1. Introduction

Almost one-quarter of adults in many industrialized countries have excessive hepatic fat accumulation (1). Although the causes of fatty liver is not clearly known, in our aging and overfed population, obesity and diabetes appear to be common and frequently associated conditions with non-alcoholic fatty liver disease (NAFLD), which includes non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH) (2). While NASH is proposed as a fatty liver disease associated with diffuse infiltration and inflammation (3), NAFL, by contrast, is characterized by only steatosis, indicating that NAFL might represent the state of 1st hit in NASH (2). NAFLD has been reported to be the most common cause of chronic liver injury and represents a spectrum of conditions that are histologically characterized by macrovesicular hepatic steatosis (3). Liver damage gradually progresses over years in patients with fatty liver, eventually resulting in liver dysfunction (4). The current model of NASH pathogenesis suggests two stages of progression. First, insulin resistance causes lipid accumulation
in hepatocytes; and second, cellular 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 (5). In most cases, however, NAFL does not progress to more severe pathological conditions, that is, 10% – 20% of patients with NAFLD eventually exhibit signs of fibrosis, necrosis, and inflammation, indicating the presence of NASH (6). 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.

Fatty acids (FAs) are vital components of essentially all known organisms. They are important substrates for oxidation and the production of cellular energy and are predominantly stored in the form of triacylglycerol (TG) within fat droplets of hepatocytes. One important aspect of the two-hit hypothesis is that steatosis per se is not causal in the development of NASH, but rather it sensitizes the liver to the damaging effects of second hits such that stressors innocuous to a healthy liver lead to the development of NASH in the steatotic liver. As will be discussed, however, an increasing body of literature suggests that the deposition of fat in the liver, and more specifically the type of fat that is deposited, may in fact directly damage the liver and precipitate the development of NASH. The purpose of this review is to focus on the current knowledge of the mechanisms of fat droplet accumulation and role of microsomal triglyceride transfer protein (MTP) in NAFLD progression.

2. Very-low-density lipoprotein (VLDL) secretion defect and NASH

From a kinetic standpoint, hepatic steatosis develops when there is an imbalance in which fatty acid uptake and de novo synthesis exceed oxidation and resecretion. The excessive/ectopic fat deposition in the liver could be initiated by a) increased fatty acid delivery from extra hepatic sources such as adipose tissue, b) increased synthesis of fatty acid via the de novo pathway, c) increased dietary fat, d) decreased mitochondrial β-oxidation, e) decreased clearance of VLDL particles, or several of these factors in combination. An understanding of hepatic fatty acid regulation should take into account all these sources; furthermore, the relative importance of each of them in determining liver fat content may differ in health and NAFLD, in the fasted and fed states, and is modulated by genetic, hormonal, and dietary factors.

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 (7) and a choline-deficient / L-amino acid–defined (CDAA) diet as a liver-specific NASH model (8). Although both are well-established animal models of NAFLD, the former involves only fatty liver, not steatohepatitis, while the latter includes both steatohepatitis and liver cirrhosis (9). 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 VLDL secretion in the latter (7, 8). These phenomena suggested that VLDL secretion defect might be a key factor in NASH pathogenesis.

3. The hepatic profiles of lipid metabolites between NAFL and NASH

The progression from simple steatosis to NASH is a key concern in the NAFLD field. In our previous study, although most of the hepatic lipid metabolite profiles were similar in the NAFL and NASH groups, the capacity for lipid outflow from the liver, especially the hepatic expression of MTP mRNA, was deteriorated to a greater extent in NAFL than in NASH, even though lipid inflow remained the same (10). The hepatic expression level of MTP 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 MTP 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. A scheme illustrating the differences in hepatic lipid metabolism in NAFL and NASH is shown in Fig. 1. The accumulation of fat, mainly TG, within the hepatocytes is a prerequisite for the development of NAFLD (11). TG is delivered to adipose tissue via intestinal CM and hepatic VLDL, and significant amounts of TGs are secreted by the liver (12). These TGs are mainly delivered to the muscle, heart, and adipose tissue (13). Fatty liver reportedly occurs when the hepatic production of TG is not matched by its secretion as VLDL or its degradation by β-oxidation (14). Our results suggested that fatty liver with NAFL was formed only for surplus FA accumulation because of energy over-inflow in the liver. On the other hand, fatty liver with NASH was formed for energy over-inflow and the impediment of MTP activity.

4. What is MTP?

Genetic variation in lipid metabolism may produce differences in the speed and extent of hepatocyte lipid accumulation, the first hit. One enzyme that has attracted
some interest is the MTP. This protein transfers triglycerides to nascent apolipoprotein B, producing VLDL and removing lipid from the hepatocyte. Patients with abetalipoproteinemia, an autosomal recessive disease caused by mutations in the MTP coding region, develop marked hepatic steatosis early in life (15, 16). MTP mRNA is down-regulated in human hepatocytes by insulin (17). A common polymorphism in the MTP gene promoter, −493 G/T, has been described, with the G allele producing less gene transcription than the T allele (18, 19).

5. Distribution of the MTP gene G/T polymorphism in NASH patients and NAFL patients

A correlation between MTP activation and NASH has been suggested in some studies using polymorphisms (20–24). On the other hand, several articles reported that hepatitis (inflammation) and hyperinsulinemia down-regulate MTP activity posteriori (25, 26). Certainly, the present finding is clear evidence that hepatic MTP expression is significantly lower in patients with NASH than in those with NAFL. It is unknown whether congenital and posteriori factors are important in MTP down-regulation in NASH. To examine the influence of a congenital factor, we analyzed single nucleotide polymorphism (SNPs) of the MTP gene in 425 healthy volunteers, 48 individuals with NAFL, and 60 individuals with NASH. NASH showed significantly higher incidence of minor allele appearance compared with NAFL (Table 1), supporting the possibility of an association between NASH pathogenesis and decreased congenital MTP activity (unpublished results). Based on our results, we hypothesize that if these two conditions, MTP disability and lipid over-inflow in the liver, were overlapped, the lipid processing performance of the liver is exceeded and leans to progression towards steatohepatitis and fibrosis.

6. Conclusions

Several potential drug therapies have been tested in animal models of NASH (9, 27). 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 MTP expression may be helpful for diagnosis. Moreover, the development of drugs capable of re-activating hepatic VLDL synthesis may provide a novel treatment for NASH.

<table>
<thead>
<tr>
<th>Table 1. Single nucleotide polymorphisms of MTP gene (−493G/T) among the control, NAFL, and NASH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Former group</td>
</tr>
<tr>
<td>G/G</td>
</tr>
<tr>
<td>NAFLD vs. control</td>
</tr>
<tr>
<td>NASH vs. control</td>
</tr>
<tr>
<td>NASH vs. NAFL</td>
</tr>
</tbody>
</table>
Acknowledgments

The skillful technical assistance of Machiko Hiraga is gratefully acknowledged. This work was supported in part by a Grant-in-Aid for Research on the Third Term Comprehensive Control Research for Cancer from the Ministry of Health, Labor, and Welfare, Japan to A.N.; a grant from the National Institute of Biomedical Innovation (NBIO) to A.N.; a grant from the Ministry of Education, Culture, Sports, Science, and Technology, Japan (KIBAN-B) to A.N.; a grant program “Collaborative Development of Innovative Seeds” from the Japan Science and Technology Agency (JST); and a Wakate-kenkyuu start-up grant (Grant No. 20890185) to K.F. from the Ministry of Education, Culture, Sports, Science, and Technology, Japan. This study was not supported by private funding. None of the authors have any conflicts of interest.

References

7. Deng XQ, Chen LL, Li NX. The expression of SIRT1 in non-alcoholic fatty liver disease induced by high-fat diet in rats. Liver Int. 2007;27:708–715.