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A novel therapeutic approach to NASH: Both polyethylene glycol 3350 and lactulose reduce hepatic inflammation in C57BL/6J mice

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Abstract

Background. The gut-liver axis is one of the most emphasized topics in the pathogenesis of non-alcoholic fatty liver disease (NAFLD). Intestinal microbiota dysbiosis has been shown to be a predictor of disease severity and progression to fatty liver disease. Therefore, research addressing gut-based therapies has become popular.

Objectives. To investigate the effect of lactulose and polyethylene glycol 3350 (PEG 3350) in mice with induced obesity and NAFLD at a non-diarrheal dose.

Materials and methods. Thirty-six C57BL/6J male mice were divided into 6 groups. The first 2 groups (n = 6 each) were used as an induced obesity model (group A) and NAFLD model (group B) for 8 weeks. The remaining 24 animals were categorized into control diet group, high-fat diet (HFD) group, HFD + lactulose group, and HFD + PEG 3350 group. Serum and liver tissue samples were obtained for biochemical and histopathological analyses, respectively.

Results. The HFD + lactulose treatment group displayed a significant decrease in liver weight (1.3 (1.3–1.4) kg compared to 1.8 (1.6–1.9) kg) and NAFLD activity score (NAS) (1.5 (1.0–3.0) compared to 5.0 (4.0–5.0), respectively; p = 0.0043, p = 0.0021) when compared with the HFD group. However, a decrease in body weight (35.0 (34.6–36.0) kg compared to 40.9 (34.7–41.9) kg) and hepatosteatosis (HS) rate (33.3% compared to 100.0%) were not statistically significant (p = 0.1796, p = 0.0606, respectively). The HFD + PEG 3350 treatment group showed a statistically significant decrease in body weight (32.4 (30.2–33.9) kg compared to 40.9 (34.7–41.9) kg), liver weight (1.5 (1.3–1.5) kg compared to 1.8 (1.6–1.9) kg), HS rate (16.7% compared to 100.0%) and NAS (0.5 (0.0–1.0) compared to 5.0 (4.0–5.0); p = 0.0086, p = 0.0086, p = 0.0151, and p = 0.0021, respectively) when compared with the HFD group.

Conclusions. We demonstrated that non-diarrheal dose of lactulose and PEG 3350 reduced hepatic inflammation in mice with induced NAFLD. It was also observed that PEG 3350 decreased HS and body weight. We believe these mechanisms can be utilized as novel therapeutic approaches in NAFLD in prospective human studies.

Key words: hepatosteatosis, lactulose, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, polyethylene glycol 3350

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Background

Non-alcoholic fatty liver disease (NAFLD) is an ever-increasing major public health problem worldwide, and due to the high rates of hepatitis B immunization and the introduction of the hepatitis C vaccine, likely the most common cause of chronic liver disease. Non-alcoholic steatohepatitis (NASH) is a histologically more aggressive subgroup of NAFLD and is more frequently associated with the development of fibrosis and cirrhosis. In the general population, the prevalence of NAFLD is 25%, and the prevalence of NASH is 3–5%.[1]

The pathogenesis of NAFLD is not yet fully understood, although the "multiple-hit" hypothesis is widely accepted. The 1st hit involves increased lipid flows to hepatocytes, which induces the liver to become more susceptible to oxidative stress and lipid peroxidation with increased lipid uptake, free fatty acid production and de novo lipogenesis. Then, mitochondrial dysfunction and the 2nd hit occurs, resulting in hepatocyte damage, inflammation and fibrosis.[2] Although the majority of NAFLD patients are overweight or obese, there is also a subgroup of patients with normal body mass index (BMI); such situation is defined as lean NAFLD. Therefore, other factors in the pathogenesis besides insulin resistance, dyslipidemia and obesity are thought to be involved.

The gut–liver axis is one of the most emphasized topics in the pathogenesis of NAFLD, with intestinal microbiota dysbiosis being shown as a predictor of disease severity and progression to fatty liver disease.[3] Therefore, research on gut-based therapies has become popular. Lactulose is a drug mainly used in the treatment of constipation, hepatic encephalopathy and salmonellosis, and it is one of the oldest prebiotics known as the 'bifidus factor'. Oral lactulose administration exerts its positive effect on gut microbiota by increasing bifidobacteriaceae and lactobacillaceae.[4] The ability of lactulose to reduce hyperammonemia in hepatic encephalopathy is thought to be mainly due to amelioration of dysbiosis.[5] Polyethylene glycol 3350 (PEG 3350) is a high-molecular-weight, water-soluble polymer and a commonly used drug for bowel lavage due to its low rate of side effects. Post-lavage changes in the microbiota composition have been shown in fecal samples of patients who had colonoscopy preparation with PEG 3350.[6]

Objectives

In our study, we developed NAFLD mice models with a high-fat diet (HFD) involving butter and aimed to investigate the effect of lactulose and PEG 3350 at non-diarrheal doses on lipid profile, obesity and NAFLD (defined as NAFLD activity score (NAS) ≥3). The NAS system reflects fundamental histological features (steatosis, ballooning and inflammation) and is a well-accepted standard used for assessing NAFLD severity and responses to treatment.[8]

Materials and methods

Animals

Thirty-six C57BL/6J male mice were used in the study. Animals were eight-week old, weighing 24–28 g, and were obtained from the Bilkent University Experimental Animals Laboratory (Ankara, Turkey). Animals were held in a twelve-hour dark/light cycle at an average temperature of 22°C, and were randomized into 6 groups (n = 6) prior to housing. Before starting the experiment, the mice were given a standard diet (5.5% of the energy from fat) and water for 2 weeks to stabilize their metabolic status. The animal study was approved by the Animal Ethics Committee of Cumhuriyet University School of Sivas (Sivas, Turkey; approval No. 295/25.06.2019). All the surgical and experimental procedures were in accordance with institutional animal care guidelines.

Experimental design

The study was designed in 2 stages (Fig. 1). The 1st stage involved inducing obesity (group A, n = 6) and NAFLD (group B, n = 6) with a HFD diet (pellets, 60% of the energy from butter). Group A and group B mice received a standard diet and HFD, 3–6 g/day for 8 weeks, respectively. Daily weights, food, and water consumption were observed during the experiment. Finally, the 2 groups were fasted for 24 h prior to euthanasia for histopathological evaluation of the liver and the acquisition of blood samples from the femoral artery. When it was seen that NAFLD was induced in group B mice, the 16-week treatment trial phase was initiated. The remaining 24 animals were categorized into 4 groups: HFD, HFD + lactulose, and HFD + PEG 3350. Lactulose and PEG 3350 were given to mice by dissolving in drinking water at a dose of 0.44 g/day and 0.3 g/kg/day, respectively.[7] The frequency and consistency of defecation were monitored. Diarrhea was identified as an undesirable side effect, although it did not affect any animals. Serum and liver tissue samples were obtained in a similar manner to the mouse model of induced NAFLD at baseline and at 8 weeks.

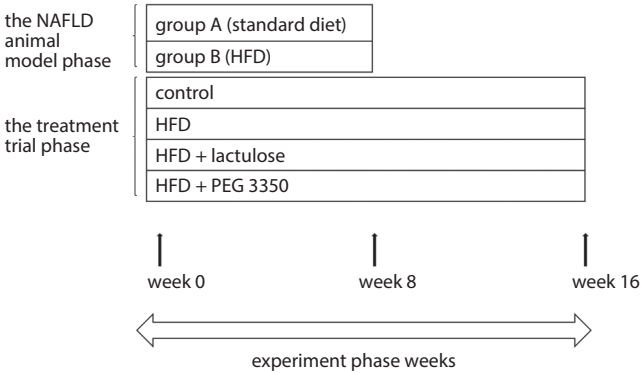

Fig. 1. Experimental study design

NAFLD – non-alcoholic fatty liver disease; HFD – high-fat diet.

Biochemical analysis and histopathological evaluation

Blood samples from euthanized mice were centrifuged for 10 min at 4000 rpm and 4°C, then were analyzed for glucose, total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein-cholesterol (HDL-C) using cobas® 8000 analyzer (Roche, Basel, Switzerland). Liver tissues were dissected, fixed with 10% neutral formalin and sent to the pathology laboratory to be examined. After the automated tissue processing and paraffin blocking steps, 5 µm-thick sections were taken, then stained with hematoxylin and eosin (H&E) in an automated stainer (Leica ST5020; Leica, Buffalo Grove, USA). Each section was assessed under light microscope by 2 independent pathologists for histopathological evaluations. Hepatosteatosis (HS) was graded based on the percentage of fat within the hepatocytes: grade 0 (<5%), grade 1 (5–33%), grade 2 (34–66%), and grade 3 (>66%). The NAFLD activity score (NAS) was calculated using the scoring system developed by Kleiner et al.[8]

Statistical methods

Statistical data were analyzed using IBM SPSS v. 23.0 (IBM Corp., Armonk, USA). Descriptive statistics were given as a median (1st quartile–3rd quartile (Q1–Q3)) for continuous variables. Mann–Whitney U test was used to compare nonparametric variables. In comparison of categorical variables, the χ^2 test was used when conditions were met; otherwise, the Fisher's exact test was used. A p-value of ≤0.05 was considered statistically significant.

Results

The NAFLD animal model at 8 weeks

Body and liver weights of group B mice fed with HFD were significantly higher than group A mice at 8 weeks (p = 0.0021, p = 0.0043). While HS was not found in group A, 5 mice had HS in group B, and their fatty liver rates were determined as 10% in 3 mice, 15% in 1 mouse and 30% in 1 mouse (p = 0.0151). The NAS was found to be 4.0 (2.0–4.0) in group B mice and 0.0 (0.0–0.0) in group A (p = 0.0021) (Table 1).

Table 1. Comparison of the animal weight, liver weight, hepatosteatosis (HS), and NAS of C57BL/6J mice receiving standard and HFD (butter) in an eight-week period

Variables	Group A (control) (n = 6)	Group B (HFD) (n = 6)	p-value
Animal weight [g], median (Q1–Q3)	24.9 (24.4–25.2)	33.5 (30.9–36.0)	0.0021
Liver weight [g], median (Q1–Q3)	0.9 (0.9–0.9)	1.2 (1.0–1.4)	0.0043
HS, n (%)	0 (0.0)	5 (83.3)	0.0151
NAS, median (Q1–Q3)	0.0 (0.0–0.0)	4.0 (2.0–4.0)	0.0021

HFD – high-fat diet; NAS – non-alcoholic fatty liver disease (NAFLD) activity score; the data are shown as median, (Q1–Q3: 1st–3rd quartile) and p-values were for the Mann–Whitney U test, except HS (χ^2 test, df = 1).

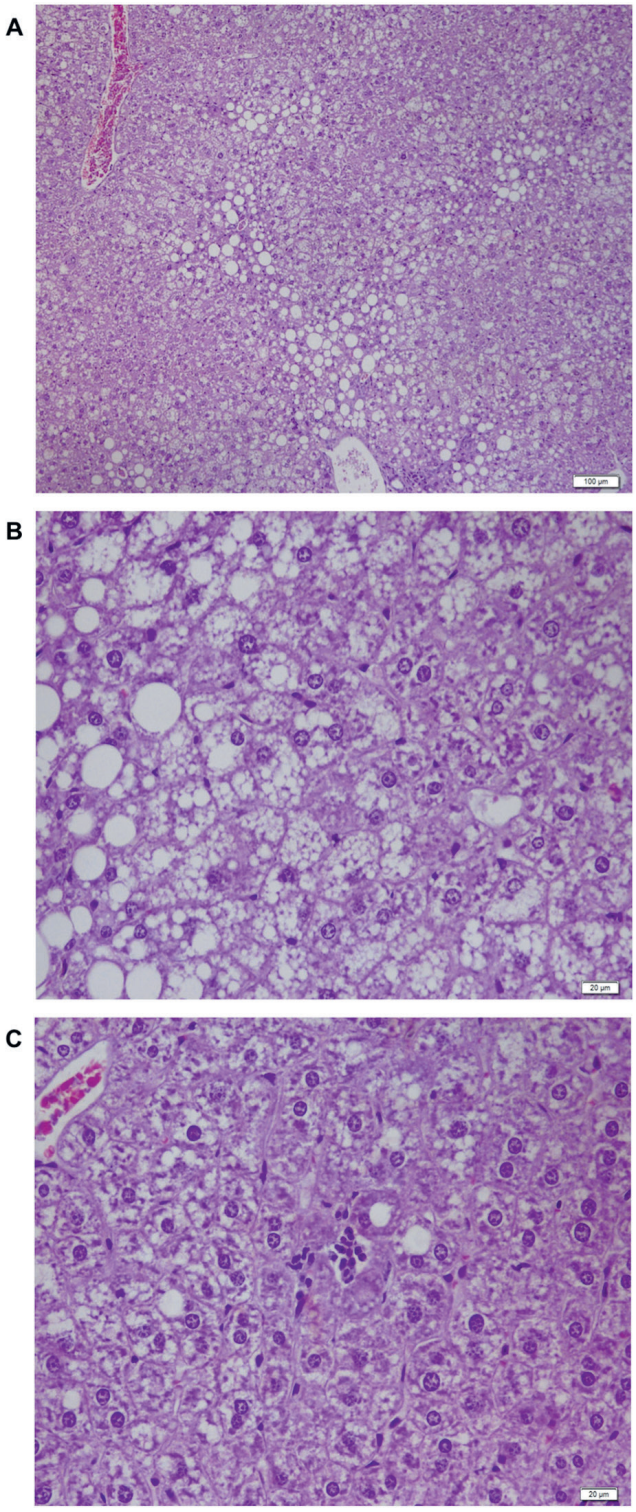

Fig. 2. Microscopic images of liver histology in high-fat diet (HFD) mice. A. Grade 2 microvesicular steatosis, ×4 magnification; B. Hepatocellular ballooning, ×10 magnification; C. Lobular inflammation, ×10 magnification; stained with hematoxylin and eosin (H&E)

Control diet compared to high-fat diet at 16 weeks

Macroscopic appearance of liver tissues was smooth and dark red in control mice. In histopathological examination of the control group, none of the mice had HS and NAS was determined to be 0.0 (0.0–0.0). Conversely, the liver tissues of HFD mice were granular, fragile and yellow-gray. All of them had HS, and fatty rates were determined as 10% in 3 mice, 30% in 1 mouse and 40% in 2 mice. The NAS in the HFD mice was calculated as 5.0 (4.0–5.0) and was significantly higher than in the controls ($p = 0.0021$). The H&E staining images samples of the liver tissue in HFD mice are displayed in Fig. 2. Also, serum lipid levels were significantly higher in HFD, except TG (Table 2).

High-fat diet compared to high-fat diet + lactulose

High-fat diet + lactulose mice had lower level of liver, but not lower body weight when compared with HFD mice ($p = 0.0043$ and $p = 0.1796$, respectively). Two of HFD + lactulose mice had HS (fatty rates were 15% and 30%; $p = 0.0606$). The NAS was 1.5 (1.0–3.0), which was significantly lower than in HFD mice ($p = 0.0021$). There was no difference between the groups in terms of body weight, glucose level and lipid parameters (Table 2).

High-fat diet compared to high-fat diet + PEG 3350

Body and liver weight of HFD + PEG 3350 mice were lower than those of HFD mice (both $p = 0.0086$). In the histopathological evaluation of the liver, only 1 mouse in the HFD + PEG 3350 group had 30% HS, and the NAS was 0.5 (0.0–1.0), which was significantly lower than in the HFD group ($p = 0.0151$, $p = 0.0021$). There was no difference between the groups in terms of glucose and lipid parameters, except TG. Higher TG levels were observed in HFD + PEG 3350 mice ($p = 0.0021$) (Table 2).

High-fat diet + lactulose compared to high-fat diet + PEG 3350

The body weight of the HFD + PEG 3350 mice was lower when compared with HFD + lactulose mice ($p = 0.0259$). However, there was no statistically significant difference between the groups in terms of liver weight, HS and NAS (Table 2).

Comparison of body weight, liver weight and NAS in all groups is shown in Fig. 3, and differences in biochemical parameters are shown in Fig. 4.

Table 2. Comparison of the liver and laboratory analysis of high-fat diet (HFD), HFD + lactulose and HFD + PEG 3350 mice in a sixteen-week period

Variables	Control (n = 6)	HFD (n = 6)	HFD + lactulose (n = 6)	HFD + PEG 3350 (n = 6)	p-value*	p-value**	p-value***	p-value****
Liver analysis								
Animal weight [g], median (Q1–Q3)	26.8 (26.7–27.2)	40.9 (34.7–41.9)	35.0 (34.6–36.0)	32.4 (30.2–33.9)	0.0021	0.1796	0.0086	0.0259
Liver weight [g], median (Q1–Q3)	1.4 (1.3–1.5)	1.8 (1.6–1.9)	1.3 (1.3–1.4)	1.5 (1.3–1.5)	0.0043	0.0043	0.0086	0.4848
HS, n (%)	0 (0.0)	6 (100.0)	2 (33.3)	1 (16.7)	0.0021	0.0606	0.0151	1.0000
NAS, median (Q1–Q3)	0.0 (0.0–0.0)	5.0 (4.0–5.0)	1.5 (1.0–3.0)	0.5 (0.0–1.0)	0.0021	0.0021	0.0021	0.1320
Laboratory analysis								
TC [mg/dL], median (Q1–Q3)	74.5 (71.0–76.0)	141.0 (136.0–157.0)	156.0 (148.0–157.0)	162.0 (159.0–165.0)	0.0021	0.2402	0.0649	0.0151
LDL-C [mg/dL], median (Q1–Q3)	9.6 (9.2–10.2)	34.2 (26.5–43.3)	26.5 (24.7–28.3)	24.7 (24.0–25.40)	0.0021	0.1796	0.0649	0.1796
HDL-C [mg/dL], median (Q1–Q3)	60.4 (57.8–61.3)	129.4 (109.0–145.3)	139.6 (136.0–142.8)	137.2 (136.7–137.6)	0.0021	0.4848	0.3939	0.3939
TG [mg/dL], median (Q1–Q3)	93.0 (86.0–133.0)	107.5 (102.0–127.0)	107.0 (102.0–108.0)	213.5 (212.0–215.0)	0.3939	0.6991	0.0021	0.0021
Glucose [mg/dL], median (Q1–Q3)	147.5 (130.0–148.0)	196.0 (191.0–218.0)	209.0 (204.0–211.0)	165.0 (155.0–170.0)	0.0095	0.2857	0.0571	0.0357

The data are shown as median (Q1–Q3: 1st–3rd quartile) and p-values were for the Mann–Whitney U test, except HS (χ^2 test, df = 1); HDL-C – high-density lipoprotein cholesterol; HFD – high-fat diet; HS – hepatosteatosis; LDL-C – low-density lipoprotein cholesterol; NAS – non-alcoholic fatty liver disease (NAFLD) activity score; PEG 3350 – polyethylene glycol 3350; TC – total cholesterol; TG – triglycerides; *between control and HFD groups; **between HFD and HFD + lactulose groups; ***between HFD and HFD + PEG 3350 groups; ****between HFD + lactulose and HFD + PEG 3350 groups.

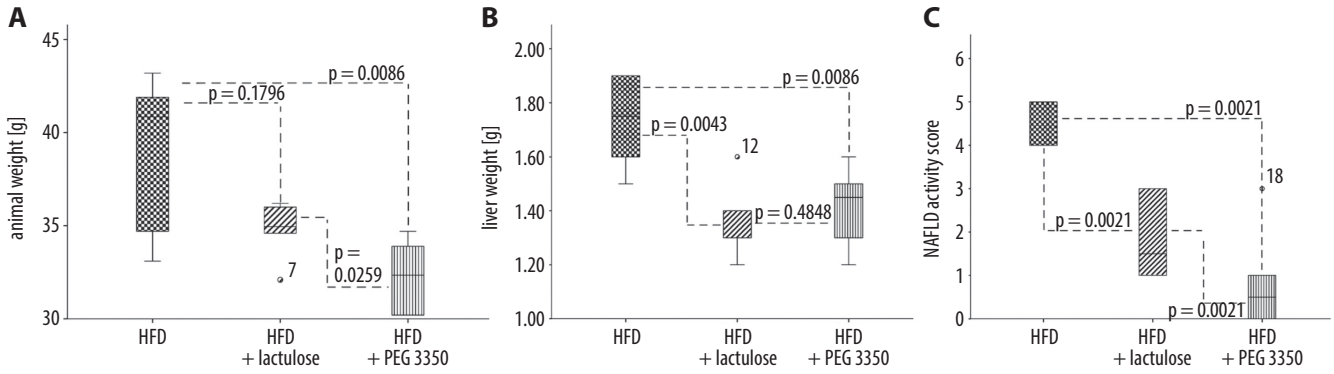

Fig. 3. High-fat diet (HFD) + lactulose and HFD + PEG 3350 mice had lower body weight (A), liver weight (B) and NAS (C) when compared with HFD mice. The data are shown as median, (Q1–Q3) (p-value for the Mann–Whitney U test)

NAFLD – non-alcoholic fatty liver disease; NAS – NAFLD activity score.

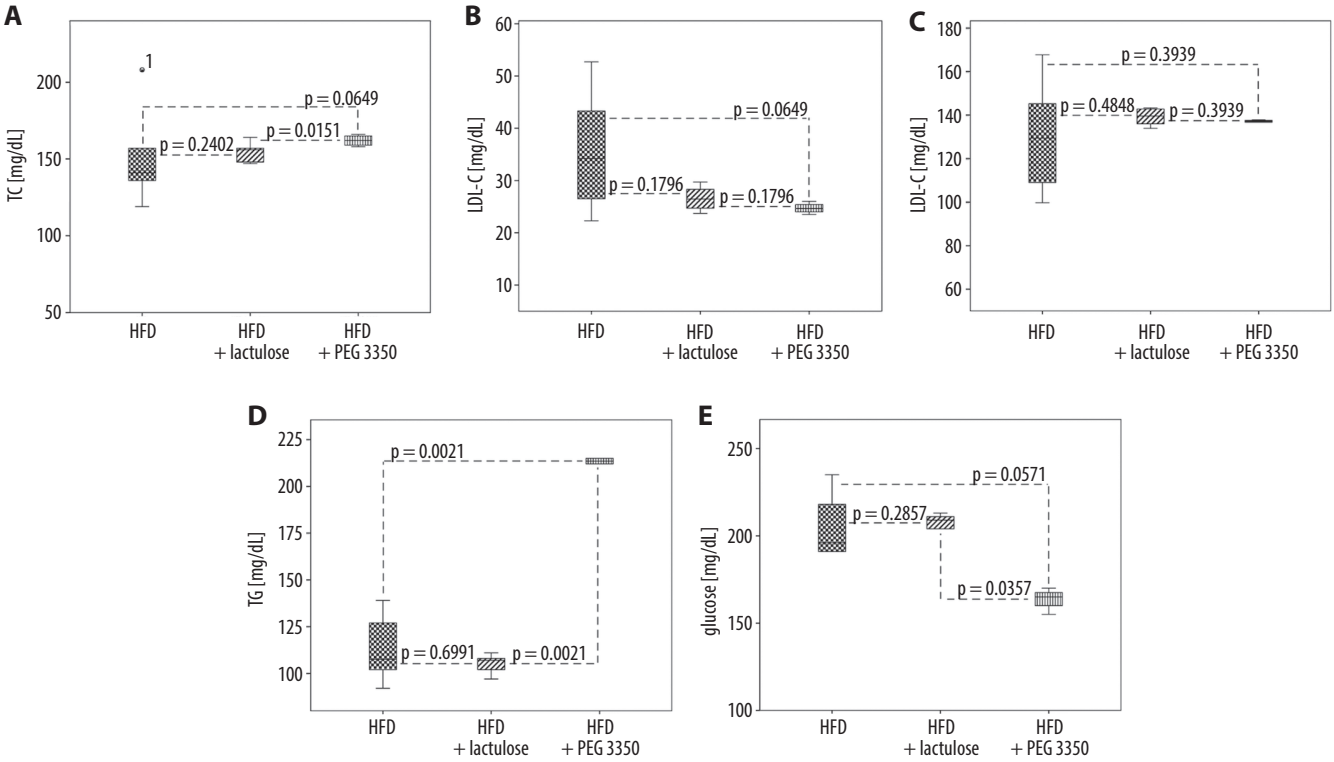

Fig. 4. Comparison of high-fat diet (HFD), HFD + lactulose and HFD + PEG 3350 mice by biochemical parameters including TC (A), LDL-C (B), HDL-C (C), TG (D), and glucose (E). The data are shown as median (Q1–Q3; p-value for the Mann–Whitney U test)

TC – total cholesterol; LDL-C – low-density lipoprotein cholesterol; HDL-C – high-density lipoproten cholesterol; TG – triglycerides.

Discussion

The pathogenesis of NAFLD is still not clearly elucidated and there are no accepted therapeutic options, except for diet and lifestyle changes. Diets lacking methionine and kaolin, and rich in sucrose and fructose or fat are often preferred to induce NAFLD. Butter is one of the well-known hyperlipidemic oils frequently included in our dietary habits. We observed that both obesity and NASH models were induced in all mice fed a HFD, which included 60% of energy provided through butter (both p = 0.0021). This is similar to a previous study in rats fed chow with a similar butter ratio, which also developed HS, although no weight gain was observed.[9] In a study investigating the effect of fatty diets on intestinal flora, fecal DNA samples of mice receiving butter were compared with mice receiving olive oil and a standard diet. It was observed that the proteobacteria family, which is frequently indicated in NASH-intestinal pathogenesis, was more prevalent in the mice receiving butter.[10] In this study, HDL-C/LDL-C ratios

were sorted as olive oil > butter > standard diet. Similarly, we observed increased HDL-C and LDL-C in mice receiving butter. Furthermore, in a human study this concurrent increase was observed in healthy volunteers given a diet containing moderate butter ratio (4.5%).[11] It is thought that HDL-C compensates for LDL-C, so there is no increased cardiovascular risk in this population. Based on this result, it is recommended that butter should be minimized in the diet of hyperlipidemic patients, but moderate butter intake can be allowed in normolipidemic individuals. We observed another interesting finding in the lipid profile of HFD mice, namely, the absence of increased TG levels. Essentially, TG accumulation in the liver is thought to be one of the fundamentals of NAFLD pathogenesis. In a rat model of NAFLD, the highest TG level was seen in the 1st week and then decreased, and this was explained by the decreased food intake in the following weeks.[12] In the present study, while the amount of food intake consumed throughout the experiment was constant, there was no correlation between TG level and NAFLD.

The liver is directly affected by changes in the intestine due to portal vein flow. Stool analysis of patients with NAFLD showed that their intestinal flora density was different from healthy individuals. For example, in the presence of NASH, bacteroidete (bacteroidaceae, prevotellaceae, rikenellaceae) density was increased, while the density of actinobacteria (bifidobacteriaceae) decreased.[13] In the same study, it was shown that *Escherichia*, which was responsible for the high endogenous serum alcohol production, increased liver damage in patients with NASH. Hence, bacteroidetes and *Ruminococcus*, which are known to produce alcohol, are considered an independent risk factor for NAFLD and fibrosis, respectively.[3] When fecal DNA analysis was performed in a patient with NAFLD, it was shown that the density of fusobacteriaceae and prevotellaceae, which are known as short chain fatty acid (SCFA) producing bacteria, increased, and the fecal SCFA concentrations were high.[14] Consequently, dysbiosis, increased SCFA production and altered permeability of the intestinal barrier are thought to lead to NAFLD and even fibrosis at later stages.

In our study, we observed that a non-diarrheal dose of lactulose improved hepatic inflammation without significant weight loss. This result suggests that lactulose may play a role in the treatment of lean NAFLD. It has been shown that *Lactobacillus plantarum* spp. NA136 and *Lactobacillus johnsonii* spp. BS15, known as probiotics, inhibited hepatic steatosis and regressed hepatic inflammation in mice.[15,16] Additionally, lactulose reduced the high concentration of SCFA seen in the stools of NAFLD patients, and caused the regression of steatosis.[4] In another study performed in rats with induced NASH, lactulose decreased both portal vein serum endotoxin levels and hepatic inflammation, but had no effect on HS.[17] Unlike in that study, we started lactulose treatment concurrent with HFD, and the duration of treatment was longer (8 weeks compared to 16 weeks). However, we observed a similar result being decreased NAS but no effect on HS.

Lactulose may contain up to 30% carbohydrates in the form of water-soluble liquid syrups, such as those used in this study. However, we observed no increase in glucose levels. In a recent randomized controlled study of healthy volunteers, neither form of lactulose was found to have a significant effect on serum glucose levels, and no hyperglycemic effect was observed in diabetic patients.[18,19] Interestingly, lactulose leads to positive changes in carbohydrate metabolism, creating fiber and acarbose effects and increasing glucose tolerance in patients with diabetes mellitus.[20] In the jejunal loops of rats given lactulose, it was observed that glucose absorption was decreased by 40%, but amino acid absorption was not.[21] It is thought that bacterial endotoxins reduce the production of pancreatic insulin, and therefore, any treatment that reduces the production of endotoxin will have an antidiabetic effect.[22] The decreased hepatic inflammation in our HFD + lactulose group could be due to the beneficial effects of lactulose on insulin resistance, as we observed no differences in terms of serum lipid levels between HFD + lactulose and HFD mice. Similarly, Mao et al. found no changes in biochemical parameters with the same dose of lactulose (15%, ~0.44 g/day) given in our study in C57BL/6J mice for 2 weeks.[7] However, we showed an approx. 23% decrease of LDL-C level with lactulose, although this was not statistically significant. Serum cholesterol levels decreased by 17% in hyperlipidemic patients receiving lactulose treatment for 4 weeks, and this decrease continued for 4 weeks following the cessation of treatment.[23]

Polyethylene glycol 3350, because of its high molecular weight and non-absorbable structure, is frequently used for intestinal perfusion studies and colonoscopy preparations, with high doses of PEG 3350 required for colonic lavage. It has also started to be preferred as a long-term treatment option for functional constipation, softening the stool without inducing diarrhea. The PEG 3350 dose that we used was calculated by adapting the lower dose (17 g/day) used in humans to the weight of a mouse. We observed decreased liver and body weight, 83.3% less HS and lower NAS in the HFD + PEG 3350 mice at non-diarrheal dose. To our knowledge, this is the first study to investigate the effect of PEG 3350 on NAFLD treatment, and we believe these results may depend on its weight-reducing effect. Essentially, the studies investigating the effect of PEG 3350 on intestinal flora were performed at a high dose, similar to that used for colonic lavage. Some of these studies showed no significant difference between prelavage and postlavage bacterial cultures,[24,25] while Drago et al. showed the decreased density of *Lactobacillus* and increased density of proteobacteria, which is known to cause severe diarrheal attacks.[6] Contrary, rikenellaceae had been shown to decrease in pediatric patients with NAFLD and increased

in individuals who had colonic lavage with PEG.[6,26] Hence, more studies are needed using formulas with different doses of PEG 3350 to determine its positive effect on the gut microbiota.

Since PEG 3350 is not considered a long-term treatment, there are few studies investigating the effects of PEG 3350 on lipid profile. In our study, we detected higher serum TG levels in the HFD + PEG 3350 treatment group despite a decrease in HS and NAS. We believe this interesting finding suggests that PEG 3350 reduces lipid uptake by the liver at the receptor level, without affecting serum lipids. For instance, it was observed that a liver-specific inhibitor that inhibited acetyl CoA carboxylase decreased hepatic steatosis, but increased serum TG levels in an insulin-resistant rat model.[27] Likewise, we observed no changes in terms of lipid parameters, except TG. It was shown that intestinal transit time decreased by 20%, but there was no change in TC and TG levels in rats given PEG 4000 at a non-diarrheal dose.[28] We also detected lower serum glucose levels in HFD + PEG 3350 mice, which was close to but not statistically significant ($p = 0.0571$). Similarly, in humans, it was observed that there was no difference between PEG 3350 and placebo in terms of glucose levels in the treatment of functional constipation for 2 weeks.[29] This provides a safe side effect profile in individuals with insulin resistance.

Limitations

This study has some limitations. First, fecal bacterial DNA analysis was not performed to evaluate the effect of drugs on the intestinal flora. Second, sequential biochemical analysis of serum lipid and glucose and aminotransferase tests were not done due to insufficient blood samples.

Conclusions

Currently, there are no approved therapies for NAFLD. Drug development efforts have focused on pathogenic mechanisms including gut–liver axis. We developed an obesity and NAFLD mouse model through the administration of a HFD. Then, we observed decreases in HS by 66.7% and 83.3% in the HFD + lactulose and HFD + PEG 3350 treatment groups, respectively. Significant decreases in NAS were found in HFD + lactulose (1.5 (1.0–3.0)) and HFD + PEG 3350 (0.5 (0.0–1.0)) treatment groups when compared with the HFD group (5.0 (4.0–5.0)). The decrease in body weight was determined as 11% and 16.5%, respectively. Considering that more than 10% weight loss reduces not only steatosis but also fibrosis in NAFLD, it seems likely that lactulose and PEG 3350 may have an effect in the treatment of obesity and NAFLD. We recommend that human studies should be conducted to address this.

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References

1. Younossi Z, Anstee QM, Marietti M, et al. Global burden of NAFLD and NASH: Trends, predictions, risk factors and prevention. *Nat Rev Gastroenterol Hepatol*. 2018;15(1):11–20. doi:10.1038/nrgastro.2017.109
2. Safari Z, Gérard P. The links between the gut microbiome and non-alcoholic fatty liver disease (NAFLD). *Cell Mol Life Sci*. 2019;76(8):1541–1558. doi:10.1007/s00018-019-03011-w
3. Boursier J, Mueller O, Barret M, et al. The severity of non-alcoholic fatty liver disease is associated with gut dysbiosis. *Hepatology*. 2016;63(3):764–775. doi:10.1002/hep.28356
4. Zhai S, Zhu L, Qin S, Li L. Effect of lactulose intervention on gut microbiota and short chain fatty acid composition of C57BL/6J mice. *Microbiologyopen*. 2018;7(6):e00612. doi:10.1002/mbo3.612
5. Wang JY, Bajaj JS, Wang JB, et al. Lactulose improves cognition, quality of life, and gut microbiota in minimal hepatic encephalopathy: A multicenter, randomized controlled trial. *J Dig Dis*. 2019;20(10):547–556. doi:10.1111/1751-2980.12816
6. Drago L, Toscano M, De Grandi R, Casini V, Pace F. Persisting changes of intestinal microbiota after bowel lavage and colonoscopy. *Eur J Gastroenterol Hepatol*. 2016;28(5):532–537. doi:10.1097/MEG.0000000000000581
7. Mao B, Li D, Ai C, Zhao J, Zhang H, Chen W. Lactulose differently modulates the composition of luminal and mucosal microbiota in C57BL/6J mice. *J Agric Food Chem*. 2016,64(31):6240–6247. doi:10.1021/acs.jafc.6b02305
8. Kleiner DE, Brunt EM, Van Natta M, et al. Nonalcoholic steatohepatitis clinical research network: Design and validation of a histological scoring system for non-alcoholic fatty liver disease. *Hepatology*. 2015;41(6):1313–1321. doi:10.1002/hep.20701
9. Nalloor TJP, Kumar N, Narayanan K, Palanimuthu VR. Long-term exposure to a butter-rich diet induces mild-to-moderate steatosis in Chang liver cells and Swiss albino mice models. *J Basic Clin Physiol Pharmacol*. 2017;28(3):257–265. doi:10.1515/jbcpp-2016-0058
10. Prieto I, Hidalgo M, Segarra AB, et al. Influence of a diet enriched with virgin olive oil or butter on mouse gut microbiota and its correlation to physiological and biochemical parameters related to metabolic syndrome. *PLoS One*. 2018;13(1):e0190368. doi:10.1371/journal.pone.0190368
11. Engel S, Tholstrup T. Butter increased total and LDL cholesterol compared with olive oil but resulted in higher HDL cholesterol compared with a habitual diet. *Am J Clin Nutr*. 2015;102(2):309–315. doi:10.3945/ajcn.115.112227
12. Wardani HA, Rahmadi M, Ardianto C, Balan SS, Kamaruddin NS, Khotib J. Development of non-alcoholic fatty liver disease model by high-fat diet in rats. *J Basic Clin Physiol Pharmacol*. 2019;30(6):/j/jbcpp.2019.30.issue-6/jbcpp-2019-0258/jbcpp-2019-0258.xml. doi:10.1515/jbcpp-2019-0258
13. Zhu L, Baker SS, Gill C, et al. Characterization of gut microbiomes in non-alcoholic steatohepatitis (NASH) patients: A connection between endogenous alcohol and NASH. *Hepatology*. 2013;57(2):601–609. doi:10.1002/hep.26093
14. Rau M, Rehman A, Dittrich M, et al. Fecal SCFAs and SCFA-producing bacteria in gut microbiome of human NAFLD as a putative link to systemic T-cell activation and advanced disease. *United European Gastroenterol J*. 2018;6(10):1496–1507. doi:10.1177/2050640618804444
15. Zhao Z, Wang C, Zhang L, et al. *Lactobacillus plantarum* NA136 improves the non-alcoholic fatty liver disease by modulating the AMPK/Nrf2 pathway. *Appl Microbiol Biotechnol*. 2019;103(14):5843–5850. doi:10.1007/s00253-019-09703-4

16. Xin J, Zeng D, Wang H, et al. Preventing non-alcoholic fatty liver disease through *Lactobacillus johnsonii* BS15 by attenuating inflammation and mitochondrial injury and improving gut environment in obese mice. *Appl Microbiol Biotechnol*. 2014;98(15):6817–6829. doi:10.1007/s00253-014-5752-1
17. Fan JG, Xu ZJ, Wang GL. Effect of lactulose on establishment of a rat non-alcoholic steatohepatitis model. *World J Gastroenterol*. 2005; 11(32):5053–5056. doi:10.3748/wjg.v11.i32.5053
18. Steudle J, Schön C, Wargenau M, et al. Blood glucose response after oral intake of lactulose in healthy volunteers: A randomized, controlled, cross-over study. *World J Gastrointest Pharmacol Ther*. 2018; 9(3):22–30. doi:10.4292/wjgpt.v9.i3.22
19. Lieberthal M, Conn HO, Bircher J. *Hepatic Encephalopathy*. Michigan, USA: Medi-Ed Press; 1988.
20. Huchzermeyer H, Schumann C. Lactulose: A multifaceted substance. *Z Gastroenterol*. 1997;35(10):945–955. PMID:9370144
21. Hosaka H, Tokunaga K, Sakumoto I. The influence of lactulose on intestinal absorption. *Gastroenterol Jpn*. 1972;7:316–317. doi:10.1007/BF02779812
22. Cornell RP. Endogenous gut-derived bacterial endotoxin tonically primes pancreatic secretion of insulin in normal rats. *Diabetes*. 1985; 34(12):1253–1259. doi:10.2337/diab.34.12.1253
23. Conte D, Bardella MT, Bernardi M, Pacetti G, Bianchi PA. Hypocholesterinemic effect of lactulose in man. *Panminerva Med*. 1977;19: 1–4. PMID:834472
24. Beck DE, Harford FJ, DiPalma JA. Comparison of cleansing methods in preparation for colonic surgery. *Dis Colon Rectum*. 1985;28(7):491–495. doi:10.1007/BF02554091
25. Morotomi M, Guillem JG, Pocsidio J, et al. Effect of polyethylene glycol-electrolyte lavage solution on intestinal microflora. *Appl Environ Microbiol*. 1989;55(4):1026–1028. doi:10.1128/AEM.55.4.1026-1028.1989
26. Del Chierico F, Nobili V, Vernocchi P, et al. Gut microbiota profiling of pediatric non-alcoholic fatty liver disease and obese patients unveiled by an integrated meta-omics-based approach. *Hepatology*. 2017;65(2):451–464. doi:10.1002/hep.28572
27. Kim CW, Addy C, Kusunoki J, et al. Acetyl CoA carboxylase inhibition reduces hepatic steatosis but elevates plasma triglycerides in mice and humans: A bedside to bench investigation. *Cell Metab*. 2017; 26(2):394–406. doi:10.1016/j.cmet.2017.07.009
28. van der Wulp MY, Cuperus FJ, Stellaard F, et al. Laxative treatment with polyethylene glycol does not affect lipid absorption in rats. *J Pediatr Gastroenterol Nutr*. 2012;55(4):457–462. doi:10.1097/MPG.0b013e3182555ba9
29. Di Palma JA, Smith JR, Cleveland MvB. Overnight efficacy of polyethylene glycol laxative. *Am J Gastroenterol*. 2002;97(7):1776–1779. doi:10.1111/j.1572-0241.2002.05840.x