Original Article

A nutritional intervention that promotes increased vegetable intake in Japanese with non-alcoholic fatty liver disease: a six-month trial

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The aim of this study was to investigate whether a nutritional intervention motivating increased vegetable consumption would be an effective treatment and diet therapy for patients with non-alcoholic fatty liver disease. We examined 15 patients with this disease (5 men and 10 women). During the 6-month intervention period, all participants received a small amount of vegetables twice a month as a nutritional education tool aimed at increasing vegetable consumption. They also received nutritional counseling and underwent ultrasound and blood biochemical examinations at baseline and 3 and 6 months after initiation of the intervention. Moreover, they were requested to submit dietary records for any 2 days. Green, white, and total vegetable intakes were significantly higher at 3 and 6 months than at baseline in 8 patients. These patients had significantly lower alanine aminotransferase and triglyceride concentrations than those whose vegetable intake did not increase. Additionally, green vegetable intake significantly negatively correlated with weight at 3 and 6 months ($r = -0.617$, $p = 0.032$ and $r = -0.848$, $p = 0.008$, respectively). These results suggest that our nutritional approach effectively increased vegetable consumption in at least some patients with non-alcoholic fatty liver disease, consequently improving their condition.

***Key Words*: non-alcoholic fatty liver disease, diet therapy, nutritional intervention, obesity, vegetable consumption**

Non-alcoholic fatty liver disease (NAFLD) is one of the most serious chronic liver diseases. It is not caused by a viral infection, an autoimmune disease, drug use, or excessive alcohol consumption.[1,2] Its pathogenesis involves visceral fat accumulation and insulin resistance and is strongly associated with obesity and type 2 diabetes mellitus (T2DM).[2] The prevalence of NAFLD has increased along with the rise in obesity in developed countries.[3–5] As reported by the Japan Study Group of NAFLD, the prevalence of NAFLD in Japan was 29.7% in 2012.[6] A previous study, which included 31 patients with non-alcoholic steatohepatitis who underwent diet and exercise therapy for 48 weeks, reported that weight loss greater than 7% significantly improves steatosis, ballooning, and liver fibrosis.[7] Therefore, weight reduction by diet and/or exercise therapy is recommended as the treatment modality for NAFLD in the clinical guidelines published by the Japanese Society of Gastroenterology.[8] However, no specific effective diet therapy for NAFLD has been established. A Japanese study showed that patients with NAFLD consume fewer vitamins, minerals, and dietary fiber than the general Japanese population, as well as fewer vegetables that are rich in these nutrients.[9] The consumption of fruits and vegetables is proposed to be important for preventing obesity.[10] A review regarding the diet of choice for NAFLD mentioned that "Vegetables can reduce the overall energy density of the diet and allow consumption of satisfying portions while reducing caloric intake".[11]

Moreover, the pathogenesis and progression of NAFLD are associated with oxidative stress;[12] thus, the antioxidant effects of vitamins, carotenoids, and some phytochemicals present in vegetables may be effective for NAFLD.[13] In support of this argument, recent studies have shown improved liver function in humans and mice receiving the phytochemicals sulforaphane and β-cryptoxanthin, respectively.[14,15] Although many studies on the intake of "fruits and vegetables" have been reported, few studies have focused only on "vegetables". Because the intake of fructose, present in fruits, increases hepatic gluconeogenesis and *de novo* lipogenesis,[16] high consumption of fruits is not recommended for patients with NAFLD. Hence, a diet therapy focusing specifically on vegetable intake should better improve NAFLD than one focusing on both fruits and vegetables.

In our previous efforts, we developed a nutritional intervention program that strongly motivated vegetable intake; after 6 months of practice, we succeeded in increasing vegetable intake in patients with NAFLD.[17] We also found that the program led to weight loss, which is an important factor in improving the condition. However, we have not been able to verify the variation of parameters related to the disease. Therefore, the purpose of this study was to determine whether a practical nutritional approach to NAFLD improved these patients' condition, and to assess

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changes in NAFLD-related parameters before and after intervention.

Materials and Methods

Participants. Patients with NAFLD who visited University Hospital, Kyoto Prefectural University of Medicine (Kyoto, Japan), were enrolled. NAFLD was diagnosed by the physician in charge via liver biopsy or transient elastography (Fibroscan®; Echosens, Paris, France). Serum-based indices and interviews were used to confirm the absence of viral infection, autoimmune disease, drug use, and excessive consumption of alcohol. Among the outpatients who visited the department between August 2016 and February 2017, 17 with NAFLD agreed to participate in the study. Two participants were lost to follow-up, resulting in a final sample of 15 participants (5 men and 10 women).

All participants provided written informed consent before study enrollment. This study was approved by the Kyoto Prefectural University Ethics Committee (No. 73) and Kyoto Prefectural University of Medicine Ethics Committee (ERB-C-242).

Nutritional intervention protocol. This interventional trial included a 1-month preparation period after consent and a 6-month intervention period (Fig. 1). All participants visited physicians and received nutritional counseling at the start of the intervention (baseline) and subsequently once every 3 months. During the intervention period, vegetables were sent to the participants' homes approximately twice a month (total: 12 packages per participant), and 6 deliveries were accompanied by a newsletter containing information about vegetables. Each package contained several kinds of vegetables and weighed approximately 1 kg. These vegetables were used as a nutritional educational tool aimed at encouraging the consumption of vegetables.

Dietary surveys. Participants were requested to submit dietary records for any 2 days between each nutritional counseling session. The daily intake amounts of food and nutrients were calculated from the completed records using nutritional analysis software (Excel Eiyokun ver. 6.0; Kenpakusha, Tokyo, Japan). The averages of the values obtained on each of the 2 days were used for the analysis. The intake of 23 nutrients were calculated: energy, protein, fat, carbohydrates, potassium, calcium, magnesium, phosphorus, iron, zinc, vitamins A, B_1, B_2, B_6, B_{12}, niacin, folic acid, vitamin C, saturated fatty acids, mono- and polyunsaturated fatty acids, total dietary fiber, and salt. To calculate intake per food group, foods were categorized into 17 groups: grains and cereals (including rice and noodles), potatoes, sugar, nuts, green vegetables, white vegetables, fruits, mushrooms, algae, pulses, fish and shellfish, meat, eggs, milk, fats and oils, confectionery, and beverages. Energy intake was adjusted per ideal weight, and the intake of all food and nutrients were adjusted per 1,000 kcal. The sum of the intake of green and white vegetables is presented as the total vegetable intake.

Data collection. Patient data (age, medical history, comorbidities, and medications) were retrieved from electronic health records. No participants changed their medications during the intervention period. Participants were asked whether they smoked, had received previous nutritional counseling, and usually cooked for themselves. The number of family members was also recorded, as was their menopause status at baseline (female participants only). Weight and skeletal muscle and body fat percentages were determined using a body composition analyzer (InBody®; InBody Japan, Tokyo, Japan).

The biochemical parameters that were examined included aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase, total bilirubin, triglycerides (TG), total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol, ferritin, platelets (PLT), fasting blood sugar, hemoglobin A1c, type IV collagen 7S, and Mac-2-binding protein glycosylation isomer.[18] The AST/ALT ratio, which indicates the progression of liver fibrosis,[19] was calculated based on the AST and ALT levels. The fibrosis (FIB)-4 index, which also predicts the progression of liver fibrosis,[20] was calculated as follows:

$$\text{FIB-4 index} = \text{age (years)} \times \text{AST (IU/L)}/\text{PLT } (10^9/\text{L}) \times \sqrt{\text{ALT (IU/L)}}.$$

The controlled attenuation parameter (CAP), which indicates liver fat accumulation, and the liver stiffness measurement (LSM), which indicates fibrosis, were determined using transient elastography. Transient elastography is a diagnostic ultrasonographic technique that noninvasively measures the amount of liver fat accumulation from the skin surface by means of a transducer attached to the tip of an ultrasound probe that measures shear wave velocity. Previous studies have demonstrated its usefulness for measuring these parameters.[21,22]

Statistical analysis. All data are presented as mean ± SD. The Kolmogorov–Smirnov and Shapiro–Wilk tests were used to assess data normality. For comparison of two independent groups, the non-paired *t* test and Mann–Whitney *U* test were used for normally and non-normally distributed data, respectively. For

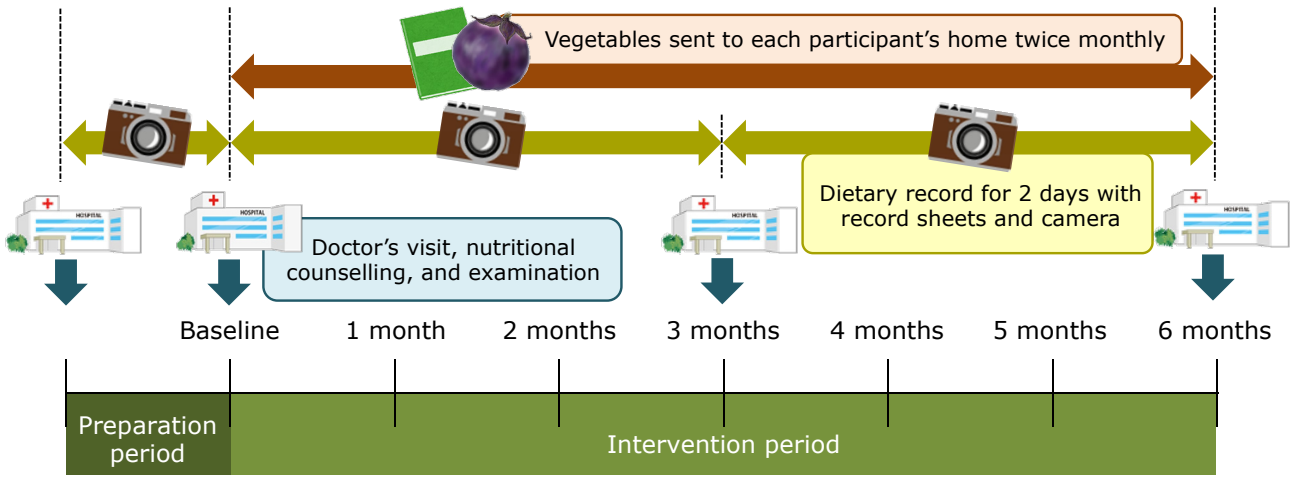

Fig. 1. The nutritional intervention protocol used in the present study.

comparison of paired samples, the paired *t* test and Wilcoxon signed-rank test were used for normally and non-normally distributed data, respectively. For comparison of rates in each group, the chi-square test was used. Bivariate correlations were assessed via partial correlation analysis adjusted for age. The significance level was 5% (two-sided test). All statistical analyses were performed using the Statistical Package for Social Sciences, ver. 22.0 (SPSS 22.0; IBM, Armonk, NY).

Results

Table 1 shows the participants' baseline characteristics. The mean body mass index of all patients exceeded 25.0 kg/m^2 (the threshold for obesity in Japan),[23] being 28.0 kg/m^2 in male and 27.6 kg/m^2 in female patients. The percentage of patients who cooked for themselves was significantly higher among women than among men ($p = 0.001$).

The trends in the patients' vegetable intake during the intervention period are presented in Fig. 2. Green, white, and total vegetable intakes were higher at 3 and 6 months of intervention than at baseline, although not significantly so (Fig. 2A). Therefore, the comparison of the uptake values for each participant revealed notable differences in their responses to the intervention (Fig. 2B). Moreover, age-adjusted partial correlation analysis was

Table 1. Characteristics of the participants

		Male (n = 5)	Female (n = 10)	p value
Age	years	40.0 ± 13.7	59.1 ± 7.4	0.032*
BMI	kg/m^2	28.0 ± 4.4	27.6 ± 2.8	0.858
†Rate of obesity	% (n)	80 (4)	90 (9)	0.591
Comorbidities				
T2DM	% (n)	20 (1)	50 (5)	0.264
Hypertension	% (n)	60 (3)	60 (6)	1.000
Dyslipidemia	% (n)	100 (5)	50 (5)	0.053
Hyperuricemia	% (n)	20 (1)	10 (1)	0.591
GERD	% (n)	20 (1)	10 (1)	0.591
A history of receiving nutritional councelling	% (n)	40 (2)	40 (4)	1.000
Lifestyle habits				
Smoking	% (n)	20 (1)	30 (3)	0.680
Number of family members	n	2.4 ± 1.1	2.6 ± 1.2	0.759
Cooking for myself	% (n)	20 (1)	100 (10)	0.001*
Menopause (only females)	% (n)	—	80 (8)	—

Values for age, BMI, and number of family members are presented as mean ± SD. The relationships between sexes were assessed using the non-paired *t* test for normally distributed data and the Mann–Whitney *U* test for non-normally distributed data. The rates were compared between the sexes using the chi-square test. †Percentage of patients with a BMI over 25.0 kg/m^2. *p<0.05. BMI, body mass index; T2DM, type 2 diabetes mellitus; GERD, gastro-esophageal reflux disease.

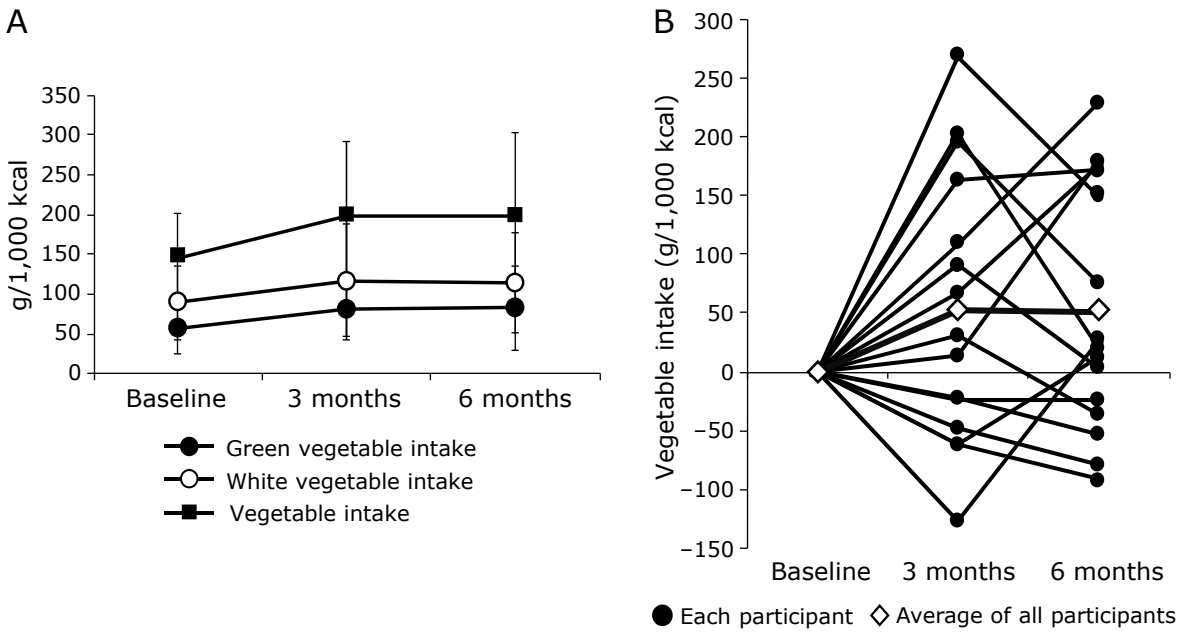

Fig. 2. The trends in the patients' vegetable intake during the intervention period. (A) Green, white, and total vegetable intakes at baseline and 3 and 6 months for all participants. Values are presented as mean ± SD. Differences in intake between baseline and 3 or 6 months were assessed using the paired *t* test for normally distributed data and the Wilcoxon signed-rank test for non-normally distributed data. (B) Total vegetable intake at baseline and 3 and 6 months for each participant and the average intake for all participants.

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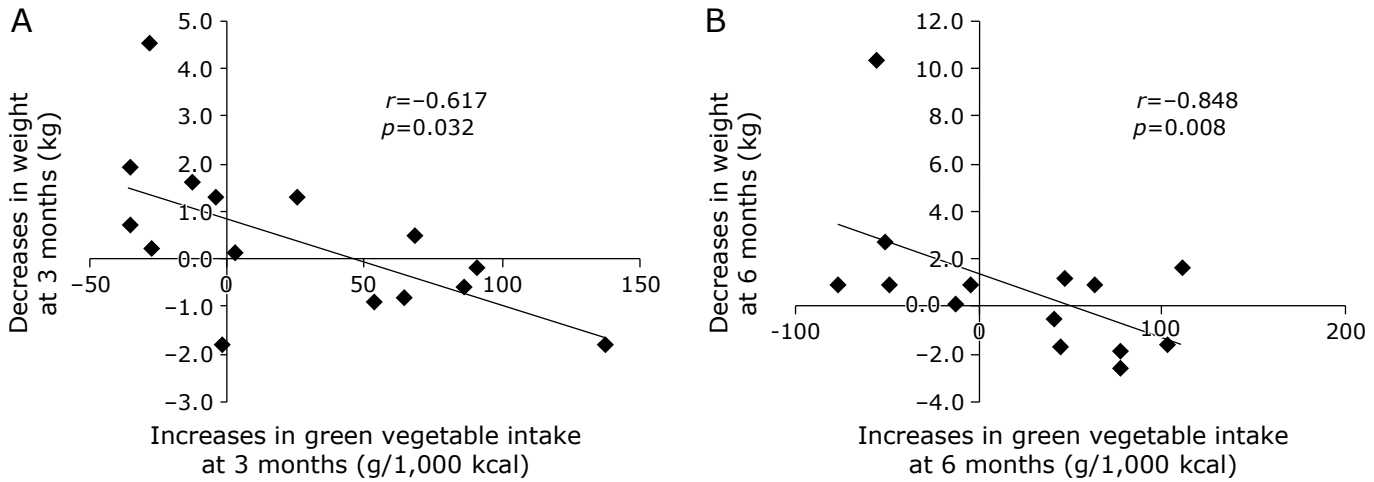

Fig. 3. Correlation between green vegetable intake and weight at 3 months (A) and 6 months (B) for all participants. Bivariate correlations were analyzed via partial correlation analysis adjusted for age.

Table 2. Characteristics of the participants stratified by vegetable intake

		Increased group (n = 8)	Non-increased group (n = 7)	p value
Male/Female	n	2/6	3/4	0.464
Age	years	55.4 ± 9.3	49.7 ± 17.0	0.955
BMI	kg/m^2	28.4 ± 4.0	27.1 ± 2.1	0.478
†Rate of obesity	% (n)	88 (7)	86 (6)	
Comorbidities				
T2DM	% (n)	63 (5)	14 (1)	0.057
Hypertension	% (n)	63 (5)	57 (4)	0.833
Dyslipidemia	% (n)	63 (5)	71 (5)	0.573
Hyperuricemia	% (n)	13 (1)	14 (1)	0.733
GERD	% (n)	0 (0)	29 (2)	0.200
A history of receiving nutritional councelling	% (n)	25 (2)	57 (4)	0.205
Lifestyle habits				
Smoking	% (n)	38 (3)	14 (1)	0.310
Number of family members	n	2.5 ± 1.4	2.6 ± 0.8	0.694
Cooking for myself	% (n)	88 (7)	57 (4)	0.185
Menopause (only females)	% (n)	67 (4)	100 (4)	0.197

Values for age, BMI, and number of family members are presented as mean ± SD. The relationships between sexes were assessed using the non-paired t test for normally distributed data and the Mann–Whitney U test for non-normally distributed data. The rates were compared between the groups using the chi-square test. †Percentage of patients with a BMI over 25.0 kg/m^2. BMI, body mass index; T2DM, type 2 diabetes mellitus; GERD, gastro-esophageal reflux disease.

performed to determine whether increased vegetable intake (green, white, and total) correlated with clinical data. As a result, increases in green vegetable intake significantly correlated with decreases in weight at 3 and 6 months ($r = -0.617$, $p = 0.032$ and $r = -0.848$, $p = 0.008$, respectively; Fig. 3). In terms of white and total vegetable intake, no significant correlations were identified.

Patients were subsequently divided into two groups based on whether their non-adjusted total vegetable intake did or did not show an increase between baseline and 3 months: the "increased" group comprised 8 patients (2 men and 6 women) and the "non-increased" group comprised 7 patients (3 men and 4 women). Table 2 presents a comparison of the characteristics of the two groups. None of the variables examined differed significantly between the groups. Table 3 presents the values for and changes in food and nutrient intakes at baseline, 3 months, and 6 months in the "increased" group. Potassium, vitamin A, vitamin B_6, folic acid, green vegetable, and total vegetable intakes were significantly higher, whereas energy intake was significantly lower at 3 and 6 months, respectively, than at baseline. Total dietary fiber and white vegetable intakes were significantly higher at 3 months than at baseline, whereas no significant changes were found in the "non-increased" group (data not shown).

Table 4 presents the examination results at baseline, 3 months, and 6 months. No baseline parameters were found to be significantly different between the "increased" and "non-increased" group. In the "increased" group, TG levels were significantly lower at 6 months than at baseline, and there was a significant increase in HDL-C at 3 months as compared to baseline. The CAP and LSM also decreased in this group, although these changes were not significant. ALT and TG levels were significantly lower in the "increased" than in the "non-increased" group at 6 months (Fig. 4). None of the parameters examined improved significantly in the "non-increased" group.

Table 3. Values for and changes in food and nutrient intake during the intervention in the "increased" group (n = 8)

	Baseline (n = 8)	3 months (n = 8)	6 months (n = 8)	p value		Changes		
				Baseline vs 3 months	Baseline vs 6 months	at 3 months	at 6 months	p value
Energy intake (kcal)	34.1 ± 5.3	27.8 ± 6.6	30.3 ± 7.2	0.015*	0.025*	−6.3 ± 5.6	−3.8 ± 3.8	0.323
Protein (g)	36.1 ± 3.0	40.2 ± 6.1	39.9 ± 5.8	0.073	0.121	4.2 ± 5.6	3.8 ± 6.1	0.901
Fat (g)	33.1 ± 4.3	32.7 ± 6.1	33.4 ± 7.2	0.867	0.852	−0.4 ± 6.7	0.3 ± 4.4	0.805
Carbohydrates (g)	135.9 ± 9.9	134.0 ± 15.2	132.8 ± 17.7	0.743	0.543	−2.0 ± 16.2	−3.1 ± 13.9	0.877
Potassium (mg)	1,132 ± 235	1,756 ± 481	1,486 ± 345	0.012*	0.012*	624 ± 516	355 ± 281	0.221
Calcium (mg)	257 ± 147	313 ± 104	338 ± 120	0.331	0.221	56 ± 152	81 ± 171	1.000
Magnesium (mg)	139 ± 29	163 ± 40	159 ± 36	0.094	0.049*	24 ± 35	20 ± 24	0.279
Phosphorus (mg)	526 ± 52	610 ± 121	591 ± 115	0.066	0.114	84 ± 109	65 ± 101	0.721
Iron (mg)	4.0 ± 0.9	4.9 ± 0.8	5.3 ± 2.9	0.072	0.123	0.9 ± 1.2	1.3 ± 2.8	0.574
Zinc (mg)	4.2 ± 0.6	4.9 ± 0.6	4.6 ± 0.5	0.007*	0.265	0.6 ± 0.5	0.4 ± 0.9	0.465
Vitamin A (μgRE)	177 ± 54	361 ± 129	353 ± 104	0.010*	0.017*	183 ± 149	175 ± 103	0.904
Vitamin B_1 (mg)	0.55 ± 0.13	0.68 ± 0.20	0.60 ± 0.21	0.140	0.475	0.12 ± 0.21	0.05 ± 0.18	0.466
Vitamin B_2 (mg)	0.52 ± 0.08	0.61 ± 0.13	0.65 ± 0.19	0.014*	0.059	0.09 ± 0.08	0.14 ± 0.17	0.512
Niacin (mg)	8 ± 2	11 ± 4	10 ± 3	0.032*	0.075	3 ± 3	2 ± 3	0.543
Vitamin B_6 (mg)	0.56 ± 0.12	0.79 ± 0.18	0.75 ± 0.21	0.003*	0.006*	0.23 ± 0.15	0.19 ± 0.14	0.582
Vitamin B_{12} (μg)	1.8 ± 0.9	2.5 ± 1.1	3.7 ± 2.8	0.123	0.123	0.7 ± 1.5	2.0 ± 3.3	0.340
Folic acid (μg)	151 ± 48	239 ± 30	225 ± 83	0.003*	0.032*	88 ± 55	74 ± 79	0.442
Vitamin C (mg)	53 ± 24	80 ± 28	84 ± 44	0.052	0.074	27 ± 33	32 ± 43	0.798
Saturated fatty acids (g)	8.8 ± 2.1	8.6 ± 2.0	9.5 ± 2.4	0.912	0.406	−0.1 ± 2.7	0.8 ± 2.5	0.503
Mono-unsaturated fatty acids (g)	11.1 ± 2.0	10.7 ± 2.5	11.3 ± 3.1	0.757	0.748	−0.3 ± 2.8	0.2 ± 2.1	0.654
Poly-unsaturated fatty acids (g)	7.7 ± 1.4	6.9 ± 1.5	6.3 ± 1.5	0.323	0.102	−0.8 ± 2.2	−1.3 ± 2.0	0.629
Total dietary fiber (g)	7.4 ± 1.8	10.3 ± 1.6	8.9 ± 2.4	0.015*	0.105	2.8 ± 2.5	1.5 ± 2.3	0.287
Salt (g)	5.3 ± 1.3	5.8 ± 1.5	6.0 ± 2.1	0.380	0.237	0.5 ± 1.5	0.6 ± 1.4	0.865
PFC-P (%E)	14.4 ± 1.2	16.1 ± 2.5	15.9 ± 2.3	0.073	0.121	1.7 ± 2.2	1.5 ± 2.4	0.901
PFC-F (%E)	29.8 ± 3.8	29.5 ± 5.5	30.1 ± 6.5	0.867	0.851	−0.4 ± 6.0	0.3 ± 4.0	0.805
PFC-C (%E)	54.4 ± 3.9	53.6 ± 6.1	53.1 ± 7.1	0.745	0.543	−0.8 ± 6.5	−1.3 ± 5.6	0.877
Grains and cereals (g)	243 ± 23	221 ± 58	223 ± 46	0.674	0.208	−22 ± 75	−20 ± 47	0.505
Potatoes (g)	14 ± 15	30 ± 23	20 ± 18	0.213	0.372	16 ± 33	6 ± 17	0.445
Sugar (g)	4 ± 3	1 ± 1	6 ± 9	0.025*	0.889	−4 ± 4	2 ± 7	0.105
Nuts (g)	3 ± 5	1 ± 1	2 ± 3	0.612	0.753	−2 ± 5	−1 ± 7	0.878
Total vegetables (g)	124 ± 47	265 ± 68	223 ± 83	0.002*	0.022*	141 ± 80	99 ± 96	0.359
Green vegetables (g)	33 ± 12	95 ± 44	93 ± 43	0.007*	0.003*	62 ± 46	60 ± 37	0.920
White vegetables (g)	91 ± 51	169 ± 53	130 ± 56	0.025*	0.198	79 ± 62	39 ± 77	0.278
Fruits (g)	62 ± 34	30 ± 37	17 ± 23	0.123	0.036*	−32 ± 53	−45 ± 46	0.620
Mushrooms (g)	8 ± 8	22 ± 19	7 ± 6	0.105	0.658	14 ± 21	−1 ± 8	0.105
Algae (g)	5 ± 7	3 ± 3	5 ± 8	0.866	0.674	−2 ± 7	1 ± 9	0.574
Pulses (g)	45 ± 26	45 ± 43	19 ± 19	0.398	0.029*	0 ± 54	−26 ± 27	0.442
Fish and shellfish (g)	18 ± 13	26 ± 22	44 ± 30	0.208	0.108	8 ± 16	26 ± 40	0.269
Meat (g)	46 ± 23	57 ± 23	46 ± 25	0.305	0.974	11 ± 28	0 ± 29	0.464
Eggs (g)	23 ± 16	16 ± 26	25 ± 18	0.327	0.790	−7 ± 20	2 ± 20	0.405
Milk (g)	52 ± 61	46 ± 65	75 ± 48	0.889	0.398	−6 ± 90	23 ± 59	0.452
Oils and fats (g)	8 ± 5	6 ± 4	6 ± 2	0.362	0.485	−2 ± 7	−1 ± 6	0.800
Confectionery (g)	3 ± 7	15 ± 31	7 ± 13	0.686	0.345	12 ± 33	4 ± 13	0.505
Beverages (g)	77 ± 55	120 ± 139	77 ± 75	0.484	0.999	42 ± 119	0 ± 68	0.398

Values are presented as mean ± SD. Values of energy intake are presented per ideal weight, and values of other foods and nutrients are presented per 1,000 kcal. The relationships between baseline and 3 months or 6 months were assessed using the paired t test for normally distributed data and the Wilcoxon signed-rank test for non-normally distributed data. The relationships between changes at 3 and 6 months were assessed using the non-paired t test for normally distributed data and the Mann–Whitney U test for non-normally distributed data. *p<0.05. PFC-P, protein-energy rate; PFC-F, fat-energy rate; PFC-C, carbohydrate rate; total vegetables, the sums of green and white vegetable.

Discussion

We examined whether a nutritional intervention method that motivates frequent and active vegetable intake could help improve NAFLD. Similar to a previous study,[17] we found that the intervention increased the intake of vegetables, although no significant difference was observed after the intervention owing to large interindividual variability. Conversely, when the correlation between the change in green vegetable intake and that in body weight was examined, a significantly strong negative corre-

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Table 4. Comparison of the clinical examination data between baseline and at 3 and 6 months

	Increased group (n = 8)					Non-increased group (n = 7)				
				p value					p value	
	Baseline	3 months	6 months	Baseline vs 3 months	Baseline vs 6 months	Baseline	3 months	6 months	Baseline vs 3 months	Baseline vs 6 months
Weight (kg)	73.6 ± 15.6	73.3 ± 16.1	73.5 ± 15.4	0.327	0.624	70.1 ± 11.4	71.3 ± 11.1	71.7 ± 12.5	0.128	0.377
BMI (kg/m^2)	28.4 ± 4.0	28.3 ± 4.2	28.3 ± 4.1	0.327	0.779	27.1 ± 2.1	27.5 ± 2.6	28.2 ± 3.5	0.237	0.387
Skeletal muscle (kg)	25.4 ± 6.6	25.4 ± 6.9	25.5 ± 6.6	0.833	1.000	25.7 ± 6.0	26.2 ± 6.2	27.5 ± 5.2	0.018*	0.134
Body fat (%)	37.1 ± 7.1	37.0 ± 7.3	36.8 ± 7.9	0.400	0.575	33.6 ± 8.1	33.6 ± 8.6	33.8 ± 8.9	0.865	0.511
AST (IU/L)	30 ± 12	29 ± 11	29 ± 11	0.916	0.944	40 ± 25	43 ± 24	48 ± 26	0.072	0.397
ALT (IU/L)	43 ± 17	38 ± 11	35 ± 8	0.233	0.123	61 ± 46	70 ± 53	75 ± 41	0.203	0.075
γ-GTP (IU/L)	52 ± 27	47 ± 24	45 ± 23	0.183	0.091	54 ± 34	52 ± 31	89 ± 88	0.499	0.173
T-bil (mg/dl)	0.9 ± 0.3	1.2 ± 0.5	1.2 ± 0.3	0.123	0.036*	0.7 ± 0.1	0.8 ± 0.4	0.9 ± 0.4	0.735	0.345
TG (mg/dl)	154 ± 54	120 ± 35	115 ± 26	0.123	0.050*	173 ± 85	147 ± 57	156 ± 45	0.237	0.708
T-cho (mg/dl)	178 ± 21	198 ± 29	188 ± 27	0.310	0.398	207 ± 36	200 ± 51	197 ± 47	0.397	0.177
HDL-C (mg/dl)	54 ± 11	59 ± 13	56 ± 12	0.049*	0.344	53 ± 13	52 ± 12	51 ± 9	0.674	0.462
LDL-C (mg/dl)	106 ± 21	129 ± 22	115 ± 22	0.075	0.116	130 ± 29	128 ± 48	125 ± 48	0.672	0.581
Ferittin (ng/ml)	147 ± 85	145 ± 69	120 ± 55	0.345	0.116	204 ± 255	214 ± 259	223 ± 253	0.345	0.345
PLT (10^3/μl)	228 ± 84	227 ± 88	242 ± 110	0.779	0.575	237 ± 73	238 ± 74	251 ± 83	0.933	0.185
FBS (mg/dl)	93 ± 5	108 ± 19	101 ± 7	0.025*	0.012*	97 ± 19	100 ± 8	103 ± 15	0.237	0.075
HbA1c (%)	6.0 ± 0.6	5.9 ± 0.5	6.0 ± 0.5	0.407	0.607	6.2 ± 1.0	6.2 ± 1.1	6.1 ± 0.9	0.480	0.386
Type 4 collagen 7S (ng/ml)	5.2 ± 1.7	5.1 ± 1.4	5.2 ± 2.4	1.000	0.715	4.7 ± 0.7	5.0 ± 1.0	4.7 ± 1.6	0.144	0.655
M2BPGi	1.3 ± 1.2	1.2 ± 1.0	1.2 ± 1.2	0.893	0.080	0.6 ± 0.2	0.8 ± 0.6	0.6 ± 0.3	0.248	0.878
AST/ALT ratio	0.7 ± 0.3	0.8 ± 0.3	0.8 ± 0.2	0.018*	0.036*	0.7 ± 0.2	0.7 ± 0.1	0.7 ± 0.1	0.310	0.450
FIB-4 index	1.5 ± 1.3	1.6 ± 1.4	1.7 ± 1.5	0.123	0.123	1.2 ± 0.6	1.3 ± 0.8	1.3 ± 1.1	0.735	0.735
CAP (dB/m)	317 ± 65	304 ± 52	292 ± 39	0.575	0.208	336 ± 40	315 ± 36	322 ± 76	0.063	0.623
LSM (kPa)	12.9 ± 15.0	7.3 ± 3.3	8.7 ± 4.9	0.674	0.612	5.3 ± 1.8	4.9 ± 2.8	5.7 ± 2.1	0.237	0.591

Values are mean ± SD. The relationships between baseline, 3 months or 6 months were assessed using the paired t test for normally distributed data and the Wilcoxon signed-rank test for non-normally distributed data. *p<0.05. BMI, body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, γ-glutamyl transpeptidase; T-bil, total bilirubin; TG, triglycerides; T-cho, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; PLT, platelets; FBS, fasting blood sugar; HbA1c, hemoglobin A1c; M2BPGi, Mac-2 binding protein glycosylation isomer; AAR, AST/ALT ratio; CAP, controlled attenuation parameter; LSM, liver stiffness measurement.

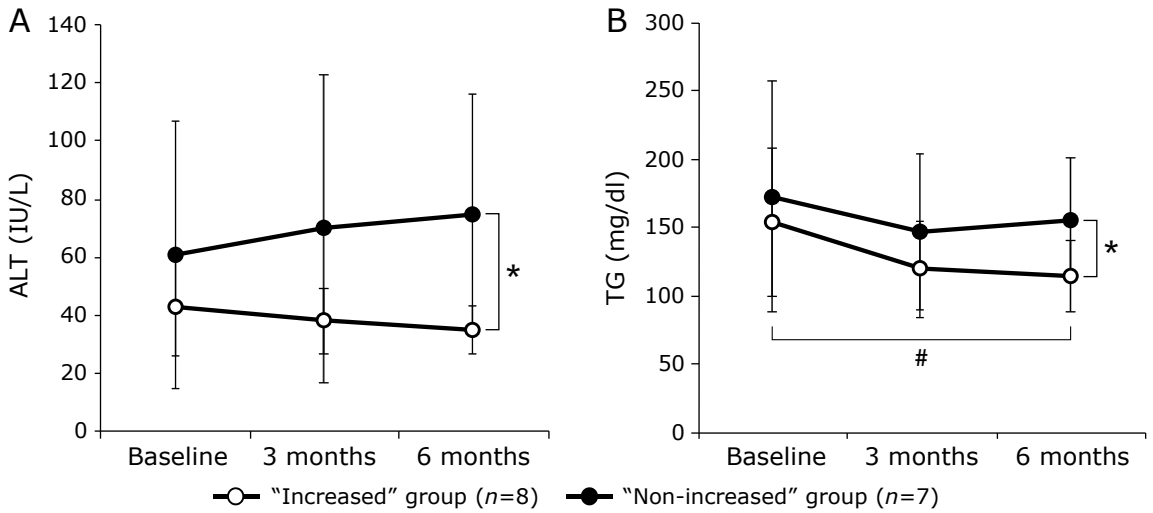

Fig. 4. Changes in ALT (A) and TG (B) levels during the 6-month intervention period in patients who had an increased vegetable consumption ("increased" group) and those who did not ("non-increased" group). Values are presented as mean ± SD. The relationships between baseline and 3 months or 6 months were assessed using the paired t test for normally distributed data and the Wilcoxon signed-rank test for non-normally distributed data. The relationships between both groups at baseline, 3 months, and 6 months were assessed using the non-paired t test for normally distributed data and the Mann–Whitney U test for non-normally distributed data. *p<0.05 between groups, $^{\#}p$<0.05 vs baseline. ALT, alanine aminotransferase; TG, triglyceride.

lation was found—this is a notable result due to weight loss being an important factor in improving NAFLD. This may be because an increased intake of vegetables, which has a low energy density, promotes a decreased intake of energy, leading to weight loss. Since previous studies have shown that patients with NAFLD have an extremely low vegetable intake compared to patients without NAFLD, the former may be expected to lose weight and improve their condition with even a small change in dietary behavior.

Due to the large individual differences in vegetable intake in this intervention study, we divided the patients into two groups—those whose vegetable intake increased after the intervention and those whose vegetable intake did not increase—and attempted to examine the effect of an increased vegetable intake on the improvement of NAFLD. The results showed that an increased intake of vegetables resulted in beneficial changes for patients with NAFLD; these include an increased intake of many vitamins, dietary fiber, and potassium, and a decreased intake of energy and fruit. Additionally, there was a significant increase in HDL-C, and a decrease in the ALT and TG concentration. Furthermore, a decreasing trend in the CAP and LSM was observed, although the trend was not significant. Vitamins that are abundant in vegetables, especially vitamins A and C, have antioxidant effects and hence may reduce oxidative stress in NAFLD.[24] In particular, a significant inverse association between vitamin C levels and NAFLD has been reported.[25] Dietary fiber has no clear direct relationship with NAFLD but is known to correlate inversely with obesity and the risk of developing T2DM.[26,27] It may also improve NAFLD by altering the intestinal microbiota and reducing oxidative stress caused by endotoxin inflow to the hepatic portal vein.[28] In a cross-sectional study involving Latino youth, consumption of nutrient-rich vegetables (dark green and deep orange/yellow) significantly reduced visceral fat levels and increased insulin sensitivity.[29] Because green vegetables contain higher amounts of carotenoids and phytochemicals than white vegetables,[30] they are more likely to be effective in improving NAFLD. We note that the software program used for nutritional analysis in this study was based on the Standard Table of Food Composition in Japan, which does not provide accurate intake values for carotenoids and phytochemicals; hence, the results for these variables were not provided here. It was not possible to determine whether the beneficial results were due to weight loss or the direct effects of the vegetable components; however, even a small change in dietary behavior may be useful in improving NAFLD.

It is necessary to understand why some of the patients in our study did not increase their consumption of vegetables during the intervention. Although the percentage of patients who cooked for themselves did not significantly differ between the "increased" and "non-increased" groups, it is possible that cooking frequency differed between the groups. Moreover, the participants might have reacted differently to the intervention depending on their socioeconomic and health conditions; these factors have been shown to influence vegetable consumption.[31] Nevertheless, diet therapy is the most important element in the treatment and prevention of NAFLD. In our study, some parameters did not significantly improve or worsen after the intervention, even in the "increased" group. In addition, our study included only a few participants, and they had various comorbidities, such as T2DM, hypertension, and dyslipidemia. Hence, vegetable intake may improve NAFLD in an indirect manner via mechanisms yet to be revealed. For the vegetable intake non-increase group, it may be necessary to combine approaches other than increasing vegetable intake, such as fish oil intake and exercise intervention, which has been reported to improve insulin resistance and lipid profile.[32]

Additional study limitations are as follows. No control group was enrolled, because this study was conducted to compare and validate results before and after intervention, and it was a trial study with a small number of participants. However, we believe that we were able to demonstrate the benefits of increased vegetable intake by comparing the group that did with the group that did not increase their vegetable consumption. Furthermore, some participants were only diagnosed by transient elastography, without undergoing liver biopsy which is the gold standard. Moreover, the dietary survey was analyzed based on records obtained on any 2 days, which might not accurately reflect habitual dietary intake. Lastly, follow-up after completion of the intervention trial was not possible in this study. Thus, future studies with more participants and long-term follow-ups are required.

In conclusion, we have developed a nutritional intervention protocol that can increase vegetable consumption in patients with NAFLD. The results suggest that increased vegetable consumption may lead to improvement in NAFLD. Our approach is advantageous in that it is simple and safe for patients to implement on a long-term basis. We expect that our intervention will be an effective and unique diet therapy and treatment for patients with NAFLD.

Author Contributions

HS, YK, and SW designed the study. HS, YK, YSu, SW, MT, YSh, KS, and YSa contributed to designing and performing the experiments. HS, YK, and TS analyzed and interpreted the data. HS and YK wrote the manuscript with input from other authors. YSu, SW, WA, YN, and MK conceived the study, researched the data, and reviewed the manuscript. All authors critically reviewed and approved the final version of manuscript.

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Abbreviations

ALT	alanine aminotransferase
AST	aspartate aminotransferase
CAP	controlled attenuation parameter
FIB-4	fibrosis-4
HDL-C	high-density lipoprotein cholesterol
LSM	liver stiffness measurement
NAFLD	non-alcoholic fatty liver disease
PLT	platelets
T2DM	type 2 diabetes mellitus
TG	triglycerides

Conflicts of Interest

YN received a scholarship from EA Pharma. Co. Ltd., collaboration research funding from Fujifilm Medical Co. Ltd., and lecture fees from Mylan EPD Co., Takeda Pharma. Co. Ltd., Mochida Pharma. Co. Ltd., EA Pharma. Co. Ltd., Otsuka Pharma. Co. Ltd., Nippon Kayaku Co. Ltd., and Miyarisan Pharma. Co. Ltd. Our study was partly financed by these funds. These companies had final approval of the manuscript but did not participate in the study design. They had no competing interests.

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