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Lactic acid-fermented Sake lees protect against nonalcoholic steatohepatitis in mice

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ABSTRACT

Background: Nonalcoholic steatohepatitis (NASH) is a common disease that may lead to hepatocellular carcinoma (HCC) through fatty liver and cirrhosis. Although the prevalence of NASH is increasing worldwide, there is no cure established thus far. Sake lees are a by-product of sake refining, with a known liver-protecting effect. Lactic acid-fermented sake lees (FSL) are a food produced by lactic acid fermentation and dealcoholization of sake lees. This product is commercially available in Japan. Although FSL has been associated with numerous functions, thus far, studies have not investigated its hepatoprotective effect.

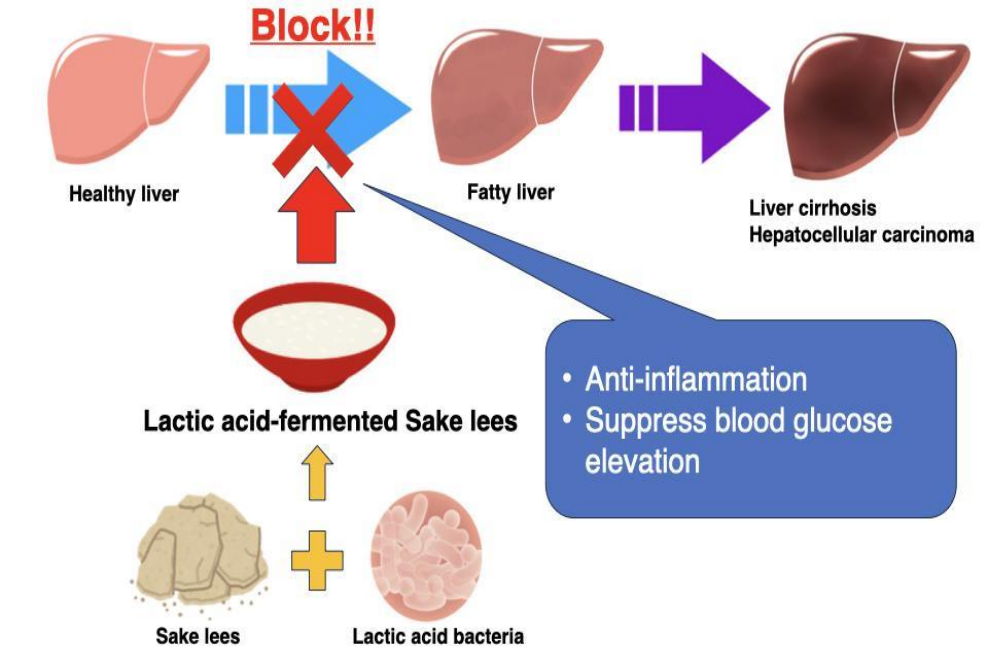
Objectives: The objectives of this study are to evaluate the hepatoprotective effects of lactic acid-fermented sake lees (FSL) in a mouse model of NASH-HCC, to assess the impact of FSL supplementation on blood glucose levels in mice with NASH, to analyze the expression of inflammatory markers in FSL-fed mice compared to controls, and to determine the overall efficacy of FSL in inhibiting the progression of NASH.

Methods: For this study, we established a mouse model of NASH-HCC. Mice were placed on a high-fat diet supplemented with FSL from 10 to 14 weeks of age. We assessed the diet's efficacy in halting NASH progression compared to a control group.

Results: The group fed with FSL exhibited a significant suppression in blood glucose levels and a notable inhibition of NASH progression compared to the control group. Protein analysis revealed a reduction in the expression of inflammatory markers in the FSL-fed group compared to controls.

Conclusion: Ingestion of FSL may exert anti-inflammatory and blood glucose-lowering effects and inhibit NASH progression.

Keywords: anti-inflammation, blood glucose, fatty liver, Sake lees



INTRODUCTION

NASH is a typical disease that causes liver dysfunction, and its increasing prevalence has become a global problem [1-3]. Increased insulin resistance, dyslipidemia, increased oxidative stress, induction of liver cell death, immune abnormalities, and changes in intestinal flora result in the progression of NASH to liver cirrhosis and HCC via fatty liver [4-6]. NAFLD (Nonalcoholic fatty liver disease) is divided into two categories: NAFL, which typically has a good prognosis, and NASH, a more severe form of NAFLD, which can progress to liver cirrhosis and liver cancer [7]. Although various drugs have been used to treat NASH, a well-established treatment based on sufficient evidence is currently lacking. Diet and exercise therapy are recommended to prevent the onset and progression of NASH. Nevertheless, few medical studies have demonstrated the effect of food on the onset and progression of NASH [8].

Sake lees are by-products of the process of sake production. Studies have revealed that sake lees improve lipid metabolism [9] and exert a hepatoprotective effect [10]. However, it is currently unclear whether Sake lees can be consumed on a daily basis to prevent the onset and progression of dyslipidemia and liver disorders. For example, since Sake lees are by-products of sake brewing, they contain alcohol. Hence, it cannot be ingested by children,

pregnant women, individuals who cannot consume alcohol, or vehicle operators. In addition, the taste of sake lees are not appealing; thus, this is not a food that can be enjoyed on a daily basis.

Lactic acid-fermented sake lees (FSL) are produced by dealcoholization and lactic acid fermentation. The production of FSL has improved the safety, taste, and functionality of sake lees. A component analysis conducted in our laboratory showed that the alcohol content in sake lees and FSL was 7.5% and 0.7%, respectively. The alcohol content in FSL is lower than common soy sauce and miso (i.e., 2%), which are often used as seasonings in Japan. FSL has a similar taste to yogurt, which is also a fermentation product of lactic acid bacteria. Therefore, FSL can be easily incorporated into foods that are ingested on a daily basis (e.g., yogurt). In a mouse model of rhinitis allergy, ingestion of FSL decreased the frequency of sneezing [11]. FSL may also exert hepatoprotective effects similar to those of sake lees; however, thus far, there are no reports of such a hepatoprotective effect. FSL is a food that can be consumed daily and might suppress the onset and progression of NASH. This offers a new direction for research on foods that may help control the progression of NASH.

The objective of this study was to determine whether FSL exerts a hepatoprotective effect in a NASH mouse model.

MATERIALS AND METHODS

Materials: The FSL used in this study were provided by Kikusui Sake Co. Ltd. (Niigata, Japan; https://www.kikusui-sake.com/home/jp/). The FSL is commercially available as food. FSL was freeze-dried at the Niigata Prefectural Agricultural Research Institute Food Research Center (Niigata, Japan; https://www.pref.niigata.lg.jp/sec/nosoken_syokuhin/). The diet preparation was performed by Oriental Yeast Co. Ltd. (Tokyo, Japan; https://www.oyc.co.jp/) using freeze-dried FSL.

Animal and experimental designs: Male newborn mice were utilized in this investigation. All procedures involving laboratory animals were conducted in accordance with institutional and national protocols and were approved by the Animal Ethics Review Committee of Niigata University (approval number: SA00876). C57BL/6J mice were bred in-house and housed under controlled conditions with a temperature range of 23 ± 2°C, humidity maintained at 55 ± 15%, and a 12-hour light/dark cycle. They were provided ad libitum access to standard laboratory food and tap water. NASH-HCC was induced in male mice via a single subcutaneous injection of 200 μg of streptozotocin (STZ; Sigma–Aldrich, St. Louis, MO, USA) at 2 days after birth. Starting at 4 weeks of age, mice were fed a high-fat diet (HFD32; CLEA Japan, Tokyo, Japan) ad libitum, which continued until either 10 or 14 weeks of age. The diets' ingredients and components are detailed in Table 1 and Table 2, respectively. The control group (n=6) received the regular diet (CE-2; CLEA Japan), while the NASH group (n=6) received STZ injection and HFD32 diet until 14 weeks of age. The NASH+FSL group (n=6) received STZ injection, HFD32 diet until 10 weeks, and then HFD32 supplemented with 20% FSL from 10 to 14 weeks of age. The concentration of FSL 20% was chosen based on previous studies [9, 12]. At 14 weeks of age, blood samples were collected from the right ventricle of the mice for serum analysis of liver function and anti-inflammatory markers. Liver tissue was also collected for histological, biochemical, and molecular biological analyses [12].

Table 1. Ingredients of HFD32 and HFD + FSL

Ingredient (%/100 g)	HFD32	HFD + FSL
Casein	24.500	19.500
Egg white powder	5.000	5.000
L-cystine	0.430	0.430
Powdered beef tallow	15.880	N/A
Beef tallow	N/A	12.704
Safflower oil	20.000	19.000
Crystalline cellulose	5.500	4.200
Maltodextrin	8.250	4.667
Lactose	6.928	3.919
Sucrose	6.750	3.818
AIN93 vitamin mix	1.400	1.400
AIN93 mineral mix	5.000	5.000
Choline bitartrate	0.360	0.360
Tert-butylhydroquinone	0.002	0.002
FSL	N/A	20.000
Total	100.00	100.000

Abbreviations: FSL, lactic acid-fermented Sake lees; HFD, high-fat diet; N/A, not applicable.

Table 2. Dietary components of the regular diet (CE-2), HFD32, and HFD + FSL

Dietary component (g/100 g)	Regular diet (CE-2)	HFD32	HFD + FSL
Water	9.05	6.2	2.17
Protein	24.80	25.5	26.61
Fat	4.60	32.0	32.84
Ash	7.00	4.0	4.64
Carbohydrate	54.55	32.3	33.70
Fiber	4.65	2.9	5.58
Energy	340.20	507.6	514.70

Abbreviations: FSL, lactic acid-fermented Sake lees; HFD, high-fat diet.

Biochemical analysis: Fasting blood glucose levels were measured using FreeStyle Freedom Lite (Abbott Diabetes Care Inc., Alameda, CA, USA). The levels of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, triglycerides, and total cholesterol were measured by the SanritsuSelkova Inspection Center Ltd. (Tokyo, Japan). All other chemicals that were used were purchased from Sigma (Kanagawa, Japan) unless indicated otherwise.

Histological examination: A histological examination

was conducted at the Histopathology Core Facility, Niigata University Faculty of Medicine (Niigata, Japan). Sections of the right lobe of each liver were obtained from mice across different groups and promptly fixed in 10% formaldehyde solution. Subsequently, they were embedded in paraffin, sliced into sections (4 µm thickness), and affixed onto glass slides. Following deparaffinization, the sections were stained with hematoxylin and eosin. Morphological assessment was performed using a computerized image analysis system, examining 10 microscopic fields per section at 20× magnification (BX-53; Olympus, Tokyo, Japan). Importantly, the observer conducting the analysis was blinded to the study groups. NAFLD activity scores (NAS) were determined as previously described based on the extent of fat content (0–3), parenchymal inflammation (0–2), and prevalence of balloon-like hepatocytes (0–2). A NAS ≥ 5 indicated the presence of definitive NASH [12].

Analysis of Liver Collagen Content: Paraffin-embedded and formalin-fixed liver sections (4 µm thickness) were subjected to deparaffinization followed by staining with Masson's trichrome (MT) stain [12]. The MT staining procedure, conducted at the Histopathology Core Facility, Niigata University Faculty of Medicine, adhered to the manufacturer's instructions (Accustain HT15; Sigma–Aldrich).

Analysis by Western Blotting: For Western blotting analysis, liver tissues were frozen, weighed, and homogenized in ice-cold buffer containing 50 mM Tris-HCl (pH 7.4), 200 mM NaCl, 20 mM NaF, 1 mM Na3VO4, 1 mM 2-mercaptoethanol, 0.01 mg/mL leupeptin, and 0.01 mg/mL aprotinin. Following homogenization, the samples were centrifuged at 3,000 × g for 10 minutes at 4°C, and the resulting supernatants were collected and stored at -80°C. Total protein concentration in the samples was determined using the bicinchoninic acid method.

Subsequently, protein samples underwent SDS polyacrylamide gel electrophoresis and were transferred to nitrocellulose membranes. The membranes were blocked with 5% bovine serum albumin in tris-buffered saline with 0.1% tween (TBST) and then incubated with antibodies against interferon gamma inducible protein-10 (IP-10), IL-1β, high-mobility group protein 1 (HMG-1), phosphorylated-NF-κB (p-NF-κB), and toll-like receptor-4 (TLR-4). Antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA) or Cell Signaling Technology, Inc. (Danvers, MA, USA) and were used at a dilution of 1:1,000.

After three washes with TBST, the membranes were incubated with appropriate horseradish-peroxidase-conjugated secondary antibodies for 1 hour at room temperature. Following another three washes with TBST, protein bands were visualized using a chemiluminescence detection system (Amersham Biosciences, Buckinghamshire, UK). Membranes were scanned, and densitometric analysis of the signals was performed using Image Studio Digits version 4 (Superior Street, Lincoln, NE, USA). Levels of glyceraldehyde 3 phosphate dehydrogenase (GAPDH) were measured to ensure equal loading of samples**.**

Statistical Analysis: The mean ± SEM represents the data. Analysis was conducted using ANOVA, followed by Tukey's method or the Kruskal–Wallis test, and Dunn's multiple comparison test, as deemed suitable. Statistically significant differences were denoted by p-values < 0.05. For statistical analysis, SPSS software version 19.0 (IBM Corp., Armonk, NY, USA) was employed.

RESULTS

Effect of NASH on Clinicopathological and Biochemical Parameters in NASH-HCC Mice: Dietary consumption

and energy expenditure displayed an incline in the NASH group versus the control cohort, albeit lacking statistical significance. Conversely, dietary consumption and energy expenditure showcased a decline in the NASH + FSL group compared to the NASH group, with no statistical significance observed. Remarkably, both body mass and the liver mass-to-body mass ratio experienced a notable reduction in both the NASH and NASH + FSL cohorts relative to the control group. Fasting blood sugar levels demonstrated a significant elevation in both the NASH and NASH + FSL groups in contrast to the control cohort. Nevertheless, a significant reduction in fasting blood sugar levels was evident in the NASH + FSL group compared to the NASH cohort. Moreover, serum levels of triglycerides and overall cholesterol exhibited a noteworthy increase in both the NASH and NASH + FSL cohorts compared with the control group. Additionally, serum alanine aminotransferase (ALT) levels demonstrated a substantial elevation in the NASH group relative to the control cohort. Although serum aspartate aminotransferase (AST) levels were higher in the NASH group compared to the control cohort, this disparity did not attain statistical significance. Notably, both ALT and AST serum levels experienced a significant reduction in the NASH + FSL group compared to the NASH cohort. Finally, the quantity of epididymal adipose tissue showcased a noticeable increase in the NASH group in comparison to the control cohort (Table 3).

Table 3. Changes in biochemical parameters after four weeks of treatment with FSL in mice with NASH-HCC

Biochemical parameter	Group		
	Control (n=6)	NASH (n=6)	NASH + FSL (n=6)
Food intake/day/mouse (g)	3.65 ± 0.57	5.83 ± 2.11	2.45 ± 0.30
Daily energy consumption per mouse (Kcal)	12.6 ± 2.52	30.5 ± 16.18	12.5 ± 2.00
BW (g)	27.0 ± 1.7	23.2 ± 3.5*	22.8 ± 1.9*
Percent of LW/BW	4.6 ± 0.1	8.7 ± 1.5*	7.4 ± 1.1#
Blood glucose (mg/dL)	154 ± 13.0	475 ± 38.8*	441.3 ± 38.8*#
Serum TG (mg/dL)	23.3 ± 8.1	66.2 ± 30.3*	98.2 ± 62.7*
Serum TC (mg/dL)	80.0 ± 6.4	131.5 ± 18.4*	134.8 ± 11.3*
Serum ALT (IU/L)	20.2 ± 9.8	69.2 ± 44.7*	27.3 ± 19.0#
Serum AST (IU/L)	106.0 ± 42.6	198.1 ± 93.7	107.8 ± 41.2#
Serum ALP (IU/L)	359.3 ± 62.0	549.7 ± 393.8	279.8 ± 111.2
Epididymis fat (g)	0.4 ± 0.1	0.5 ± 0.3*	0.2 ± 0.2

Values are expressed as means ± SEM. *$p < 0.05$ vs. normal group; #$p < 0.05$ vs. NASH group. Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BW, body weight; FSL, lactic acid-fermented Sake lees; LW, liver weight; TC, total cholesterol; TG, triglycerides.

Impact of FSL on Hepatic Fibrosis in NASH-HCC Mice: Macroscopically, mice treated with STZ-HFD exhibited liver swelling, accompanied by hepatic lipid accumulation and tumor protrusion within the NASH group. Conversely, administration of FSL resulted in improved liver architecture with reduced tumor protrusion and a moderate degree of lipid accumulation (Figure 1A). Histological examination using hematoxylin and eosin staining revealed severe steatosis, substantial hepatocyte enlargement, and widespread infiltration of inflammatory cells in the NASH group. In contrast, these histological changes were less pronounced in the NASH + FSL group. Notably, the NAFLD Activity Score (NAS) was significantly elevated in the NASH group compared to the control group, while a marked decrease in NAS was observed in the NASH + FSL group relative to the NASH cohort (Figures 1B and 1C). Additionally, Masson's trichrome staining depicted pericellular fibrosis surrounding central veins in the NASH group, whereas fibrotic deposition was markedly reduced in the NASH + FSL group (Figure 1D).

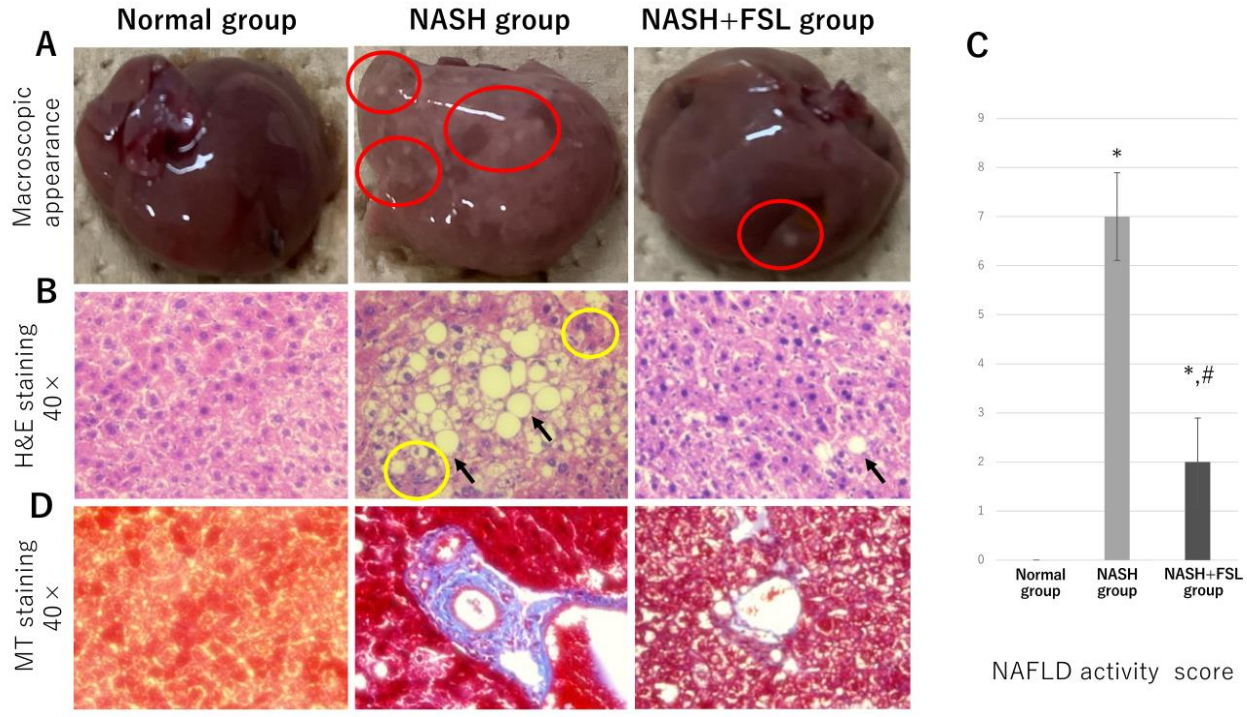


Figure 1. Effect of FSL on Clinicopathology in NASH-HCC Mice: (A) Macroscopic examination showcasing liver appearance (highlighted in red circles: liver tumors). (B) Hematoxylin and eosin (H&E) staining (indicated by black arrows: lipid droplets, yellow circles: inflammatory cells). (C) Representation of NAFLD activity score via histogram. (D) Fibrotic deposition assessed through Masson's trichrome (MT) staining (depicted in blue). Data are expressed as mean ± SEM. Statistical analysis was performed using one-way ANOVA followed by Tukey's method. *p < 0.05 compared to the normal group; #p < 0.05 compared to the NASH group. Abbreviations: FSL, fermented Sake lees enriched with lactic acid; H&E, hematoxylin and eosin; MT, Masson's trichrome.

Effect of FSL on hepatic inflammatory markers in mice with NASH-HCC: We assessed the hepatic concentrations of IP-10, IL-1β, HMG-1, p-NF-κB, and TLR-4 through western blot analysis. The levels of these proteins exhibited a notable elevation in the NASH group. Conversely, these concentrations demonstrated a significant reduction in the NASH + FSL cohort, aligning with mice afflicted with NASH (Figure 2).

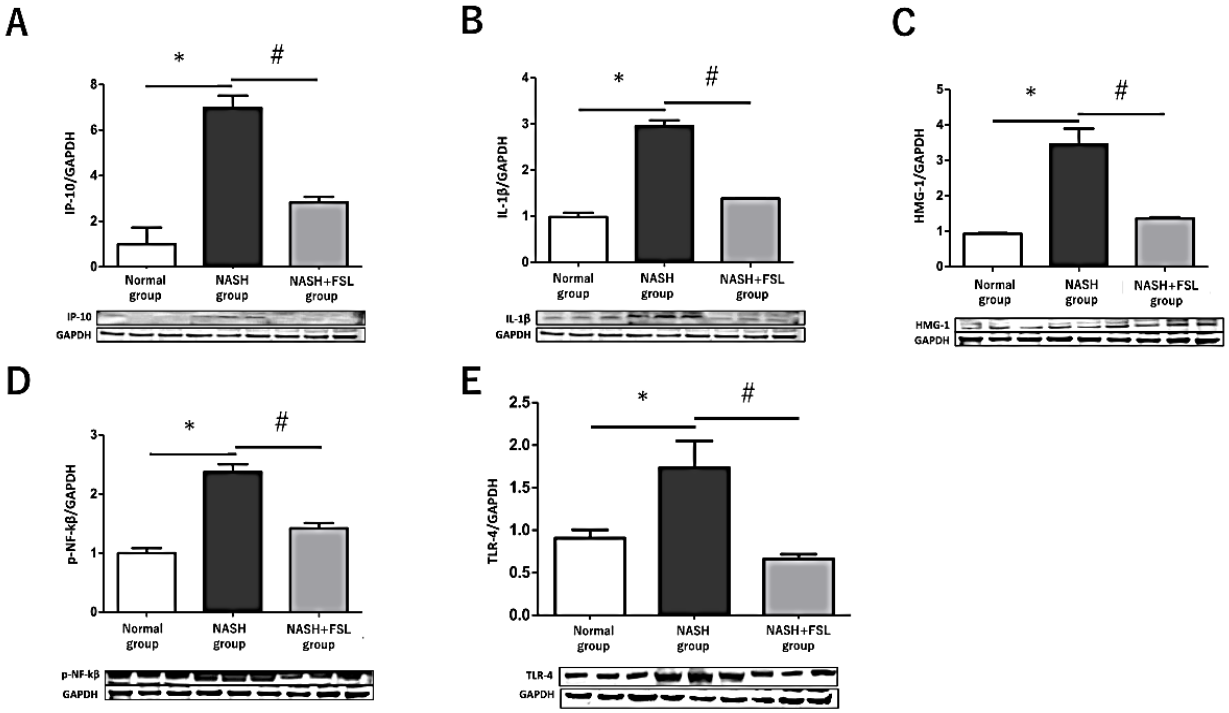


Figure 2. Impact of FSL on Inflammatory Markers in NASH-HCC Mice: Western blot analysis revealed distinct bands corresponding to hepatic IP-10 (A), IL-1β (B), HMG-1 (C), p-NF-κB (D), and TLR-4 (E). Representative histograms illustrate band densities normalized to that of GAPDH. Each bar represents the mean ± SEM. Statistical evaluation was conducted utilizing one-way ANOVA followed by Tukey's method. *p < 0.05 compared to the control group; #p < 0.05 compared to the NASH group. Abbreviations: FSL, fermented Sake lees enriched with lactic acid; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HMG-1, high-mobility group 1; IP-10, interferon-γ inducible protein 10; p-NF-κB, phosphorylated-nuclear factor-kappa B; TLR-4, toll-like receptor 4.

DISCUSSION

this study, the NASH + FSL group tended to have lower food and energy intake than the NASH group. It has been reported that dietary fiber delays the absorption of glucose in the intestine and alters the intestinal flora to suppress the rise in blood glucose levels [13-14]. Sorghum, which is a cereal grain and contains rich dietary fiber, suppress postprandial blood glucose [15]. In our laboratory, glucose levels and incremental areas under curve after intake were significantly lower with dietary fiber-enriched brown rice crackers than with white rice crackers [16]. In recent years, it has also been suggested that dietary fiber exerts appetite-suppressing and anti-obesity effects [17]. Sake lees are rich in dietary fiber. Notably, the HFD + FSL used in this study also have a higher dietary fiber content than HFD32. In addition, sake lees contain a type of starch that remains undigested in the body (i.e., resistant

In

starch), which has the same function as dietary fiber [18]. In FSL, it is speculated that dietary fiber and resistant starch contribute to the suppressive effect on blood glucose elevation and appetite. As already stated in this article, FSL is produced by fermentation of lees with lactic acid bacteria, which suppress blood glucose elevation, improve intestinal flora, and reduce inflammation. Hence, the observed effects may be attributed to lactic acid bacteria [19]. In this study, we were unable to analyze the intestinal flora. IP-10 is a marker associated with the progression of NASH [20]. IL-1β is a marker involved in any stage of NASH, such as the promotion of fibrosis and inflammation [20-21]. It has been reported that HMG-1 causes inflammatory diseases through chronic action [22]. Studies have also shown that NF-κB contributes to NASH activity [23]. Furthermore, TLR-4 contributes to liver inflammation,

fibrosis, and carcinogenesis [24]. Sake lees contain antioxidants and, thus, may also have anti-inflammatory properties [25]. In our investigation, the concentrations of inflammatory indicators demonstrated a noteworthy elevation in the NASH group in contrast to the normal cohort. Nevertheless, this elevation was mitigated in the NASH + FSL group. It is plausible that in the NASH + FSL group, the advancement of NASH was hindered through the attenuation of blood glucose escalation. As a result, the increase in the levels of inflammatory markers was also suppressed. Nonetheless, it is also possible that the progression of NASH was suppressed by the anti-inflammatory effect of FSL.

The small intestine releases incretin hormones, namely gastric inhibitory peptide (GIP) and glucagon-like peptide-1 (GLP-1). Recent findings have demonstrated the significant roles played by these hormones in the modulation of appetite and blood glucose levels [26-27]. Notably, these incretins are strongly influenced by diet. In recent reports, Unbaria pinnatifida sporophylls (Mekabu) suppressed blood glucose level elevation and promoted GLP-1 secretion [28]. In animal experiments, the use of a GLP-1 preparation enhances the secretion of incretins suppressed liver fibrosis [29]. While this study did not assess the levels of incretins, it is conceivable that the consumption of FSL could have stimulated the secretion of these hormones. This effect may have led to the reduction of appetite, inhibition of blood glucose elevation, and suppression of NASH progression (Figure 3).

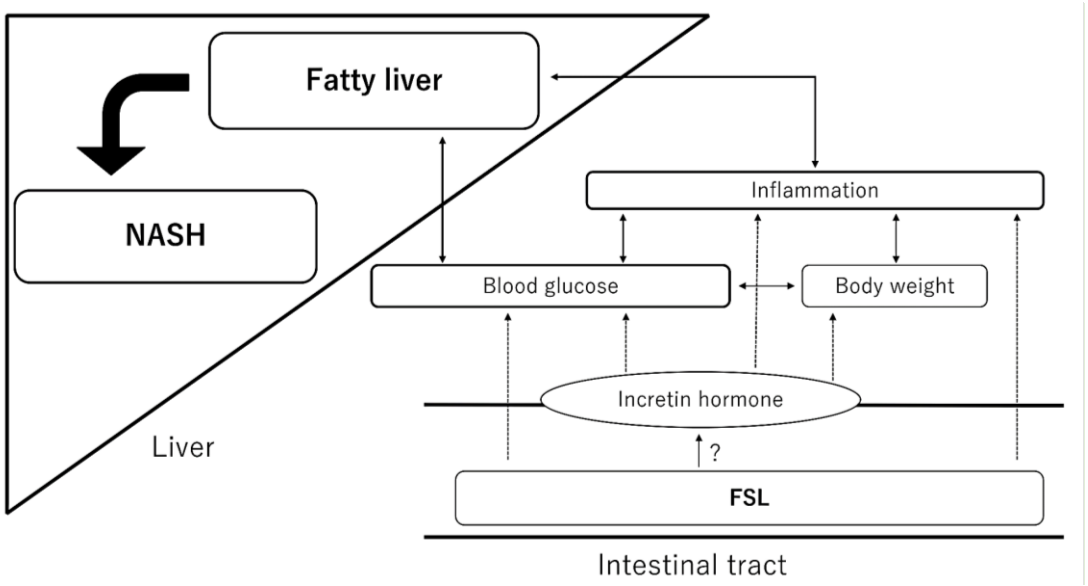


Figure 3. Role of blood glucose and inflammation in the progression of NASH. Solid and dotted lines indicate enhancement and suppression, respectively. Elevated blood glucose levels exacerbate fatty liver, cause inflammation, and lead to weight gain. Inflammation also raises blood glucose levels, leads to weight gain, and exacerbates fatty liver. Weight gain increases blood glucose levels and inflammation. Incretin decreases blood glucose levels, results in weight loss, and reduces inflammation. Fatty liver raises blood glucose levels and causes inflammation; these effects exacerbate fatty liver and result in progression to NASH. FSL may prevent the exacerbation of fatty liver by suppressing the rise in blood glucose levels and inflammation. The relationship between FSL and incretin hormones is unknown. Abbreviations: FSL, lactic acid-fermented Sake lees.

This study is the first to examine the suppressive effect of FSL on the progression of NASH. Compared to the NASH group, the increase in blood glucose levels, expression of inflammatory markers, and progression of NASH were suppressed in the NASH + FSL group. It is considered that the dietary fiber, resistant starch, and lactic acid bacteria contained in FSL contributed to these effects as active ingredients. One limitation to

this study is a lack of intestinal flora analysis and measurement of incretin hormone levels. Consequently, it was not possible to elucidate the mechanism underlying the hepatoprotective effect of FSL. Future research should focus on detecting the active ingredients of FSL, determining the levels of incretin hormones, analyzing the intestinal flora, and elucidating the underlying mechanism. When it comes to dietary consumption, although no significant differences were observed, there seemed to be a tendency towards lower consumption in the NASH + FSL group compared to the control group. Significant variations in food intake among individuals were evident, and FSL might exert an appetite-suppressing influence; nevertheless, the exact cause remains uncertain since activity levels were not assessed. It is necessary to investigate the effects of FSL after adjusting the food intake by pair feeding. The sample size criteria for the study might be unclear, as having six animals in each group (control, HFD, HFD+FSL) may not be sufficient for achieving statistical significance in the analysis between the groups.

CONCLUSION

The findings of this study indicate that intake of FSL exerts blood glucose-lowering and anti-inflammatory effects, as well as suppresses the progression of NASH. FSL has the potential to be a functional food as defined by Functional Foods Center [30].

Abbreviations: AST, aspartate aminotransferase; ALP, alkaline phosphatase; ALT, alanine aminotransferase; BW, body weight; FSL, lactic acid-fermented Sake lees; GAPDH, glyceraldehyde three phosphate dehydrogenase; GIP, gastric inhibitory peptide; GLP-1, glucagon-like peptide-1; H&E, hematoxylin-and-eosin; HCC, hepatocellular carcinoma, HMG-1, high-mobility group 1; HRP, horseradish-peroxidase; IP-10, interferon-γ inducible protein 10; LW, liver weight; MT, Masson's trichrome; NAS, NAFLD activity scores; NASH, nonalcoholic steatohepatitis; p-NF-κB, phosphorylated-nuclear factor-kappa B; STZ, streptozotocin; TBST, tris-buffered saline with 0.1% tween; TC, total cholesterol; TG, triglyceride; TLR-4, toll-like receptor 4.

Authors' contributions: Hirohito Sone and Kenichi Watanabe: designed and supervised the project; revised the manuscript. Hiroshi Suzuki: performed the experiments, analyzed data, and wrote the manuscript. Somasundaram Arumugam, Rejina Afrin, Masahiko Yamamoto, Yasuhiro Matsubayashi: analyzed data. All authors have read and approved the final version of the article and agree with the order in which the authors are listed.

Competing Interests: No author has any conflict of interest, except that we received the FSL for free from Kikusui Sake Co., Ltd. No authors received research funding from Kikusui Sake Co., Ltd.

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REFERENCES

1. Huang DQ, Terrault NA, Tacke F, Gluud LL, Arrese M, Bugianesi E, Loomba R. Global epidemiology of cirrhosis – aetiology, trends and predictions. Nat Rev Gastroenterol Hepatol 2023;20(6):388–398. DOI: https://doi.org/10.1038/s41575-023-00759-2
2. Younossi ZM, Wong G, Anstee QM, Henry L. The global burden of liver disease. Clin Gastroenterol Hepatol 2023;21(8):1978–1991. DOI: https://doi.org/10.1016/j.cgh.2023.04.015
3. Wong VW, Ekstedt M, Wong GL, Hagström H. Changing epidemiology, global trends and implications for outcomes of NAFLD. J Hepatol 2023;79(3):842–852. DOI: https://doi.org/10.1016/j.jhep.2023.04.036
4. Llovet JM, Willoughby CE, Singal AG, Greten TF, Heikenwälder M, El-Serag HB, Finn RS, et al. Nonalcoholic

steatohepatitis-related hepatocellular carcinoma: pathogenesis and treatment. Nat Rev Gastroenterol Hepatol 2023;20(8):487–503.
DOI: https://doi.org/10.1038/s41575-023-00754-7

5. Kuang J, Wang J, Li Y, Li M, Zhao M, Ge K, Zheng D, et al. Hyodeoxycholic acid alleviates non-alcoholic fatty liver disease through modulating the gut-liver axis. Cell Metab 2023;35(10):1752–1766.
DOI: https://doi.org/10.1016/j.cmet.2023.07.011
6. Hammerich L, Tacke F. Hepatic inflammatory responses in liver fibrosis. Nat Rev Gastroenterol Hepatol 2023;20(10):633–646.
DOI: https://doi.org/10.1038/s41575-023-00807-x
7. Sharma B, John S. Nonalcoholic steatohepatitis (NASH). In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024.
8. Wang LL, Zhang PH, Yan HH. Functional foods and dietary supplements in the management of non-alcoholic fatty liver disease: A systematic review and meta-analysis. Front Nutr 2023; 10:1014010.
DOI: https://doi.org/10.3389/fnut.2023.1014010
9. Ashida Y, Saito Y, Kawato A, Suginami K, Imayasu S. Effects of dietary sake cake on cholesterol metabolism in rats. Nippon Nogeikagaku Kaishi 1997;71(2):137–143.
DOI: https://doi.org/10.1271/nogeikagaku1924.71.137
10. Izu H, Goto K, Iefuji H. Effect of Sake Cakes on D-Galactosamine-induced Liver Injury in Mice. J Brew Soc Japan 2006;101(11):893–899.
DOI: https://doi.org/10.6013/jbrewsocjapan1988.101.893
11. Kawamoto K, Kaneoke M, Ohkouchi K, Amano Y, Takaoka Y, Kume K, Aki T, et al. Sake lees fermented with lactic acid bacteria prevents allergic rhinitis-like symptoms and IgE-mediated basophil degranulation. Biosci Biotechnol Biochem 2011;75(1):140–144.
DOI: https://doi.org/10.1271/bbb.100541
12. Suzuki H, Watanabe K, Arumugam S, Yellurkar ML, Sreedhar R, Afrin R, Sone H. Meal ingestion of ceraceomyces tessulatus strain BDM-X (agaricomycetes) protects against nonalcoholic steatohepatitis in mice. Int Jour of Medicinal Mushrooms 2022;24(1):41–52.
DOI: https://doi.org/10.1615/IntJMedMushrooms.2021041928
13. Niero M, Bartoli G, De Colle P, Scarcella M, Zanetti M. Impact of dietary fiber on inflammation and insulin resistance in older patients: a narrative review. Nutrients 2023;15(10):2365.
DOI: https://doi.org/10.3390/nu15102365
14. Lu K, Yu T, Cao X, Xia H, Wang S, Sun G, Chen L, et al. Effect of viscous soluble dietary fiber on glucose and lipid metabolism in patients with type 2 diabetes mellitus: a systematic review and meta-analysis on randomized clinical trials. Front Nutr 2023; 10:1253312.
DOI: https://doi.org/10.3389/fnut.2023.1253312
15. Miyazaki H, Nagae M, Uchida H, Shimizu K. Effect of sorghum intake on postprandial blood glucose levels: A randomized, double-blind, crossover study. Func Foods in Health Dis 2024;14(1):87–95.
DOI: https://doi.org/10.31989/ffhd.v14i1.1266
16. Suzuki H, Watanabe K, Ikeda I, Takeda Y, Hatta M, Horikawa C, Ferreira ED, et al. Effect of dietary fiber-enriched brown rice crackers on suppressing elevation of blood glucose level. Func Foods in Health Dis 2023;13(11):595–604.
DOI: https://doi.org/10.31989/ffhd.v13i11.1231
17. Triffoni-Melo AT, Castro M, Jordão AA, Leandro-Merhi VA, Dick-DE-Paula I, Diez-Garcia RW. High-fiber diet promotes metabolic, hormonal, and satiety effects in obese women on a short-term caloric restriction. Arq Gastroenterol 2023;60(2):163–171.
DOI: https://doi.org/10.1590/s0004-2803.202302022-96
18. Watanabe T. Ingredients in “Sake Cake” contribute to health and beauty. Journal of the Brewing Society of Japan 2012;107(5):282–291.
DOI: https://doi.org/10.6013/jbrewsocjapan.107.282
19. Guo W, Mao B, Tang X, Zhang Q, Zhao J, Zhang H, Chen W, et al. Improvement of inflammatory bowel disease by lactic acid bacteria-derived metabolites: a review. Crit Rev Food Sci Nutr 2023; 11:1–18.
DOI: https://doi.org/10.1080/10408398.2023.2291188
20. Mounika N, Mungase SB, Verma S, Kaur S, Deka UJ, Ghosh TS, Adela R. Inflammatory protein signatures as predictive disease-specific markers for non-alcoholic steatohepatitis (NASH). Inflammation 2024.
DOI: https://doi.org/10.1007/s10753-024-02035-0
21. Wang X, He Q, Zhou C, Xu Y, Liu D, Fujiwara N, Kubota N, et al. Prolonged hypernutrition impairs TREM2-dependent efferocytosis to license chronic liver inflammation and NASH development. Immunity 2023;56(1):58–77. e11.
DOI: https://doi.org/10.1016/j.immuni.2022.11.013
22. Tamber SS, Bansal P, Sharma S, Singh RB, Sharma R. Biomarkers of liver diseases. Mol Biol Rep 2023;50(9):7815–7823.
DOI: https://doi.org/10.1007/s11033-023-08666-0
23. Stiglund N, Hagström H, Stål P, Cornillet M, Björkström NK. Dysregulated peripheral proteome reveals NASH-specific signatures identifying patient subgroups with distinct liver biology. Front Immunol 2023;14:1186097.
DOI: https://doi.org/10.3389/fimmu.2023.1186097
24. Samy AM, Kandeil MA, Sabry D, Abdel-Ghany AA, Mahmoud MO. Exosomal miR-128, miR-200, miR-298,

and miR-342 as novel diagnostic biomarkers in NAFL/NASH: impact of LPS/TLR-4/FoxO3 pathway. Arch Pharm (Weinheim) 2024;357(4): e2300631.
DOI: https://doi.org/10.1002/ardp.202300631

25. Takenouchi A, Enomoto R, Horikawa Y, Koyama C, Yoshioka M, Iguchi T, Yamashita K, et al. Effects of sake-lees-derived profine supplementation in rat models of acute hepatic injury. Func Food Res 2021;17:1–11.
DOI: https://doi.org/10.32153/ffr.ffr20-0430
26. Hong SH, Choi KM. Gut hormones and appetite regulation. Curr Opin Endocrinol Diabetes Obes 2024;31(3):115–121.
DOI: https://doi.org/10.1097/MED.0000000000000859
27. Angelini G, Russo S, Mingrone G. Incretin hormones, obesity and gut microbiota. Peptides 2024;178:171216.
DOI: https://doi.org/10.1016/j.peptides.2024.171216.
28. Takano S, Yoshizumi K, Kobayashi H, Iwamoto N, Taga M. Suppression of blood glucose level elevation and promotion of GLP-1 secretion by ingestion of Undaria pinnatifida sporophylls (Mekabu): Open-label crossover design. Func Foods in Health Dis 2022;12(2):93–102.
DOI: https://doi.org/10.31989/ffhd.v12i2.891
29. Xue H, Xing HJ, Wang B, Fu C, Zhang YS, Qiao X, Guo C, et al. Cinchonine, a potential oral small-molecule glucagon-like peptide-1 receptor agonist, lowers blood glucose and ameliorates non-alcoholic steatohepatitis. Drug Des Devel Ther 2023; 17:1417–1432.
DOI: https://doi.org/10.2147/DDDT.S404055
30. Martirosyan D, Stratton S. Advancing functional food regulation. Bio Compounds in Health and Dis 2023;6(7):166–171.
DOI: https://doi.org/10.31989/bchd.v6i7.1178