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No association between fruits or vegetables and non-alcoholic fatty liver disease in middle-aged men and women

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ABSTRACT

Objective: It has been hypothesized that fruit and vegetable intake is inversely associated with non-alcoholic fatty liver (NAFLD). However, some studies have speculated that fruit intake might be positively associated with NAFLD owing to the fructose content of the fruit. This might cause consumers to hesitate consuming fruit. The aim of this study was to assess the association between fruit and vegetable consumption and NAFLD.
Methods: This was a cross-sectional study of 977 men and 1467 women, 40 to 69 y of age without current liver disease other than NAFLD and who did not report excess alcohol intake (i.e., $\geq$30 g/d in men and $\geq$20 g/d in women). Dietary intake was assessed using a validated diet history questionnaire. NAFLD was diagnosed from abdominal ultrasonography results. The association between quartiles of fruit or vegetable consumption and NAFLD prevalence was assessed using logistic regression analysis, with lowest category as reference.
Results: The prevalence of NAFLD was 34.9% in men and 11.7% in women. Adjusted for age and lifestyle factors, fruit intake was inversely associated with NAFLD in both sexes. However, these associations disappeared after further adjustment for body mass index. Consumption of total vegetables was not associated with NAFLD. In women, a linear inverse association was demonstrated between green and yellow vegetable intake and NAFLD in the final model ($P_{trend} = 0.04$), but odds ratios for any intake category did not reach significance.
Conclusions: No obesity-independent association was found between fruit or vegetable intake and NAFLD. According to the findings of this study, Japanese do not need to restrict fruit consumption to limit fructose intake as a means of preventing NAFLD.

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Introduction

Non-alcoholic fatty liver disease (NAFLD) is characterized by fat accumulation in the liver not caused by excess alcohol intake. NAFLD is a common liver disease both in Western [1,2] and Asian countries [3] and can proceed to more severe liver diseases such as liver cirrhosis or hepatocellular carcinoma [4]. Thus, it is important to clarify the lifestyle factors that relate to NAFLD. Although the

pathogenesis of NAFLD has not been fully revealed, it is strongly associated with increased peripheral fatty acid flux in the insulin-resistant state and also with de novo lipogenesis [5].

It is well known that fruit and vegetable intake is inversely associated with several insulin resistance-related diseases. This has naturally led to the hypothesis that fruit and vegetable intake also is associated with a low prevalence of NAFLD. Fruits and vegetables supply potentially protective dietary factors, such as antioxidant vitamins, for NAFLD [6]. In fact, the Mediterranean diet, which is rich in antioxidants from fruits and vegetables, is considered a potential dietary regimen for NAFLD prevention, although studies regarding its efficacy are limited cross-sectional studies and intervention studies in high-risk populations [7]. On the contrary, one study showed that fruit intake was positively associated with

NAFLD prevalence in Chinese women [8]. Another study in Korea showed that vegetable intake, but not fruit intake, was inversely associated with NAFLD prevalence, and the authors speculated that the lack of an inverse relation of fruits to NAFLD might be due to fructose [9]. These findings are partly supported by studies using dietary pattern analysis: NAFLD prevalence was positively associated with a "fruits" pattern [10] and inversely with a "grain and vegetables" pattern [11].

Despite the failure of epidemiologic studies to demonstrate an association of fructose with NAFLD pathogenesis independently of excess energy intake [12], fructose still has received attention as a potential risk factor for NAFLD, mainly based on biochemical knowledge [13,14]. Some consumers might not accurately understand recommendations for regulating added sugar intake or distinguish food sources of added versus naturally occurring sugar [15]. Indeed, even patients with diabetes educated by health professionals incorrectly believe that they need to avoid foods containing any sugar [16]. Thus, misunderstanding the relationship between health and sugar is considered common and might interfere with the global health campaign recommending fruit and vegetable consumption [17]. Additional studies examining the relationship between the intake of fruits and vegetables with NAFLD may help develop dietary strategies to prevent NAFLD. To our knowledge, such studies are few and none have been conducted in a Japanese population.

In the present study, we assessed the relationship between the consumption of fruits and vegetables and the prevalence of NAFLD in middle-aged men and women. Considering that each subtype of vegetable might be differently associated with insulin resistance [18], we also assessed the relationship by vegetable subtype.

Material and methods

Study setting and participant selection

The participants in this cross-sectional study were recruited at the Center for Preventive Medicine at St. Luke's International Hospital in Tokyo, Japan. All individuals who planned to undergo a health examination during the investigation period (from January to April 2015) received a brief-type self-administered diet questionnaire (BDHQ) and a document informing them of the objectives of the study. Of the eligible participants (4758 men and 5112 women), 3163 men and 3662 women consented to participate. Further information about this study can be found in a previous report [19]. This study was conducted under approval of the Institutional Review Boards of Ochanomizu University and St. Luke's International Hospital.

To distinguish NAFLD from alcoholic fatty liver, hepatotoxic alcohol consumption was defined as ≥30 g/d in men and ≥20 g/d in women [20]. To minimize the influence of over- and underreporting alcohol intake, we adjusted alcohol intake for energy intake under the assumption that each individual takes their estimated energy requirement (EER) [21], rather than their reported energy intake. Energy-adjusted alcohol intake was calculated using following formula: reported alcohol intake (g/d) × EER for the second physical activity level (kcal/d)/energy intake estimated from the diet questionnaire (kcal/d). Individuals under suspicion of misreporting their energy intake were identified according to the following definition: having <50% of the EER for the lowest physical activity level (underreporting) or >1.5 times the EER for the highest physical activity level (overreporting) [21].

Finally, individuals meeting the following criteria were excluded from the analysis:

- non-Japanese (n = 33);
- self-reported possibility of pregnancy (n = 5);
- <40 y of age (n = 656) or ≥70 y of age (n = 787);
- hepatotoxic alcohol consumption in men (n = 1083) and women (n = 550);
- other self-reported liver diseases (n = 27);
- positive or unknown hepatitis B or C serology (n = 34);
- history of gastrectomy (n = 30);
- use of medication for hyperlipidemia or diabetes (n = 457);
- use of hepatotoxic drugs (n = 43);
- use of hormone replacement therapy (only in women, n = 426);

- evidence of type 2 diabetes mellitus (fasting blood glucose ≥126 mg/dL, or hemoglobin [Hb]A1c ≥6.5%; n = 56);
- dietary control with health professionals (n = 59);
- logical errors in BDHQ (n = 10);
- suspicion of over- (n = 3) or underreporting (n = 108) of energy intake; and
- BDHQ answered outside of the period from January to April 2015 (n = 14).

The remaining 977 men and 1467 women were included in the analysis.

Dietary assessment

Dietary factors were assessed using the BDHQ, which was validated using 16-d dietary records as reference [22,23]. Total vegetable intake was calculated based on 11 items that could be classified into the following subtypes: "green and yellow vegetables" (e.g., carrots and pumpkins, tomatoes, and green leafy vegetables), "other vegetables" (e.g., raw vegetables used in salad, cabbages and Chinese cabbage, radishes and turnips, and other root vegetables), "pickled vegetables" (e.g., salted green and yellow vegetable pickles and other salted vegetable pickles), "mushrooms," and "seaweeds." Fruit intake was estimated based on the three categories of "citrus fruits," "strawberries, persimmons, and kiwi fruits," and "other fruits." Vegetable juice, potatoes, and pulses were not considered as vegetables, and fruit juice was not considered as fruit. Juices were excluded from the calculation of fruit and vegetable intake because they were considered to be less satiating compared with whole fruits and vegetables [24].

The subtype classification of vegetables was done according to the food grouping method in the validation study of the BDHQ [17]. Although total vegetable intake included intake of pickled vegetables, mushrooms, and seaweed, the association between these subtypes of vegetables and NAFLD prevalence was not assessed in this study because these vegetables accounted only for a small part of total vegetable intake in both sexes. In Japan, "green and yellow vegetables" are defined as vegetables rich in β-carotene (≥600 μg/100 g vegetable) or vegetables significantly contributing to total β-carotene consumption because of their frequent consumption. β-carotene is one of the major antioxidants in the habitual diet and its intake is reported to be inversely associated with type 2 diabetes [19]. Thus, we assessed green and yellow vegetable intake and its association with NAFLD separately from other vegetables.

Spearman's correlation coefficient between fruits, total vegetables, green and yellow vegetables, and other vegetables estimated from dietary records and those estimated from the BDHQ were 0.55, 0.51, 0.28, and 0.50 in men and 0.41, 0.55, 0.37, and 0.46 in women, respectively [22]. These values are considered to be comparable to those of other questionnaires in Japan [25].

Ascertainment of NAFLD

Fatty liver was diagnosed by abdominal ultrasonography based on four common criteria:

1. enhanced liver echogenicity,
2. greater echogenicity in liver than kidneys,
3. deep attenuation, and
4. vascular blurring.

Examinations were performed by trained ultrasound technicians and reviewed by board-certified radiologists and physician for ultrasonography. These personnel were blinded to both the answers to items in the BDHQ and the results of the annual health checkup.

All fatty liver observed in this study was considered as NAFLD because participants with the possibility of alcoholic fatty liver or secondary fatty liver (positive results of hepatitis B or C serology, history of gastrectomy, and hepatotoxic drug use) were excluded.

Other variables

Height, body weight, HbA1c, blood glucose, hepatitis B antigen, and hepatitis C antibody were measured as standard items of the health check. Body mass index (BMI) was calculated by dividing body weight (kg) by the square of height (m^2). Information about medical history, present diseases and treatments, smoking habit, and habitual exercise was collected using a medical interview sheet. Habitual exercise was defined as the number of days per week spent doing ≥20 min of sports or walking and categorized as *almost every day, 3 to 5 d/wk, 1 to 2 d/wk,* or *almost never.*

Statistical analysis

All analyses were conducted separately by sex. All dietary factors were adjusted for total energy intake by the nutrient density method. Participants were

divided into quartiles according to fruit intake, total vegetable intake, green and yellow vegetable intake, and other vegetable intake. Linear associations between these intake categories and other variables were evaluated using a linear-by-linear association test for categorical variables and regression analysis with assignment of median values to each category for continuous variables. Associations between NAFLD prevalence and quartiles of fruit and vegetable intake were assessed using logistic regression analysis with the lowest quartile as the reference. The following possible confounding factors were adjusted: age (continuous), exercise <1 d/wk (binomial), current smoking (binomial), total energy intake (continuous), alcohol intake (continuous), eicosapentaenoic acid + docosahexaenoic acid (EPA + DHA) intake (continuous), coffee intake (continuous), soft drink intake (continuous), total vegetable intake (continuous, for odds ratios [ORs] for fruits), fruit intake (continuous, for ORs for total vegetable, green and yellow vegetable, and other vegetable), and BMI (kg/m^2, continuous). Dietary factors were considered in accordance with previous studies [26–28]. Adjustment for intakes of α-linolenic acid or total ω-3 fatty acids instead of EPA + DHA intake did not change the results.

Statistical analyses were conducted using SPSS, version 24 (IBM, Armonk, NY, USA). $P < 0.05$ was considered statistically significant.

Results

Mean (SD) fruit intake was 48.8 (40.6) g/1000 kcal in men and 67.2 (47.5) g/1000 kcal in women. Mean (SD) total vegetable intake was 136.6 (69.8) g/1000 kcal in men and 182.1 (89.4) g/1000 kcal in women. Fruits contributed to 21.7% of total vitamin C intake in men and 25% in women. Vegetables were a more important dietary source of vitamin C (41.9% in men and 44.3% in women) than fruits. A substantial part of dietary fiber was derived from vegetables (41% in men and 46.5% in women), as well as fruits (22.2% in men and 22.9% in men). Green and yellow vegetables provided a large part of total β-carotene intake (85.7% in men and 89.1% in women). Individuals with high intake of fruits were older, had a lower BMI, were more engaged in exercise, and consumed more fat (women only), protein, EPA + DHA, and total vegetables (Tables 1 and 2). Individuals with high intake of total vegetables were older, had a lower BMI (women only), were more likely to be engaged in exercise, and consumed more fat, protein, EPA + DHA, and fruits.

NAFLD was diagnosed in 34.9% (341 of 977) of men and 11.7% (171 of 1467)) of women. In both sexes, fruit intake was inversely associated with NAFLD prevalence in the age-adjusted model (Tables 3 and 4). Although the association did not change after further adjustment for lifestyle factors, it became non-significant after adjustment for BMI. In women but not in men, total vegetable intake was inversely associated with NAFLD prevalence in the age-adjusted model, but the association became non-significant after

adjustment for lifestyle factors and BMI. Also in women only, there was a linear inverse association between quartiles of green and yellow vegetable intake and NAFLD (P_{trend} = 0.04), but ORs for upper categories did not reach significance. Other vegetable intake was not associated with NAFLD prevalence in any model in either sex.

Discussion

In this study, fruit and total vegetable intakes were not inversely associated with NAFLD prevalence. In women, a linear inverse association between green and yellow vegetables was observed in the obesity-adjusted model, but ORs for any upper intake quartiles were not significant.

Mainly based on animal studies, fructose is considered to be one of the most important dietary factors in the pathogenesis of NAFLD [13,14]. In Chinese women, NAFLD prevalence was positively associated with a dietary pattern characterized by high intake of foods rich in fructose and with fruit intake itself [8]. However, in the female participants in the present study, an inverse association was observed between fruit intake and NAFLD prevalence in the age-adjusted and lifestyle factor–adjusted model. Consistent with our studies, fructose intake was inversely associated with fatty liver index in older Finnish adults whose dietary fructose was derived mainly from fruits [29]. Those authors mentioned that the possible protective effect of fruits on NAFLD may have been overcome by the possible harmful effect of soft drinks, another fructose supplier, on NAFLD [29]. For example, fruits supply antioxidants such as vitamin C, which is reported to be inversely associated with NAFLD prevalence [6]. In the present study, however, the inverse association was highly attenuated after adjustment for BMI. As is well known, overweight or obesity is the most important predictive factor for NAFLD. Fruit intake was reported to be inversely associated with becoming overweight or obese [30,31]. Fruit intake might be inversely associated with NAFLD via suppression of weight gain rather than supply of antioxidants. Nevertheless, we saw no obesity-independent association between fruit intake and NAFLD prevalence in this study. Median fruit intake (75.7 g/d in men and 93.7 g/d in women, energy adjusted by the residual method) was lower than in previous studies (∼180 g/d in Chinese women [8] and 230 g/d in Finnish adults [29]), although median fruit intake calculated from the BDHQ appeared to be

Table 1
Characteristics of participants according to quartiles of fruit and vegetable intake in 977 men*

	Fruit				P_{trend}†	Vegetable				P_{trend}†
	Q1	Q2	Q3	Q4		Q1	Q2	Q3	Q4	
Age (y)	49.1 (6.8)	51.6 (7.9)	55.3 (8.6)	57.6 (7.5)	<0.001	50.8 (8)	53 (8.1)	53.8 (8.6)	56 (8)	<0.001
Exercise; almost never (%)	41.4	31.1	25.3	19.7	<0.001	38.7	26.4	31.5	20.9	<0.001
Current smoker (%)	21.7	16.4	10.6	5.7	<0.001	18.9	17.4	10.9	7.4	<0.001
BMI (kg/m^2)	23.3 (3.4)	23.5 (3)	23.7 (2.9)	23 (2.8)	0.003	23.6 (2.9)	23.8 (3.5)	23.1 (2.8)	23.4 (2.8)	0.27
Nutrients intake										
Fat (% energy)	27.4 (5.7)	27.2 (4.6)	27.4 (4.6)	27.1 (4.9)	0.67	25.7 (4.6)	26.9 (4.4)	27.8 (4.9)	28.8 (5.2)	<0.001
Protein (% energy)	15.1 (2.6)	15.7 (2.5)	15.8 (2.5)	16.1 (2.7)	<0.001	14.3 (2)	15 (1.8)	16.1 (2.5)	17.3 (2.9)	<0.001
Carbohydrate (% energy)	54.8 (7.7)	54.3 (6.8)	53.7 (6.7)	53.9 (6.4)	0.17	57.5 (6.1)	55 (6)	53.2 (6.9)	51 (7)	<0.001
Alcohol (% energy)	2.8 (2.7)	2.8 (2.7)	2.8 (2.7)	2.9 (2.6)	0.56	2.5 (2.5)	3.1 (2.8)	2.9 (2.8)	2.9 (2.6)	0.26
EPA + DHA (% energy)	0.36 (0.20)	0.44 (0.22)	0.45 (0.23)	0.45 (0.24)	<0.001	0.35 (0.17)	0.39 (0.17)	0.46 (0.24)	0.51 (0.27)	<0.001
Dietary fiber (g/1000 kcal)	5.8 (1.6)	6.2 (1.4)	6.8 (1.6)	8.2 (1.9)	<0.001	5.1 (1)	6 (0.9)	6.9 (1)	8.9 (1.7)	<0.001
Vitamin C (mg/1000 kcal)	47.6 (20.8)	55.4 (19.3)	67.5 (25.1)	93.7 (31.2)	<0.001	42.9 (16.8)	57.1 (19.1)	67.5 (19.8)	96.6 (32.2)	<0.001
Foods intake (g/1000 kcal)										
Fruits	9.6 (5.4)	28.6 (5.9)	52.5 (7.8)	104.6 (37.4)	—	33.1 (29.9)	43.6 (34.5)	52.1 (37.9)	66.3 (50)	<0.001
Total vegetables	115.3 (63.5)	122.3 (56.4)	140.6 (67.8)	168.1 (77.8)	<0.001	66.0 (16.9)	106.8 (8.5)	141.3 (12.9)	232.1 (62.9)	—
Green and yellow vegetables	38.6 (27.5)	40.5 (25)	48.3 (31.5)	61.7 (35.3)	0.03	20.5 (9.2)	34.8 (11.1)	49.3 (15.5)	84.3 (35.4)	—
Other vegetables	60 (34.4)	63.9 (32.5)	72.8 (36.5)	84.6 (42.7)	0.04	34.7 (11.9)	55.4 (11.8)	72.1 (15.6)	118.9 (37.1)	—

BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid
*Continuous variables are shown using mean (SD).
†Based on regression analysis with assignment of median values to each category for continuous variables and the linear-by-linear association test for categorical variables.

Table 2
Characteristics of participants according to quartiles of fruit and vegetable intake in 1467 women*

	Fruit				P_{trend}†	Vegetable				P_{trend}†
	Q1	Q2	Q3	Q4		Q1	Q2	Q3	Q4	
Age (y)	48.5 (6.3)	51.5 (7.8)	53.1 (8)	54.7 (8.1)	<0.001	49.1 (6.9)	51.3 (7.5)	52.8 (8.1)	54.7 (8)	<0.001
Exercise; almost never (%)	46.4	33	26.7	23.7	<0.001	43	36.2	29.9	20.7	<0.001
Current smoker (%)	3.6	3.5	2.2	3	0.45	3.8	2.2	2.7	3.5	0.93
BMI (kg/m²)	21.4 (3.1)	21.5 (3.2)	20.8 (2.8)	20.6 (2.5)	<0.001	21.6 (3.2)	20.8 (2.5)	21.1 (3)	20.9 (2.9)	0.045
Nutrients intake										
Fat (% energy)	29.4 (5)	28.9 (4.8)	28.9 (4.5)	28.2 (4.5)	0.002	27.7 (4.8)	28.9 (4.6)	29.3 (4.4)	29.5 (4.9)	<0.001
Protein (% energy)	15.7 (2.4)	16.1 (2.5)	16.7 (2.5)	16.9 (2.7)	<0.001	14.8 (2.1)	15.8 (2.2)	16.8 (2.2)	17.9 (2.6)	<0.001
Carbohydrate (% energy)	53.3 (6.7)	53.4 (6.6)	53 (6.3)	53.8 (6.1)	0.39	56 (6.3)	53.7 (6.1)	52.6 (5.8)	51.2 (6.4)	<0.001
Alcohol (% energy)	1.6 (2.1)	1.6 (2)	1.4 (2)	1.1 (1.7)	<0.001	1.5 (2)	1.6 (2)	1.3 (1.9)	1.4 (2)	0.3
EPA + DHA (% energy)	0.37 (0.23)	0.42 (0.20)	0.46 (0.24)	0.46 (0.25)	<0.001	0.34 (0.18)	0.41 (0.23)	0.46 (0.23)	0.51 (0.26)	<0.001
Dietary fiber (g/1000 kcal)	6.6 (1.9)	7.4 (1.9)	8.2 (1.9)	9.7 (2.2)	<0.001	5.8 (1.1)	7.1 (1)	8.4 (1.2)	10.7 (2)	<0.001
Vitamin C (mg/1000 kcal)	58.9 (23.4)	72.4 (25.2)	88.5 (25.8)	115.2 (31)	<0.001	55.5 (22.3)	71.8 (20.8)	89.4 (23.4)	118.1 (30.6)	<0.001
Foods intake (g/1000 kcal)										
Fruits	15.8 (8.5)	45.5 (8.2)	75 (9.6)	132.5 (36.8)	–	45.9 (36.5)	63.1 (43)	73.2 (47.4)	86.6 (52.2)	<0.001
Total vegetables	149.9 (75)	168.4 (85.2)	185.7 (83)	224.2 (96.4)	<0.001	89.2 (22.8)	141.4 (13.2)	193.7 (16.7)	303.5 (76.8)	–
Green and yellow vegetables	50.4 (33.7)	59.5 (37)	68.9 (38.8)	85.8 (43.1)	0.03	29.8 (14)	48.1 (16.4)	72.3 (21.8)	114.3 (40.1)	–
Other vegetables	79.8 (42.5)	86.9 (47.8)	93.1 (45.8)	110.5 (54)	0.03	45.8 (15.3)	73.9 (16.2)	96.5 (21.3)	153.9 (48.3)	–

BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid
*Continuous variables are shown using mean (SD).
†Based on regression analysis with assignment of median values to each category for continuous variables and the linear-by-linear association test for categorical variables.

Table 3
OR and 95% CIs of non-alcoholic fatty liver disease according to quartiles of fruit intake and vegetable intake in 977 men

	Q1	Q2	Q3	Q4	P_{trend}*
Fruit (g/1000 kcal), median (range)	10.4 (0.0–18.1)	28.2 (18.2–39.3)	52.6 (39.4–67.9)	93.9 (68.3–301.6)	
n cases/n participants	99/244	89/244	90/245	63/244	
Age-adjusted OR (95% CI)	1.00	0.84 (0.58–1.22)	0.86 (0.59–1.25)	0.51 (0.34–0.78)	0.002
+lifestyle factors†	1.00	0.88 (0.60–1.28)	0.92 (0.62–1.37)	0.59 (0.38–0.91)	0.02
+BMI	1.00	0.90 (0.58–1.38)	0.88 (0.56–1.37)	0.68 (0.42–1.11)	0.12
Total vegetables (g/1000 kcal), median (range)	68.1 (18.6–92)	106.2 (92.3–121.9)	139.3 (122.1–167.7)	212.5 (167.9–488)	
n cases/n participants	96/243	86/242	82/248	77/244	
Age-adjusted OR (95% CI)	1.00	0.86 (0.59–1.24)	0.77 (0.53–1.12)	0.73 (0.50–1.08)	0.11
+lifestyle factors†	1.00	0.95 (0.65–1.39)	0.86 (0.59–1.27)	0.92 (0.61–1.39)	0.68
+BMI	1.00	0.86 (0.55–1.33)	1.00 (0.64–1.55)	0.87 (0.55–1.39)	0.68
Green and yellow vegetables (g/1000 kcal), median (range)	16.8 (0.0–26.6)	32.7 (26.7–40)	48.7 (40.3–59.9)	80.5 (60–238)	
n cases/n participants	94/244	99/246	73/243	75/244	
Age-adjusted OR (95% CI)	1.00	1.07 (0.74–1.54)	0.69 (0.47–1.01)	0.72 (0.50–1 06)	0.03
+lifestyle factors†	1.00	1.23 (0.85–1.80)	0.75 (0.51–1.11)	0.91 (0.60–1.37)	0.27
+BMI	1.00	1.25 (0.81–1.92)	0.79 (0.51–1.24)	0.93 (0.58–1.49)	0.40
Other vegetables (g/1000 kcal), median (range)	33.7 (2–44.4)	53.8 (44.5–62.6)	73.0 (62.7–87.1)	111.0 (87.4–275.8)	
n cases/n participants	99/244	82/244	83/245	77/244	
Age-adjusted OR (95% CI)	1.00	0.75 (0.52–1.09)	0.77 (0.53–1.11)	0.70 (0.48–1.02)	0.10
+lifestyle factors†	1.00	0.85 (0.58–1.24)	0.86 (0.58–1.27)	0.86 (0.57–1.28)	0.54
+BMI	1.00	0.78 (0.50–1.21)	0.87 (0.55–1.35)	0.79 (0.49–1.25)	0.43

BMI, body mass index; CI, confidence interval; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; OR, odds ratio
*Based on logistic regression analysis with assignment of median intake values to each category.
†Adjusted for habitual exercise, current smoking, intakes of total energy, alcohol, EPA + DHA, coffee, soft drink, total vegetables (for ORs for fruit), and fruit (for ORs for total vegetables, green and yellow vegetables, and other vegetables).

overestimated [22]. Therefore, it can be assumed that fruits have neither harmful nor protective effects on NAFLD prevalence independently of obesity in Japanese, who have relatively low consumption of fruits.

Meanwhile, a global campaign against chronic diseases has recommended a minimum daily intake of 400 g of fruits and vegetables [17]. Following this, the Japanese national health promotion movement aimed to prevent chronic diseases by decreasing the proportion of individuals with a daily fruit intake <100 g [32]. Several meta-analyses reported nonlinear inverse associations between fruit intake and coronary heart disease and certain types of cancer, and a remarkable risk reduction was observed up to an intake of 200 g of fruits per day [33–35]. Considering the present results, we concluded that Japanese, with a daily fruit intake of

<100 g, can increase their fruit intake without worrying about the possible harmful effects of fruit-derived fructose on NAFLD.

As food composition tables in Japan did not include fructose until December 2015, no Japanese diet questionnaire has been validated for fructose intake. Thus, we were unable to directly assess the association between fructose intake from fruits and NAFLD prevalence. Future studies should assess the contribution of each food group to total fructose intake and the association between fructose from fruits or other foods and NAFLD prevalence.

In the present study, no association was found between total vegetable intake and NAFLD. The female participants showed a linear inverse association between the prevalence of NAFLD and quartiles of green and yellow vegetable intake in the final model, but ORs for upper intake categories did not indicate statistical significance.

Table 4
OR and 95% CIs of non-alcoholic fatty liver disease according to quartiles of fruit intake and vegetable intake in 1467 women

	Q1	Q2	Q3	Q4	P_{trend}*
Fruit (g/1000 kcal), median (range)	16.3 (0−30.5)	45.4 (30.8−59.9)	74.4 (60−93.1)	121 (93.2−329.5)	
n cases/n participants	50/366	52/367	37/367	32/367	
Age-adjusted OR (95% CI)	1.00	0.90 (0.59−1.38)	0.57 (0.35−0.90)	0.45 (0.27−0.73)	<0.001
+lifestyle factors[2]	1.00	0.97 (0.63−1.50)	0.63 (0.39−1.03)	0.53 (0.31−0.89)	0.006
+BMI	1.00	0.85 (0.49−1.47)	0.90 (0.49−1.63)	0.82 (0.44−1.55)	0.60
Total vegetables (g/1000 kcal), median (range)	93.6 (8.2−119.2)	140.9 (119.3−165.8)	191.7 (166−225.2)	277.8 (225.3−686.1)	
n cases/n participants	51/365	43/367	38/368	39/367	
Age-adjusted OR (95% CI)	1.00	0.74 (0.48−1.15)	0.60 (0.38−0.95)	0.58 (0.36−0.92)	0.02
+lifestyle factors[†]	1.00	0.83 (0.53−1.30)	0.73 (0.45−1.18)	0.75 (0.46−1.25)	0.28
+BMI	1.00	1.48 (0.85−2.57)	0.67 (0.36v1.24)	0.76 (0.40−1.45)	0.14
Green and yellow vegetables (g/1000 kcal), median (range)	26.5 (0.0−36.3)	47.4 (36.6−58.5)	70.4 (58.6−86.8)	111.6 (87−286.5)	
n cases/n participants	53/365	51/369	40/367	27/366	
Age-adjusted OR (95% CI)	1.00	0.89 (0.59−1.35)	0.62 (0.40−0.97)	0.39 (0.24−0.65)	<0.001
+lifestyle factors[†]	1.00	0.98 (0.63 −1.51)	0.76 (0.47−1.23)	0.49 (0.29−0.85)	0.006
+BMI	1.00	1.35 (0.79−2.32)	1.09 (0.59−2.00)	0.54 (0.27−1.07)	0.04
Other vegetables (g/1000 kcal), median (range)	44.9 (6.6−58.5)	70.5 (58.6−83.3)	97.9 (83.4−114.7)	143.3 (115−394.2)	
n cases/n participants	42/367	47/366	44/368	38/366	
Age-adjusted OR (95% CI)	1.00	1.06 (0.67−1.65)	0.94 (0.60−1.48)	0.75 (0.47−1.21)	0.17
+lifestyle factors[†]	1.00	1.15 (0.73−1.82)	1.06 (0.66−1.70)	0.95 (0.58−1.57)	0.71
+BMI	1.00	1.49 (0.84−2.64)	1.11 (0.62−2.00)	0.81 (0.42−1.53)	0.27

BMI, body mass index; CI, confidence interval; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; OR, odds ratio
*Based on logistic regression analysis with assignment of median intake values to each category.
[†]Adjusted for habitual exercise, current smoking, intakes of total energy, alcohol, EPA + DHA, coffee, soft drink, total vegetables (for ORs for fruit), and fruit (for ORs for total vegetables, green and yellow vegetables, and other vegetables).

Although β-carotene has been inversely associated with elevated serum alanine aminotransferase (ALT), there was no association between fruit and vegetable intake and elevated ALT [36]. The authors mentioned that the results might have affected by a limitation in the validity of the food frequency questionnaire [36]. Here, we assessed dietary intake using the BDHQ, despite its relatively low correlation with dietary records for green and yellow vegetables ($r = 0.28$ in men and $r = 0.37$ in women) compared with total vegetables ($r = 0.51$ in men and $r = 0.55$ in women) [22]. This difference might mean that consumption of green and yellow vegetables is more vulnerable to misclassification than that of total vegetables. Accordingly, evidence suggesting that green and yellow vegetables as subtypes of total vegetables are important components in the relationship between vegetables and NAFLD is insufficient.

Several limitations of the present study warrant noting. First, the study was based on cost-associated health checks and most participants were referred to the hospital via employer-sponsored programs. Thus, the participants might be more affluent and health conscious than the general population. However, the prevalence of NAFLD in this hospital was did not differ from that in a general health care association [37]. Second, fatty liver was diagnosed using abdominal ultrasonography, although the gold standard is liver biopsy. Meta-analysis showed that the overall sensitivity and specificity of ultrasonography in the detection of moderate to severe fatty liver compared with biopsy was 84.8% (95% confidence interval [CI], 79.5−88.9) and 93.6% (95% CI, 87.2−97), respectively [38]. However, most epidemiologic studies avoid biopsies because of their invasiveness. Although new tools such as the computed hepatorenal index have been developed to improve the accuracy of ultrasound [39,40], it is difficult to use for screening in the setting of large populations [41]. Nevertheless, the use of ultrasonography would be meaningful in studying populations at preclinical risk for fatty liver. Third, it is not appropriate to use the present findings to discuss adequate levels of fruit and vegetable intake to prevent NAFLD because the BDHQ cannot be used to accurately assess median intake level in a population [22,23]. However, the main aim of the present study was- to evaluate whether fruit and vegetable intake was associated with NAFLD prevalence. Regarding its ranking ability [22], BDHQ was considered to be valid in classifying individuals into categories according to vegetable and fruit intake. Fourth, we could not completely distinguish NAFLD from alcoholic fatty liver because true individual alcohol intake was not determinable by dietary assessment. However, we used alcohol intake calculated by a validated diet questionnaire and adjusted alcohol intake for energy intake to minimize measurement error. Finally, the observed inverse relationships of intakes of fruits and green and yellow vegetables to NAFLD might be due to residual confounding by inappropriately measured or adjusted factors. For example, habitual exercise was assessed by a simple question, and occupational physical activity was not covered. Other potential protective factors for NAFLD, such as dietary fiber [42], might also require adjustment. However, we could not discern dietary fiber as a confounder or mediator because a substantial part of it was derived from fruits and vegetables.

Conclusions

Results from the present study demonstrated that no relationship exists between fruit and vegetable intake and NAFLD prevalence in middle-aged Japanese men and women. These populations do not need to restrict fructose intake from fruits to prevent NAFLD. Moreover, we found no evidence to recommend a higher intake of certain types of vegetable to prevent NAFLD. Further studies are needed to detail the association between the intake of fruits and NAFLD prevalence, particularly using dietary assessment methods that can estimate fructose intake from specified types of fruit.

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