Article

Effect of Fermented Rice Drink "Amazake" on Patients with Nonalcoholic Fatty Liver Disease and Periodontal Disease: A Pilot Study

Yumiko Nagao [1,2,*], Hirokazu Takahashi [2,3], Atsushi Kawaguchi [4] and Hiroshi Kitagaki [5]

1 Department of Public Health, Graduate School of Medicine, Juntendo University, Bunkyo-ku, Tokyo 113-8421, Japan
2 Liver Center, Saga University Hospital, Nabeshima, Saga 849-8501, Japan; takahas2@cc.saga-u.ac.jp
3 Division of Metabolism and Endocrinology, Faculty of Medicine, Saga University, Nabeshima, Saga 849-8501, Japan
4 Education and Research Center for Community Medicine, Faculty of Medicine, Saga University, Nabeshima, Saga 849-8501, Japan; akawa@cc.saga-u.ac.jp
5 Faculty of Agriculture, Saga University, Honjo-cho, Saga 840-8502, Japan; ktgkhrs@cc.saga-u.ac.jp
* Correspondence: y.nagao.qd@juntendo.ac.jp; Tel.: +81-03-5802-1049

Abstract: The worldwide increase in nonalcoholic fatty liver disease (NAFLD) is a major public health problem. Obesity and diabetes are risk factors for NAFLD and the development of liver fibrosis is a risk factor for liver cancer. Periodontal disease bacteria can also exacerbate NAFLD. We previously reported that amazake, a traditional Japanese fermented food, improves the quality of life (QOL) of patients with liver cirrhosis. In this study, we investigated the effect of amazake intake on NAFLD patients with periodontal disease. Ten patients (mean age: 57.1 ± 19.2 years) consumed 100 g of amazake daily for 60 days. On days 0 and 60, their body mass index (BMI), body fat percentage, serum biochemical parameters, periodontal disease bacteria in saliva, and ten visual analog scales (VASs), namely, sense of abdomen distension, edema, fatigue, muscle cramps, loss of appetite, taste disorder, constipation, diarrhea, depression, and sleep disorder, were measured. For periodontal bacteria, the numbers of six types of bacteria in saliva (*Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola*, *Aggregatibacter actinomycetemcomitans*, *Prevotella intermedia*, and *Fusobacterium necleatum*) and *P. gingivalis*-specific *fimA* genotype were determined. After 60 days of amazake consumption, eosinophils ($p < 0.05$), immune reactive insulin (IRI) ($p < 0.01$), and HOMA-IR ($p < 0.05$) had significantly increased and tumor necrosis factor α (TNFα) ($p < 0.01$), muscle cramps ($p < 0.05$), and depression ($p < 0.05$) had significantly decreased. All subjective symptoms improved after amazake intake. No change was observed in the periodontal bacteria. In conclusion, amazake significantly decreased TNFα and improved the QOL of the patients with NAFLD and periodontitis. However, caution should be exercised because amazake, which is manufactured using techniques that lead to concentrations of glucose from the saccharification of rice starch, may worsen glucose metabolism in NAFLD patients. Amazake may be an effective food for improving the symptoms of a fatty liver if energy intake is regulated.

Keywords: amazake; NAFLD; periodontal disease bacteria; quality of life

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is a global public health issue since it is the most common liver disease worldwide [1]. In recent years, the number of patients with NAFLD has been increasing [2–4]. In Japan, the prevalence of NAFLD among screened individuals increased from 18% in 2001 [5] to 29.7% in 2009–2010 [6]. In 2016, it was estimated that there were approximately 20 million people with NAFLD and 4 million people with non-alcoholic steatohepatitis (NASH) in Japan, and this number is expected to increase further by 2030 [7].

Obesity is the most important factor in the development of NAFLD/NASH [8] and weight loss through diet and exercise therapy can improve the pathogenesis [9,10]. The main causes of NAFLD/NASH are the exacerbation of insulin resistance, metabolic syndrome, and related diseases, such as type 2 diabetes mellitus, dyslipidemia, and hypertension, among which, type 2 diabetes mellitus is closely related to the development of NAFLD/NASH [11]. In addition, according to the results of the NASH Clinical Research Network in the United States, which assessed health-related quality of life in patients with NAFLD/NASH, patients with NAFLD had a lower quality of life (QOL) in both physical and mental aspects compared with the general population [12].

Periodontal disease is known to be associated with cardiovascular disease [13], type 2 diabetes mellitus [14], and other systemic diseases [15]. Periodontal disease homeostatically increases the production of tumor necrosis factor α (TNFα) and exacerbates the pathogenesis of diabetes mellitus through insulin resistance [16]. Among the periodontal pathogens, *Porphyromonas gingivalis* (*P. gingivalis*) was reported to infect many organs, including the brain, lungs, bronchi, liver, blood vessels, placenta, and coronary arteries [17]. *P. gingivalis* was shown to be a potential cause and risk factor for NAFLD/NASH [18,19]. According to Arimatsu et al., the proposed mechanisms by which periodontal disease induces systemic inflammation are as follows: (i) the direct effect of infectious agents and their products; (ii) increased expression of cytokines, chemokines, and cell adhesion molecules produced in periodontitis lesions; (iii) translocation of swallowed *P. gingivalis* from the gut to the circulatory system; and (iv) alteration of gut-microbial-composition-induced increases in gut epithelial permeability by swallowed *P. gingivalis* [20]. Arimatsu et al. revealed that the population of Bacteroidales in the gut microbiota was significantly elevated in mice that were administered *P. gingivalis*, which coincided with increases in insulin resistance and systemic inflammation [20]. We reported that the periodontal inflammatory response could be associated with liver fibrosis and obesity in hepatitis C virus (HCV) and hepatitis B virus (HBV) patients with liver disease [21] and that the red complex (*P. gingivalis*, *Tannerella forsythia*, and *Treponema denticola*) that is involved in the pathogenesis of periodontal disease was associated with cirrhosis [22].

We previously reported that amazake, which is a traditional fermented beverage produced in Japan for more than several hundred years, was effective in late evening snacks (LES) therapy for patients that were infected with HCV or HBV and with cirrhosis [23]. Amazake is a nutritious drink that contains essential amino acids and vitamins, as well as a high concentration of glucose. Recently, it has also been called Japanese yogurt, and its health benefits are attracting attention [24]. Hamajima et al. revealed for the first time that glycosylceramide, which is present in large amounts in the *koji* used for the fermentation of amazake, has the effect of improving the profile of intestinal bacteria [25]. They showed that the *koji* glycosylceramide is degraded in the intestine, is absorbed and transported to the liver by lymphatic vessels, and activates peroxisome proliferator-activated receptor γ (PPARγ) in hepatocytes and lowers liver cholesterol. Furthermore, Hamajima et al. showed that the *koji* glycosylceramide decreases liver cholesterol in obese mice by altering the expression of genes in the metabolism of cholesterol, such as cholesterol 7-alpha-hydroxylase (CYP7A1, which is the rate-limiting enzyme for cholesterol catabolism) and ATP binding cassette subfamily G member 8 (ABCG8, which is the protein that is implicated in the biliary excretion of cholesterol) involved in liver cholesterol metabolism [26].

There are still no drugs that are approved for NAFLD/NASH in Japan. This study was designed as a first-time, pilot study to investigate how the intake of amazake affects NAFLD patients and whether this may also indirectly affect periodontal pathogens. Hypothesizing the effect of amazake on NAFLD and periodontal pathogens, we measured the amount of periodontal bacteria in this study. The purpose of this study was to examine whether amazake consumption in NAFLD patients with periodontal disease reduces inflammatory cytokine markers and *P. gingivalis* levels, as well as improves the QOL.

2. Materials and Methods

2.1. Subjects

The subjects were those who were diagnosed with a fatty liver with liver dysfunction and periodontal disease, who were visiting the Saga University Hospital, and who met the following eligibility criteria: (i) at least 20 years old at the time of obtaining consent, (ii) any gender, (iii) obtained written consent for this study, (iv) had not consumed amazake for more than one month, (v) had abnormal liver functions such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels in blood tests, (vi) diagnosed as NAFLD using abdominal ultrasonography, (vii) diagnosed with periodontal disease, and (viii) negative for HCV antibody and negative for hepatitis B surface antigen. NAFLD was defined as the presence of fatty deposits in the liver and the exclusion of other causes, such as alcoholic liver disease.

The exclusion criteria were (i) subjects who had not given consent, (ii) subjects who had been taking amazake continuously for more than one month, (iii) subjects who were pregnant or lactating, (iv) complications and/or history of other liver diseases (chronic hepatitis C, chronic hepatitis B, autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, Wilson's disease, etc.), (v) presence of HCC (except for patients with no recurrence for more than one year after treatment for HCC), (vi) severe liver dysfunction and liver failure (encephalopathy, ascites, ruptured varicose veins, hyperbilirubinemia, etc.), (vii) diabetic patients on insulin therapy (excluding patients that managed their condition using only oral hypoglycemic agents or diet/exercise therapy with good glycemic control and HbA1c below 7.0%), (viii) severe organic passage disorders, (ix) severe renal dysfunction, (x) serum creatinine greater than or equal to 2.0 mg/dL, and (xi) serum total bilirubin of 3.0 mg/dL or higher.

Prohibited concomitant medications and concomitant dietary supplements were as follows: (i) antiviral drugs for liver disease (oral direct-acting antiviral drugs "DAA," interferon, peginterferon, ribavirin, nucleic acid analog, etc.), (ii) corticosteroids, and (iii) new dietary supplements including probiotics (however, intake of dietary supplements that were taken before the study was acceptable).

A total of 11 patients with NAFLD and periodontitis were enrolled in the study at Saga University Hospital from May 2018 to November 2018, of whom, one was excluded during screening. The reason for exclusion was a contravention of the exclusion criteria. The patients ranged in age from 24 to 76 years, with an average age of 57.1 ± 19.2 years. The subjects' background information is shown in Table 1. The diagnosis of liver disease was based on clinical, serological, imaging, and/or histological evidence that was analyzed by a hepatologist. One case of liver cirrhosis was found among the 10 cases. The diagnosis of periodontal disease was made by an oral surgeon, who confirmed bleeding from the gingiva, plaque-stained calculus, and gingival recession. Salivary occult blood testing was performed using the commercial kit PerioScreen® (Sunstar Co. Ltd., Osaka, Japan), which uses a monoclonal antibody against human hemoglobin. Patients were followed as outpatients and no therapeutic interventions, such as lifestyle changes, physical activity, or treatment of periodontal disease, were performed after the study. One subject had a history of treatment for colorectal cancer and another had a history of treatment for breast cancer. The diagnosis of diabetes mellitus was made in accordance with the diagnostic criteria of Standards of Medical Care in Diabetes 2016 [27].

Table 1. Characteristics of the study population (*n* = 10).

Characteristics	Variable	Total
Sex	Male/female	2/8
Age	Mean ± SD, years	57.1 ± 19.2
Diagnosis of liver diseases	NAFLD	10 (100%)
Diabetes mellitus	Positive (%)	2 (20%)
Dyslipidemia	Positive (%)	7 (70%)

Table 1. *Cont.*

Characteristics	Variable	Total
Hypertension	Positive (%)	4 (40%)
Depression [a]	Positive (%)	2 (20%)
History of extrahepatic malignant tumor [b]	Positive (%)	2 (20%)
A habit of tooth brushing after meals	Positive (%)	2 (20%)
Anti-HCV	Positive (%)	0 (0%)
HBsAg	Positive (%)	0 (0%)

Data are presented as *n* or the mean $\pm$ SD. [a] None of the patients were taking antidepressants. [b] One patient had a history of treatment for colorectal cancer and another had a history of treatment for breast cancer. Anti-HCV, antibodies to hepatitis C virus; HBsAg, hepatitis B surface antigen; SD, standard deviation.

2.2. Study Assessment Protocol

The intervention schedule is shown in Figure 1. Each patient drank 100 g of amazake (equivalent to 112 kcal) (Hashimoto Shoyu Co. Ltd., Kumamoto, Japan) daily for 60 days. One hundred grams of amazake contains 25.6 g carbohydrates, 1.7 g protein, 0.3 g fat, 0.66 g salt, and trace ingredients (vitamins, essential amino acids, etc.). Amazake does not contain any alcohol. The study period comprised 60 days with evaluations at baseline (visit 1) and 60 days (visit 2). During the intervention, patients were instructed to reduce their rice or snack intake by about 100 kcal per day.

Day 0   Day 60

Amazake intake 100 g/day

Visit 1   Visit 2

Measurements

- Body mass index
- Clinical and biochemical measurements
- Identification of periodontitis bacteria in saliva
- Evaluation of VAS

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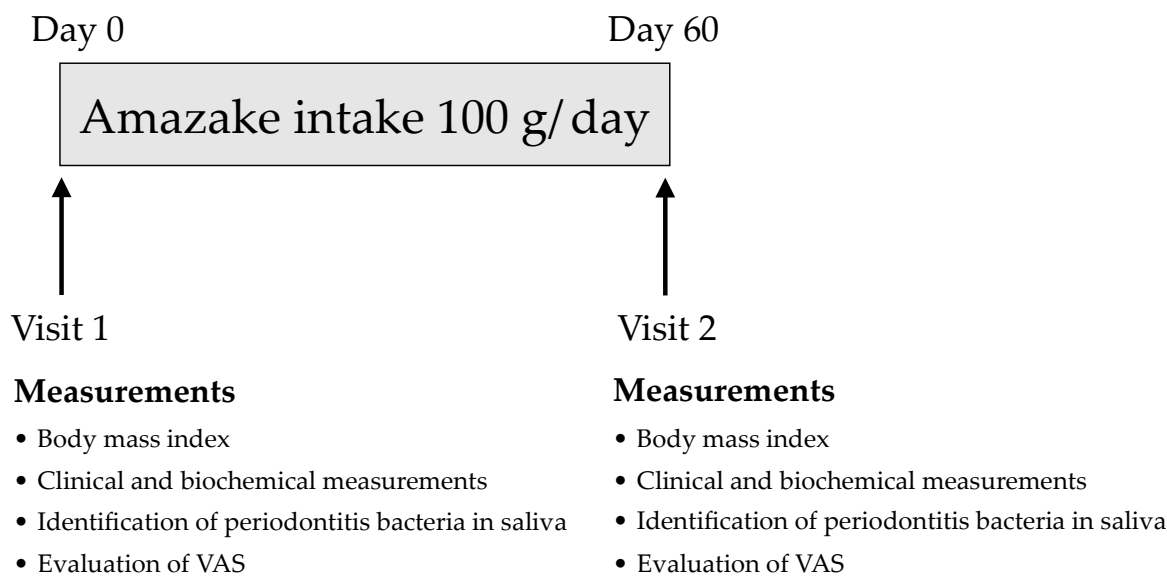
Figure 1. Study design.

On days 0 and 60, we evaluated the patients' body mass index (BMI), as well as their subjective symptoms (sense of abdomen distension, edema, fatigue, muscle cramps, loss of appetite, taste disorder, constipation, diarrhea, depression, and sleep disorder) using a visual analog scale (VAS) (Figure 2). A VAS is a horizontal line, 100 mm in length, anchored by word descriptors for subjective symptoms at each end. The patients were asked to mark the line at the point that they felt represented their perception of their current state. The VAS score was determined by measuring in millimeters from the left-hand end of the line to the point that the patient had marked, where 0 mm indicated an absence of symptoms and 100 mm indicated the worst symptom.

The following blood biochemical parameters were measured in all the patients on days 0 and 60: white blood cell (WBC) count, red blood cell (RBC) count, hemoglobin (Hb), platelets (PLT) count, AST, ALT, gamma-glutamyl transpeptidase (γ-GTP), lactic dehydrogenase (LDH), alkaline phosphatase (ALP), total protein, albumin, total cholesterol (T. cho), low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglyceride, total

bilirubin (T. Bil), direct bilirubin (D. Bil), creatinine (Crea), blood urea nitrogen (BUN), serum iron (Fe), ferritin, zinc, fasting blood glucose, hemoglobin A1c (HbA1c), fasting immune reactive insulin (IRI), HOMA-IR, AFP, total procollagen type 1 amino-terminal propeptide (Total P1NP), type III procollagen-N-peptide (P-III-P), type IV collagen 7s domain, mac-2 binding protein glycosylated isomers (M2BPGi) [28], tartrate-resistant acid phosphatase-5b (TRACP-5b), high molecular weight adiponectin, leptin, interleukin-6 (IL6), high-sensitivity TNFα, and high-sensitivity C-reactive protein (CRP). The formula for the HOMA-IR is as follows: HOMA-IR = fasting blood glucose × fasting insulin/405.

Patient Name: Date:

Q1. Sense of abdomen distension
Not at all ▬▬▬▬▬▬▬▬ Very much

Q2. Edema
Not at all ▬▬▬▬▬▬▬▬ Very much

Q3. Fatigue
Not at all ▬▬▬▬▬▬▬▬ Very much

Q4. Muscle cramps
Not at all ▬▬▬▬▬▬▬▬ Very much

Q5. Loss of appetite
Not at all ▬▬▬▬▬▬▬▬ Very much

Q6. Taste disorder
Not at all ▬▬▬▬▬▬▬▬ Very much

Q7. Constipation
Not at all ▬▬▬▬▬▬▬▬ Very much

Q8. Diarrhea
Not at all ▬▬▬▬▬▬▬▬ Very much

Q9. Depression
Not at all ▬▬▬▬▬▬▬▬ Very much

Q10. Sleep disorder
Not at all ▬▬▬▬▬▬▬▬ Very much

Figure 2. Visual analog scale (VAS) of 10 items. A VAS is a horizontal line, 100 mm in length, anchored by word descriptors at each end.

2.3. Saliva Sample Collection

Whole saliva (1 mL) was collected from the patients following chewing a non-flavored gum for 5 min. All samples were immediately sent to the Health Examination Laboratory (BML Inc., Tokyo, Japan) for bacterial testing [29].

2.4. Identification of Periodontitis Bacteria and Detection of P. gingivalis fimA Genotypes

The total number of bacteria in saliva and the counts of 6 types of periodontal pathogens (*Aggregatibacter actinomycetemcomitans*, *Prevotella intermedia*, *P. gingivalis*, *T. forsythia*, *T. denticola*, *Fusobacterium necleatum*) were quantified using the modified poly-

merase chain reaction (PCR) Invader assay, as described previously [30]. *P. gingivalis*-specific *fimA* genotypes, ranging from types I–V, were detected in all patients.

2.5. Safety Monitoring

Safety was assessed in terms of the physical examination results, laboratory test results, and clinical adverse events. When the ten patients had completed the study, the data monitoring committee evaluated the safety and disadvantages of the subjects and confirmed that there were no severe adverse events or disadvantages observed in the subjects.

2.6. Statistical Analysis

All data are expressed as the number or mean ± standard deviation. Statistical comparisons between before and 60 days after intake of amazake were performed using a paired *t*-test. A *p*-value <0.05 was considered to indicate a statistically significant difference. All analyses were performed using JMP 13 (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Patient Characteristics

Patient characteristics prior to the amazake intake are summarized in Table 2. The mean values of BMI and body fat percentage were above normal, indicating obesity. Serum AST, ALT, γ-GTP, ferritin, HOMA-IR, P-III-P, and high-sensitivity CRP were elevated compared to normal.

Table 2. Effects of amazake intake on biochemical examinations, VASs, and periodontitis disease bacteria.

Variable	Baseline	60 Days after Amazake Intake	Difference in Means	LCL	UCL	*p*-Value
	(Mean ± SD)	(Mean ± SD)				
BMI, kg/m^2	27.39 ± 4.1	27.45 ± 4.1	0.06	−0.37	0.49	NS
Body fat percentage, %	31.18 ± 5.2	31.99 ± 5.6	0.81	−0.92	2.54	NS
WBC, 10^3/μL	5.55 ± 1.9	5.14 ± 1.8	−0.41	−1.07	0.25	NS
Neutrophil count, %	55.87 ± 11.7	56.85 ± 9.5	0.98	−2.61	4.57	NS
Lymphocyte counts, %	33.90 ± 10.6	32.67 ± 8.5	−1.23	−4.30	1.84	NS
Monocyte count, %	6.85 ± 1.7	6.46 ± 1.5	−0.39	−1.44	0.66	NS
Eosinophil count, %	2.72 ± 1.4	3.27 ± 1.4	0.55	0.11	0.99	0.0203
Basophil count, %	0.66 ± 0.3	0.75 ± 0.4	0.09	−0.06	0.24	NS
RBC, 10^6/μL	4.50 ± 0.5	4.49 ± 0.5	−0.003	−0.05	0.05	NS
Hb, g/dL	14.22 ± 1.7	14.12 ± 1.6	−0.10	−0.31	0.10	NS
PLT, 10^4/μL	22.39 ± 9.1	21.95 ± 9.1	−0.44	−1.21	0.33	NS
AST, U/L	47.6 ± 28.0	51.5 ± 40.1	3.9	−7.91	15.71	NS
ALT, U/L	58.4 ± 38.1	56.2 ± 36.7	−2.2	−13.03	8.63	NS
γ-GTP, U/L	44.5 ± 29.9	47.8 ± 33.7	3.3	−4.47	11.07	NS
LDH, U/L	196.2 ± 17.2	200.4 ± 26.4	4.2	−8.16	16.56	NS
ALP, U/L	251.5 ± 83.6	252.8 ± 82.4	1.3	−14.9	17.5	NS
Total protein, g/dL	7.47 ± 0.2	7.46 ± 0.3	−0.01	−0.25	0.23	NS
Albumin, g/dL	4.26 ± 0.3	4.26 ± 0.3	0	−0.22	0.22	NS
T. cho, mg/dL	187.7 ± 25.2	190.3 ± 34.6	2.6	−14.51	19.71	NS
LDL, mg/dL	125.3 ± 24.5	126.9 ± 31.6	1.6	−13.6	16.8	NS
HDL, mg/dL	48.7 ± 9.9	49.6 ± 10.1	0.9	−3.1	4.9	NS
Triglyceride, mg/dL	143.2 ± 86.6	130.2 ± 51.9	−13	−44.57	18.57	NS
T.Bil, mg/dL	0.80 ± 0.3	0.85 ± 0.3	0.05	−0.12	0.22	NS
D.Bil, mg/dL	0.10 ± 0	0.11 ± 0.1	0.01	−0.03	0.05	NS
Crea, mg/dL	0.64 ± 0.2	0.63 ± 0.2	−0.01	−0.05	0.03	NS
BUN, mg/dL	12.80 ± 3.1	13.19 ± 2.8	0.39	−1.57	2.35	NS
Fe, μg/dL	126.3 ± 37.8	117.6 ± 25.2	−8.7	−39.07	21.67	NS
Ferritin, μg/dL	231.9 ± 206.7	231.2 ± 196.2	−0.7	−22.38	20.98	NS
Zinc, μg/dL	80.6 ± 14.6	70.7 ± 7.6	−9.9	−20.74	0.94	NS
Fasting plasma glucose, mg/dL	108.2 ± 19.6	109.6 ± 28.4	1.4	−14.48	17.28	NS
HbA1c, %	5.9 ± 0.5	6.1 ± 0.7	0.2	−0.02	0.42	NS
Fasting IRI, μU/mL	16.18 ± 7.3	24.42 ± 10.0	8.24	2.87	13.61	0.0070
HOMA-IR	4.47 ± 2.5	6.72 ± 3.6	2.25	0.62	3.87	0.0122
AFP, ng/mL	4.54 ± 1.6	4.60 ± 1.9	0.06	−0.88	1	NS

Table 2. *Cont.*

Variable	Baseline	60 Days after Amazake Intake	Difference in Means	LCL	UCL	*p*-Value
	(Mean ± SD)	(Mean ± SD)				
Total P1NP, ng/mL	58.88 ± 15.3	54.75 ± 19.0	−4.13	−9.17	0.91	NS
P-III-P, ng/mL	11.65 ± 5.3	11.98 ± 5.0	0.32	−1.22	1.86	NS
Type IV collagen 7s domain, ng/mL	5.86 ± 2.3	6.00 ± 2.1	0.14	−0.26	0.54	NS
M2BPGi, COI	1.61 ± 1.3	1.64 ± 1.3	0.03	−0.23	0.3	NS
TRACP−5b, mU/dL	320.56 ± 147.5	354.40 ± 140.9	33.84	−11.67	79.35	NS
High molecular weight adiponectin, μg/dL	2.65 ± 1.3	2.68 ± 1.5	0.03	−0.17	0.22	NS
Leptin, ng/mL	25.61 ± 13.0	31.09 ± 19.9	5.48	−3.06	14.02	NS
IL-6, pg/mL	2.16 ± 0.8	2.50 ± 1.2	0.34	−0.35	1.03	NS
High-sensitivity TNFα, pg/mL	1.41 ± 0.4	1.21 ± 0.4	−0.20	−0.31	−0.09	0.0025
High-sensitivity CRP, ng/mL	2344.5 ± 2266.7	2311.6 ± 3442.9	−32.9	−1191.25	1125.45	NS
Total number of bacteria in saliva, copy/10 μL	13,290,000 ± 7,130,131.7	11,560,000 ± 6,794,147.8	−1,730,000	−4,700,000	1,246,368	NS
Number of *A. actinomycetemcomitans* in saliva, copy/10 μL	<10	<10	0	0	0	NS
Number of *P. intermedia* in saliva, copy/10 μL	17.8 ± 22.6	34.7 ± 75.7	16.9	−21.08	54.88	NS
Number of *P. gingivalis* in saliva, copy/10 μL	144.0 ± 294.4	54.0 ± 113.8	−90	−267.1	87.10	NS
Number of *T. forsythia* in saliva, copy/10 μL	330.0 ± 292.0	703.5 ± 1242	373.5	−433.98	1180.98	NS
Number of *T. denticola* in saliva, copy/10 μL	369.0 ± 492.1	391.5 ± 609.3	22.5	−188.40	233.40	NS
Number of *F. necleatum* in saliva, copy/10 μL	283,090 ± 274,895.9	243,044 ± 342,379.7	−40,046	−233,951	153,859	NS
P. gingivalis fimA genotype II, positive (%)	2 (20%)	2 (20%)	*n*/a	*n*/a	*n*/a	NS
P. gingivalis fimA below detection sensitivity limits, positive (%)	8 (80%)	8 (80%)	*n*/a	*n*/a	*n*/a	NS
Sense of abdominal distension (VAS), mm	25.8 ± 25.0	18.6 ± 28.9	−7.2	−20.1	5.7	NS
Edema (VAS), mm	27.2 ± 30.9	31.6 ± 37.7	4.4	−6.48	15.28	NS
Fatigue (VAS), mm	37.2 ± 28.7	36.8 ± 29.6	−0.4	−7.02	6.22	NS
Muscle cramps (VAS), mm	24.1 ± 29.6	3.4 ± 7.2	−20.7	−39.62	−1.78	0.0353
Loss of appetite (VAS), mm	24.4 ± 25.2	12.1 ± 9.8	−12.3	−31.47	6.87	NS
Taste disorder (VAS), mm	3.0 ± 6.9	0	−3	−7.93	1.93	NS
Constipation (VAS), mm	31.4 ± 30.0	18.2 ± 29.6	−13.2	−29.34	2.94	NS
Diarrhea (VAS), mm	16.6 ± 29.8	7.3 ± 12.5	−9.3	−28.42	9.82	NS
Depression (VAS), mm	30.8 ± 24.7	18.2 ± 21.6	−12.6	−23.52	−1.68	0.0282
Sleep disorder (VAS), mm	25.3 ± 24.2	19.6 ± 24.1	−5.7	−25.06	13.66	NS

Data are presented as *n* (%) or the mean ± SD. Statistical comparisons between before and after 60 days of amazake intake were performed using *t*-tests. BMI, body mass index; WBC, white blood cell; RBC, red blood cell; Hb, hemoglobin; PLT, platelets; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, gamma-glutamyl transpeptidase; LDH, lactic dehydrogenase; ALP, alkaline phosphatase; T. cho, total cholesterol; TG, triglyceride; T.Bil, total bilirubin; D.Bil, direct bilirubin; Crea, creatinine; BUN, blood urea nitrogen; Fe, serum iron; HbA1c, hemoglobin A1c; IRI, immune reactive insulin; AFP, alpha-fetoprotein; Total P1NP, total procollagen type 1 amino-terminal propeptid; P-III-P, type III procollagen-N-peptide; M2BPGi, mac-2 binding protein glycosylated isomers; TRACP-5b, tartrate-resistant acid phosphatase-5b; IL6, interleukin-6; TNFα, high-sensitivity tumor necrosis factor α; CRP, C-reactive protein; VAS, visual analog scale; *A. actinomycetemcomitans, Aggregatibacter actinomycetemcomitans*; *P. intermedia, Prevotella intermedia*; *P. gingivalis, Porphyromonas gingivalis*; *T. forsythia, Tannerella forsythia*; *T. denticola, Treponema denticola*; *F. necleatum, Fusobacterium necleatum*; SD, standard deviation; LCL, Lower confidence limit; UCL, Upper confidence limit; *n*/a, not applicable; NS, not significant.

3.2. Effects of Amazake Intake on Biochemical Examinations, VAS, and Periodontitis Disease Bacteria

We analyzed any differences in laboratory data, VAS, and periodontitis disease bacteria before and after the intake of amazake (Table 2). After 60 days of amazake intake, eosinophils, IRI, and HOMA-IR were significantly elevated, while high-sensitivity TNFα, muscle cramps, and depression were significantly decreased. All subjective symptoms were improved after the amazake intake. Taste disorder disappeared after 60 days of amazake intake. There were no significant changes in liver function values, such as AST and ALT; renal function values; fasting plasma glucose; HbA1c; cholesterol; number of periodontal

disease bacteria (*A. actinomycetemcomitans*, *P. intermedia*, *P. gingivalis*, *T. forsythia*, *T. denticola*, and *F. necleatum*); and *P. gingivalis*-specific *fimA* genotypes after the amazake intake.

3.3. M2BPGi Levels and Effects of Amazake Intake

M2BPGi is a useful biomarker in diagnosing the fibrosis stage of the liver and assessing the risk of hepatocarcinogenesis [28]. We compared the effects of amazake by dividing the M2BPGi values into three groups: less than 1.00 cut-off index (C.O.I) (negative, group A), 1.00–3.00 C.O.I (positive 1+, group B), and more than 3.00 C.O.I (positive 2, group C) (Table 3). As shown in Table 3, eosinophils and HOMA-IR increased, and high-sensitivity TNFα, cramps, and depression decreased in all groups.

Table 3. Comparison of the effects of amazake according to M2BPGi levels: differences in biochemical tests, VAS, and periodontal disease.

M2BPGi	Group A	Group B	Group C
	Negative (*n* = 5)	Positive 1 (*n* = 3)	Positive 2 (*n* = 2)
	Difference in Means	Difference in Means	Difference in Means
BMI, kg/m^2	−0.08	0.23	0.15
Body fat percentage, %	1.76	0.03	−0.40
WBC, 10^3/μL	−0.76	0.20	−0.45
Neutrophil count, %	−1.9	5.2	1.8
Lymphocyte counts, %	1.6	−4.6	−3.2
Monocyte count, %	−0.2	−1.1	0.2
Eosinophil count, %	0.38	0.57	0.95
Basophil count, %	0.1	−0.1	0.3
RBC, 10^6/μL	0.01	0	0
Hb, g/dL	0.03	−0.2	−0.4
PLT, 10^4/μL	0.02	−1.7	0.3
AST, U/L	−3.8	15.3	6.0
ALT, U/L	−6.2	2.0	1.5
γ-GTP, U/L	3.0	6.7	−1.0
LDH, U/L	−1.0	13.7	3.0
ALP, U/L	−9.0	16.7	4.0
Total protein, g/dL	0.16	−0.13	−0.25
Albumin, g/dL	0.08	−0.03	−0.15
T. cho, mg/dL	5.8	10.0	−16.5
LDL, mg/dL	3.8	6.7	−11.5
HDL, mg/dL	1.8	1.3	−2.0
Triglyceride, mg/dL	−23.0	−9.7	7.0
T.Bil, mg/dL	0.08	−0.03	0.10
D.Bil, mg/dL	0	0.03	0
Crea, mg/dL	0	−0.02	−0.03
BUN, mg/dL	0.92	1.93	−3.25
Fe, μg/dL	−21.2	4.0	3.5
Ferritin, μg/dL	−15.2	20.3	4.0
Zinc, μg/dL	−6.6	−18.3	−5.5
Fasting plasma glucose, mg/dL	−3.4	1.7	13.0
HbA1c, %	0.1	0.1	0.7
Fasting IRI, μU/mL	8.98	12.57	−0.10
HOMA-IR	2.06	3.29	1.15
AFP, ng/mL	−0.10	0.57	−0.30
Total P1NP, ng/mL	−8.48	0.47	−0.15
P-III-P, ng/mL	−0.17	2.56	−1.80
Type IV collagen 7s domain, ng/mL	0.26	0	0.05
TRACP-5b, mU/dL	68.88	4.00	−9.00

Table 3. *Cont.*

M2BPGi	Group A	Group B	Group C
	Negative (*n* = 5)	Positive 1 (*n* = 3)	Positive 2 (*n* = 2)
	Difference in Means	Difference in Means	Difference in Means
High molecular weight adiponectin, μg/dL	−0.06	0.31	−0.18
Leptin, ng/mL	5.40	11.27	−3.00
IL-6, pg/mL	−0.30	0.77	1.30
High-sensitivity TNFα, pg/mL	−0.24	−0.17	−0.16
High-sensitivity CRP, ng/mL	305.40	−208.00	−616.00
Total number of bacteria in saliva, copy/10 μL	−1,220,000	−2,533,333	−1,800,000
Number of *A. actinomycetemcomitans* in saliva, copy/10 μL	0	0	0
Number of *P. intermedia* in saliva, copy/10 μL	0.2	0	84
Number of *P. gingivalis* in saliva, copy/10 μL	−18	−263	−10
Number of *T. forsythia,* in saliva, copy/10 μL	760	−20	−3
Number of *T. denticola* in saliva, copy/10 μL	−14	96	4
Number of *F. necleatum* in saliva, copy/10 μL	64,200	−216,567	−35,880
P. gingivalis fimA genotype II, positive (%)	*n*/a	*n*/a	*n*/a
P. gingivalis fimA below detection sensitivity limits, positive (%)	*n*/a	*n*/a	*n*/a
Sense of abdominal distension (VAS), mm	−15.2	−8.3	14.5
Edema (VAS), mm	9.4	7.7	−13.0
Fatigue (VAS), mm	2.6	−1.7	−6.0
Muscle cramps (VAS), mm	−11.4	−22.3	−41.5
Loss of appetite (VAS), mm	−13.6	3.0	−32.0
Taste disorder (VAS), mm	−5.4	−1.0	0
Constipation (VAS), mm	−28.8	1.3	4.0
Diarrhea (VAS), mm	−17.8	−1.3	0
Depression (VAS), mm	−16.0	−13.0	−3.5
Sleep disorder (VAS), mm	1.2	−14.3	−10.0

BMI, body mass index; WBC, white blood cell; RBC, red blood cell; Hb, hemoglobin; PLT, platelets; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, gamma-glutamyl transpeptidase; LDH, lactic dehydrogenase; ALP, alkaline phosphatase; T. cho, total cholesterol; TG, triglyceride; T.Bil, total bilirubin; D.Bil, direct bilirubin; Crea, creatinine; BUN, blood urea nitrogen; Fe, serum iron; HbA1c, hemoglobin A1c; IRI, immune reactive insulin; AFP, alpha-fetoprotein; Total P1NP, total procollagen type 1 amino-terminal propeptid; P-III-P, type III procollagen-*n*-peptide; M2BPGi, mac-2 binding protein glycosylated isomers; TRACP-5b, tartrate-resistant acid phosphatase-5b; IL6, interleukin-6; TNFα, high-sensitivity tumor necrosis factor α; CRP, C-reactive protein; VAS, visual analog scale; *A. actinomycetemcomitans*, *Aggregatibacter actinomycetemcomitans*; *P. intermedia*, *Prevotella intermedia*; *P. gingivalis*, *Porphyromonas gingivalis*; *T. forsythia*, *Tannerella forsythia*; *T. denticola*, *Treponema denticola*; *F. necleatum*, *Fusobacterium necleatum*; *n*/a, not applicable.

3.4. Adverse Events

No adverse events other than elevated IRI or HOMA-IR were observed. All patients completed the intake of amazake as scheduled.

4. Discussion

This study investigated the effect of amazake consumption on NAFLD patients with periodontal disease. After 10 patients consumed 100 g of amazake daily for 60 days, eosinophils ($p < 0.05$), IRI ($p < 0.01$), and HOMA-IR ($p < 0.05$) were significantly elevated, and TNFα ($p < 0.01$), muscle cramps ($p < 0.05$), and depression ($p < 0.05$) were significantly decreased. This result showed a similar tendency in cases with advanced liver fibrosis, as seen in the subgroups in Table 3. However, since the number of cases was small, the results of statistical tests should be interpreted with caution.

Amazake is a traditional Japanese sweetened beverage that is made from rice, malted rice, and water. The culture of drinking amazake dates back to the early 7th century and is mentioned in the "Nihon Shoki," the second-oldest book of classical Japanese history. The drink was commonly consumed during the Edo period (1603–1868 AD) [31,32]. *Koji* amazake, a sweet non-alcoholic beverage, is made by the fermentation of rice by filamentous fungi called *Aspergillus oryzae*, which is known as "*koji*" in Japanese. In recent years, various effects of rice malt and sake lees, which are ingredients of amazake,

were reported as follows: improvement of the intestinal environment [25,33], obesity control [34,35], suppression of cholesterol increase [36], suppression of blood pressure increase [37], prevention of amnesia [34], a whitening effect [38], a moisturizing effect [39], an effect on the elasticity of the skin [40], increased bifidobacteria in the intestine with acid protease derived from *koji* mold [41], and improved bowel movement in healthy people [42].

In the present study, amazake intake increased eosinophils, IRI, and HOMA-IR, while high-sensitivity TNFα, muscle cramps, and depression were significantly decreased. Amazake intake did not cause increases in fasting blood glucose and HbA1c. However, increased IRI and HOMA-IR indicate that amazake intake may have worsened insulin resistance and glucose metabolism. Because the concentration of glucose contained in amazake can be regulated by manufacturing procedures, such as via the saccharifying time, temperature, and the extent and form of propagation of the fungus *Aspergillus oryzae*, the glucose concentration of amazake should be decreased by manufacturers to attain its health benefits.

The fact that TNFα was significantly reduced by amazake intake suggested an improvement in the pathogenesis of fatty liver with periodontitis, although the mechanism is unknown. Inflammatory cytokines, such as TNFα, IL-6, and CRP, are significantly elevated in obesity and visceral adiposity [43] and are associated with insulin resistance [44]. TNFα was reported to be associated with the development and progression of NAFLD [45,46] and Crespo et al. reported that the expression of TNF receptor 1 was increased in liver tissue in NASH, especially in patients with advanced fibrosis [47].

Periodontitis is the most common infectious disease worldwide [48]. Periodontal pockets are inhabited by periodontopathogenic bacteria, such as *A. actinomycetemcomitans*, *P. intermedia*, *P. gingivalis*, *T. forsythia*, *T. denticola*, and *F. necleatum* [49]. Yoneda et al. reported that a high rate of *P. gingivalis* was detected in the oral cavities of NASH patients and that the removal of *P. gingivalis* via dental treatment improved AST and ALT levels [18]. It was reported that *P. gingivalis* infection exacerbates fibrosis in NASH by stimulating the activation of hepatic stellate cells (HSCs) through the production of transforming growth factor β1 (TGF-β1) and galectin-3 [50]. *P. gingivalis fimA* fimbriae are classified into six genotypes (types I–V and Ib) [51], with types II/IV/Ib being invasive and types I/III/V being non-invasive [52]. In this study, there was no change in the number of periodontal bacteria and *fimA* genotype due to amazake, but the two patients with *fimA* both had type II, which is associated with liver fibrosis [21,22].

The fact that amazake consumption significantly elevated eosinophils may be an effective phenomenon for a fatty liver with obesity. Recently, eosinophils were reported to play a protective role against obesity [53,54]. In 2011, Wu et al. first reported on the potential of eosinophils to prevent obesity [53], feeding a high-fat diet to transgenic C57BL/6 mice lacking eosinophils (△dblGATA mice) resulted in increased body fat and worsened insulin resistance in adipose tissue compared with wild-type mice. On the other hand, they showed that mice with increased eosinophils and mice with eosinophils that were increased physiologically by infection with helminths had reduced body fat gain and improved insulin sensitivity and glucose tolerance. These results suggest that eosinophils play an important role in the regulation of glucose metabolism by maintaining the function of endogenous macrophages in adipose tissue.

This study showed that the intake of amazake improved subjective symptoms, especially muscle cramps and depression, in all patients. Muscle cramps are a common comorbidity of chronic liver disease and were reported to be associated with female sex, diabetes and chronic kidney disease in chronic liver disease, and with decreased muscle mass in NAFLD [55]. Mehta et al. reported that muscle cramps are common in patients with liver disease and adversely influence their QOL [56]. Increased TNFα is known to be associated with muscle damage and inflammation processes in the extremities [57], as well as depression [58,59]. Although the precise mechanism by which amazake improved QOL is not clear, in the present study, the reduction in TNFα by amazake seems to have

improved muscle cramps and depressed moods. Another reason may be that the BCAAs contained in amazake may have influenced the results [60–65]. The administration of BCAA supplements might reduce the frequency of muscle cramps in patients with cirrhosis [66,67]. Indeed, our previous study on the efficacy of amazake as an LES therapy in cirrhotic patients infected with HCV and HBV ameliorated muscle cramps [23].

The transfer of intestinal bacteria and their metabolites to the liver via the portal vein contributes to the pathogenesis of NAFLD. In patients with NAFLD, changes in the intestinal microbiota were shown to be closely related to the pathogenesis of the disease, especially an increase in *Firmicutes* [68] and a decrease in *Bacteroidetes* [69]. It was also reported that patients with NAFLD not only have changes in the composition of the intestinal microbiota but also increased intestinal permeability [70]. It was confirmed that ingestion of the glycosylceramide contained in *Aspergillus oryzae* improved the intestinal microbiota of mice, including an increase in *Bacteroidetes* [25]. Therefore, as a mechanism, the intake of amazake containing glycosylceramide to NAFLD patients may have induced changes in the intestinal microbiota, although the details are unknown.

The present study has several limitations. First, the sample size was small. Second, the study was not conducted as a controlled trial. Large-scale case–control studies are needed in the future to clarify the effects of amazake. Third, no assessment of periodontitis, such as measuring the probing depth or bleeding on probing, was conducted.

5. Conclusions

In this pilot study, it was confirmed that consuming 100 g/day of amazake made from rice malt for 60 days improved TNFα and subjective symptoms, although it exacerbated IRI and HOMA-IR in patients with NAFLD and periodontal disease. It should be emphasized that due to the small sample size in this study, these observations require further study. Because NAFLD patients often have complications of diabetes and obesity, it is desirable to consume amazake with an adequate adjustment to their energy intake.

Author Contributions: Y.N. designed the study, interpreted the data, and wrote the manuscript. H.T. collected data from participants. A.K. performed the statistical analysis. H.K. conducted the monitoring to assess the study's safety. All authors have read and agreed to the published version of the manuscript.

Abbreviations

The abbreviations including in the text are reported alphabetically.

AFP	α-fetoprotein
ALP	alkaline phosphatase
ALT	alanine aminotransferase
Anti-HCV	antibodies to HCV
AST	aspartate aminotransferase
BCAA	branched-chain amino acids
BMI	body mass index
BUN	blood urea nitrogen
Crea	creatinine
CRP	C-reactive protein
D.Bil	direct bilirubin
DAA	direct-acting antiviral drug
Hb	hemoglobin
HbA1c	hemoglobin A1c
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
HCC	hepatocellular carcinoma
HCV	hepatitis C virus
HDL	high-density lipoprotein
IL6	interleukin-6
IRI	immune reactive insulin
LDH	lactic dehydrogenase
LDL	low-density lipoprotein
LES	late evening snacks
M2BPGi	mac-2 binding protein glycosylated isomers
NAFLD	nonalcoholic fatty liver disease
NASH	nonalcoholic steatohepatitis
P-III-P	type III procollagen-N-peptide
PLT	platelets
QOL	quality of life
RBC	red blood cell
T. Bil	total bilirubin
T. cho	total cholesterol
TNFα	tumor necrosis factor α
Total P1NP	total procollagen type 1 amino-terminal propeptide
TRACP-5b	tartrate-resistant acid phosphatase-5b
VAS	visual analog scale
WBC	white blood cell
γ-GTP	gamma-glutamyl transpeptidase

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