31. Koibuchi Y, Lee WS, Gibbons GH, Pratt RE. Role of transforming growth factor-beta 1 in the cellular growth response to angiotensin II. Hypertension 1993;21(6 Pt 2):1046-50.
32. Gibbons GH, Pratt RE, Dzau VJ. Vascular smooth muscle cell hypertrophy vs. hyperplasia. Autocrine transforming growth factor-beta 1 expression determines growth response to angiotensin II. J Clin Invest 1992;90:456-61.
33. Hirose A, Ono M, Saibara T, Nozaki Y, Masuda K, Yoshioka A, et al. Angiotensin II type 1 receptor blocker inhibits fibrosis in rat nonalcoholic steatohepatitis. Hepatology 2007;45:1375-81.
34. Moreno M, Gonzalo T, Kok RJ, Sancho-Bru P, van Beuge M, Swart J, et al. Reduction of advanced liver fibrosis by short-term targeted delivery of an angiotensin receptor blocker to hepatic stellate cells in rats. Hepatology 2010;51:942-52.
35. Munshi MK, Uddin MN, Glaser SS. The role of the renin-angiotensin system in liver fibrosis. Exp Biol Med (Maywood) 2011;236:557-66.
36. Powell EE, Edwards-Smith CJ, Hay JL, Clouston AD, Crawford DH, Shorthouse C, et al. Host genetic factors influence disease progression in chronic hepatitis C. Hepatology 2000;31:828-33.
37. Iizuka K, Horikawa Y. ChREBP: a glucose-activated transcription factor involved in the development of metabolic syndrome. Endocr J 2008;55:617-24.
38. Bi L, Jiang Z, Zhou J. The role of lipin-1 in the pathogenesis of alcoholic fatty liver. Alcohol Alcohol 2015;50:146-51.
39. Xu Y, Zalzala M, Xu J, Li Y, Yin L, Zhang Y. A metabolic stress-inducible miR-34a-HNF4α pathway regulates lipid and lipoprotein metabolism. Nat Commun 2015;6:7466.
40. Yang T, Poenisch M, Khanal R, Hu Q, Dai Z, Li R, et al. Therapeutic HNF4A mRNA attenuates liver fibrosis in a preclinical model. J Hepatol 2021;75:1420-33.
41. Barchetta I, Del Ben M, Angelico F, Di Martino M, Fraioli A, La Torre G, et al. No effects of oral vitamin D supplementation on non-alcoholic fatty liver disease in patients with type 2 diabetes: a randomized, double-blind, placebo-controlled trial. BMC Med 2016;14:92.
42. Geier A, Eichinger M, Stirnimann G, Semela D, Tay F, Seifert B, et al. Treatment of non-alcoholic steatohepatitis patients with vitamin D: a double-blinded, randomized, placebo-controlled pilot study. Scand J Gastroenterol 2018;53:1114-20.
43. Kitson MT, Pham A, Gordon A, Kemp W, Roberts SK. High-dose vitamin D supplementation and liver histology in NASH. Gut 2016;65:717-8.

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