Study on NASH mouse model with different proportions of high-fat and high-sugar diets

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Abstract: *Nonalcoholic steatohepatitis (NASH) is characterized by varying degrees of steatosis, inflammatory and fibrotic changes. The pathophysiological mechanism of NASH is complex, and there is no rapid and effective method to establish Nash model. In this study, NASH mice were modeled by feeding different proportions of high-fat and high-sugar diets, and blood glucose and body weight were measured. After the modeling, HE and Masson staining were performed on the liver tissues of mice to detect the liver function, and the modeling situation of NASH on the three diets was comprehensively evaluated in combination with the liver function, which provided ideas for the development of a more rapid and effective method of NASH modeling and laid a foundation for revealing the pathogenesis of NASH.*

Keywords: *Nonalcoholic steatohepatitis, NASH, high-fat diet*

1. Background

1.1 Non-alcoholic fatty liver disease

Non-alcoholic Fatty Liver Disease (NAFLD) is one of the most common liver diseases in the world.In recent years, with the improvement of people's living standards and the change of living habits and diet structure, the incidence of obesity and diabetes increases, and the incidence of NAFLD is also on the rise, reaching 25%-30% in the general population. There are many adverse reactions to liver morphology and function in patients with NAFLD. These adverse reactions are a series of continuous pathological processes, including simple fatty liver disease (FLD), steatohepatitis (NASH) and its associated liver fibrosis and cirrhosis, and may even develop into liver cancer (HCC). Of these, 10% to 20% of NAFLD patients develop non-alcoholic steatohepatitis (NASH).

1.2 Discussion on the pathogenesis of non-alcoholic steatohepatitis

NASH is a severe liver disease with extensive inflammatory cell infiltration and necrosis. It is an important transitional stage in the evolution of nonalcoholic fatty liver disease into other types of severe liver disease, characterized by hepatocyte balloon degeneration and inflammation, and may evolve into liver fibrosis, cirrhosis, hepatocellular carcinoma, and liver failure. However, there is still no scientific and systematic explanation of the pathogenesis of NASH. Many years ago, scholars suggested that the "two-strike" hypothesis could be applied to explain the gradual transition from steatosis to NASH.The theory is that the first shock triggers steatosis, followed by a second shock that leads to inflammation, damage to liver cells and progression to fibrosis.Although the two-strike hypothesis is generally accepted, the sequence of lipid deposition and inflammatory response in NASH, and the causal relationship between the two remains unclear.Later studies have proposed an improved version of the "multiple hits" theory.This theory suggests that NASH is the result of multiple concurrent conditioning effects, including genetic predisposition, abnormal lipid metabolism, oxidative stress, lipid toxicity, mitochondrial dysfunction, changes in cytokines and adipokines, intestinal ecological disorders, and endoplasmic reticulum stress.According to many years of research, non-alcoholic steatohepatitis is a complex and multifactorial disease[1-2].

1.3 Construction of the NASH model

At present, many disease animal models have been established in the medical community at home and abroad, but most of them are in-depth studies on the pathophysiology and molecular biological

pathogenesis of NAFLD, while there are few studies on the establishment of NASH animal models.According to the existing research results, the dietary animal model, that is, the animal model of dystrophic fatty liver disease, is relatively common.It refers to the addition of excessive lipids, cholesterol and carbohydrates in the process of giving high fat feed, so that lipids accumulate in the liver and cause fatty liver, further hepatitis and liver fibrosis.Existing NASH models at home and abroad show that in the establishment of MCD model (high sucrose, high fat and lacking methionine and choline), inflammation, fibrosis and hepatocyte apoptosis develop rapidly and seriously, and endoplasmic reticulum stress and oxidative stress are also very active.However, this model results in weight loss and no insulin resistance in animals, which is significantly different from the level of glycolipid metabolism of human NASH.The Ch model (high cholesterol and high cholic acid) can stimulate the production of inflammatory factor TNF-α in the liver, enhance the inflammatory response and promote the progression of NASH. Although insulin resistance is produced in this process, there are clinical manifestations opposite to human NASH characteristics, such as weight loss, epididymal fat reduction and reduced plasma triglyceride level.In addition, the simple high-fat diet model or high-fructose diet model can cause weight gain, lipid accumulation, insulin resistance and inflammation, but can not cause liver fibrosis and obvious NASH characteristics, and the modeling time is relatively long.However, adding a certain proportion of high-energy substances such as high fructose or high cholesterol into the ordinary high-fat diet to form high-fat and high-sugar diet models and high-fat and high-cholesterol diet models, these models are more likely to cause significant obesity, insulin resistance, liver inflammation, oxidative stress, autophagy, apoptosis and fibrosis in animals. This process is highly similar to the development of NASH in humans, and is the most commonly used method of modeling[3-5].

In recent years, most experiments on NASH modeling methods are single, lack of timeliness, and can not clarify the advantages and disadvantages of various models and their mechanisms at the same time.Therefore, in our laboratory, we studied the time and characteristics of NASH formation in mice by using ordinary high-fat diet, high-fat high-sucrose diet and high-fat high- fructose diet at the same period, and compared the duration of NASH formation and the success rate of modeling among the three groups of models by means of phenotypic data, blood glucose analysis, pathological tissue comparison, biochemical detection and other experimental methods.

2. Materials and methods

2.1 Selection and grouping of experimental animals

A total of 120 8-week-old SPF male C57BL/6J mice with a body weight of 18-22 g were randomly divided into 4 groups: normal group (CNTR), common high-fat group (HFD), high-fat and high-fructose group (HFD+F) and high–fat and high–sucrose group (HFD+S), with 30 mice in each group.The high-fat group was given a Rodent diet with 60 Kcal% Fat same as D12492 (60% Fat caloric diet), and the high-fat high-fructose group was given a Rodent diet with 60 Kcal% fat and 15% Fructose (60% Fat calories +15% fructose Diet), and high-fat high-sucrose group were given a Rodent Diet with 60 Kcal% Fat and 15% Sucrose (60% fat calories +15% sucrose diet).The feeding environment temperature was 22–28°C, with free eating and drinking[6].

2.2 Detection of glucose and lipid metabolism

GTT (glucose tolerance test), ITT (insulin tolerance test) and random blood glucose tests were performed one week before execution.

2.3 Biochemical tests

The serum of mice was collected, and TC (total cholesterol), TG (triglyceride), HDL -C (high density lipoprotein cholesterol), LDL-C (low density lipoprotein cholesterol) in the serum of mice were detected by biochemical analyzer.

2.4 Pathological staining

HE staining and Masson staining were used to detect liver pathological changes

3. Experimental results

3.1 Changes of body weight of mice after modeling with different proportions of high-fat and high-sugar diets

Our research team established NASH mouse models induced by HFD, HFD+S and HFD+F in February 2022, and monitored their body weight changes every two weeks.The results showed that compared with the normal group (CNTR), the body weight of mice in each group was significantly different.Compared with the high-fat group (HFD), there were significant differences in body weight in the high-fat + high-fructose group (HFD+F) and the high-fat + high-sucrose group (HFD+S), and there were also significant differences between the HFD+F group and the HFD+S group. The experiment showed that HFD+F feed may have the best effect on inducing obesity in mice.However, from 11 months of feeding, the body weight of mice in the model group did not increase significantly or decreased slightly, which may be related to the fact that the mice fed high-fat diet for a long time may enter the late stage of NASH and cause tumors or other metabolic diseases[7-9].

3.2 Changes in tissue weight of mice after modeling with different proportions of high-fat and high-sugar diets

HFD, HFD+S and HFD+F models were prepared for 6 months, 8 months and 12 months, respectively, and then sacrificed after fasting for 14h.Blood, heart, liver, kidney, pancreas and adipose tissue were collected and weighed.After weighing, it was found that compared with CNTR group, all tissues and organs in each model group had different degrees of weight gain after 6 months, 8 months and 12 months, and the weight gain in HFD+S and HFD+F groups was significantly higher than that in HFD group.

3.3 Results of sugar metabolism in mice modeled after different proportions of high-fat and high-sugar diets

Mice in experimental group were fed GTT (glucose tolerance test) and ITT (insulin tolerance test) for 6 months, 8 months and 12 months, respectively.After fasting for 14h, the fasting blood glucose of mice was measured by tail vein. The results showed that the fasting blood glucose concentration of mice in each model group was significantly higher than that in CNTR group.After 4h of fasting, postprandial blood glucose was detected, and the results showed that the postprandial blood glucose concentration of mice in all model groups was also significantly higher than that in CNTR group.In the GTT and ITT experiments, the area under the blood glucose curve (AUC) of all experimental groups was significantly higher than that of CNTR group.The results showed that three kinds of high-fat diets for six months could significantly reduce insulin sensitivity and produce insulin resistance in mice, but there was no significant difference among model groups.

3.4 Pathological changes of liver tissue in mice after different proportions of high-fat and high-sugar diets

The tissue blocks of the right lobe of the liver were fixed in 4% paraformaldehyde and then embedded after dehydration for pathological observation.H&E staining showed that after eight months of feeding, the structure of liver cells in CNTR group was complete, the liver cells were closely arranged, and the liver cords were neatly arranged.The structure of hepatocytes in each model group was irregular, the arrangement of hepatocytes was disordered, hepatocytes we're enlarged, the boundary was blurred, hepatocytes showed slight steatosis, a few vacuoles, and inflammatory infiltration, and HFD+F group had serious hepatocellular lesions, and the effectwas the most obvious[10].

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