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Association between Inflammatory Biomarkers and Nutritional Status in Fatty Liver

Hee-Sook Lim [iD],[1] **Joungyun Choi** [iD],[2] **Bora Lee** [iD],[3] **Sang Gyune Kim** [iD],[4]
Young Seok Kim [iD],[4] **Jeong-Ju Yoo** [iD] [4]

[1]Department of Food Sciences & Nutrition, Yeonsung University, Anyang 14011, Korea
[2]Department of Health and Nutrition Survey, Korean Centers for Disease Control & Prevention, Cheongju 28159, Korea
[3]Department of Biostatistics, Graduate School of Chung-Ang University, Seoul 06974, Korea
[4]Division of Gastroenterology and Hepatology, Department of Internal Medicine, Soonchunhyang University Bucheon Hospital, Bucheon 14854, Korea

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Correspondence to
Jeong-Ju Yoo
Division of Gastroenterology and Hepatology, Department of Internal Medicine, Soonchunhyang University Bucheon Hospital, 170 Jomaru-ro, Wonmi-gu, Bucheon 14584, Korea.
E-mail: puby17@naver.com

ORCID iDs
Hee-Sook Lim [iD]
https://orcid.org/0000-0003-0745-8906
Joungyun Choi [iD]
https://orcid.org/0000-0002-3471-6782
Bora Lee [iD]
https://orcid.org/0000-0002-6322-5712
Sang Gyune Kim [iD]
https://orcid.org/0000-0001-8694-777X
Young Seok Kim [iD]
https://orcid.org/0000-0002-7113-3623
Jeong-Ju Yoo [iD]
https://orcid.org/0000-0002-7802-0381

ABSTRACT

The prevalence and progression of non-alcoholic fatty liver disease (NAFLD) is mediated via several factors correlating with hepatic necroinflammation (adipokines/cytokines). This study was performed to analyze the level of inflammatory markers according to the presence of NAFLD and to identify related nutritional factors. A total of 80 adults were classified into 2 groups (healthy and NAFLD), and their body composition, blood tests, and eating habits were evaluated. In addition, inflammatory markers (adiponectin, high-sensitivity C-reactive protein [CRP], and tumor necrosis factor-alpha [TNF-α]), nutrient intake status, and dietary quality were compared. The quality of diet was assessed according to the nutrient adequacy ratio and the mean adequacy ratio (MAR). The NAFLD group had a higher body mass index (p < 0.001) than the healthy group and also carried significantly higher CRP levels (p < 0.001) but lower adiponectin (p = 0.001). TNF-α levels increased significantly with fatty liver grade (p = 0.023). The NAFLD group showed significantly higher intake of energy, carbohydrates, iron, sodium, vitamin A and saturated fatty acids, but significantly lower intake of zinc and vitamin E than the healthy group. The MAR values were slightly higher in the NAFLD group but without any significant difference. The levels of adiponectin and vitamin E showed a significant inverse correlation (p < 0.05). Nutritional management of NAFLD patients is important, and the intake of antioxidant and anti-inflammatory nutrients such as zinc and vitamin E should be emphasized.

Keywords: Non-alcoholic fatty liver disease; Adipokines; Adiponectin; Nutrients; Vitamin E

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is defined as the excessive accumulation of triglycerides within the liver cells [1]. Accumulation of fat induces oxidative stress, peroxidation, cytokine response or other secondary damages, which may lead to non-alcoholic steatohepatitis or cirrhosis [2]. The high prevalence of NAFLD in metabolic disorders such as hypertension, dyslipidemia, abdominal obesity and type II diabetes is related to insulin resistance. Insulin resistance is an important mechanism underlying NAFLD [3]. Recently, the roles of various inflammatory markers have been reported. High-

sensitivity C-reactive protein (hs-CRP), an acute phase protein, is an inflammatory marker that leads to endothelial cell damage, which has also been associated with NAFLD [4]. Even when corrected for various pathological traits of NAFLD, hs-CRP is still an independent risk factor for NAFLD [5].

Adiponectin is secreted by adipose tissue. As the amount of adipose tissue in the body increases, the amount of adiponectin in the blood decreases [6]. Therefore, the level of adiponectin in blood is inversely correlated with the fat content and insulin resistance of the liver tissue [7]. Tumor necrosis factor-alpha (TNF-α) is an inflammatory cytokine, and the concentrations of which are increased in patients with NAFLD. The levels of TNF-α and TNF-α type 1 receptor increase in the liver tissue of NAFLD patients, coupled with the increased fibrosis in the liver tissue [8]. Similarly, the abnormal expression of various adipokines can be used to explain the relationship between metabolic and liver disorders.

The liver is an essential organ in the human digestive system. Patients with a defective liver often present with nutritional imbalance because the liver plays a significant role in metabolism and nutritional balance [9]. The risk factors for NAFLD include lack of exercise and westernized diet; however, the progression of a fatty liver disease can be slowed by combining weight control with healthy diet [10]. Preventative factors include a healthy intake of nuts, ω-3 fatty acids and antioxidant vitamins. The higher the intake of carbohydrates and simple sugars, the higher the NAFLD [11,12]. Micronutrient deficiencies also play a key role in fatty liver disease, and especially zinc, copper, iron, selenium, magnesium, vitamins A, C, D, and E and carotenoids have antioxidant, antifibrotic and immunomodulatory effects [13]. NAFLD patients showed decreased levels of vitamin A and E in their blood. Therefore, vitamin E supplementation may be used to treat fatty liver [14]. In specific cases, Mediterranean diet significantly ameliorated fatty liver disease without weight loss, suggesting the need for a customized dietary plan for individual patients [15].

The relationship between NAFLD, nutrition and food has been investigated; however, the effect of nutrition must be elucidated in the future. Studies investigating the role of dietary intake in Korean patients with NAFLD and thus the correlation between NAFLD and inflammatory markers have yet to be reported. Therefore, this study analyzed the adipokine levels in NAFLD patients, determined their nutritional intake and quality of diet, and established the correlation between adipokines and nutrition from various angles.

MATERIALS AND METHODS

Subjects
The subjects of this study were healthy adults over the age of 30 years who visited the university hospital health screening examination center between April and September 2013. Subjects with a previous history of viral or toxic hepatitis, chronic liver disease, significant alcohol consumption, those currently on medications for fatty liver disease and those taking health supplements or trying folk remedies, were excluded. A total of 92 patients were recruited, but subjects with chronic liver disease (n = 5), examination refusal (n = 4), and missing data (n = 3) were excluded. All potential subjects were informed of the study objectives, and the study included 80 participants finally. The subjects were divided into the healthy and the NAFLD groups depending on their liver conditions. NAFLD has been diagnosed as a moderate fatty liver disease via abdominal ultrasound while consuming less

than 140 g/week or 20 g/day of alcohol. The study protocol was approved by the Institutional Review Board (IRB) of Soonchunhyang University Bucheon Hospital (IRB No. SCHBC IRB 2013-08). Written informed consent was obtained from all patients. All the participants understood the study, and voluntarily agreed to participate in the study. The study protocol conformed to ethical guidelines of the World Medical Association Declaration of Helsinki.

Data collection

Parameters such as height, weight, body mass index (BMI) and the percentage of body fat were evaluated using the bio-electrical impedance fatness analyzer (Inbody 720; Bio-space, Seoul, Korea). The waist circumference was measured from the mid-point between the lowest rib and the highest part of the iliac spine, with the subject exhaling lightly. The hip circumference was considered as the largest part of the iliac bone. Both circumferences were measured twice, and the average value was used to calculate the waist/ hip ratio. The tests were conducted using blood samples drawn from venous blood in the upper arm after 12 hours of fasting. An automatic serum analyzer (Hitachi 7170; Hitachi Ltd., Tokyo, Japan) was used to conduct the basic blood tests and lipid analyses, while cytokines were measured using the automatic biochemical analyzer (Fuji DRI-CHEM 3500i; Fujifilm, Tokyo, Japan) and enzyme-linked immunosorbent assay test. The systolic and diastolic blood pressure levels were measured from the upper arm using an automatic blood pressure measurement tool, after 10 minutes of rest, and the presence of metabolic disorder was diagnosed based on NCEP ATP III criteria [16].

Dietary habits were identified via a survey of meal frequency, skipping meals, regularity of mealtime, eating speed, snacking frequency, frequency of eating out, exercise, smoking and drinking habits. Dietary intake was measured based on a 24-hours recall method, which was used to survey a 3-day meal. The consumed food was analyzed using a Computer Aided Nutritional Analysis Program for Professionals 4.0 (CAN-Pro 4.0; The Korean Nutrition Society, Seoul, Korea) based on the intake of individual nutrients. The quality of the diet was assessed according to the nutrient adequacy ratio (NAR) and mean adequacy ratio (MAR). The NAR is based on the intake recommended by the 2015 Dietary Reference Intakes for Koreans as a reference value [17]. The nutrients identified were protein, calcium, phosphorous, iron, zinc, vitamin A, vitamin B_1, vitamin B_2, vitamin B_6, niacin, vitamin C, and folic acid.

Definitions

The formula used to calculate NAR and MAR are as follows [18].

$$NAR = \text{nutrient intake/recommended nutrient intake}$$
$$MAR = \text{sum of NAR of nutrients/the number of nutrients}$$

The NAR calculated from the recommended nutrient intake represents the gender-specific and age-specific requirements of daily intake. A NAR close to 1 indicates that the amount of nutrient intake is close to the daily recommended intake. A value higher than 1 suggests that the intake exceeds the daily recommended intake, while a value less than 1 implies that the daily intake is not adequate to meet the daily recommended intake [18]. A fatty liver was diagnosed when the liver parenchymal echogenicity was higher than the kidney cortex. The severity of the fatty liver was graded as normal, mild, moderate, or severe according to echogenicity of the liver parenchyma [19]. The metabolic syndrome was defined by the presence of three or more of the following five risk factors: central obesity (men > 102 cm,

women > 88 cm), triglycerides ≥ 150 mg/dL, low high-density lipoprotein (HDL) cholesterol (men < 40 mg/dL, women < 50 mg/dL), high blood pressure (≥ systolic 130 mmHg or ≥ diastolic 85 mmHg), and impaired fasting glucose (≥ 110 mg/dL) [20].

Statistical analyses

The clinical and nutrient-related factors of the subjects were summarized as means and standard deviations for continuous variables, and frequency and percentage (%) for categorical variables. The significant differences between the healthy and the NAFLD groups were identified by independent 2-sample t-test and Wilcoxon's rank-sum test for continuous data and Fisher's exact test for categorical data. Multiple linear regression analysis was also carried out to identify the risk factors for fatty liver disease. All the analyses were conducted using R (version 3.6.1; The R Foundation for Statistical Computing, Vienna, Austria) and the statistical significance was set at 0.05 based on the 2-sided test.

RESULTS

Demographic status of the subjects

The average age of the subjects was 47.80 years, and the average age of the NAFLD group was significantly higher than that of the healthy group (50.91 years vs. 45.72 years). The NAFLD group also had a higher percentage of male subjects (68.2% vs. 37.5%). The average BMI was 24.50 ± 3.45 kg/m^2, which falls in the overweight category, whereas the NAFLD group had a BMI of 26.60 ± 3.53 kg/m^2, which falls in the obese category. The BMI differed significantly between the 2 groups. The waist-hip ratio was also significantly higher in the NAFLD group. Subjects with metabolic disorders constituted 12.5% of the healthy group and 50.0% of the NAFLD group, and were significantly higher in the NAFLD group (**Table 1**).

Comparative biochemistry of healthy and NAFLD groups

The laboratory test results of the subjects are shown in **Table 2**. The NAFLD group showed significantly higher levels of hemoglobin, hematocrit, glucose, aspartate aminotransferase, alanine aminotransferase (ALT), gamma glutamyl transferase, total cholesterol, triglyceride, and low-density lipoprotein (LDL) cholesterol, while the HDL cholesterol levels were significantly lower. In particular, the levels of blood glucose, ALT, triglyceride, and LDL cholesterol in the NAFLD group were higher than the normal range. Compared to a healthy

Table 1. Demographic data of the subjects

Variable	Total (n = 80)	Healthy (n = 48)	NAFLD (n = 32)	p value
Age (yr)	47.80 ± 9.62	45.72 ± 10.13	50.91 ± 7.98	0.017
Sex				0.012
Male	40 (50.0)	18 (37.5)	22 (68.8)	
Female	40 (50.0)	30 (62.5)	10 (31.2)	
BMI (kg/m^2)	24.50 ± 3.45	23.09 ± 2.60	26.60 ± 3.53	< 0.001
Body fat (%)	25.58 ± 6.41	24.85 ± 6.35	26.68 ± 6.44	0.211
WC (cm)	85.75 ± 12.13	83.89 ± 6.70	88.53 ± 17.14	0.094
WHR	0.90 ± 0.06	0.88 ± 0.05	0.94 ± 0.05	< 0.011
SBP (mmHg)	122.89 ± 16.75	118.00 ± 12.47	130.22 ± 19.68	0.003
DBP (mmHg)	73.71 ± 11.43	71.46 ± 10.02	77.09 ± 12.71	0.030
Metabolic syndrome (yes)	22 (27.5)	6 (12.5)	16 (50.0)	< 0.001

Data represented as mean ± standard deviation and number (%). The p value by χ^2 test (categorical variables) and independent t-test (continuous variables).
NAFLD, non-alcoholic fatty liver disease; BMI, body mass index; WC, waist circumference; WHR, waist-hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Table 2. Biochemical data of the subjects

Variable	Normal range	Total (n = 80)	Healthy (n = 48)	NAFLD (n = 32)	p value
Hgb (g/dL)	13–17	14.42 ± 1.45	14.07 ± 1.54	14.94 ± 1.19	0.008
Hct (%)	36–52	42.89 ± 3.61	42.00 ± 3.79	44.20 ± 2.92	0.007
Albumin (g/dL)	3.3–5.2	4.56 ± 0.24	4.54 ± 0.22	4.59 ± 0.27	0.409
Glucose (mg/dL)	60–108	102.28 ± 20.10	97.79 ± 15.40	109.00 ± 24.32	0.025
AST (IU/L)	5–40	25.38 ± 12.51	21.38 ± 8.04	31.41 ± 15.44	0.002
ALT (IU/L)	0–40	30.01 ± 24.50	21.59 ± 13.84	42.66 ± 31.06	< 0.001
ALP (IU/L)	44–119	81.23 ± 46.53	80.55 ± 57.82	82.25 ± 21.26	0.874
LDH (IU/L)	219–480	310.41 ± 66.39	299.98 ± 71.73	326.06 ± 54.87	0.085
γGT (IU/L)	12–73	44.05 ± 37.38	31.48 ± 31.92	62.91 ± 37.48	< 0.001
Calcium (mg/dL)	8.3–10	9.12 ± 0.38	9.11 ± 0.33	9.13 ± 0.45	0.841
Phosphorous (mg/dL)	2.5–4.5	3.67 ± 0.42	3.67 ± 0.44	3.67 ± 0.40	0.957
Cholesterol (mg/dL)	90–250	205.93 ± 43.07	197.33 ± 40.69	218.81 ± 43.94	0.028
Triglyceride (mg/dL)	0–200	153.50 ± 89.36	110.38 ± 68.81	218.19 ± 77.29	< 0.001
HDL-cholesterol (mg/dL)	35–50	53.64 ± 12.27	56.63 ± 12.18	49.16 ± 11.14	0.007
LDL-cholesterol (mg/dL)	0–140	134.31 ± 41.24	124.48 ± 40.06	149.06 ± 39.08	0.008
CRP (mg/dL)	0–0.5	0.20 ± 0.17	0.12 ± 0.19	0.34 ± 0.22	< 0.001
Adiponectin (μg/mL)	5–25	5.08 ± 3.81	7.16 ± 4.21	3.37 ± 2.28	0.001
TNF-α (pg/mL)		2.42 ± 1.98	2.13 ± 2.00	2.86 ± 1.90	0.109

Data represented as mean ± standard deviation and number (%). The p value by χ^2 test (categorical variables) and independent t-test (continuous variables).
NAFLD, non-alcoholic fatty liver disease; Hgb, hemoglobin; Hct, hematocrit; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; γGT, gamma-glutamyl transferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; CRP, C-reactive protein; TNF-α, tumor necrosis factor-alpha.

group, the NAFLD group showed significantly higher CRP levels (p < 0.001) but lower adiponectin (p = 0.001) (**Table 2**).

Healthy habits and eating behaviors of healthy group and NAFLD group

The dietary habits and lifestyles of the 2 groups are shown in **Table 3**: 34.5% of the total subjects ate 2 or fewer meals per day, with breakfast being the most frequently skipped meal; 73.7% had regular mealtimes, whereas 57.5% ate rapidly. One snack per day was the highest frequency of snacking, while 61.3% of the overall subjects never exercised. There were no significant differences in drinking frequency; and the highest frequency of eating out was once or twice a week. The healthy habits and eating behaviors of the 2 groups showed no significant differences, except for smoking, whereas the NAFLD group showed a significantly lower frequency of smoking (p < 0.007) (**Table 3**).

Comparison of nutrient intake and dietary quality between healthy and NAFLD groups

The results of nutrient intake and dietary quality of the healthy and the NAFLD groups are shown in **Tables 4** and **5**. Compared to the healthy group, the NAFLD group showed significantly higher intakes of energy, carbohydrate, iron, sodium, vitamin A and saturated fatty acids, while the intake of zinc and vitamin E was significantly lower. The dietary quality analysis of nutritional intake was based on gender and age. NAR showed that the NAFLD group showed significantly higher levels of iron and vitamin A intake, while the intake of zinc was significantly lower. MAR values were slightly higher in the NAFLD group: 1.24 ± 0.32 compared to 1.18 ± 0.29 in the healthy group with no significant difference between the 2 groups. However, when the nutrients that do not meet the daily intake recommendations were analyzed, the healthy group showed a lack of calcium, vitamin A, vitamin B_2 and folic acid, while the NAFLD group was deficient in calcium, zinc, vitamin B_2 and folic acid (**Tables 4** and **5**).

Table 3. Health habit and eating behavior of the subjects

Variable	Total (n = 80)	Healthy (n = 48)	NAFLD (n = 32)	p value
Frequency of meal				0.460
One time	1 (1.3)	1 (2.1)	0 (0.0)	
Twice	27 (33.2)	118 (37.5)	9 (28.1)	
Three times	52 (65.0)	29 (60.4)	23 (71.9)	
Skipping meal				0.299
Breakfast	18 (22.4)	14 (29.2)	4 (12.4)	
Lunch	7 (8.8)	4 (8.3)	3 (9.4)	
Dinner	3 (3.8)	1 (2.1)	2 (6.3)	
None	52 (65.0)	29 (60.4)	23 (71.9)	
Regularity of mealtime				0.381
Regular	59 (73.7)	33 (68.7)	26 (81.3)	
Irregular	21 (26.3)	15 (31.3)	6 (18.7)	
Eating speed				0.534
Fast	46 (57.5)	30 (62.4)	16 (50.0)	
Normal	28 (35.0)	15 (31.3)	13 (40.6)	
Slow	6 (7.5)	3 (6.3)	3 (9.4)	
Frequency of snack				0.196
1 time/day	38 (47.5)	23 (47.9)	15 (46.9)	
2 times/day	10 (12.4)	7 (14.6)	3 (9.4)	
3 times/day	21 (26.3)	12 (25.0)	9 (28.1)	
≥ 4 times/day	4 (5.0)	4 (8.3)	0 (0.0)	
None	7 (8.8)	2 (4.2)	5 (15.6)	
Exercise				0.244
None	49 (61.3)	29 (60.4)	20 (62.4)	
3–4 times/week	21 (26.3)	15 (31.3)	6 (18.8)	
Daily	10 (12.4)	4 (8.3)	6 (18.8)	
Smoking				0.007
Nonsmoker	48 (60.0)	35 (72.9)	13 (40.6)	
Smoker at past	14 (17.5)	4 (8.3)	10 (31.3)	
Smoker	18 (22.5)	9 (18.8)	9 (28.1)	
Drinking				0.831
Nondrinker	31 (38.8)	19 (39.6)	12 (37.5)	
Drinker at past	8 (10.0)	4 (8.3)	4 (12.5)	
Drinker	41 (51.2)	25 (52.1)	16 (50.0)	
Frequency of eating out				0.124
Daily	5 (6.3)	3 (6.3)	2 (6.3)	
≥ 4 times/week	23 (28.7)	18 (37.5)	5 (15.6)	
1–2 times/week	34 (42.5)	20 (41.6)	14 (43.7)	
1–2 times/month	17 (21.2)	7 (14.6)	10 (31.3)	
None	1 (1.3)	0 (0.0)	1 (3.1)	

Data represented as number (%). The p value by χ^2 test (categorical variables).
NAFLD, non-alcoholic fatty liver disease.

Correlation between inflammatory markers and vitamin E level

The nutritional factors associated with inflammatory markers that showed a significant difference in the nutrient intake between the healthy and the NAFLD groups were identified. As mentioned earlier, the CRP levels in the fatty liver group were significantly higher than in the healthy group. Comparing TNF-α according to fatty liver grade, TNF-α level was 2.17 ± 0.86 in mild grade, 3.01 ± 1.04 in moderate grade, and 5.50 ± 0.92 in severe grade. In addition, the TNF-α levels increased significantly with the fatty liver grade (p = 0.023). A significant inverse correlation was found between adiponectin and vitamin E (p < 0.05) (**Figures 1** and **2**).

Factors affecting fatty liver disease development

Univariate analysis was conducted to confirm the factors related to NAFLD occurrence. Variables identified in this analysis were further subjected to multivariate analysis. The results

Table 4. Nutrients intake of the subjects

Variable	Total (n = 80)	Healthy (n = 48)	NAFLD (n = 32)	p value
Energy (kcal)	1,897.44 ± 408.38	1,820.00 ± 357.03	2,028.61 ± 449.59	0.018
Protein (g)	81.42 ± 23.37	77.60 ± 20.14	87.15 ± 26.84	0.074
Fat (g)	50.94 ± 19.10	49.8 ± 18.2	52.6 ± 20.5	0.520
Carbohydrate (g)	273.46 ± 65.94	258.85 ± 62.04	295.37 ± 66.46	0.014
Fiber (g)	22.10 ± 7.52	21.90 ± 7.70	22.41 ± 7.32	0.766
Calcium (mg)	576.28 ± 231.08	577.72 ± 264.80	574.1 ± 172.5	0.946
Phosphorous (mg)	1,133.90 ± 324.34	1,096.94 ± 339.12	1,189.34 ± 297.38	0.214
Iron (mg)	15.41 ± 5.21	14.14 ± 4.45	17.32 ± 5.74	0.007
Sodium (mg)	5,435.70 ± 1,851.07	4,868.42 ± 1,840.64	6,286.63 ± 1,532.85	0.001
Potassium (mg)	2,984.46 ± 976.88	2,827.25 ± 983.61	3,220.27 ± 932.41	0.078
Zinc (mg)	10.89 ± 6.89	12.43 ± 8.30	8.58 ± 2.73	0.014
Vitamin A (µgRE)	817.89 ± 423.52	721.91 ± 389.82	961.85 ± 437.09	0.012
Retinol (µg)	99.44 ± 976.88	105.70 ± 136.24	90.04 ± 63.09	0.546
β Carotene (µg)	3,665.69 ± 2,242.78	3,648.34 ± 2,189.41	3,691.72 ± 2,355.79	0.933
Vitamin B_1 (mg)	1.19 ± 0.38	1.14 ± 0.38	1.24 ± 0.38	0.289
Vitamin B_2 (mg)	1.13 ± 0.40	1.08 ± 0.42	1.21 ± 0.35	0.139
Vitamin B_6 (mg)	2.35 ± 0.90	2.44 ± 0.93	2.21 ± 0.85	0.276
Niacin (mgNE)	17.56 ± 6.14	16.71 ± 5.26	18.83 ± 7.16	0.130
Vitamin C (mg)	109.68 ± 79.29	106.07 ± 76.18	115.08 ± 84.69	0.621
Folic acid (µg)	283.33 ± 121.50	291.09 ± 134.83	271.67 ± 99.11	0.487
Vitamin E (mg α-TE)	14.98 ± 6.93	16.37 ± 7.55	12.89 ± 5.32	0.027
Cholesterol (mg)	330.68 ± 187.34	299.48 ± 168.48	377.47 ± 206.48	0.068
Total fatty acids (g)	29.86 ± 16.73	27.64 ± 15.42	33.18 ± 18.27	0.147
SFA (g)	9.76 ± 6.22	8.21 ± 4.48	12.08 ± 7.69	0.013
MUFA (g)	11.36 ± 8.00	10.30 ± 7.38	12.94 ± 8.69	0.148
PUFA (g)	8.95 ± 4.92	9.15 ± 5.07	8.65 ± 4.76	0.656

Data represented as mean ± standard deviation. The p value by independent t-test (continuous variables).
NAFLD, non-alcoholic fatty liver disease; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Table 5. Diet quality of the subjects

Variable	Total (n = 80)	Healthy (n = 48)	NAFLD (n = 32)	p value
NAR				
Protein	1.48 ± 0.43	1.41 ± 0.37	1.58 ± 0.49	0.074
Calcium	0.77 ± 0.31	0.77 ± 0.35	0.77 ± 0.23	0.946
Phosphorous	1.62 ± 0.46	1.57 ± 0.48	1.70 ± 0.42	0.216
Iron	1.54 ± 0.52	1.41 ± 0.44	1.73 ± 0.57	0.007
Zinc	1.21 ± 0.77	1.39 ± 0.92	0.95 ± 0.30	0.004
Vitamin A	1.09 ± 0.56	0.96 ± 0.52	1.28 ± 0.58	0.015
Vitamin B_1	1.19 ± 0.38	1.15 ± 0.39	1.25 ± 0.38	0.289
Vitamin B_2	0.87 ± 0.31	0.83 ± 0.32	0.93 ± 0.27	0.136
Vitamin B_6	1.81 ± 0.70	1.88 ± 0.72	1.70 ± 0.66	0.279
Niacin	1.10 ± 0.38	1.04 ± 0.33	1.18 ± 0.45	0.133
Vitamin C	1.10 ± 0.79	1.06 ± 0.76	1.15 ± 0.85	0.618
Folic acid	0.71 ± 0.31	0.73 ± 0.34	0.67 ± 0.27	0.389
MAR	1.21 ± 0.38	1.18 ± 0.29	1.24 ± 0.32	0.398

Data represented as mean ± standard deviation. The p value by independent t-test (continuous variables).
NAFLD, non-alcoholic fatty liver disease; NAR, nutrient adequacy ratio; MAR, mean adequacy ratio.

showed that age (odds ratio [OR], 1.09; 95% confidence interval [CI], 1.01–1.19), CRP (OR, 1.55; 95% CI, 1.21–2.26) and metabolic disorders (OR, 4.36; 95% CI, 1.24–16.84) significantly correlated with the occurrence of fatty liver. The Hosmer-Lemeshow goodness-of-fit test for the final model yielded a value of 5.801 (p = 0.67) which indicated a non-significant differences between observed and predicted occurrences (**Table 6**).
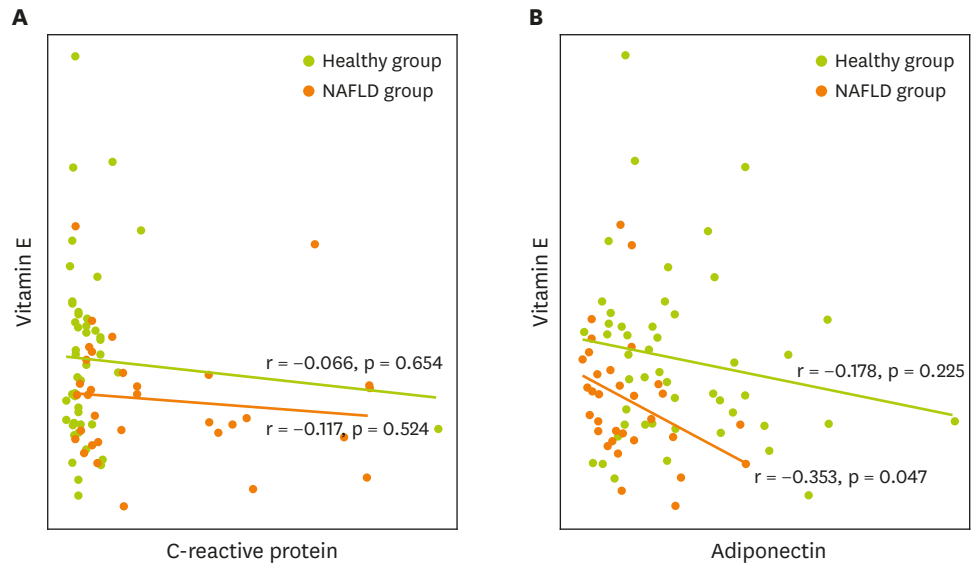
Figure 1. Correlation of adipokines and vitamin E in the subjects.
(A) Correlation of C-reactive protein and vitamin E, (B) Correlation of adiponectin and vitamin E.
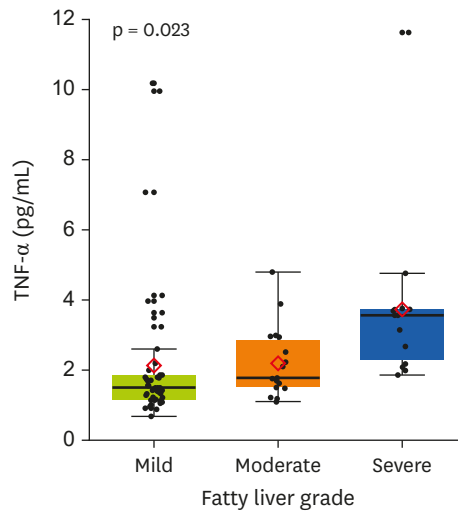

Figure 2. Differences in the level of TNF-α by fatty liver grade.
TNF-α, tumor necrosis factor-alpha.

DISCUSSION

In this study, we found that inflammation was closely related not only with the occurrence of fatty liver disease, but also its severity, which is probably related to inadequate micronutrient intake such as vitamin E or zinc.

NAFLD which accounts for a high proportion of chronic liver disease, is increasing in frequency in Korea due to the westernized diet, imbalance nutrition and lifestyle changes. Males are known to be more susceptible to NAFLD and the disease is closely related with metabolic disorders and visceral obesity [21]. The results of this study also showed a higher number of males in the NAFLD group, with higher percentage of body fat and abdominal obesity

Table 6. Logistic regression analysis for NAFLD

Variable	Univariable		Multivariable	
	OR (95% CI)	p value	OR (95% CI)	p value
Demographic factor				
Age	1.07 (1.01–1.14)	0.023	1.09 (1.01–1.19)	0.042
Male	3.67 (1.45–9.79)	0.007	2.45 (0.58–13.34)	0.267
Inflammatory marker				
CRP	1.55 (1.2–2.29)	0.006	1.55 (1.21–2.26)	0.003
Adiponectin	0.73 (0.58–0.88)	0.002	0.78 (0.55–1.03)	0.119
TNF-α	1.21 (0.96–1.61)	0.131		
Lifestyle-related factor				
Obesity	3.93 (1.55–10.46)	0.005		
Metabolic syndrome	7.00 (2.43–22.56)	0.001	4.36 (1.24–16.84)	0.025
Regularity	1.97 (0.69–6.17)	0.217		
Exercise	0.92 (0.36–2.29)	0.851		
Smoking	1.70 (0.58–4.95)	0.328		
Drinking	0.92 (0.37–2.26)	0.855		

Goodness-of-fit: Hosmer-Lemeshow statistic = 5.801, DF = 8, p = 0.67.
NAFLD, non-alcoholic fatty liver disease; OR, odds ratio; CI, confidence interval; CRP, C-reactive protein; TNF-α, tumor necrosis factor-alpha.

and higher frequency of metabolic disorders. The prevalence of NAFLD increases to 58% in overweight people (BMI ≥ 25 kg/m^2), rising to 74%–98% in obese people (BMI ≥ 30 kg/m^2), who underwent bariatric surgery [22]. The role of intestinal microbiota has recently been identified as the leading cause of obese NAFLD, but factors other than endocrine disease such as excessive energy intake, imbalanced diet and sedentary lifestyles are implicated in obesity and metabolic disorders, which leads to a stronger correlation between NAFLD and obesity.

The accumulation of fat in the liver occurs due to abnormal fatty acid metabolism, including not only synthesis but hepatic uptake of free fatty acids and triglyceride export [23,24]. These changes may lead to inflammation, oxidative stress and even abnormal adipokine synthesis, which has implications for downstream signaling pathways [25]. The analyses of subjects showed that the NAFLD group had higher triglyceride and LDL cholesterol levels, but lower HDL cholesterol. Thus, various strategies including weight management, diet therapy, exercise and medication should be implemented for NAFLD patients with abnormal lipid metabolism.

The pathogenesis of NAFLD is strongly mediated via dysregulation of adipokines and cytokines [26]. Adiponectin is hepatoprotective and increases the viability of hepatic cells and hepatic insulin sensitivity, and attenuates liver inflammation and fibrosis [27]. However, the effect is only transient and may be affected or altered by genetic or external factors, which requires customized treatment strategies targeting adipokines in the NAFLD [28]. In this study, the NAFLD group showed a normal range of adipokines, but significantly lower levels of adiponectin and higher hs-CRP, which underscored the need to validate the interactions between various factors.

The subjects' lifestyles were also compared. The NAFLD group had similar dietary habits compared to the healthy group but showed lower proportion of subjects who exercised and a higher percentage of smoking subjects. The management of NAFLD recommends that lifestyle management is the primary option, with medications playing only a supplementary role as needed [29]. In this context, diet therapy may help to improve insulin resistance and hepatic steatosis in NAFLD patients [30]. Exercise may decrease hepatic steatosis, and the cohort study reported that adequate strength exercises improved liver enzymes and insulin resistance independent of weight loss [31].

A study investigating the dietary habits and actions of NAFLD patients identified excessive intakes of energy, carbohydrate, and fat but lack of necessary vitamin and mineral intake as key risk factors [32]. This study also identified higher levels of energy, carbohydrate, sodium and saturated fatty acid intake and lower zinc and vitamin E intake in the NAFLD group compared to the healthy group, suggesting a similar trend. A recent study proposed that excessive intake of simple sugars such as sucrose and fructose is the main risk factor for NAFLD. The average daily intake of calories and soft drinks by the NAFLD patients was twice as high as that of non-NAFLD subjects [33]. Excessive intake of carbohydrates activates sterol regulatory element-binding protein-1c, which leads to the synthesis of enzymes needed for fatty acid production, which is also related to obesity [34]. Intake of an abundance of carbohydrates leads to a rapid increase in the serum glucose level and response hypoglycemia, resulting in feelings of empty stomach, increased appetite and overeating [35]. This study failed to identify the causative food behind increased carbohydrate intake, but it did identify the need to control the carbohydrate and fat intake of NAFLD patients.

The increased oxidative stress due to vitamin E deficiency is considered as one of the key factors in the onset and development of NAFLD, and vitamin E is a key therapeutic agent [36]. Progression from NAFLD to nonalcoholic steatohepatitis is influenced by oxidative stress. The fibrosis scores of NAFLD patients are inversely correlated with total antioxidant status, which points to the importance of antioxidants [37]. Well-known antioxidant nutrients include vitamins E, C, and A, zinc and selenium. In this study, the NAFLD group had a significantly lower intake of vitamin E and zinc, while vitamin E showed an inverse correlation with adiponectin among all adipokines. Adiponectin, an endogenous adipokine, increases insulin sensitivity via PPARγ-mediated upregulation. Since vitamin E increases the expression of adiponectin, it improves insulin resistance in the body [38]. Vitamin E is also implicated in the expression of genes that regulate the broad cellular functions such as cell cycle, inflammation and cell adhesion. It is a fat-soluble vitamin with antioxidant property, and is present in high concentrations in fish, some oils and nuts as well as green vegetables [39]. NAFLD patients need more vitamin E in an effort to combat oxidative stress; however, it was evident that these patients actually suffer from insufficient intake. Therefore, management strategies must include ways to meet the daily recommended intake of vitamin E.

The study limitations include a relatively small number of patients in a retrospective study, and the possibility of recall bias. Despite the small sample size, this study measured the adipokine levels in NAFLD patients and established their correlation with nutrient levels. In contrast, large-scale nutrition studies often excluded key nutritional details, whereas our study presented more comprehensive nutritional information.

CONCLUSION

Our study showed that a balanced diet rich in antioxidant and anti-inflammatory nutrients is more effective dietary management for NAFLD patients. In addition, NAFLD patients must be managed via a multidisciplinary approach closely monitoring their weight, blood lipid levels and other factors that affect metabolic status, which underscores the need for further studies in the field of diet and nutrition.

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