Dysfunctional Very-Low-Density Lipoprotein Synthesis and Release Is a Key Factor in Nonalcoholic Steatohepatitis Pathogenesis

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The specific mechanisms of nonalcoholic fatty liver (NAFL) and nonalcoholic steatohepatitis (NASH) pathogenesis remain unknown. In the present study we investigated the differences between NAFL and NASH in terms of liver lipid metabolites and serum lipoprotein. In all, 104 Japanese subjects (50 men and 54 postmenopausal women) with histologically verified NAFL disease (NAFLD) (51 with NAFL, 53 with NASH) were evaluated; all diagnoses were based on liver biopsy findings and the proposed diagnostic criteria. To investigate the differences between NAFL and NASH in humans, we carefully examined (1) lipid inflow in the liver, (2) lipid outflow from the liver, (3) very-low-density lipoprotein (VLDL) synthesis in the liver, (4) triglyceride (TG) metabolites in the liver, and (5) lipid changes and oxidative DNA damage. Most of the hepatic lipid metabolite profiles were similar in the NAFL and NASH groups. However, VLDL synthesis and lipid outflow from the liver were impaired, and surplus TGs might have been produced as a result of lipid oxidation and oxidative DNA damage in the NASH group. Conclusion: A growing body of literature suggests that a deterioration in fatty acid oxidation and VLDL secretion from the liver, caused by the impediment of VLDL synthesis, might induce serious lipid oxidation and DNA oxidative damage, impacting the degree of liver injury and thereby contributing to the progression of NASH. Therefore, dysfunctional VLDL synthesis and release may be a key factor in progression to NASH. (HEPATOLOGY 2009;50:772-780.)

Although NASH represents steatosis with ballooning and/or fibrosis (Type 3-4 NAFLD), NAFL is characterized by simple steatosis or steatosis with only inflammation (Type 1-2 NAFLD),3 indicating that NAFL might represent the first stage of NASH pathogenesis.4 The current model of NASH pathogenesis suggests two stages of progression. First, insulin resistance causes lipid accumulation in hepatocytes; and second, cellular...

Abbreviations: ADRP, adipose differentiation-relative protein; ALT, alanine aminotransferase; apoB100, apolipoproteinB100; BMI, body mass index; CDAA, choline-deficient 1-amino acid-defined; CM, chylomicron; DGAT, diacylglycerol acyltransferase; FFA, free fatty acid; fCh, free choline; HDL, high-density lipoprotein; HF/HC, high-fat/high-calorie; HOMA-IR, homeostasis assessment insulin resistance; HPLC, high-performance liquid chromatography; LDL, low-density lipoprotein; MDA, malondialdehyde; MTTP, microsomal triglyceride transfer protein; NAFL, nonalcoholic fatty liver; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; PC, phosphatidylcholine; PET, positron emission tomography; PL, phospholipid; PPAR-α, peroxisome proliferator-activated receptor alpha; SFA, subcutaneous fat area; SREBP, sterol regulatory element binding protein; TC, total cholesterol; TG, triglycerides; TNF-α, tumor necrosis factor alpha; VFA, visceral fat area; VLDL, very-low-density lipoprotein.

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insults such as oxidative stress, lipid oxidation, direct lipid toxicity, mitochondrial dysfunction, and/or bacterial endotoxins from the gut cause hepatic inflammation, resulting in NASH. In most cases, however, NAFL does not progress to more severe pathological conditions, i.e., 10%-20% of patients with NAFLD eventually exhibit signs of fibrosis, necrosis, and inflammation, indicating the presence of NASH. The factors involved in the progression of NAFL to NASH are not fully understood. Moreover, the real percentage of NAFL that progresses to NASH is unknown, and some researchers have suggested that NAFL and NASH are two distinct entities. Therefore, it is critically important to clarify this issue.

In general, two experimental animal models are often used to study NAFLD: a high-fat/high-calorie (HF/HC) diet as a generalized NAFL model, and a choline-deficient/1-amino acid-defined (CDAA) diet as a liver-specific NASH model. Although both are well established animal models of NAFLD, the former involves only fatty liver, not steatohepatitis, whereas the latter includes both steatohepatitis and liver cirrhosis. The mechanisms of fatty liver pathogenesis also differ in that lipids accumulate in the liver because of increased fatty acid (FA) inflow in the former model and because of the blockage of very-low-density lipoprotein (VLDL) secretion in the latter. The specific mechanism by which lipids accumulate in the liver in patients with NAFLD remains unknown, and identifying this mechanism would advance efforts toward the prevention and reversal of this condition. Based on the findings of the above-mentioned animal models, we hypothesized that blocking VLDL secretion might be a key factor in NASH pathogenesis in humans. The hepatic profiles of lipid metabolites, especially FA inflow and VLDL secretion, have not been previously compared in human patients with NAFL or NASH. In the present study, we investigated the differences between NAFL and NASH in terms of liver lipid metabolites and serum lipoprotein levels. We compared the expression patterns, activities, and functional roles of hepatic lipid metabolites in patients with NAFL and those with NASH.

**Patients and Methods**

**Subjects.** The study protocol was reviewed and approved by an institutional ethical review committee. Written informed consent was obtained from all the subjects before examination. In all, 104 consecutive subjects, who were restricted to men and postmenopausal women over 20 years of age, were analyzed in the present study. Subjects with a history of excessive alcohol consumption (weekly consumption: men, >210 g; women, >140 g), other liver diseases, use of drugs associated with fatty liver, weight reduction, etc., were excluded. A liver biopsy was performed and three specimens obtained from the same lobe and on the same occasion were obtained from each subject using a 16-gauge needle biopsy kit according to a standard protocol; three specimens were obtained to enable a sufficient sample size for analysis and to reduce histological errors. All the subjects were histologically confirmed as having NAFLD based on the liver biopsy findings, and the diagnoses were further confirmed in accordance with the categories defined by Matteoni et al. As a control group, 88 healthy subjects with a mean age and sex ratio comparable to that of the NAFL and NASH groups were also analyzed. All control subjects were confirmed to have normal liver enzyme levels, a liver/spleen ratio of greater than 1.0 according to a computed tomography (CT) examination, and no other liver disease or metabolic disorder.

**Measurement of Serum Biochemical Markers.** Venous blood samples were obtained after the subjects had fasted overnight (12 hours) and were subsequently used to measure the serum alanine aminotransferase (ALT), glucose, insulin, total cholesterol (TC), low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglyceride (TG), hyaluronic acid, free fatty acid (FFA), free choline (fCh), chylomicron (CM)-TG, and VLDL-TG levels. The serum TC, TG, FFA, and plasma lipoprotein levels were analyzed using an online dual-enzymatic method for the simultaneous quantification of cholesterol and triglycerides using high-performance liquid chromatography (HPLC) at Skylight Biotech (Akita, Japan), according to the procedure described by Usui et al. CM (80-1,000 nm), VLDL1 (50-75 nm), VLDL2 (35-50 nm), large buoyant LDL

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(25.5-30 nm), small dense LDL (17-25.5 nm), and HDL (7-15 nm) were defined according to particle size.

We previously developed a simple and reliable method for the quantitative detection of serum fCh using HPLC, with a slight modification.10 Briefly, 4 μL of sample was automatically injected and carried over the column’s surface using a buffer that had first been delivered by way of a pump at a flow rate of 0.24 mL/min; the column effluent was then mixed with an fCh reagent. The enzymatic reagent was pumped at a flow rate of 0.12 mL/min, and the mixture was passed through a 15M reaction-coil in a postcolumn reactor at 37°C. Finally, the absorption was monitored using an ultraviolet-visible (UV-VIS) detector at 550 nm, and data were collected every 300 ms. The fCh concentrations were calculated by comparing the results with the fCh area under the chromatographic curves of a calibration material (choline chloride; TOYOB) of known concentration. The degree of insulin resistance (IR) was calculated using the homeostasis model for the assessment of insulin resistance (HOMA-IR) according to the following formula: [fasting serum insulin (μU/mL) \times fasting plasma glucose (mg/dL)/405]. However, the HOMA-IR assessment was only conducted in the 166 subjects with a fasting glucose level of less than 140 mg/dL, because HOMA-IR has been reported to be a suitable method for evaluating insulin resistance only in such patients.11

**Anthropometry and Abdominal Fat Distribution.**
Abdominal fat distribution was determined using a CT scanning method with the subjects in a supine position, in accordance with a described procedure.12 The subcutaneous fat area (SFA) and the intraabdominal visceral fat area (VFA) were measured at the level of the umbilicus and determined using a standardized method based on the CT number. The liver/spleen (L/S) ratio was also calculated. A histogram representing the fat tissue was computed based on the mean attenuation +2 SD.

**[11C]Choline Dynamic Positron Emission Tomography (PET) for Assessing Hepatic Phospholipid Metabolites.** [11C]Choline was synthesized according to the loop methylation method of Wilson et al.13 [11C]Choline PET was performed as described.14 In brief, [11C]choline PET was performed after 8 hours of fasting using an integrated PET scanner following the intravenous injection of 370 MBq of [11C]choline. After a transmission scan, [11C]choline was injected and the first emission scan was started at 30 seconds after injection. The scans were performed continuously every 30 seconds for a total of 1,500 seconds. Once the scans were completed, the PET images were reconstructed and the time-activity count was calculated for the appropriate liver sections.

**Measurement of Hepatic TG Content.** One and one-half liver biopsy samples per subject (25 mg of liver tissue) were used to measure the hepatic triglyceride content. The liver biopsy samples were homogenized in 50 mM Tris/HCl buffer (pH 7.4) containing 150 mM NaCl, 1 mM ethylene diamine tetraacetic acid (EDTA), and 1 μM phenylmethylsulfonyl fluoride (PMSF). TGs were analyzed enzymatically using a diagnostic kit (Infinity; Thermo DMA, Arlington, TX) and measured using spectrophotometry (Beckman Coulter, Fullerton, CA).15

**Histopathologic and Immunohistochemical Evaluations.** Liver biopsy samples were excised and embedded in Tissue-Tek OCT compound (Sakura Finetek, Torrance, CA) and paraffin for histological analysis. Five-micrometer-thick sections were processed routinely using hematoxylin-eosin staining. The presence of collagen, as an index of fibrosis in the lesions, was examined in Masson’s trichrome-stained preparations. OCT-embedded samples were serially sectioned at 4 μm. To evaluate fat deposition and to determine the fat droplet size, the liver sections were stained with Oil Red O and the diameters of the fat droplets were measured using a microscope at a 40× magnification using a grid of 0.0625 mm² with 100 points. All histopathological findings were scored by the same two pathologists (Y.N. and S.M.), who were unaware of the other biochemical and anthropometric parameters.

**RNA Isolation, Reverse Transcription, and Quantification of Gene Expression Using Real-time Reverse-Transcription Polymerase Chain Reaction (RT-PCR).** One-half of the liver biopsy samples per subject (10 mg of liver tissue) was used to measure gene expression. Total RNA was isolated from the samples using an RNeasy Mini Kit (Qiagen, Hilden, Germany; Cat. No. 74126). Reverse transcription to produce cDNA was performed using a TaqMan Gold RT-PCR Kit (Applied Biosystems, Foster City, CA), according to the manufacturer’s instructions. The reaction mixtures (100 μL) contained 2.5 μg of total RNA, and the reaction was carried out for 50 minutes at 48°C; reverse transcriptase was then inactivated by heating the samples to 95°C for 5 minutes.16 The messenger RNA (mRNA) levels of liver microsomal triglyceride transfer protein (MTTP), apolipoproteinB100 (apoB100), peroxisome proliferator activated receptor alpha (PPAR-α), sterol regulatory element binding protein-1c (SREBP-1c), SREBP-2, diacylglycerol acyltransferase 1 (DGAT1), diacylglycerol acyltransferase 2 (DGAT2), perilipin, and adipose differentiation-related protein (ADRP) were determined using the liver tissue using fluorescence-based RT-PCR and an ABI PRISM 7700 Sequence Detection System (Applied Biosystems). RT-PCR was performed using the TaqMan Universal PCR Master
Mix reagent (Applied Biosystems).16,17 The values in all the samples were normalized to the expression level of the endogenous control, GAPDH.16,18

**Statistical Analysis.** All results are expressed as the mean ± SD. A one-way Student's t test was performed for comparisons between two groups, and an analysis of variance (ANOVA) was performed to compare the means of multiple groups, followed by an unpaired Student's t test for comparisons between two groups. All analyses were two-sided, and P-values < 0.05 were considered statistically significant.

**Results**

**Subject Characteristics.** In all, 192 subjects (104 subjects with NAFLD, 88 control subjects) were analyzed in this study. The main features of the NAFL, NASH, and control groups are shown in Table 1. Compared with the control group, both the NAFL and NASH groups had high body mass index (BMI), VFA, SFA, fasting blood sugar, insulin, HOMA-IR, ALT, TG, LDL-cholesterol, hyaluronic acid scores, percentage of diabetes mellitus, hyperlipidemia, hypertension, obesity, and IR values and low L/S ratios and HDL-cholesterol scores (all \( P < 0.05 \)) (Table 1). Most of the parameters, such as BMI, VFA, SFA, L/S ratios, HDL-cholesterol, TG, ALT, percent of obesity, diabetes mellitus, hyperlipidemia, and hypertension, showed no significant difference between the NAFL and NASH groups. Compared with the NAFL group, however, the NASH group had a higher fasting blood sugar, insulin, HOMA-IR, LDL-cholesterol, hyaluronic acid score, fibrosis stage, and percentage of IR (all \( P < 0.05 \)) (Table 1).

**Lipid Inflow (FA Inflow and Synthesis) in the Liver.** We analyzed the serum levels of CM-TG, glucose, and FFA and the hepatic expressions of SREBP1c and SREBP2 mRNA as markers of lipid inflow in the liver. The serum levels of CM-TG, glucose, and FFA were significantly higher in both the NAFL and NASH groups than in the control group, in spite of the serum samples having been obtained after fasting (Fig. 1A-C). We next compared the serum levels of CM-TG, glucose, and FFA between the NAFL and NASH groups, but no significant differences were observed (Fig. 1A-C). In support of these data, the hepatic expressions of SREBP1c and SREBP2 mRNA, which are markers of FA inflow and synthesis, were similar in the NAFL and NASH groups (Fig. 1D,E). Moreover, the liver steatosis levels (L/S ratio, an index of the hepatic fat content) and the TG content were almost equal in the NAFL and NASH groups (Table 1; Fig. 1F).

**Lipid Outflow (VLDL Secretion and FA Oxidation) from the Liver.** We analyzed the serum levels of VLDL-TG, which is an index of lipid outflow from the liver, and the hepatic expression of PPAR-\( \alpha \) mRNA, which stimulates FA oxidation in the liver. The serum VLDL-TG levels were significantly higher in both the NAFL and NASH groups than in the control group (Fig. 1F).  

| Table 1. Clinical and Biochemical Characteristics of NAFL, NASH, and Control Groups |
|---------------------------------|--------|--------|
|                                | Control | NAFL   | NASH   |
| Number (n)                     | 88     | 51     | 53     |
| Age (years)                    | 49.2 ± 13.3 | 47.1 ± 13.6 | 53.6 ± 12.8 |
| Gender (male; female)          | 41:47  | 24:27  | 26:27  |
| BMI (kg/m²)                    | 21.1 ± 2.4 | 28.6 ± 6.1 | 28.2 ± 5.7 |
| Visceral fat area (cm²)        | 76.9 ± 14.2 | 121.6 ± 38.2* | 119.4 ± 35.3* |
| Subcutaneous fat area (cm²)    | 134.8 ± 42.4 | 217.3 ± 63.8* | 207.3 ± 61.4* |
| Liver/spleen ratio             | 1.08 ± 0.08 | 0.70 ± 0.26* | 0.71 ± 0.29* |
| Fasting blood sugar (mg/dL)    | 82.3 ± 20.1 | 108.5 ± 33.9* | 120.8 ± 28.1† |
| Insulin (µg/L)                 | 4.22 ± 1.83 | 9.87 ± 3.51* | 11.21 ± 3.89† |
| HOMA-IR                        | 0.83 ± 0.29 | 2.88 ± 1.14* | 3.32 ± 1.53† |
| LDL-cholesterol (mg/dL)        | 75.6 ± 20.7 | 133.1 ± 37.7* | 143.3 ± 32.9† |
| HDL-cholesterol (mg/dL)        | 64.8 ± 14.6 | 51.2 ± 12.4* | 47.3 ± 11.4* |
| Triacylglycerol (mg/dL)        | 82.4 ± 38.1 | 159.5 ± 71.1* | 173.3 ± 62.6* |
| ALT (U/mL)                     | 17.2 ± 6.1 | 49.2 ± 33.8* | 58.1 ± 42.2* |
| Hyaluronic acid (ng/dL)        | 6.0 ± 3.9 | 13.2 ± 8.4 | 46.5 ± 31.2† |
| Fibrosis stage F0/F1/F2/F3/F4   | -      | 51/0/0/0/0 | 0/32/15/6/0† |
| Obesity (BMI > 25) (%)         | 0      | 60.8* | 60.4* |
| Diabetes mellitus (%)          | 0      | 31.4* | 39.6* |
| Hyperlipidemia (%)             | 0      | 37.3* | 37.7* |
| Hypertension (%)               | 0      | 25.5* | 30.2* |
| IR (HOMA-IR > 2.5) (%)         | 0      | 53* | 64.2† |
| Metabolic syndrome (%)         | 0      | 33.3* | 37.7* |

Several parameters were measured and are expressed as the mean ± SD. *P < 0.05, compared with control group. †P < 0.05, compared with NAFL group.
Interestingly, the NASH patients had significantly lower serum VLDL-TG levels \( (P = 0.035) \) (Fig. 2A), a lower expression of liver PPAR-\( \alpha \) mRNA \( (P = 0.039) \) (Fig. 2B) than the NAFL group.

**VLDL Synthesis in the Liver.** We analyzed the hepatic expression of apoB100 mRNA, which is a structural protein of VLDL and controls the synthesis of VLDL in the liver and the release of lipoprotein from the liver. The NASH patients had a significantly lower expression of liver apoB100 mRNA \( (P = 0.026) \) (Fig. 3A). In addition, we analyzed the scores for serum fCh, which is required for lipoprotein synthesis. Contrary to our expectations, the serum fCh level was significantly higher in both the NAFL and NASH groups than in the control group \( (P < 0.05) \); moreover, the serum fCh level was significantly higher in the NASH group than in the NAFL group \( (P = 0.022, \text{Fig. 3B}) \).

To investigate the levels of choline-phospholipid (PL) metabolites in the liver, we measured \([^{11}\text{C}]\)choline uptake in the liver using \([^{11}\text{C}]\)choline dynamic PET imaging. As shown in Fig. 3C,D, \([^{11}\text{C}]\)choline accumulation in the liver was significantly lower in the NASH group (at all timepoints) than in the NAFL or control groups (both \( P < 0.05 \)). We also analyzed TG transportation by way of MTTP mRNA expression,
because MTTP is a key factor for TG transport in the liver. The hepatic expression of MTTP mRNA was significantly lower in the NASH group than in the NAFL group ($P < 0.001$) (Fig. 3E). The hepatic expression level of MTTP mRNA for differentiating between NAFL and NASH, as determined using a receiver operating characteristic (ROC) curve, was 0.775 (area under the curve, 0.910). This hepatic MTTP mRNA expression level had a sensitivity of 91.3%, a specificity of 82.3%, a positive predictive value of 87.5%, and a negative predictive value of 87.5% for the differentiation of NAFL and NASH.

**TG Metabolites in the Liver.** We analyzed the hepatic expressions of PAT family proteins (perilipin and ADRP), which control lipid droplet accumulation and decomposition. The NASH patients had significantly higher expressions of hepatic perilipin1 and perilipin2 mRNA ($P < 0.001$, respectively) (Fig. 4A,B), whereas the hepatic expressions of ADRP mRNA were similar in the NAFL and NASH groups (Fig. 4C). Next we analyzed the hepatic expressions of DGAT1 and DGAT2 mRNA, which control TG synthesis in the liver. No significant differences were observed in the NAFL and NASH groups (Fig. 4D,E).

**Lipid Changes and Oxidative DNA Damage.** Based on the results mentioned above, we measured the changes in the liver lipid droplet sizes and numbers, the serum TG rich VLDL (VLDL1) ratio and small dense LDL, and the liver expression of markers of oxidative stress-induced DNA damage (malondialdehyde [MDA], 8-hydroxydeoxyguanine [8-OHdG]). The liver droplet sizes were significantly higher in the NASH group than in the NAFL group ($P = 0.019$, Fig. 5A), whereas the liver droplet numbers tended to be lower in the NASH group than in the NAFL group ($P = 0.062$, Fig. 5B). Both the serum VLDL1 ratio and small dense LDL, a serum marker of oxidation, were significantly higher in the NASH group than in the NAFL group ($P = 0.043$, Fig. 5C,D). Moreover, the NASH group had significantly higher expressions of liver MDA levels ($P < 0.001$) (Fig. 5E) and liver 8-OHdG levels ($P = 0.014$) (Fig. 5F) than the NAFL group.
Discussion

The key findings of this study are that NAFL and NASH have distinct hepatic lipid metabolite profiles, and different patterns of lipogenesis are seen in NAFL and NASH. A scheme illustrating the differences in hepatic lipid metabolism in NAFL and NASH is shown in Fig. 6. The accumulation of fat, mainly TG, within the hepatocytes is a prerequisite for the development of NAFLD.19 The primary metabolic abnormalities leading to lipid accumulation are not well understood. TG is delivered to adipose tissue by way of intestinal CM and hepatic VLDL, and significant amounts of TGs are secreted by the liver.20 These TGs are mainly delivered to the muscle, heart, and adipose tissue.21 Fatty liver reportedly occurs when the hepatic production of TG is not matched by its secretion as VLDL or its degradation by \( \beta \)-oxidation.22 In the present study, patients with NAFL and NASH exhibited highly similar tendencies and degrees of TG pooling in the liver, but the manner of lipogenesis differed. Namely, the capacity for lipid outflow from the liver was deteriorated to a greater extent in NAFL than in NASH, even though lipid inflow remained the same. These results suggest that the blockage of VLDL-TG secretion and the reduction in FA oxidation might be key factors in the pathogenesis of NASH.

The blockage of VLDL-TG secretion, which can be caused by a dietary choline deficiency, reportedly caused steatohepatitis in an animal model.8,9 Choline deficiency in animal models may be responsible for a lack of PLs, more specifically of phosphatidylcholine (PC), which are necessary for VLDL synthesis and secretion.23 However, VLDL secretion from the liver was lower in the NASH group than in the NAFL group in the present study, contrary to our expectations. The NASH group also had a significantly higher serum fCh level than the NAFL group. These phenomena seem to contradict each other, because fCh is essential to VLDL synthesis by way of phosphatidylcholine formation in the liver.24 To clarify this discrepancy, we compared the ability to synthesize VLDL in NAFL and NASH under the condition of a high serum fCh level in NASH patients. In the present study, \([^{11}C] \) choline accumulation in the liver was significantly lower in the NASH group than in the NAFL and control groups (all \( P < 0.05 \)), in spite of the presence of abundant fCh in the NASH group. Moreover, the hepatic expression of apoB100 mRNA and MTTP mRNA were impaired in the NASH group. A correlation between MTTP activation and NASH has been suggested in some studies using polymorphisms or MTTP knockout mice,25 but the present finding is clear evidence that hepatic MTTP expression is significantly lower in patients with NASH than in those with NAFL. Charlton et al.26 reported that NASH is associated with a markedly altered hepatic synthesis of apoB100, compared with obese (BMI-matched) controls without NASH. This report is in accordance with our observation that the hepatic expression of apoB100 mRNA was significantly lower in the NASH group than in the NAFL group (\( P = 0.026 \)). These results suggest that VLDL synthesis is impaired in NASH, in spite of the presence of abundant fCh in the serum, as a result of some obstacle in choline utilization.

In the present study the hepatic TG content was found to be similar between NAFL and NASH in spite of the fact that VLDL synthesis and secretion was impaired to a greater extent in NASH patients than in NAFL patients. We next examined TG metabolism in the liver to elucidate how the surplus TGs were used. A drastic increase in the mRNA expression of one member of the PAT family of proteins, perilipin, might have led to the formation of larger lipid droplets in NASH. Moreover, the surplus TGs

Fig. 4. TG metabolism in the liver. The hepatic mRNA expressions of perilipin1 (A) and perilipin2 (B) were significantly higher in the NASH group than in the NAFL group (both \( P < 0.001 \)). The hepatic mRNA expressions of ADRP (C), DGAT1 (D), and DGAT2 (E) were similar in the NAFL and NASH groups. All data are expressed as the mean ± SD.
would have been transported as a TG-rich lipoprotein (VLDL1), leading to the formation of small dense LDL in NASH. These alterations in lipid packaging might have been caused by lipid oxidation and DNA oxidative damage, which are typically observed in patients with NASH rather than patients with NAFL. The deposition of fat in the liver, and, more specifically, the type of fat that is deposited might in fact directly damage the liver and precipitate the development of NASH. Moreover, the accumulation of oxidative lipids in the liver is thought to play a key role in the pathogenesis of NASH, as such increases would activate hepatic nonparenchymal cells, including Kupffer cells and hepatic stellate cells, leading to liver injury during both the early stage of NASH and the late fibrotic stage.

Certainly, whether patients with NASH have had an antecedent period of time in which they had NAFL or whether these two populations diverge at the inception of fatty liver remains unclear. A growing body of literature suggests that deterioration in FA oxidation and VLDL
secretion from the liver, caused by the impediment of VLDL synthesis, might result in serious lipid oxidation and DNA oxidative damage, impacting the degree of liver injury and therefore contributing to the progress of NASH. Therefore, dysfunctional VLDL synthesis and release might be a key factor in NASH pathogenesis.

Several potential drug therapies have been tested in animal models of NASH. Although large studies (multicenter clinical trials, case-control studies, and family studies) along with detailed analyses using animal models are needed to define the precise mechanism involved in the development and progression of NASH, the measurement of hepatic MTTP expression may be helpful for diagnosis. Moreover, the development of drugs capable of reactivating hepatic VLDL synthesis may provide a novel treatment for NASH.

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