Meal ingestion of *Ceraceomyces tessulatus* (*Agaricomycetes*) protects against NASH in mice

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SHORT TITLE: Effect of Basidiomycetes-X in NASH mice

ABSTRACT: Non-alcoholic steatohepatitis (NASH) is becoming the most common cause of hepatocellular carcinoma (HCC) in developed countries. Oxidative stress plays a major role in the pathogenesis of NASH due to steatosis and, hence, novel therapeutic approaches might include natural antioxidants. *Ceraceomyces tessulatus* (strain BDM-X), a novel edible mushroom, possesses potent antioxidant activity. This study aimed to investigate the hepatoprotective effect of BDM-X in a novel NASH-HCC mouse model. To prepare this animal model, 2-day-old C57BL/6J male pups were exposed to low-dose streptozotocin (STZ), and at 4 weeks of age they were randomly divided into 2 groups: the NASH group (NASH) received a high-fat diet (HFD32) up to 14 weeks of age; the BDM-X group (BDM-X) received HFD32 up to age 10 weeks, followed by HFD32 + 20% BDM-X (percentage W/W in the diet) up to age 14 weeks. Mice not treated with STZ fed a normal diet served as a control group. We found that BDM-X improved serum aminotransferase levels as well as histopathological features such as steatosis, inflammatory foci, and pericellular fibrosis in NASH mice. Hepatic protein expression of SREBP-1 and PPARα was significantly increased in NASH mice. BDM-X treatment normalized the expression of both proteins. Our data suggest the BDM-X may protect the liver against lipogenesis in NASH-HCC mice.

KEY WORDS: Functional food; Lipogenesis; Non-alcoholic Fatty Liver Disease; Medicinal mushrooms and fungi

ABBREVIATIONS: NASH, non-alcoholic steatohepatitis; **HCC**, hepatocellular carcinoma; **NAFLD**, non-alcoholic fatty liver disease; **BDM-X**, Basidiomycetes-X; **STZ**, streptozotocin; **HFD**, high-fat diet; **ALT**, alanine aminotransferase; **AST**, aspartate aminotransferase; **ALP**, alkaline phosphatase; **TG**, triglyceride; **TC**, total cholesterol; **H&E**, hematoxylin and eosin; **MT**, Masson's trichrome; **PPARα**, peroxisome proliferator-activated receptor-α; **SREBP**, sterol regulatory element binding protein isoform, **GAPDH**, glyceraldehyde 3 phosphate dehydrogenase; **SEM**, standard error of the mean; **ANOVA,** one-way analysis of variance

Ⅰ. INTRODUCTION

NASH is the main cause of chronic liver disease worldwide and a major health problem due to its close association with obesity, diabetes, and metabolic syndrome.[1] NASH is characterized by steatosis as well as the presence of inflammation, hepatocellular ballooning, leading to fibrosis and HCC.[2] Multiple case reports of NASH patients support a link between diabetes and HCC, and between NASH and the progression of fibrosis.[3] Since complications associated with NASH, including cirrhosis and HCC, are expected to increase with the growing epidemic of diabetes, our study utilized an model of NASH-HCC under diabetic conditions in mice. Although the pathogenesis of NASH has not been fully elucidated, it has recently been proposed that increased levels of toxic lipids such as free fatty acids and cholesterol lead to hepatic injury and inflammation, which may result from oxidative stress.[4] To date is no established

pharmacological treatment for NASH. Current treatment strategies for NASH aim to improve insulin sensitivity, modify underlying metabolic risk factors, or protect the liver from further insult by reducing oxidative stress or inflammation.[5]

Mushrooms have been reported to contain many bioactive compounds such as ergosterol, pyroglutamic acid, oleanolic acid and β-glucan.[5] It has been reported that several active components of various species of mushrooms have a beneficial impact on human health by modulating the immune system, and by reducing lipid levels in blood and liver and tumor growth.[6-10] Recently, mukitake mushroom (*Panellus serotinus*) was reported to alleviate NAFLD in db/db mice.[8] β-glucans, the major component of mushrooms, are reported to be effective against NASH. In a mouse model of NASH induced by a methionine-choline deficient diet, β-(1,3)-(1,6)-glucan was shown to improve hepatic steatosis and inflammation possibly via modulating endoplasmic reticulum (ER) stress.[11] Antrodan, a purified β-glucan, alleviated high-fat and high-fructose diet-induced fatty liver disease in a mouse model via the AMPK/Sirt1/SREBP-1c/PPARγ pathway.[12] Additionally, the anti-fibrotic activity of paramylon, another β-glucan, was demonstrated in a mouse model of NASH.[13]

Ceraceomyces tessulatus (Cooke) Julich strain (BDM-X, Echigoshirayukidake, Corticiaceae, Agaricomycetes) is a novel edible mushroom cultivated by Mycology-Techno Co., Ltd. (Niigata, Japan). It has been reported that a hot aqueous extract of BDM-X possesses potential antioxidant activity both in vitro and in vivo.[14,15] Antioxidant activity plays an important role in the biological response modifier action of mushrooms.[16,17] Another study has reported that BDM-X with high β-glucan content improved atopic satisfaction related to pruritus among dermatitis patients. Improved skin condition was found in 73% of patients who ingested BDM-X.[18] In comparison with other mushrooms, namely

Agaricus, *Shelf fungus* and *Ganoderma lucidum,* BDM-X has a higher content of ergosterol (121 mg/100 g). An ergosterol-containing extract from *Agaricus bisporus* was shown to lower hepatic triglycerides and modify the mRNA expression of cholesterol-related genes.[19] *Agaricus brasiliensis* KA21 has been reported to prevent diet-induced NASH through its antioxidant, anti-inflammatory, and anti-fibrotic activity in the liver.[20] Similarly, Fontes et al. highlighted the antioxidant and anti-tumorigenic effects of mushroom-enriched diets on mitochondria in liver disease.[21] Since oxidative stress and inflammation are associated with tumor pathogenesis and other inflammatory diseases, in the present study, we focused our attention on the hepatoprotective effect of BDM-X in NASH-HCC mice. PPARα is an enzyme involved in β-oxidation and is an important factor for converting fatty acids to ATP.[22] SREBP-1 regulates PPARα gene expression resulting in increased de novo lipogenesis, which is associated with NASH.[23] We previously reported the that oral ingestion of BDM-X had a hepatoprotective effect,[24] in which elevation of SREBP-1 in NASH model mice was suppressed in by BDM-X treatment.

The purpose of the present study was to determine whether BDM-X mixed in the diet could have a hepatoprotective effect in NASH model mice.

Ⅱ. MATERIALS AND METHODS

A. Animals and experimental designs

All institutional and national guidelines for the care and use of laboratory animals were followed. C57BL/6J mice were bred in our laboratory. This study was approved by the animal ethics review committee of Niigata University (approval number: SA00622). Animals were housed at a temperature of 23 ± 2℃ and humidity of 55 ± 15% with a 12-hour light/dark cycle, and

allowed free access to standard laboratory chow and tap water. NASH-HCC was induced in male mice by a single subcutaneous injection of 200μg STZ (Sigma, MO, USA) at 2 days after birth. The animals were started on HFD32 (CLEA Japan) ad libitum at the age of 4 weeks, and feeding was continued up to 10 or 14 weeks age of mice. The ingredients of the diets are shown in Table 1 (Table 1), and the diet components are shown in Table 2 (Table 2). We used CE-2 as a normal diet, which is a standard rodent diet consisting mainly of vegetable protein (soybean meal) with a proper balance of animal protein. This diet is also used for the production and rearing. The ingredients of this diet include a fat source, a fiber source, a carbohydrate source, and vitamins and minerals, but the ratio of the its ingredients has not been disclosed. Mice were randomly assigned to 3 groups: the normal group (Normal: n=6) consisted of normal mice fed normal diet (CE-2, CLEA Japan, Inc., Japan); the NASH group (NASH: n=7) was injected with STZ and fed HFD32 up to 14 weeks of age; the BDM-X group (BDM-X: n=7) was injected with STZ and fed HFD32 up to 10 weeks, and HFD32 + 20% BDM-X up to 14 weeks. A BDM-X concentration of 20% was used based on previous studies in the same kind of research.[25,26] All mice were sacrificed at the age of 14 weeks and blood samples were collected from the right ventricle. Serum was separated from blood and used for measuring liver function and lipogenesis. Liver tissue was isolated for histological, biochemical and molecular biological analyses.[27]

B. Preparation of BDM-X

BDM-X used in the present study was generously provided by Mycology-Techno Co., Ltd. Niigata, Japan. All other chemicals used were purchased from Sigma, Japan unless indicated otherwise.

C. Biochemical analysis

Fasting blood glucose was measured using FreeStyle Freedom Lite (Abbott Diabetes Care Inc., USA). Serum ALT, AST, ALP, TG and TC were measured by SanritsuSelkova Inspection Center Ltd. (Tokyo, Japan).

D. Histological examination

Slices of the right lobe of the liver from different groups of mice were immediately fixed in 10% formaldehyde solution, embedded in paraffin, cut into several sections (4μm) and mounted on glass slides. The sections were then deparaffinized and stained with H&E. Morphological analysis was done using a computerized image analysis system on 10 microscopic fields per section at 20-times magnification (Olympus, Tokyo, Japan), with the observer blind to the study group.[28] The calculation of NAFLD activity scores were calculated using a method similar to that described previously. Scoring was performed according to the degree of fat content (0-3), the degree of parenchymal inflammation (0-2) and the frequency of appearance of balloon-like hepatocytes (0-2).[28] An NAFLD score ≥5 was regarded as definite NASH.

E. Determination of liver collagen content

Formalin-fixed and paraffin-embedded liver sections (4μm) were deparaffinized and stained with Masson's trichrome (MT) stain.[29] MT staining was performed following the manufacturer's instructions (Accustain HT15, Sigma-Aldrich, St. Louis, MO).

F. Western blot analysis

Frozen liver tissues were weighed and homogenized in an ice-cold buffer (50mM Tris-HCl, pH 7.4, 200mM NaCl, 20mM NaF, 1mM Na_3VO_4, 1mM 2-mercaptoethanol, 0.01 mg/mL leupeptin and 0.01 mg/mL aprotinin). Homogenates were then centrifuged (3000 × g, 10min, 4℃) and the supernatants were collected and stored at -80℃. The total protein concentration in samples was measured by the bicinchoninic acid method.[27] Samples with equal amounts of protein were separated in polyacrylamide gel, transferred and identified on nitrocellulose membrane with specific antibodies followed by HRP-labelled secondary antibodies. PPARα and SREBP-1 antibodies were used as primary antibodies. Levels of GAPDH were measured in every sample to check for equal loading of samples.

G. Measurement of hepatic triglyceride concentration

The hepatic triglyceride concentration was measured using a by Triglyceride Assay Kit (ab65336, Abcam Plc., UK).

H. Statistical analysis

Data are shown as mean ± SEM and were analyzed using ANOVA followed by Tukey's method or Kruskal-Wallis test followed by Dunn's multiple comparison test when appropriate. A p-value of $p<0.05$ was considered statistically significant. SPSS (IBM Ltd., USA) was used for the statistical analysis.

Ⅲ. RESULTS

A. Effect of BDM-X on clinicopathology and biochemical parameters in NASH-HCC mice

Food intake and energy consumption were higher in the NASH group than those in the normal group; however, these differences were not statistically difference. In the BDM-X group, food intake and energy consumption were significantly lower than in the normal group and NASH group. Body weight was significantly lower in the NASH and BDM-X groups than in the normal group, and body weight in the BDM-X group was significantly lower than in the NASH group. The relative liver weight (LW)/BW was significantly higher in the NASH and BDM-X groups. The fasting blood glucose level was significantly elevated in the NASH and BDM-X groups as compared with the normal group. Serum triglycerides were higher in the NASH group than in the normal group, and were lower, although not significantly, in the BDM-X group than in the NASH group. Serum total cholesterol was higher in the NASH and BDM-X groups than in the normal group. Serum ALT and AST levels, indicators of liver damage, were significantly higher in the NASH group versus the normal group. The serum ALT level was lower in the BDM-X group than in both the normal and NASH groups, and the serum AST level was lower in the BDM-X group than in the NASH group. The serum ALP level was significantly higher in the NASH group than in the normal group, and was significantly lower in the BDM-X group versus the NASH group (Table 3).

B. Effect of BDM-X on hepatic fibrosis in NASH-HCC mice

Macroscopically, STZ-HFD treated mice showed swelling with fat in the livers and tumor protrusion in the NASH group while the BDM-X treated group mice showed improved liver architecture without tumor protrusion and moderate fat (Fig. 1A). H&E staining of liver tissue showed extreme steatosis, enlarged hepatocytes, and scattered lobular inflammatory cells in the NASH

group, and these changes were quite severe. In comparison, the changes were less severe in the BDM-X group. In this histopathological study, the NAFLD activity score of the NASH group was significantly increased in comparison with the normal group, and significantly lower in the BDM-X group than in the NASH group (Fig. 1B&C).

Lastly, MT staining demonstrated pericellular fibrosis around central veins in the NASH group, while in the BDM-X group, fibrosis were greatly reduced (Fig. 1D).

C. Effect of BDM-X on hepatic lipogenesis in NASH-HCC mice

The hepatic protein expression of SREBP-1, which is a lipogenic controller, was significantly higher in the NASH group compared to the normal group. In contrast, expression was significantly lower in the BDM-X group versus the NASH group (Fig. 2A). The expression of PPARα protein was significantly increased in the NASH group, while in the BDM-X group, it was significantly lower than in the NASH group (Fig. 2B). Hepatic triglyceride levels were increased in the NASH group compared with the normal group, and were significantly lower in the BDM-X group versus the NASH group (Fig. 3).

Ⅳ. DISCUSSION

The term "NASH" was first coined by Dr. Ludwig in 1980. While there is no specific drug to treat NASH, dietary and lifestyle modifications may help to improve the disease.[30] Several studies have suggested that mushrooms can play an important role in improving human health, and that they possess a broad spectrum of physiological activities including anti-tumor, immune-modulating, cholesterol-lowering, anti-inflammatory and anti-oxidative effects.[6,9,18] Although, in recent

years, *Agaricus blazei* murill, *Phellinus linteus* Tehg and other mushrooms have attracted attention for their anticancer effects, none of them have been decisively shown to be effective, and would be desirable to identify of mushrooms species with high efficacy. In this study, we focused our interests on the hepatoprotective effect of the selected mushroom BDM-X.

BDM-X contains large amounts of polysaccharides (β-D-glucan), which are food factor that have immunomodulatory activity while lacking adverse effects, in addition to high antioxidant and OH radical scavenging activity. The antioxidant activity of BDM-X was shown to be superior to that of *Agaricus blazei* Murill.[14,15] The mechanism by which BDM-X protects against liver damage in NASH-HCC mice with a diabetic background is investigated in the present study. In this model, injection of low-dose STZ early after birth drives both islet injury and regenerative responses, leading to diabetic conditions.[25] Steady dietary intervention then promotes fat deposition in the liver with increased lipogenesis and reduced fatty acid oxidation, leading to hepatocellular injury.[1, 31]

We previously reported the hepatoprotective effect of BDM-X in NASH model mice.[24] In this study, NASH model mice were treated to be administered BDM-X (500mg/kg/day) dissolved in distilled water via oral gavage from 12 to 16 weeks of age. In the present study, BDM-X was shown to have a hepatoprotective effect when ingested by mixing it in the diet of NASH model mice from 10 to 14 weeks of age. For example, mice with a body weight of 20 g received 10 mg BDM-X in our prior study, whereas the dose was 400 mg of BDM-X in this study. In the previous study, dietary intake and body weight trended to increase in mice treated with BDM-X, whereas in this study, those parameters decreased in BDM-X treated mice. In the present study, BDM-X was also found to have a hepatoprotective effect when administration was started

earlier than in the previous study. Taking high doses of BDM-X may also have anti-obesity effects.

In this study, HFD feeding was continued for 3 months in the NASH and NASH + 20%BDM-X groups. We observed that BDM-X prevented the liver steatosis in NASH mice by decreasing the fatty acids and reducing lipogenesis. BDM-X treatment decreased the expression of PPARα in NASH liver, and PPAR α plays an important role in fatty acid metabolism.[32] Similarly, SREBP-1 regulates gene expression resulting in an increased de novo lipogenesis, which is associated with NASH.[33] Interestingly, the hepatic expression of PPARα and SREBP-1 were increased in the NASH group, while in the BDM-X group the expression of both was reduced. In present study, food intake and energy consumption increased more in the NASH group than in the normal group, and in the BDM-X group, the factors showed a significantly greater decrease than in the NASH group. Mice prefer to eat a diet high in fat,[34] and despite preparing the HFD32 and HFD32 + 20% BDM-X diets to contain the same number of calories, the groups showed different levels of food intake. Mixing BDM-X in the diet may have led to appetite suppression and reduced food intake. As a result, mice in the BDM-X group consumed a lower amount of dietary fatty acids, and as a consequence, triglyceride levels in the BDM-X group were lower, which probably contributed to the lack of NASH progression. We presumed that the decrease in SREBP-1 and PPARα in the BDM-X group was due to the decrease in free fatty acids.

In the present study, fasting began at 8 AM and dissection was performed after 3 PM. Thus, the duration of fasting after prior to sacrifice was at least 7 hours. SREBP-1 is an important marker involved in lipid synthesis, and it is known that insulin stimulates the secretion of SREBP-1 and synthesizes

triglycerides from fatty acids.[35] On the other hand, when insulin secretion decreases during fasting, SREBP-1 secretion also decreases significantly, and triglycerides are converted to fatty acids and used as ATP. PPARα is an enzyme involved in β-oxidation and is an important factor for converting fatty acids to ATP. In our experiment, SREBP-1 was elevated to a significantly greater degree in the NASH group versus the normal group. This suggests that triglyceride synthesis from abundant fatty acids proceeded despite fasting in the NASH group. In contrast, SREBP-1 expression was significantly suppressed in the BDM-X group as compared with the NASH group, indicating that fasting suppressed triglyceride synthesis. Similarly, PPARα was significantly increased in the NASH group compared with the normal group. It is presumed that β-oxidation was enhanced during fasting and ATP production from fatty liver was promoted. In the BDM-X group, the increase in PPARα was significantly suppressed as compared with the NASH group. We therefore concluded that PPARα activation did not occur even during fasting due to the small pool of fatty acids. In the study, epididymal fat trended to be decreased more in the BDM-X group than in the NASH group (0.13 ± 0.11 g vs. 0.33 ± 0.20 g), and was significantly lower than in the normal group (0.13 ± 0.11 g vs. 0.42 ± 0.04 g). Other researchers have also reported that BDM-X may have an anti-obesity effect.[36] In one study, the BDM-X group showed a trend for lower dietary intake and significantly reduced body weight compared with the control group. BDM-X may reduce visceral fat accumulation by reducing dietary intake (Fig. 4). It has been reported that dietary intake has a significant effect on hepatic enzymes involved in fatty acid synthesis, and we plan to use postprandial blood to study lipid synthases and to determine the effect of BDM-X by measuring postprandial lipid synthase in a future study. We will also measure hormones related to appetite such as leptin and ghrelin to

study the relationship between BDM-X and appetite. In addition, we aim to proceed with additional research on palatability.

The results of the present study suggest that BDM-X has a protective effect against liver steatosis and obesity in NASH mice. Serum ALT and AST levels were measured, as these are sensitive parameters used in the detection of liver steatosis and clinical diagnosis[1]; they are also linked with liver fat content.[37] Our results showed that serum ALT and AST were significantly reduced in the BDM-X group. Histologically, suppression of fat droplet accumulation was observed along with reduced fibrosis in the BDM-X group as compared with the NASH group.

In summary, our data reveal that BDM-X has a beneficial effect in NASH, which might be related in part to improvement of metabolic abnormalities as a result of enhanced lipid profiles in NASH liver. Although further investigation is required to detect the active ingredient in BDM-X responsible for its hepatoprotective and anti-obesity effects, our results strongly suggest that BDM-X has a high potential for the management of NASH.

AUTHOR STATEMENT

HSu: Conceptualization, Methodology, Formal analysis, Investigation, Writing-Original Draft, Visualization. **KW:** Conceptualization, Writing-Review & Editing, Supervision, Project administration, Funding acquisition. **SA:** Methodology, Formal analysis, Investigation, Writing-Review & Editing. **MLY:** Investigation. **RS:** Investigation. **RA:** Methodology, Investigation. **HSo:** Conceptualization, Writing-Review & Editing, Supervision.

CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest.

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FIGURE LEGENDS

Fig.1: BDM-X attenuates the clinicopathology in NASH HCC mice. (A), Representative macroscopic appearance of livers (circles: liver tumors). (B), H&E staining (black arrow, fat droplets; circles, inflammatory cells). (C), Histogram for the NAFLD activity score. (D), Fibrosis deposition by Masson's trichrome staining (blue area). Data present mean ± SE. Normal (n=6), age-matched normal mice fed a normal diet; NASH (n=7), STZ-injected mice fed HFD up to 14 weeks of age; BDM-X (n=7), STZ-injected mice fed HFD up to 10 weeks of age and fed HFD+20% BDM-X from the age of 10 weeks to 14 weeks. Statistical analysis was carried out using one-way ANOVA followed by Tukey's method; *p<0.05 vs normal, $^{\dagger}p$<0.05 vs NASH.

Fig.2: Effect of BDM-X on hepatic lipogenesis in NASH-HCC mice. Western blots show specific bands for hepatic SREBP-1 (A) and PPARα (B); the representative histograms show the band densities with relative to that GAPDH. Each bar represents mean ± SE. Normal (n=6), age-matched normal mice fed normal diet; NASH (n=7), STZ-injected mice fed HFD up to 14 weeks of age; BDM-X (n=7), STZ-injected mice fed HFD up to 10weeks of age and fed HFD+20% BDM-X from the age of 10 weeks to 14 weeks. Statistical analysis

was carried out using one-way ANOVA followed by Tukey's method; *p<0.05 vs normal, $^{\dagger}p$<0.05 vs NASH.

Fig.3: Hepatic triglyceride concentration. Each bar represents mean ± SE. Normal (n=6), age-matched normal mice fed normal diet; NASH (n=7), STZ-injected mice fed HFD up to 14 weeks of age; BDM-X (n=7), STZ-injected mice fed HFD up to 10weeks of age and fed HFD+20% BDM-X from the age of 10 weeks to 14 weeks. Statistical analysis was carried out using one-way ANOVA followed by Tukey's method; *p<0.05 vs normal, $^{\dagger}p$<0.05 vs NASH.

Fig.4: Lipogenesis centered on the accumulation of free fatty acids. The accumulation of fatty acids in the liver is due to the following 4 factors: (1) dietary origin, (2) de novo synthesis from carbohydrates and amino acids, (3) uptake of LDL and (4) adipose tissue origin. (1) Dietary lipids pass through chylomicrons to become liver fatty acids. (2) Glucose and amino acids become fatty acids by de novo synthesis. (3) LDL is taken up by the liver and converted to triglycerides and fatty acids. (4) In adipose tissue, triglycerides are converted to fatty acids and transported to the liver. SREBP1c is an enzyme involved in converting triglycerides into fatty acids. PPARα is involved in the production of ATP by causing β-oxidation of fatty acids in the liver. In diabetes, excess glucose accumulates as triglycerides and insulin resistance exacerbates fatty liver. BDM-X may prevent exacerbation of fatty liver by reducing dietary fatty acids.
FFA: free fatty acid; TG: triglyceride; LDL: low density lipoprotein; VLDL: very low density lipoprotein.

Table 1: Diet ingredients; HFD32, HFD32 + BDM-X.

Ingredients (%/100g)	HFD32	HFD32 + BDM-X
Casein	24.5000	21.226
Egg white powder	5.000	4.332
L-cystine	0.430	0.372
Powdered beef tallow	15.880	-
Beef tallow	-	12.316
Safflower oil	20.000	19.388
Crystalline cellulose	5.500	-
Maltodextrin	8.250	5.871
Lactose	6.928	4.930
Sucrose	6.750	4.803
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
BDM-X	-	20.000
Total	100.00	100.00

Table 2: Dietary components; normal diet (CE-2), HFD32, BDM-X, HFD32 + BDM-X.

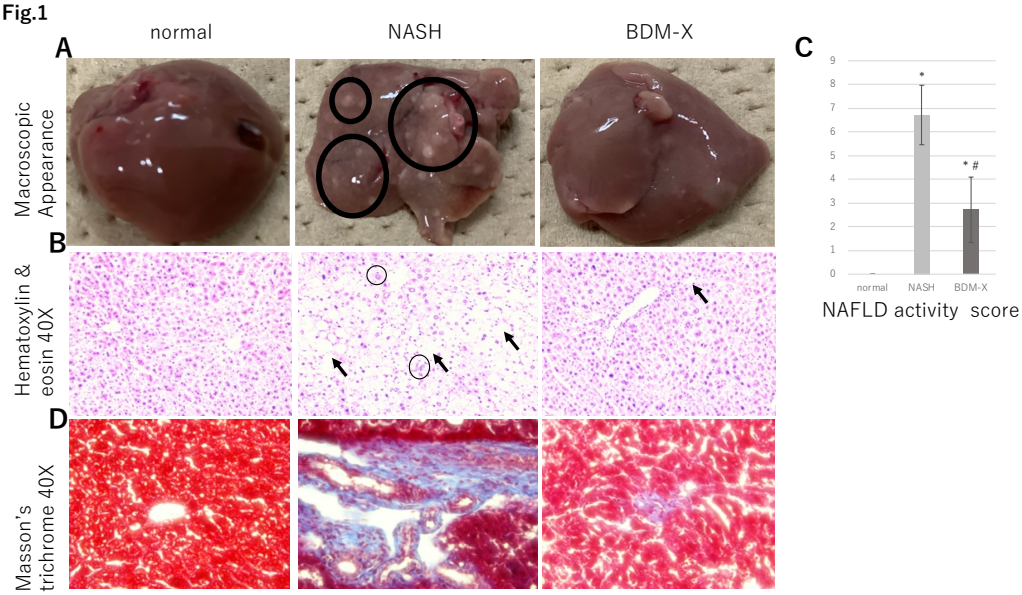
Dietary components	Normal diet (CE-2)	HFD32	BDM-X	HFD32 + BDM-X
Water (g/100g)	9.05	6.2	8.2	6.2
Protein (g/100g)	24.8	25.5	16.0	25.63
Fat (g/100g)	4.65	32.0	1.9	31.38
Ash (g/100g)	7.00	4.0	4.5	5.26
Carbohydrate (g/100g)	54.5	32.3	69.4	31.53
Fiber (g/100g)	5.05	2.9	32.7	6.43
Energy (kcal/100g)	340.2	507.6	179.0	485.3
β-glucan (g/100g)	-	-	13.5	2.7

Table 3. Changes in biochemical parameters after 4 weeks of treatment with BDM-X in NASH-HCC mice.

Biochemical parameters	normal (n=6)	NASH (n=7)	BDM-X (n=7)
Food intake/day/mouse (g)	3.9 ± 0.2	5.7 ± 1.7	2.4 ± 0.4*†
Energy consumption /day/mouse (kcal)	13.2 ± 0.7	28.9 ± 8.6	11.6 ± 1.9*†
Body weight (BW) (g)	29.2 ± 2.3	25.0 ± 1.1*	20.3 ± 5.0*†
% of relative liver weight (LW)/BW	4.1 ± 0.6	7.9 ± 0.9*	7.2 ± 0.8*
Blood glucose (mg/dL)	120.5 ± 29.1	463.6 ± 37.2*	431.9 ± 67.2*
Serum TG (mg/dL)	39.9 ± 8.0	97.0 ± 44.0*	47.7 ± 28.9
Serum TC (md/dL)	74.4 ± 12.6	131.0 ± 24.0*	148.0 ± 21.8*
Serum ALT (IU/L)	25.8 ± 6.0	123.0 ± 83.7*	22.0 ± 6.2*†
Serum AST (IU/L)	79.2 ± 6.7	328.9 ± 163.0*	127.3 ± 26.4*†
Serum ALP (IU/L)	304.5 ± 22.6	436.1 ± 72.0	310.9 ± 80.3†

Normal, age-matched normal mice; NASH, untreated NASH-HCC mice; BDM-X, NASH-HCC mice treated with BDM-X. TG, triglyceride; TC, total cholesterol; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase. Values are expressed as means ± SEM. *$p<0.05$ vs. normal. †$p<0.05$ vs. NASH.

Fig.1

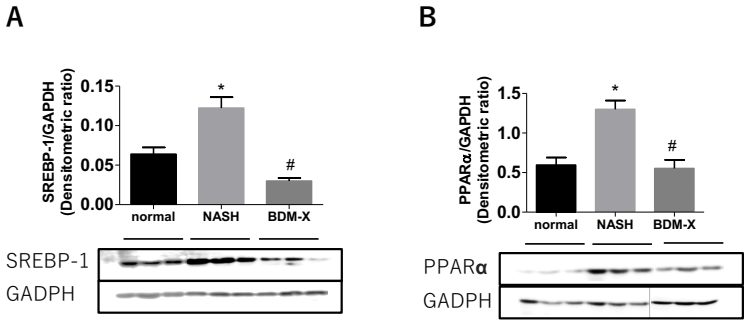


*P < 0.05 vs. normal group.

#P < 0.05 vs. NASH group.

One-way ANOVA followed by Tukey's Test.

Fig.2

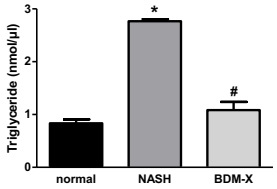


*P < 0.05 vs. normal group.

#P < 0.05 vs. NASH group.

One-way ANOVA followed by Tukey's Test.

Fig.3

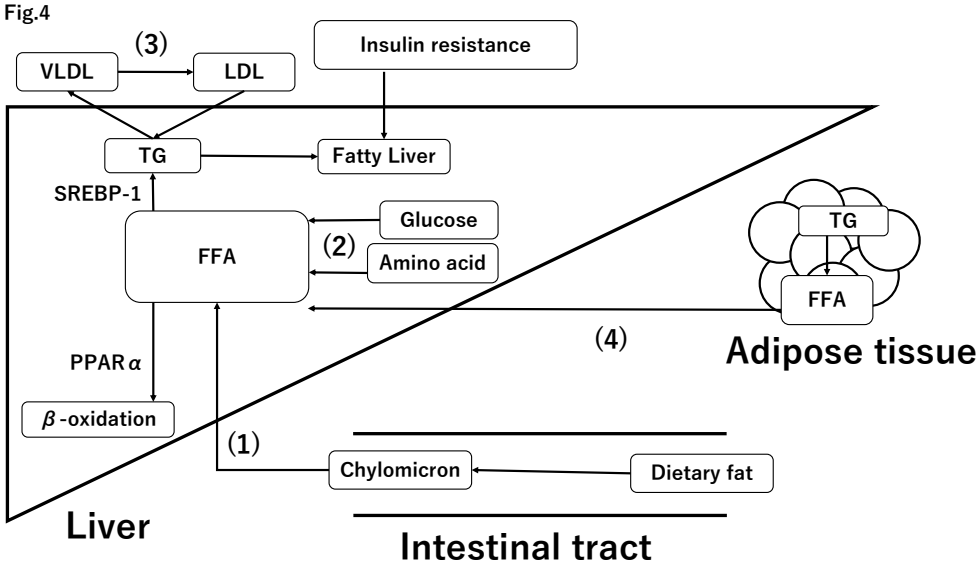


*P < 0.05 vs. normal group.

#P < 0.05 vs. NASH group.

One-way ANOVA followed by Tukey's Test.

Fig.4


(3)
VLDL
LDL
Insulin resistance
TG
Fatty Liver
SREBP-1
FFA
Glucose
(2)
Amino acid
TG
FFA
(4)
Adipose tissue
PPARα
β-oxidation
(1)
Chylomicron
Dietary fat
Liver
Intestinal tract