Dietary lipids, such as cholesterol and triglycerides (TGs), have been shown to exacerbate adipose tissue inflammation and non-alcoholic fatty liver disease in animal models [9], [10]. Histologically, the main feature of non-alcoholic fatty liver disease is benign steatosis, developing in 6%–55% of patients with nonalcoholic steatohepatitis (NASH) [11]. Apart from steatosis, NASH is mainly characterized by the presence of inflammatory cells in the liver parenchyma cells, activation of resident macrophages (Kupffer cells [KCs]), and the fibrotic process resulting in activation of hepatic stellate cells (HSCs) [12]. Based on previous research, obesity is closely linked with nonalcoholic fatty liver disease (NAFLD), and HFD can induce obesity and steatosis [13]. While a normal liver has oxidative stress-resistant, fatty liver is vulnerable to oxidative stress. As a result of obesity resulting in fatty liver, inflammation and also liver fibrosis, and steatohepatitis [14].

Our research aimed to determine the level of steatosis in Wistar rats given a standard diet, a high-fat diet, and a modified high-fat diet and evaluate weight gain and metabolic markers in the three groups of rats.
Materials and Methods

**Animal models**

This research was conducted with an accurate experimental study with a post-test control group design. The research was conducted at the Pharmacology Universitas Sumatera Utara, with the approval of Law No. 726/KEP/USU/2021 ethics committee of experimental animals. All animals were cared for by the principles and guidelines of animals. The experimental animal samples were white male rats of the Wistar strain, aged 10 weeks, weighing 150–250 g, kept in separate cages in the Pharmacology Laboratory, Faculty of Medicine, Universitas Sumatera Utara. The treatment of rats was started by adapting (acclimation) rats for 7 days in a cage with a constant temperature, a standardized 12/12 h light/dark cycle with a laboratory atmosphere for observation of health and changes in behavior during the adaptation period given standard food, animal feed, and drinking-water ad libitum [15].

**Animal intervention**

In this study, using 18 rats after acclimatization, rats were randomly selected (randomized) and then grouped into groups by feeding a standard diet (Group 1), a high-fat diet (Group 2), and a modified high-fat diet (Group 3). Each group of rat samples consisted of 6 rats/cage with a cage size of 50 × 40 cm². The composition of the feed given to each group of rats is as shown in the Table 1. We measured weight, body length, and abdominal circumference in all groups before diet administration, and every week. We measured the body weight. We measure the body length and abdominal circumference once every 2 weeks. We were feeding the diet for 9 weeks.

**Statistical analysis**

Before carrying out statistical tests, all sample variables were tested for normality the Shapiro Wilk test and found p > 0.05, meaning that the sample variables were typically distributed, tested by the ANOVA test and if not normally distributed the Kruskal–Wallis test. Values are expressed as the arithmetic mean ± standard error of the mean (SEM). One-way ANOVA was used to determine the main effects of diet (St vs. HF diet vs. HF modification diet) and their interaction. Tukey’s multiple comparison test was used to determine differences between all experimental groups whenever identified a significant interaction. The difference was considered statistically significant when p < 0.05.

**Results**

In this study, there was an increase in body weight in three groups of rats every week. Still, the average increase in body weight in these three groups was not significantly different, where the average increase in body weight in the group of rats fed a diet before and after dieting for 9 weeks by 29.19% in the first group of rats (187–264.40 g), in the second group of 19.12% (219.33–275 g), and by 24.53% (213.33–275 g) in the third group for 9 weeks, Figure 1.

Feeding diet for 9 weeks in the three groups of rats increased abdominal circumference. Still, the addition of abdominal circumference in the three groups of the rats showed no significant difference in the 2nd week. Still, in the 4th week, there was a significant difference in the addition of abdominal circumference in Group 1 and Group 3 (p < 0.05). In contrast, in group 1 and group 2,
In this study, the three groups of rats experienced weight gain every week until the 9th week. The process of steatosis in Group 1 experienced steatosis of 57.50%, which was moderate grade steatosis. In contrast, Groups 2 and 3 had severe grade steatosis; another study in animals given High carbohydrates for 24 weeks indicates obesity with severe hepatic steatosis [16], [17], [18]. The occurrence of fatty liver (steatosis) in this study showed a significant difference in the group of rats fed a high-fat diet compared to the group of rats fed a standard diet wherein the group of rats fed a standard diet, hepatocyte cells were still partially normal. Other researchers say that steatosis is caused by consuming a high-fat diet [19].

This study showed that the average weight gain during dieting in the first group of rats was 29.19% (187–264.40 g), more significant than in the other two groups, namely, the second group of 19.12% (219.33–275 g) and 24.53 g in the third group. % (213.33–275 g), this is in line with other studies that found that a high-fat diet did not cause weight gain but caused a more significant increase in liver fat than controls [20].

In addition to, hepatocyte cells experiencing steatosis, central venous congestion was also found in this study, and there were also necrotic cells. This process could be distinguished in rats with a regular and high-fat diet. Fatty liver is characterized by excessive accumulation of lipids due to excessive consumption of fat or carbohydrates [21]. Many studies have been conducted on obese rat models with various dietary compositions such as Feeding high fat and high cholesterol diet for 10 weeks showed an increased significance of the weight, epididymal fat, and steatosis. The study showed that diet with sphingomyelin attenuates hepatic steatosis and adipose tissue inflammation in high-fat-diet-induced obese mice [22]. In this study, we also found that the average liver weight was higher in Group 3 (10.01 g) compared to Groups 1 and 2, where a high-fat diet caused a more significant increase in liver fat than controls [20].

**Table 4: Microscopic state of the liver of obese model rat**

| Group   | Steatosis (%) Mean ± SD |
|---------|-------------------------|
| Group 1 | 57.50 ± 29.28           |
| Group 2 | 93.33 ± 2.58            |
| Group 3 | 95 ± 3.16               |

**Table 2: The weight of the liver of the group (gram)**

| Group   | Mean ± SD |
|---------|-----------|
| Group 1 | 8.66 ± 4.20 |
| Group 2 | 8.94 ± 1.54 |
| Group 3 | 10.01 ± 1.63 |

**Table 3: Abdominal adipose tissue of the groups (gram)**

| Group   | Mean ± SD |
|---------|-----------|
| Group 1 | 6.66 ± 0.82 |
| Group 2 | 9.53 ± 0.99 |
| Group 3 | 9.54 ± 0.91 |

In this study, there was a significant difference in the occurrence of steatosis in groups 1 with groups 2 and 3 (p < 0.05), but there was no significant difference in the process of steatosis in groups 2 and 3 (p > 0.05). The average steatosis process in group 1 was around 57.50%, while in group 2, it was 93.33% and in group 3 was around 95%. In the process of steatosis groups, 2 and 3 followed by process of congestive and necrosis compared to group 1 (Table 4).

**Discussion**

In this study, the three groups of rats experienced weight gain every week until the 9th week. The process of steatosis in Group 1 experienced steatosis of 57.50%, which was moderate grade steatosis. In contrast, Groups 2 and 3 had severe grade steatosis; another study in animals given High carbohydrates for 24 weeks indicates obesity with severe hepatic steatosis [16], [17], [18]. The occurrence of fatty liver (steatosis) in this study showed a significant difference in the group of rats fed a high-fat diet compared to the group of rats fed a standard diet wherein the group of rats fed a standard diet, hepatocyte cells were still partially normal. Other researchers say that steatosis is caused by consuming a high-fat diet [19].

This study showed that the average weight gain during dieting in the first group of rats was 29.19% (187–264.40 g), more significant than in the other two groups, namely, the second group of 19.12% (219.33–275 g) and 24.53 g in the third group. % (213.33–275 g), this is in line with other studies that found that a high-fat diet did not cause weight gain but caused a more significant increase in liver fat than controls [20].

In addition to, hepatocyte cells experiencing steatosis, central venous congestion was also found in this study, and there were also necrotic cells. This process could be distinguished in rats with a regular and high-fat diet. Fatty liver is characterized by excessive accumulation of lipids due to excessive consumption of fat or carbohydrates [21]. Many studies have been conducted on obese rat models with various dietary compositions such as Feeding high fat and high cholesterol diet for 10 weeks showed an increased significance of the weight, epididymal fat, and steatosis. The study showed that diet with sphingomyelin attenuates hepatic steatosis and adipose tissue inflammation in high-fat-diet-induced obese mice [22]. In this study, we also found that the average liver weight was higher in Group 3 (10.01 g) compared to Groups 1 and 2, where a high-fat diet caused a more significant increase in liver fat than controls [20].

In this study, there was a significant difference in the occurrence of steatosis in groups 1 with groups 2 and 3 (p < 0.05), but there was no significant difference in the process of steatosis in groups 2 and 3 (p > 0.05). The average steatosis process in group 1 was around 57.50%, while in group 2, it was 93.33% and in group 3 was around 95%. In the process of steatosis groups, 2 and 3 followed by process of congestive and necrosis compared to group 1 (Table 4).
considerable liver weight than a regular diet; this is by the other study in the results of the study found that liver weight was more significant in the HFD group compared to the Low Fat Diet. And according to the other study, giving HFD to Sprague–Dawley rats for 12 weeks showed hepatocyte cells in liver tissue of different sizes of lipid droplets in the cytoplasm compared to controls, which did not have any characteristics associated with steatosis [23]. Administration with HFD for 12 weeks resulted in steatosis with a 6.5-fold increase in steatosis score compared to the CD group, which showed normal liver morphology [24].

Another study established a rat model of nonalcoholic fatty liver disease in Sprague–Dawley rats by giving a high fat diet for 10 weeks, and this study also stated that Animal models of NAFLD can be divided into two types: Those caused by genetic mutations and those induced by dietary or pharmacological modifications [23]. Another study suggested that the degree of steatosis was exacerbated by the induction of dietary HFD in combination with ethanol, indicating the involvement of ethanol in the development of steatosis. This study by Souza also showed that oxidative stress could also worsen the state of steatosis [25].

In the study, we conducted on mice induced with a high-fat diet and a modified high-fat diet, in addition to the occurrence of severe grade steatosis, it also resulted in necrosis of hepatocytes and also central vein congestion, in contrast to mice induced with a standard diet, which still showed some hepatocyte cells were still normal, this is by the study that SD rats with regular diet showed that liver tissue from mice with normal diet showed standard structure, without degeneration and necrosis of liver cells. However, the liver tissue of animals from a group of mice fed a high-fat diet showed swollen liver cells, scattered cytoplasm, and large fat droplets were visualized, showing accumulation of liver fat and signs of vacuolar degeneration and necrosis [26].

In another study, giving HFD to male rats with a composition of 60% energy from fat (lard), 20% from carbohydrates, and 20% protein for 10 weeks caused steatohepatitis [27]. And feeding high-fat–high-fructose (HFHFR) diet at male Wistar developed mildly overweight, associated with increased adipose tissue weight, hepatic steatosis, hyperglycemia, and hyperinsulinemia after 8 weeks of HFHFR diet [28].

In our study that we conducted on three groups of mice where steatosis occurred in these three groups.
but based on the degree of steatosis based on the non-alcoholic steatohepatitis clinical research network scoring system by Kleiner et al. 2005, the occurrence of steatosis in group 1 on average occurs in Grade 2 or grade moderate which the occurrence of steatosis in liver cells is around 34–66% while in Groups 2 and 3 the occurrence of steatosis in Grade 3 or severe grade occurs steatosis in liver cells > 66%. This showed that a high-fat diet causes a greater degree of steatosis in liver cells than a diet containing less fat (Figure 3).

**Conclusion**

A consumed high-fat diet does not fully increase body weight but results in a higher percentage of hepatocyte cells experiencing steatosis than a standard diet.

**References**

1. Nobili V, Alisi A, Newton KP, Schwimmer JB. Comparison of the phenotype and approach to pediatric vs adult patients with nonalcoholic fatty liver disease. Gastroenterology. 2016;150(8):1798-810. https://doi.org/10.1053/j.gastro.2016.03.009 PMid:27003600

2. Aydos LR, do Amaral LA, de Souza RS, Jacobowski AC, Dos Santos EF, Rodrigues Macedo ML. Nonalcoholic fatty liver disease induced by high-fat diet in C57Bl/6 models. Nutrients. 2019;11:3067. https://doi.org/10.3390/nu11123067 PMid:31888190

3. Pompili S, Vetuschis A, Gaudio E, Tessitore A, Capelli R, Alesse E, et al. Long-term abuse of a high-carbohydrate diet is as harmful as a high-fat diet for development and progression of liver injury in a mouse model of NAFLD/NASH. J Physiol. 2020;715:110782. https://doi.org/10.1101/j.nut.2020.110782 PMid:32268264

4. Ekstedt M, Franzen LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodnar G, et al. Long-term follow-up of patients with NAFLD and elevated liver enzymes. Hepatology. 2006;44(4):865-73. PMid:17006923

5. El-Kader SM, El-Den Ashmawy EM. Non-alcoholic fatty liver disease: The diagnosis and management. World J Hepatol. 2015;7(6):846-58. https://doi.org/10.4254/wjh.v7.i6.846 PMid:25937862

6. Al Rifai M, Silverman MG, Nasir K, Budoff MJ, Blankstein R, Szlko M, et al. The association of nonalcoholic fatty liver disease, obesity, and metabolic syndrome, with systemic inflammation and subclinical atherosclerosis: The multi-ethnic study of atherosclerosis (MESA). Atherosclerosis. 2015;239(2):629-33. https://doi.org/10.1016/j.atherosclerosis.2015.02.011 PMid:25683387

7. Mirrman P, Amirhamidi Z, Ejtaheh HS, Bahadoran Z, Azizi F. Relationship between diet and non-alcoholic fatty liver disease: A review article. Iran J Public Health. 2017;46(8):1007. PMid:28894701

8. Umemoto T, Subramanian S, Ding Y, Goodspeed L, Wang S, Han CY, et al. Inhibition of intestinal cholesterol absorption decreases atherosclerosis but not adipose tissue inflammation. J Lipid Res. 2012;53(11):2380-9. https://doi.org/10.1194/jlr.m029264 PMid:22956784

9. Ma Z, Chu L, Liu H, Wang W, Li J, Yao W, et al. Beneficial effects of paeonol on non-alcoholic fatty liver disease induced by high-fat diet in rats. Sci Rep. 2017;7(1):1-10. https://doi.org/10.1038/srep44819 PMid:28300221

10. Brunt EM, Wong VW, Nobili V, Da CP, Sooskan S, Maher JJ, et al. Nonalcoholic fatty liver disease. Nat Rev Dis Primers. 2015;1:15080. https://doi.org/10.1038/nrdp.2015.80

11. Polyzos SA, Kountouras J, Zavos C, Tsiacou S. The role of adiponectin in the pathogenesis and treatment of non-alcoholic fatty liver disease. Diabetes Obes Metab. 2010;12(5):365-83. https://doi.org/10.1111/j.1463-1326.2009.01176.x PMid:20415685

12. Baffy G. Kupffer cells in non-alcoholic fatty liver disease: The emerging view. J Hepatol. 2009;51(1):212-23. https://doi.org/10.1016/j.jhep.2009.03.008 PMid:19447517

13. Diehl AM, Day C. Cause, pathogenesis, and treatment of nonalcoholic steatohepatitis. N Engl J Med. 2017;377(21):2063-72. https://doi.org/10.1056/nejmra1503519 PMid:29166236

14. Friedman SL, Neuschwander-Tetri BA, Rinella M, Sanyal AJ. Mechanisms of NAFLD development and therapeutic strategies. Nat Med. 2018;24(7):908-22. https://doi.org/10.1038/s41591-018-0104-9 PMid:29967350

15. Chen X, Acquah-Mensah GK, Denning KL, Peterson JM, Wang K, Dervir J, Lu Y. High-fat diet induces fibrosis in mice lacking CYP2A5 and PPARG: A new model for steatohepatitis-associated fibrosis. Am J Physiol Gastroint Liver Physiol. 2020;319(5):G626-35. https://doi.org/10.1152/ajpgi.00213.2020 PMid:32877213

16. Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology. 2005;41(6):1313-21. https://doi.org/10.1002/hep.20701 PMid:15915461

17. Santhekadur PK, Kumar DP, Sanyal AJ. Preclinical models of non-alcoholic fatty liver disease. J Hepatol. 2018;68(2):230-7. https://doi.org/10.1016/j.jhep.2017.10.031 PMid:29128391

18. Semiane N, Foufelle F, Ferré P, Hainault I, Ameddah S, Mallek A, et al. High carbohydrate diet induces non-alcoholic steatohepatitis (NASH) in a desert gerbil. Comptes Rendus Biol. 2017;340(1):25-36. https://doi.org/10.1016/j.crvi.2016.09.002

19. Lian CY, Zhai ZZ, Li ZF, Wang L. High fat diet-triggered non-alcoholic fatty liver disease: A review of proposed mechanisms. Chem Biol Interact. 2020;330:109199. https://doi.org/10.1016/j.cbii.2020.109199

20. Picchi MG, Mattos AM, Barbosa MR, Duarte CP, Gandini MD, Bodenar G, et al. A high-fat diet as a model of fatty liver disease in rats. Acta Cir Bras. 2011;26(1):25-30. https://doi.org/10.1590/s0102-8650201100000006 PMid:22030811

21. Xin X, Cai BY, Chen C, Tian HJ, Wang X, Hu YY, et al. High-trans fatty acid and high-sugar diets can cause mice with non-alcoholic steatohepatitis with liver fibrosis and potential pathogenesis. Nutr Metab. 2020;17(1):1-12. https://doi.org/10.1038/s41394-020-00352-y
22. Norris GH, Porter CM, Jiang C, Millar CL, Blesso CN. Dietary sphingomyelin attenuates hepatic steatosis and adipose tissue inflammation in high-fat-diet-induced obese mice. J Nutr Biochem. 2017;40:36-43. https://doi.org/10.1016/j.jnutbio.2016.09.017
PMid:27855315

23. Ji G, Zhao X, Leng L, Liu P, Jiang Z, et al. Comparison of dietary control and atorvastatin on high fat diet induced hepatic steatosis and hyperlipidemia in rats. Lipids Health Dis. 2011;10(1):1-10. https://doi.org/10.1186/1476-511x-10-23
PMid:21269482

24. Echeverría F, Valenzuela R, Bustamante A, Álvarez D, Ortiz M, Soto-Alarcon SA, et al. Attenuation of high-fat diet-induced rat liver oxidative stress and steatosis by combined hydroxytyrosol-(HT)-eicosapentaenoic acid supplementation mainly relies on HT. Oxid Med Cell Longev. 2018;2018:5109503. https://doi.org/10.1155/2018/5109503
PMid:30057681

25. de Souza CE, Stolf AM, Dreifuss AA, dos Reis Livero F, de Oliveira Gomes L, Petiz L, et al. Characterization of an alcoholic hepatic steatosis model induced by ethanol and high-fat diet in rats. Braz Arch Biol Technol. 2015;58:367-78. https://doi.org/10.1590/s1516-8913201500294

26. Cheng H, Xu N, Zhao W, Su J, Liang M, Xie Z, et al. (-)-Epicatechin regulates blood lipids and attenuates hepatic steatosis in rats fed high-fat diet. Mol Nutr Food Res. 2017;61(11):1700303. https://doi.org/10.1002/mnfr.201700303
PMid:28734036

27. Ibrahim SH, Hirsova P, Malhi H, Gores GJ. Animal models of nonalcoholic steatohepatitis: eat, delete, and inflame. Digestive Dis Sci. 2016;61(5):1325-36. https://doi.org/10.1007/s10620-015-3977-1
PMid:26626909

28. Fouret G, Gaillet S, Lecomte J, Bonafos B, Djohan F, Barea B, et al. 20-Week follow-up of hepatic steatosis installation and liver mitochondrial structure and activity and their interrelation in rats fed a high-fat-high-fructose diet. Br J Nutr. 2018;119(4):368-80. https://doi.org/10.1017/s0007114517003713
PMid:29498345