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EDITORIAL

Is the control of dietary cholesterol intake sufficiently effective to ameliorate nonalcoholic fatty liver disease?

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Abstract

In our examination of the distribution of abdominal fat, dietary intake and biochemical data in patients with nonalcoholic fatty liver disease (NAFLD), non-obese NAFLD patients without insulin resistance presented a characteristic pattern of dietary intake. Dietary cholesterol intake was superabundant in non-obese patients compared with obese patients, although total energy and carbohydrate intake was not excessive. Namely, excess cholesterol intake appears to be one of the main factors associated with NAFLD development and liver injury. Therefore, the control of dietary cholesterol intake may lead to an improvement in NAFLD, and the NPC1L1 inhibitor ezetimibe might be a promising treatment for NAFLD. We review one pathogenic aspect of lipid metabolism dysregulation in NAFLD and survey new strategies for NAFLD treatment based on the modification of cholesterol metabolism.

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Key words: Cholesterol; Ezetimibe; Nonalcoholic fatty liver disease; NPC1L1; Polyunsaturated fatty acids

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD), which encompasses a broad spectrum of liver disorders ranging from simple hepatic steatosis to steatohepatitis and cirrhosis, is currently the most common cause of chronic liver disease and abnormal liver function tests in Western countries. The development of hepatic steatosis is considered to be associated with an excess intake of calories, visceral obesity and insulin resistance, which result in an increased release of free fatty acids from adipocytes and increased rates of fatty acid synthesis in the liver[1,2]. However, the mechanisms involved in the pathogenesis of NAFLD in humans have not been thoroughly investigated. Because of the associated triglyceride accumulation in hepatocytes, NAFLD has been mainly investigated as a lipogenic disorder. Indeed, fatty acid overload because of the acceleration of *de novo* synthesis and cellular uptake results in mitochondrial dysfunction, oxidative stress and impaired VLDL formation, which lead to disease progression[1,2]. These changes related to lipid metabolism were positively linked to transcriptomic and metabolomic profiles in rats with NAFLD induced by a high fat diet[3]. In addition, from our analyses of the expression profile of fatty acid metabolism-associated genes in

biopsy samples from NAFLD liver, a similar expression pattern was seen, which indicated that the expression of sterol regulatory element-binding protein-1c (SREBP-1c), a positive regulator of fatty acid synthesis, was still upregulated and the expression of AMP-activated protein kinase, a negative regulator of fatty acid synthesis, was down-regulated despite the increased uptake of free fatty acids and intracellular accumulation of fatty acids and triglycerides[4-9]. These results suggest a breakdown of the feedback regulation from the increased level of intracellular fatty acids. Recently, it has been considered that cholesterol metabolism has a significant role in the pathogenesis of NAFLD. In the examination of cholesterol metabolism-associated genes, despite cholesterol overload in hepatocytes, *de novo* synthesis of cholesterol is still activated in the NAFLD liver, meaning that cholesterol metabolism is dysregulated in NAFLD[10]. This review focuses on the intrahepatic cholesterol dysregulation in NAFLD and potential emerging therapies for NAFLD.

To understand the nature of NAFLD, a nutritional approach provided helpful information. Among NAFLD patients, a large percentage of patients have obesity with insulin resistance, however, many non-obese individuals are also included[11,12]. Considering visceral fat and insulin resistance, which are evident in obese patients, the distribution of abdominal fat, dietary intake and biochemical data were compared between obese (BMI > 25 kg/m^2) and non-obese patients (BMI < 25 kg/m^2) to identify potential nutritional factors that affect NAFLD[13,14]. Visceral fat and dietary intake of total energy and carbohydrates were at overtly higher levels in the obese group as a matter of course. In contrast, in non-obese patients, dietary cholesterol was significantly higher and dietary polyunsaturated fatty acids (PUFA) were significantly lower than those in obese patients. Mean concentrations of serum total cholesterol, LDL-cholesterol and triglycerides were near the upper limit of the normal range, and serum levels of adipocytokines were not in the abnormal range in either group.

Namely, superabundant dietary cholesterol and decreased dietary PUFA intake may contribute to NAFLD development without the presence of obesity or insulin resistance. These findings are supported by some animal models fed a high-cholesterol diet, which show hepatic steatosis without obesity[15-17]. However, these animals had obvious hypercholesterolemia in contrast to NAFLD patients. This might be because the dietary cholesterol levels are considerably higher (0.2%-1.25%) in animal models than in our examined NAFLD patients. Furthermore, in these patients, hypercholesterolemia might be masked by the overwork of hepatocytes, resulting in cholesterol overload in tissues. Cholesterol supply and fatty acid synthesis are associated on a stream of the liver X receptor α (LXRα)-SREBP-1c pathway. In hepatocytes, LXRα is a key regulator of cholesterol and fatty acid metabolism, and its endogenous agonistic ligands are oxysterols, which are metabolites of cholesterol. Surplus cholesterol produces increased levels of oxysterols, resulting in activation of the LXRα-SREBP-1c pathway and enhancement of fatty acid synthesis. Furthermore, upregulation of LXRα expression was more noticeable in non-obese than in obese NAFLD patients[8]. Also, in the study of PUFA, patients with NAFLD were found to have lower levels of hepatic n-3 and n-6 PUFA, and n-3 PUFA dietary intake had therapeutic effects on fatty liver in patients with NAFLD[18-20]. n-3 PUFAs, such as eicosapentaenoic acid, which function as suppressors of SREBP-1c, are considered to reduce hepatic levels of triglycerides. However, clinically, a drug containing eicosapentaenoic acid does not have a high enough efficacy in many cases to overcome NAFLD (our own data).

Until now, investigations of therapeutic interventions have largely focused on agents that modify oxidative stress and insulin sensitivity, but clearly, an effective therapy for NAFLD has not been proven. If excess cholesterol plays a key role in the onset and progression of NAFLD, the control of dietary cholesterol intake should be a beneficial treatment strategy. Niemann-Pick C1 like 1 (NPC1L1), found in the proximal jejunum and canalicular aspect of hepatocytes, is essential for the absorption/reabsorption of cholesterol from the intestines and liver. Accordingly, the NPC1L1 inhibitor ezetimibe is expected to decrease intracellular cholesterol levels and to down-regulate/inactivate the LXRα-SREBP-1c pathway, and may be a suitable candidate for NAFLD treatment. In animal models, knocking out NPC1L1 or treatment with a NPC1L1 inhibitor provides resistance against steatosis[21,22]. Clinically, we encountered and reported a patient with NAFLD in whom ezetimibe clearly provided an improvement against liver injury and steatosis[23]. In a clinical study, to reduce cholesterol load, ezetimibe was administered (10 mg/d, orally) to non-obese NAFLD patients (n = 12) without any other treatments and any lifestyle modifications (unpublished data). In fact, ezetimibe was effective for liver injury because significant improvements were seen in serum aminotransferase levels, with 75% of subjects normalizing their transaminases. Six months after the treatment, alanine aminotransferase levels decreased by nearly 60% on average. However, a steatotic appearance remained as determined by liver echotexture in many of the patients (9/12) after 12 mo of treatment, indicating that a significant attenuation of fat content was not necessarily found. Of note, suppression of dietary cholesterol absorption may be a feasible option to successfully treat NAFLD, particularly in non-obese patients.

Considering the above findings, cholesterol-modifying treatments are favorable for NAFLD patients, and ezetimibe is expected to show a prompt clinical effect on laboratory findings for at least non-obese patients. Hence, the following should be examined and determined: (1) Are HMG-CoA reductase (HMGR) inhibitors (statins), which suppress *de novo* cholesterol synthesis, effective for NAFLD as well as ezetimibe? In recent reports, some

affirm but some deny the effect[24-26]. However, statins, with the exception of pravastatin, have generally shown promising results with improved serum aminotransferase levels. Combination therapy with an HMGR inhibitor plus ezetimibe might be more effective than monotherapy, although several cases of hepatic injury as an adverse effect have been reported in patients with pre-existing chronic liver disease[27,28]; (2) Is the control of cholesterol levels also effective in obese NAFLD patients with insulin resistance? Because dietary cholesterol intake was also significantly higher in obese patients than in normal individuals[13], ezetimibe is possibly effective for obese patients. However, in obese patients, it is difficult to remove the impact of other factors such as lifestyle modifications and other baseline agents; therefore, a study in obese patients requires circumspection; (3) Does the control of cholesterol levels improve steatosis in long-term observations? In the studies of NAFLD treatment by statins, a consistent opinion has not been drawn on the matter of the improving effect in hepatic steatosis[24,25]; (4) Further studies are required to determine whether cholesterol modifications are effective for both types of NAFLD, simple steatosis and steatohepatitis; and (5) Does the control of cholesterol levels show an additive therapeutic effect with any other treatments such as antioxidants, hepatoprotective agents or insulin sensitizers?

CONCLUSION

According to our nutritional examinations, increased cholesterol intake may be one of the main causes of an increase in the prevalence of NAFLD. Therefore, as a potential treatment, cholesterol-lowering agents look promising. Indeed, several recent studies endorse the clinical indication of statin therapy for NAFLD. Ezetimibe has recently been viewed as an alternative to statin therapy in patients with hypercholesterolemia. Ezetimibe targets the cholesterol absorption/reabsorption step, and accordingly ezetimibe may be a suitable treatment for NAFLD. Larger trials are needed to confirm whether ezetimibe or statins are really efficacious as monotherapeutic agents and, to maximize clinical benefits while minimizing side effects, further trials may be required to investigate the best combination partners for the treatment of NAFLD.

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