ORIGINAL

# Association of lipids in lipoprotein subfractions with liver fibrosis in a mouse model of metabolic dysfunction–associated steatohepatitis

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**Abstract : Metabolic dysfunction–associated steatohepatitis (MASH) exhibits inflammation and fibrosis in addition to lipid accumulation in the liver, which may progress to cirrhosis and liver failure. This study investigated whether the serum lipoprotein subfraction reflects fibrosis severity in a MASH mouse model. Nine-week-old male A/J and C57BL6/J mice were fed a high-fat/cholesterol/cholate-based diet to induce fibrotic MASH. To generate fibrosis of varying severity, mice were fed two diets with different cholesterol concentrations (1.25% and 2.5%). After 9 weeks of feeding, serum cholesterol and triglyceride levels of each lipoprotein were comprehensively analyzed, including chylomicron, very-large low-density lipoprotein, low-density lipoprotein (LDL), and high-density lipoprotein (HDL), with 20 subclasses according to particle size. Serum levels of very-large HDL-cholesterol, very-small HDL-cholesterol, very-small HDL-triglycerides, and very-small LDL-cholesterol were significantly higher in the stage 2 fibrosis group than the stage 1 fibrosis group. Serum very-small LDL-cholesterol levels were correlated with histological severity of MASH, which reportedly increases with the progression of MASH in humans. In conclusion, the serum lipoprotein subfraction reflects liver fibrosis severity even in early phase, independent of the severity of other MASH lesions in MASH model mice. Fractionating HDL, which have been measured in clinical practice, may help establish noninvasive markers of liver fibrosis. J. Med. Invest. 72 : 375-384, August, 2025**

***Keywords*** **:** *metabolic dysfunction-associated steatohepatitis (MASH), liver fibrosis, animal model, high-density lipoprotein cholesterol, high-density lipoprotein triglycerides*

## INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD), now referred to as metabolic dysfunction–associated steatotic liver disease (MASLD) (1), is characterized by hepatic steatosis and represents one of the most common hepatic diseases worldwide (2). The more aggressive form of MASLD, metabolic dysfunction–associated steatohepatitis (MASH), shows lobular inflammation, hepatocyte ballooning and fibrosis, as well as steatosis (3). MASH can progress to cirrhosis and hepatocellular carcinoma (4), especially in patients developing fibrosis. The presence of fibrosis is strongly correlated with the prognosis of MASH (5). Patients with fibrosis of stage 3 or higher are at substantial risk of death from end-stage liver failure and hepatocellular carcinoma (6). In addition, stage 2 fibrosis is reportedly associated with critical consequences such as cirrhosis and extra-hepatic complications, including cardiovascular disease (7).

The gold-standard method for assessing liver fibrosis is histological staging of liver biopsy specimens (8), but this is not available for all patients due to its invasiveness (9). Several noninvasive serum biomarkers to identify liver fibrosis have been reported to date, including platelets, hyaluronic acid, type 4 collagen 7s, Mac2-binding protein glycosylation isomer (10), and the glycoprotein autotaxin (11, 12). Moreover, a scoring system that combines single biomarkers was developed and validated against liver biopsy parameters, such as FIB-4 index, enhanced liver fibrosis test, and NAFLD fibrosis score (13). These tests provide improved diagnostic performance for fibrosis and can be used to identify patients who have advanced liver fibrosis and/or cirrhosis in both primary and secondary care. However, there are currently few markers that provide an indication of fibrosis at earlier stages (stages 1 and 2). Given that 20% of patients diagnosed with stage 1-2 fibrosis will progress to stage 3 or 4 within 5 years (14), liver fibrosis markers that can be monitored multiple times and that reflect the early stages of disease are desirable.

MASH represents a hepatic phenotype of metabolic syndrome and is thus complicated by a high frequency of dyslipidemia (15-17). High serum levels of small dense low-density lipoprotein (sdLDL) have been reported in MASH patients as well as patients with cardiovascular disease (18-20). Levels of serum non–high-density lipoprotein cholesterol (HDL-C), which is expressed as the difference between total cholesterol and HDL-C, are also reportedly associated with MASH. Although demonstration of the dynamic changes in lipoproteins and lipids in lipoproteins could be used to distinguish between MASH and non-MASH, few studies have addressed the association between the lipid and lipoprotein composition and liver fibrosis in MASH. The aim of the present study, therefore, was to evaluate the composition of the lipoprotein fraction and serum lipids that characterize the early stages of fibrosis using a mouse model of MASH.

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## MATERIALS AND METHODS

*Animal models*

Eight-week-old male C57BL6/J (BL6) and A/J mice were purchased from Japan SLC (Hamamatsu, Japan). The mice were maintained on a 12-hour light and 12-hour dark cycle, housed individually in a temperature- and humidity-controlled room. After 1 week of acclimation with feeding of standard rodent chow (MF ; Oriental Yeast, Tokyo, Japan), the BL6 and A/J mice were each randomly assigned to the following two groups : low-dose cholesterol (LC) or high-dose cholesterol (HC), in which the mice were fed a high-fat/-cholesterol/-cholate–based (iHFC) diet supplemented with 1.25% or 2.5% cholesterol, respectively. The iHFC diet was developed to induce MASH-fibrosis (Hayashi Kasei, Osaka, Japan). The number of mice in the BL6-LC, BL6-HC, A/J-LC, and A/J-HC groups was 6, 4, 5, and 5, respectively. All mice were allowed *ad libitum* access to diet and water. All mice were euthanized under anesthesia with isoflurane at 18 weeks of age. Blood samples were collected from the inferior vena cava and used to prepare serum, which was stored at –20°C. The liver was removed from each mouse and fixed in 10% neutral buffered formalin. All processes were approved by the Animal Use Committee of Nara Women's University.

*Histopathological assessment of the liver*

After fixation in neutral buffered formalin, liver tissues were embedded in paraffin and processed into 2-μm sections. The sections were then stained with hematoxylin-eosin as well as Azan-Mallory and Sirius Red according to standard procedures. Stained liver tissue sections were assessed in a blinded manner by two pathologists (K.T. and M.I-S.) for histological steatosis (grade 0 to 3), lobular inflammation (grade 0 to 3), hepatocyte ballooning (grade 0 to 2), and fibrosis (stage 0 to 4). The NAFLD activity score (NAS) was defined as the unweighted sum of the scores for steatosis, lobular inflammation, and hepatocyte ballooning (6).

*Serum lipoprotein subfraction analysis*

The concentrations of cholesterol and triglycerides in lipoprotein subclasses were determined by Liposearch service (Skylight Biotech, Akita, Japan) using gel filtration high-performance liquid chromatography. The lipoproteins were divided into the following four main classes : HDL, low-density lipoprotein (LDL), very-low-density lipoprotein (VLDL), and chylomicrons (CM). Lipoproteins were also classified into 20 subfractions according to particle size (Table 1). Serum total cholesterol and triglyceride levels were analyzed similarly.

*Statistical analysis*

Data are presented as mean ± SD. The mice were divided into two groups according to fibrosis stage 1 or 2 because two-way analysis of variance (ANOVA) indicated that neither the interaction between strain and diet nor their individual effects were significant in the histological severity of fibrosis (strain : P=0.262, diet : P=0.087, interaction : P=0.870). Concentration of lipid levels of the two groups were analyzed using one-way ANOVA and Welch's *t*-tests with Metaboanalyst 6.0 software (https://www.metaboanalyst.ca). For categorical variables, the statistical significance was determined using Pearson's chi-squared test. Correlation coefficients were determined using Spearman's correlation coefficient with IBM SPSS Statics software (version 24). All statistical tests were two-sided. $P < 0.05$ was considered to be statistically significant.

Table 1. Lipoprotein subclasses segregated by particle diameter.

| Major class | Subclass | No. | Particle diameter (nm) |
|---|---|---|---|
| CM | CM | 1 | >90 |
| | | 2 | 75 |
| VLDL | Large VLDL | 3 | 64 |
| | | 4 | 53.6 |
| | | 5 | 44.5 |
| | Medium VLDL | 6 | 36.8 |
| | Small VLDL | 7 | 31.3 |
| LDL | Large LDL | 8 | 28.6 |
| | Medium LDL | 9 | 25.5 |
| | Small LDL | 10 | 23 |
| | Very-small LDL | 11 | 20.7 |
| | | 12 | 18.6 |
| | | 13 | 16.7 |
| HDL | Very-large HDL | 14 | 15 |
| | | 15 | 13.5 |
| | Large HDL | 16 | 12.1 |
| | Medium HDL | 17 | 10.9 |
| | Small HDL | 18 | 9.8 |
| | Very-small HDL | 19 | 8.8 |
| | | 20 | 7.6 |

CM, chylomicron ; HDL, high-density lipoprotein ; LDL, low-density lipoprotein ; VLDL, very-low-density lipoprotein.

## RESULTS

*Histopathological observation*

The results of liver histology and assessments are shown in Figure 1 and Table 2. All mice developed MASH-like lesions, with liver histology showing steatosis, lobular inflammation, hepatocyte ballooning, and fibrosis. Steatosis was identified in 17 of 19 animals (89%), with 8 (42%) cases of grade 1 and 9 (47%) of grade 2. Lobular inflammation was observed in 16 of 19 mice (84%), of which 5 (26%) were grade 1, 10 (53%) were grade 2, and 1 (5%) was grade 3. Hepatocyte ballooning was identified in 17 (89%) of the 19 animals, 7 (37%) of which were grade 1 and 10 (53%) grade 2. Fibrosis was present in all 19 animals, with 14 (74%) having stage 1 and 5 (26%) with stage 2. Stage 1 was defined by mild fibrotic deposition predominantly perivenular and pericellular pattern, while stage 2 was characterized by progression of fibrosis into the portal areas, in addition to pericellular fibrosis, representing a moderate degree of hepatic pathology. With regard to NAS, 11 of the 19 mice (58%) had a score of 5 or higher (i.e., these mice were considered to have MASH). The severity of steatosis, lobular inflammation, and NAS were higher in A/J mice than BL6 mice ($P = 0.036$). There were no significant differences in terms of histological severity of the liver between the high- and low-cholesterol diet groups.

*Association between progression of liver fibrosis and lipids of serum lipoprotein subfractions*

The relationship between the progression of liver fibrosis and the profile of lipids in lipoprotein subfractions was also examined. Compared to the control group fed a normal diet, the fibrosis group fed the iHFC diet tended to have lower TG levels and higher cholesterol levels (Table 3). No significant differences in total TG or cholesterol levels were observed in any of the

serum lipoprotein subclasses between groups F1 and F2. Nevertheless, serum levels of very-small LDL-C, very-large HDL-C, very-small HDL-C, and very-small HDL-TG were significantly higher in the F2 group than the F1 group (Fig. 2A-G and Supplemental Table 1).

*Association between MASH severity and lipids of serum lipoprotein subfractions*

Next, we examined the association between the severity of MASH lesions and dynamic changes in serum lipids. Mice were classified into two groups, one group with NAS ≤4 (non-MASH

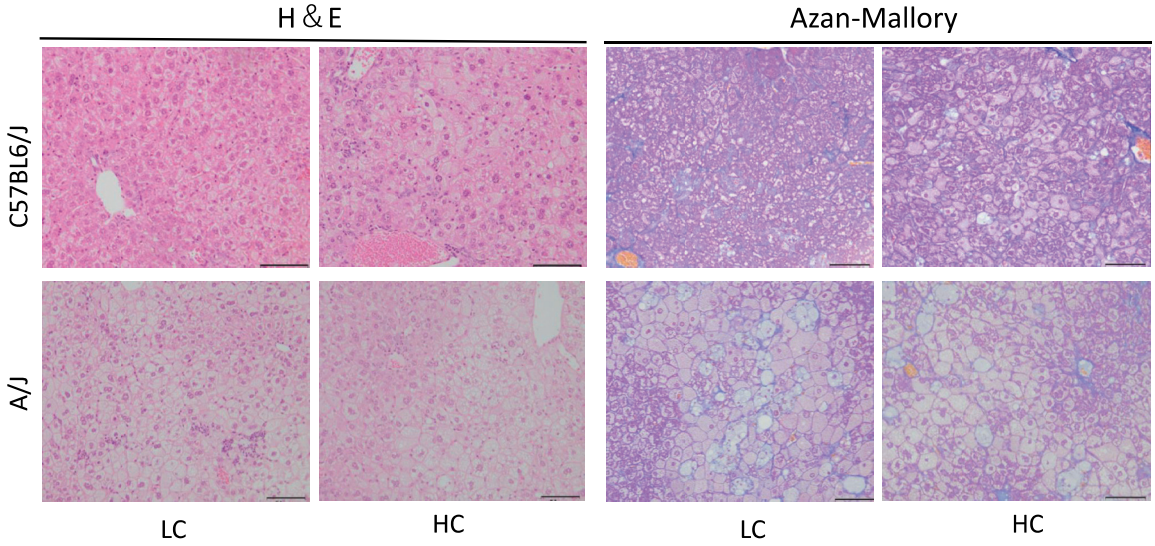


Figure 1. Representative results of histopathologic analysis of liver tissue from C57BL6/J and A/J mice fed an iHFC diet supplemented with 1.25% (LC) and 2.5% (HC) cholesterol for 9 weeks. Hematoxylin and eosin (H&E) and Azan-Mallory staining. Scale bars = 100 μm. All mice developed MASH-like lesions, such as steatosis, lobular inflammation, hepatocyte ballooning, and fibrosis.

Table 2. Histopathological assessment of the liver in MASH model mice.

| Group | Mouse no. | Steatosis (0-3) | Lobular inflammation (0-3) | Hepatocyte ballooning (0-2) | NAS (0-8) | Fibrosis (0-4) |
|---|---|---|---|---|---|---|
| C57BL6/J -LC | 1 | 1 | 1 | 0 | 2 | 1 |
| | 2 | 2 | 2 | 2 | 6 | 1 |
| | 3 | 1 | 0 | 0 | 1 | 1 |
| | 4 | 1 | 3 | 2 | 6 | 1 |
| | 5 | 0 | 0 | 1 | 1 | 1 |
| | 6 | 0 | 1 | 1 | 2 | 1 |
| C57BL6/J -HC | 1 | 2 | 0 | 1 | 3 | 1 |
| | 2 | 1 | 1 | 2 | 4 | 1 |
| | 3 | 1 | 2 | 2 | 5 | 2 |
| A/J-LC | 1 | 1 | 2 | 2 | 5 | 1 |
| | 2 | 1 | 2 | 2 | 5 | 2 |
| | 3 | 1 | 2 | 2 | 5 | 1 |
| | 4 | 2 | 2 | 1 | 5 | 1 |
| | 5 | 2 | 1 | 1 | 4 | 1 |
| A/J-HC | 1 | 2 | 2 | 1 | 5 | 2 |
| | 2 | 2 | 1 | 1 | 4 | 1 |
| | 3 | 2 | 2 | 2 | 6 | 2 |
| | 4 | 2 | 2 | 2 | 6 | 2 |
| | 5 | 2 | 2 | 2 | 6 | 1 |

Histopathological evaluation was performed according to the NASH Clinical Research Network Scoring System (6).
LC, iHFC diet with low-dose cholesterol ; HC, iHFC diet with high-dose cholesterol.

Table 3. Serum levels of total triglycerides and cholesterol in each lipoprotein subclass in the stage 0, 1 and stage 2 fibrosis groups.

| | Fibrosis | | |
|---|---|---|---|
| | Stage 0 n = 9 | Stage 1 n = 14 | Stage 2 n = 5 |
| Total TG | 32.20 ± 11.40 | 9.75 ± 8.54* | 4.24 ± 2.08* |
| CM-TG | 2.17 ± 2.01 | 1.49 ± 1.54 | 0.78 ± 0.52 |
| VLDL-TG | 21.91 ± 9.38 | 5.65 ± 6.55* | 1.12 ± 0.90* |
| LDL-TG | 7.12 ± 1.95 | 1.02 ± 0.82* | 0.28 ± 0.23* |
| HDL-TG | 1.00 ± 0.29 | 1.59 ± 0.62 | 2.06 ± 0.75* |
| Total cholesterol | 60.0 ± 6.0 | 156.1 ± 25.0* | 161.1 ± 26.0* |
| CM-C | 0.43 ± 0.23 | 6.55 ± 3.70* | 6.38 ± 3.09* |
| VLDL-C | 5.76 ± 2.00 | 77.98 ± 20.89* | 72.89 ± 22.04* |
| LDL-C | 7.55 ± 1.23 | 30.91 ± 9.45* | 35.88 ± 2.42* |
| HDL-C | 46.31 ± 5.84 | 40.66 ± 8.13 | 45.98 ± 4.40 |

Values are mean ± SD (mg/dL). *P<0.05 vs. stage 0 group by ANOVA. No significant differences was seen between the stage 1 and 2 groups.
C, cholesterol ; CM, chylomicron ; HDL, high-density lipoprotein ; LDL, low-density lipoprotein ; TG, triglyceride ; VLDL, very-low-density lipoprotein.

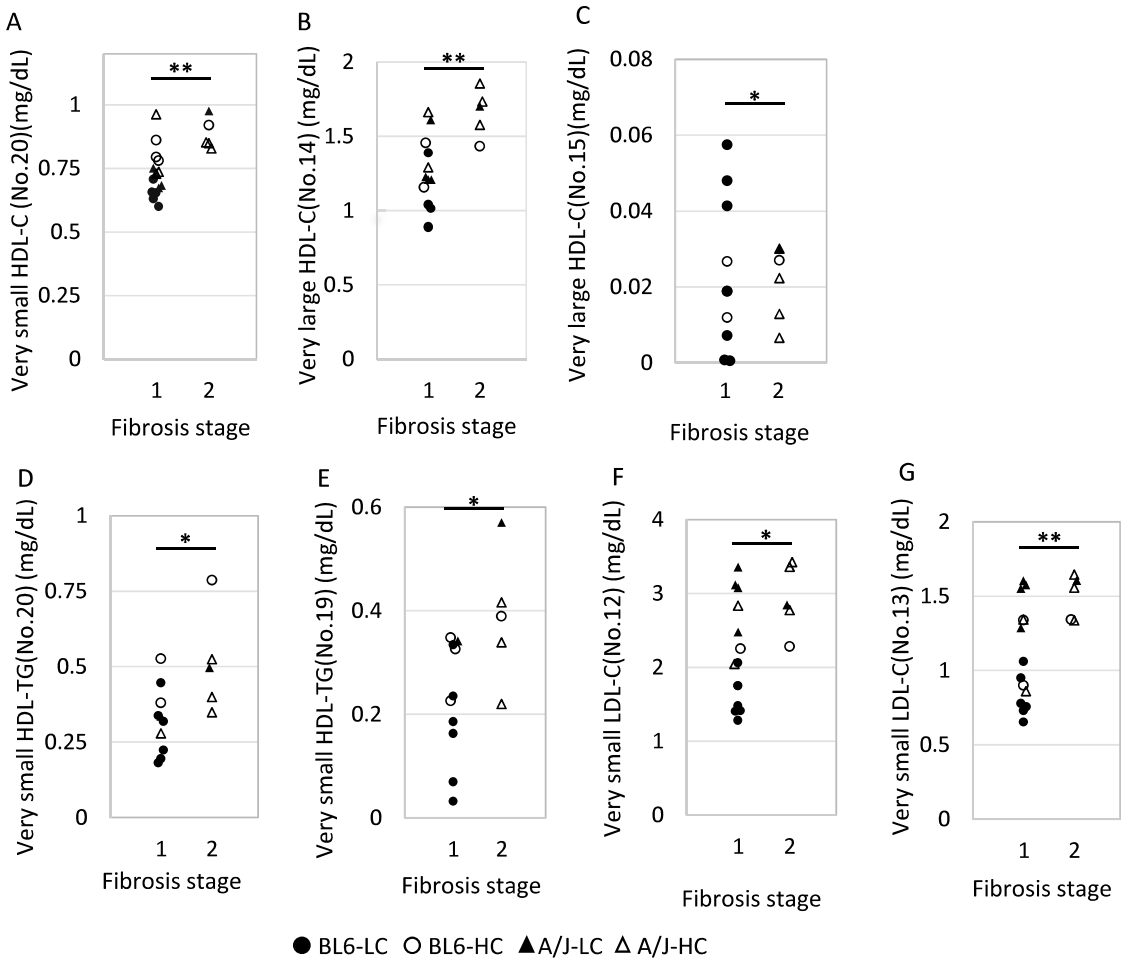


Figure 2. Levels of serum cholesterol and TGs in lipoprotein subfractions. (A) Cholesterol levels in very-small HDL (No. 20). (B) Cholesterol levels in very-large HDL (No. 14). (C) Cholesterol levels in very-large HDL (No. 15). (D) TG levels in very-small HDL (No. 20). (E) TG levels in very-small HDL (No. 19). (F) Cholesterol levels in very-small LDL (No. 12). (G) Cholesterol levels in very-small LDL (No. 13). *$P$<0.05 and **$P$<0.01 by Welch's t-test. HDL : high-density lipoprotein, LDL : low-density lipoprotein.

group) and another with NAS ≥5 (MASH group). Similar to the comparison of fibrosis progression, no significant differences in total TG or cholesterol levels were observed in any of the serum lipoprotein subclasses between the MASH and non-MASH groups (Table 4). Levels of very-small LDL-C were higher in the MASH group (Supplemental Table 2), consistent with a previously reported human study (19). In addition, these very-small LDL-C levels were significantly correlated with NAS (Fig. 3A and B), suggesting that changes in very-small LDL-C levels might not depend solely on fibrosis progression but could be affected by any type of liver lesion, including steatosis and inflammation.

Table 4. Serum levels of total triglycerides and cholesterol in each lipoprotein subclass in the MASH and non-MASH groups.

| | Non-MASH (NAS ≤ 4) n=8 | MASH (NAS ≥ 5) n=11 | *P*-value |
|---|---|---|---|
| Total TG | 11.56 ± 9.43 | 5.48 ± 4.95 | 0.277 |
| CM-TG | 1.64 ± 1.58 | 1.00 ± 1.12 | 0.526 |
| VLDL-TG | 7.20 ± 7.49 | 2.09 ± 3.23 | 0.203 |
| LDL-TG | 1.21 ± 0.75 | 0.48 ± 0.64 | 0.166 |
| HDL-TG | 1.51 ± 0.74 | 1.90 ± 0.63 | 0.334 |
| Total cholesterol | 155.4 ± 21.21 | 158.9 ± 27.81 | 0.754 |
| CM-C | 7.05 ± 3.64 | 6.11 ± 3.46 | 0.579 |
| VLDL-C | 77.10 ± 17.83 | 76.31 ± 23.41 | 0.935 |
| LDL-C | 28.80 ± 8.26 | 34.70 ± 7.99 | 0.140 |
| HDL-C | 42.40 ± 8.67 | 41.81 ± 7.16 | 0.878 |

Values are mean ± SD (mg/dL). *P<0.05 vs. non-MASH group by Welch's t-test. C, cholesterol ; CM, chylomicron ; HDL, high-density lipoprotein ; LDL, low-density lipoprotein ; TG, triglyceride ; VLDL, very-low-density lipoprotein.

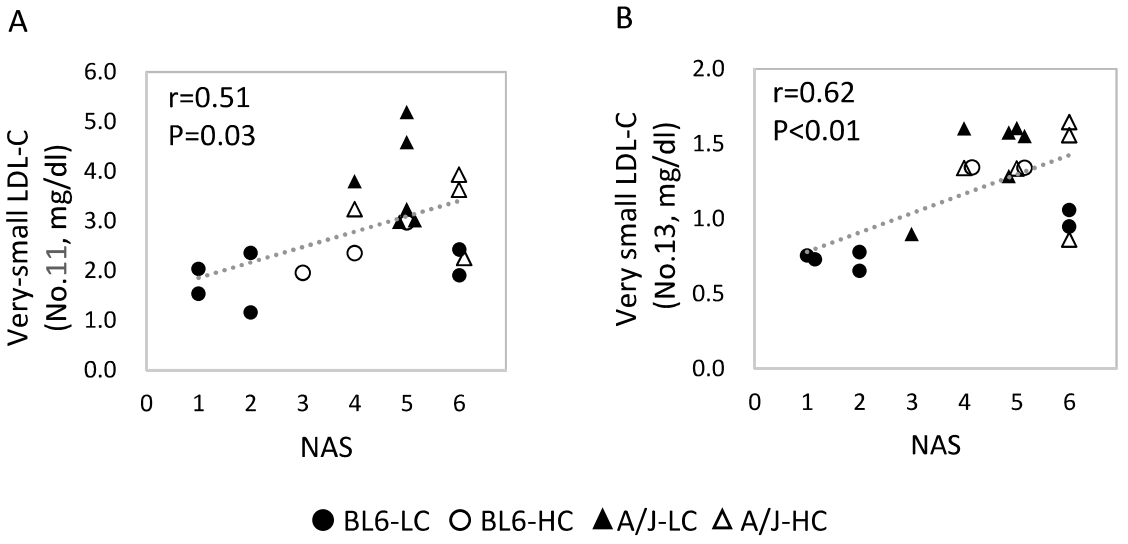


Figure 3. Association between serum levels of cholesterol and TGs in lipoprotein subfractions and NAFLD activity score (NAS). NAS was defined as the unweighted sum of the scores for steatosis, lobular inflammation, and hepatocyte ballooning, according to criteria proposed by Kleiner *et al*. (6). (A) Cholesterol levels in very-small LDL (No. 11). (B) Cholesterol levels in very-small LDL (No. 13). HDL, high-density lipoprotein ; LDL, low-density lipoprotein.

## DISCUSSION

Analyses of serum lipoprotein lipids in the present study using a mouse model of MASH-related fibrosis revealed that the levels of cholesterol in very-small LDL and very-large HDL and levels of both TGs and cholesterol in very-small HDL are associated with the severity of fibrosis. Levels of these lipoprotein subfractions increased in a manner dependent on fibrosis severity.

The concentrations of very-small LDL subfractions that showed dynamic changes in this study were identical to changes in levels of lipids known to increase with progression to simple steatosis and MASH in humans. These results suggest that the serum lipid profiles observed in the present study might be similar to those in humans and not specific only to mice. Previous reports of studies conducted in humans and animal models have mainly focused on analyses of lipid profiles during the phase of progression from steatosis to steatohepatitis, and few studies have focused on fibrosis progression. This is the first study to comprehensively examine the relationship between MASH-related fibrosis and serum lipid dynamics in lipoprotein subfractions.

LDL is composed of cholesterol, TGs, and phospholipids. In this study, LDL was classified into large, medium, small, and very-small LDL subfractions. sdLDL is readily oxidized and reportedly useful as a marker of coronary atherosclerosis (21-23). In human clinical studies, sdLDL levels are reportedly higher in MASH patients than in patients with simple steatosis (18, 19). The size of sdLDL particles (<25.5 nm) in those studies corresponds to the middle, small, and very-small LDL subfractions of the present study. In line with a previous study, high concentrations of very-small LDL-C are associated with high NAS (19). We also found a positive correlation between liver fibrosis progression and very-small LDL-C levels, which indicates that very-small LDL-C plays a role in liver fibrosis in MASH.

Serum levels of total HDL-C are known to be low in dyslipidemia in patients with MASLD (24). HDL plays a role in the reverse cholesterol transport, transferring excess cholesterol from the periphery to the liver (25), and its dynamics may be affected by cholesterol in extrahepatic tissues. The formation of HDL initially involves ApoA1 from the liver and small intestine together with cholesterol and phospholipids as small HDL disks. The volume of these disk-shaped HDL particles increases via esterification of the surrounding cholesterol by lecithin-cholesterol acyltransferase to form spherical HDL (26). In the present study, discoidal HDL was classified as very-small HDL, and spherical HDL particles were classified as very-large, large, medium, and small HDL. Our results showed no significant changes in serum total HDL-C concentrations with progression of liver fibrosis, but the subfraction levels of very-large and very-small HDL-C increased markedly with fibrosis progression. It is interesting to note that the HDL subfractions in which the lipid concentrations increased with liver fibrosis progression were those with the largest and smallest diameter. The levels of neither very-large HDL-C nor very-small HDL-C differed significantly when grouped based on NAS. This suggests that the lipids in these two lipoprotein subfractions that increased in level are fibrosis specific.

Cholic acid, a primary bile acid, is commonly used to promote absorption of lipids in diets high in fat and cholesterol, and it also decreases serum TG levels (27, 28). As expected, TG levels in most lipoprotein subclasses except for HDL-TG decreased in the present study ; however, it is noteworthy that only TG levels in very-small HDL increased with fibrosis. HDL-TG is associated with metabolic syndrome, diabetes, and obesity (29). HDL3, which represents very-small HDL, is inherently anti-inflammatory, but TG-rich HDL3 is less anti-inflammatory than HDL with a lower TG content (30). Therefore, it is possible that the increased levels of very-small HDL-TGs indicate a lack of anti-inflammatory activity by HDL3 in mice with severe fibrosis in the present study. These results suggest that focusing on both the quality and quantity of lipoproteins could aid in elucidating the mechanism of pathogenesis for conditions such as liver inflammation and fibrosis and facilitate the identification of new drug targets.

A limitation of this study must be considered. Mice were fed a cholesterol-added iHFC diet to induce fibrosis. An effect of dietary cholesterol on the increase in serum cholesterol cannot be completely excluded, although dose-dependent increase in serum cholesterol was not observed in the LC and HC group in the present study (Supplemental Figure 1). Further studies will be necessary utilizing a diet-independent fibrosis induction model.

In summary, cholesterol levels in small LDL, large HDL, and very-small HDL as well as TG levels in very-small HDL were higher in model mice with advanced fibrosis than in those with mild fibrosis. In particular, the dynamic changes in lipids in the HDL subfraction were significantly related to progression of liver fibrosis, and these data could aid in elucidating the pathogenesis of liver fibrosis and facilitate the discovery of new markers.

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## DISCLOSURE OF CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

## AUTHOR CONTRIBUTIONS

M.I-S., S.M., and K.T. designed the research and secured funding ; H.N., M.I-S., S.T., J.O., and Q.C., performed the experiments ; H.N., M.I-S., M.Y., and H.O. performed data validation and analysis ; H.N., and M.I-S. prepared the original draft of the manuscript ; and S.M., and K.T edited the manuscript. All authors read and approved the manuscript.

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Supplemental Table 1. Lipid levels of serum lipoprotein subfractions in stage 1 and 2 fibrosis groups

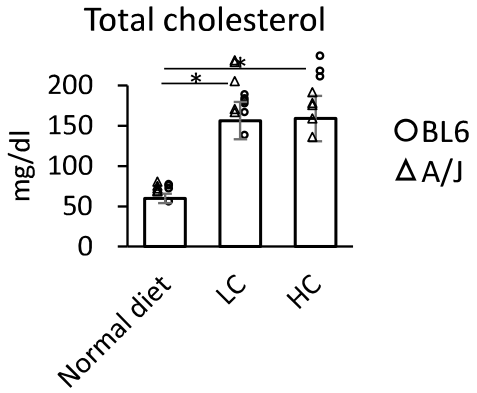
| | Fibrosis | | | | | | P-value |
|---|---|---|---|---|---|---|---|
| | Stage 1 *N*=14 | | | Stage 2 *N*=5 | | | |
| CM-TG (No.1) | 1.05 | ± | 1.04 | 0.61 | ± | 0.40 | 0.654 |
| Total CM-TG (No.2) | 0.44 | ± | 0.52 | 0.17 | ± | 0.12 | 0.287 |
| Large-VLDL-TG (No.3) | 0.96 | ± | 1.31 | 0.23 | ± | 0.17 | 0.179 |
| Large-VLDL-TG (No.4) | 1.46 | ± | 2.05 | 0.26 | ± | 0.20 | 0.138 |
| Large-VLDL-TG (No.5) | 1.76 | ± | 2.07 | 0.28 | ± | 0.24 | 0.082 |
| Medium VLDL-TG (No.6) | 1.12 | ± | 0.93 | 0.25 | ± | 0.22 | 0.058 |
| Small-VLDL-TG (No.7) | 0.35 | ± | 0.27 | 0.10 | ± | 0.08 | 0.078 |
| Large-LDL-TG (No.8) | 0.42 | ± | 0.32 | 0.11 | ± | 0.10 | 0.072 |
| Medium-LDL-TG (No.9) | 0.31 | ± | 0.23 | 0.08 | ± | 0.07 | 0.066 |
| Small-LDL-TG (No.10) | 0.15 | ± | 0.13 | 0.04 | ± | 0.04 | 0.094 |
| Very small LDL-TG (No.11) | 0.06 | ± | 0.06 | 0.01 | ± | 0.01 | 0.082 |
| Very small LDL-TG (No.12) | 0.05 | ± | 0.06 | 0.02 | ± | 0.01 | 0.263 |
| Very small LDL-TG (No.13) | 0.03 | ± | 0.03 | 0.02 | ± | 0.01 | 0.389 |
| Very large HDL-TG (No.14) | 0.04 | ± | 0.04 | 0.01 | ± | 0.01 | 0.240 |
| Very large HDL-TG (No.15) | 0.02 | ± | 0.02 | 0.02 | ± | 0.01 | 0.646 |
| Large HDL-TG (No.16) | 0.10 | ± | 0.04 | 0.09 | ± | 0.02 | 0.541 |
| Medium HDL-TG (No.17) | 0.33 | ± | 0.16 | 0.36 | ± | 0.17 | 0.246 |
| Small HDL-TG (No.18) | 0.55 | ± | 0.31 | 0.68 | ± | 0.35 | 0.168 |
| Very small HDL-TG (No.19) | 0.23 | ± | 0.11 | 0.39 | ± | 0.13* | **0.012** |
| Very small HDL-TG (No.20) | 0.32 | ± | 0.11 | 0.51 | ± | 0.17* | **0.018** |
| CM-C (No.1) | 3.75 | ± | 2.30 | 3.75 | ± | 1.780 | 1.000 |
| CM-C (No.2) | 2.80 | ± | 1.44 | 2.63 | ± | 1.34 | 0.820 |
| Large-VLDL-C (No.3) | 7.71 | ± | 3.34 | 6.82 | ± | 3.32 | 0.621 |
| Large-VLDL-C (No.4) | 13.98 | ± | 4.99 | 12.07 | ± | 5.18 | 0.500 |
| Large-VLDL-C (No.5) | 21.41 | ± | 6.75 | 19.00 | ± | 6.50 | 0.502 |
| Medium VLDL-C (No.6) | 24.72 | ± | 7.88 | 24.21 | ± | 5.43 | 0.877 |
| Small VLDL-C (No.7) | 10.16 | ± | 3.09 | 10.80 | ± | 1.78 | 0.587 |
| Large LDL-C (No.8) | 11.68 | ± | 3.35 | 12.77 | ± | 1.79 | 0.383 |
| Medium LDL-C (No.9) | 7.99 | ± | 2.41 | 9.08 | ± | 0.61 | 0.138 |
| Small LDL-C (No.10) | 5.25 | ± | 1.91 | 6.29 | ± | 0.62 | 0.091 |
| Very small LDL-C (No.11) | 2.71 | ± | 1.16 | 3.30 | ± | 0.45 | 0.128 |
| Very small LDL-C (No.12) | 2.17 | ± | 0.71 | 2.94 | ± | 0.47* | **0.020** |
| Very small LDL-C (No.13) | 1.10 | ± | 0.34 | 1.50 | ± | 0.15* | **0.003** |
| Very large HDL-C (No.14) | 1.22 | ± | 0.24 | 1.66 | ± | 0.16* | **0.001** |
| Very large HDL-C (No.15) | 1.03 | ± | 0.62 | 1.63 | ± | 0.38* | **0.025** |
| Large-HDL-C (No.16) | 13.41 | ± | 5.08 | 16.80 | ± | 2.70 | 0.083 |
| Medium HDL-C (No.17) | 17.29 | ± | 2.70 | 17.95 | ± | 1.72 | 0.542 |
| Small HDL-C (No.18) | 5.62 | ± | 0.87 | 5.60 | ± | 0.94 | 0.971 |
| Very small HDL-C (No.19) | 1.36 | ± | 0.17 | 1.45 | ± | 0.20 | 0.423 |
| Very small HDL-C (No.20) | 0.73 | ± | 0.10 | 0.88 | ± | 0.06* | **0.002** |

Values are mean±SD (mg/dL). *P<0.05 vs. stage 0 group by Welch's t-test. No. 1-20 are indicated the order of lipoprotein particle size. C, cholesterol ; CM, chylomicron, HDL, high-density lipoprotein ; LDL, low-density lipoprotein ; TG, triglyceride ; VLDL, very-low-density lipoprotein.

Supplemental Table 2. Lipid levels of lipoprotein subfraction in MASH and non-MASH groups.

| | Non-MASH (NAS ≤ 4) N=8 | | | MASH (NAS ≥ 5) N=11 | | | P-value |
|---|---|---|---|---|---|---|---|
| CM-TG (No.1) | 1.11 | ± | 1.01 | 0.77 | ± | 0.82 | 0.629 |
| CM-TG (No.2) | 0.53 | ± | 0.58 | 0.23 | ± | 0.30 | 0.359 |
| Large-VLDL-TG (No.3) | 1.22 | ± | 1.57 | 0.38 | ± | 0.57 | 0.284 |
| Large-VLDL-TG (No.4) | 1.92 | ± | 2.46 | 0.49 | ± | 0.80 | 0.244 |
| Large-VLDL-TG (No.5) | 2.28 | ± | 2.35 | 0.59 | ± | 1.01 | 0.182 |
| Medium-VLDL-TG (No.6) | 1.36 | ± | 0.90 | 0.48 | ± | 0.67 | 0.140 |
| Small-VLDL-TG (No.7) | 0.42 | ± | 0.26 | 0.16 | ± | 0.21 | 0.145 |
| Large-LDL-TG (No.8) | 0.51 | ± | 0.29 | 0.19 | ± | 0.24 | 0.131 |
| Medium-LDL-TG (No.9) | 0.38 | ± | 0.21 | 0.14 | ± | 0.18 | 0.126 |
| Small-LDL-TG (No.10) | 0.17 | ± | 0.12 | 0.07 | ± | 0.11 | 0.220 |
| Very small LDL-TG (No.11) | 0.07 | ± | 0.05 | 0.03 | ± | 0.05 | 0.238 |
| Very small LDL-TG (No.12) | 0.05 | ± | 0.06 | 0.03 | ± | 0.05 | 0.523 |
| Very small LDL-TG (No.13) | 0.04 | ± | 0.03 | 0.02 | ± | 0.03 | 0.548 |
| Very large HDL-TG (No.14) | 0.04 | ± | 0.04 | 0.02 | ± | 0.03 | 0.514 |
| Very large HDL-TG (No.15) | 0.02 | ± | 0.02 | 0.02 | ± | 0.02 | 0.932 |
| Large HDL-TG (No.16) | 0.11 | ± | 0.03 | 0.09 | ± | 0.03 | 0.642 |
| Medium HDL-TG (No.17) | 0.33 | ± | 0.19 | 0.35 | ± | 0.15 | 0.721 |
| Small HDL-TG (No.18) | 0.52 | ± | 0.35 | 0.64 | ± | 0.30 | 0.447 |
| Very small HDL-TG (No.19) | 0.19 | ± | 0.13 | 0.34 | ± | 0.11 | 0.066 |
| Very small HDL-TG (No.20) | 0.30 | ± | 0.13 | 0.44 | ± | 0.15 | 0.159 |
| CM-C (No.1) | 4.02 | ± | 2.26 | 3.56 | ± | 2.11 | 0.656 |
| CM-C (No.2) | 3.03 | ± | 1.42 | 2.56 | ± | 1.38 | 0.480 |
| Large-VLDL-C (No.3) | 8.30 | ± | 3.40 | 6.87 | ± | 3.20 | 0.368 |
| Large-VLDL-C (No.4) | 14.53 | ± | 5.08 | 12.71 | ± | 4.99 | 0.447 |
| Large-VLDL-C (No.5) | 20.80 | ± | 5.95 | 20.75 | ± | 7.32 | 0.987 |
| Medium VLDL-C (No.6) | 23.53 | ± | 5.84 | 25.35 | ± | 8.19 | 0.578 |
| Small VLDL-C (No.7) | 9.93 | ± | 2.80 | 10.62 | ± | 2.85 | 0.605 |
| Large LDL-C (No.8) | 11.32 | ± | 3.05 | 12.44 | ± | 3.04 | 0.444 |
| Medium LDL-C (No.9) | 7.55 | ± | 2.23 | 8.81 | ± | 1.98 | 0.225 |
| Small LDL-C (No.10) | 4.64 | ± | 1.55 | 6.17 | ± | 1.58 | 0.052 |
| Very small LDL-C (No.11) | 2.30 | ± | 0.86 | 3.28 | ± | 0.99* | **0.035** |
| Very small LDL-C (No.12) | 1.97 | ± | 0.77 | 2.66 | ± | 0.57 | 0.054 |
| Very small LDL-C (No.13) | 1.01 | ± | 0.36 | 1.34 | ± | 0.28* | **0.049** |
| Very large HDL-C (No.14) | 1.22 | ± | 0.31 | 1.42 | ± | 0.27 | 0.159 |
| Very large HDL-C (No.15) | 1.11 | ± | 0.77 | 1.25 | ± | 0.5 | 0.654 |
| Large HDL-C (No.16) | 13.84 | ± | 5.68 | 14.64 | ± | 4.22 | 0.741 |
| Medium HDL-C (No.17) | 18.12 | ± | 2.64 | 16.98 | ± | 2.31 | 0.342 |
| Small HDL-C (No.18) | 5.98 | ± | 0.86 | 5.36 | ± | 0.80 | 0.132 |
| Very small HDL-C (No.19) | 1.40 | ± | 0.17 | 1.37 | ± | 0.19 | 0.724 |
| Very small HDL-C (No.20) | 0.74 | ± | 0.12 | 0.79 | ± | 0.11 | 0.325 |

Values are mean±SD (mg/dL). *P<0.05 vs. NAS≤4 group by Welch's t-test. No. 1-20 are defined in order of lipoprotein particle diameter.. C : cholesterol, TG : triglyceride, CM : chylomicron, HDL : high-density lipoprotein, LDL : low-density lipoprotein, VLDL : very-low-density lipoprotein.




Supplemental Figure 1. Serum total cholesterol levels in mice fed with the normal, low-cholesterol (LC), and high-cholesterol (HC) diets. *P<0.001 vs. normal diet by one-way ANOVA with Bonfferoni's post hoc analysis.