



Applied nutritional investigation

Association between serum β -carotene-to-retinol ratio and severity of hepatic steatosis in non-alcoholic fatty liver disease in Japan: A cross-sectional study

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ABSTRACT

Objectives: Retinol and β -carotene have been reported to be involved in the pathogenesis of non-alcoholic fatty liver disease (NAFLD). However, clinical studies are limited. The aim of this study was to investigate the relationship between serum the ratio of β -carotene to retinol (SC/SR) and hepatic steatosis in NAFLD diagnosed by ultrasonography.

Methods: The participants were 606 Japanese adults who were enrolled in a health survey. Clinical profile, dietary nutrition intake, blood biochemistry, serum retinol, and carotenoids were analyzed. NAFLD was defined as fatty liver on ultrasonography in the absence of other causes of steatosis.

Results: Women had higher daily intake of α - and β -carotene, although there were no differences in daily retinol and carotenoid intake between participants with or without NAFLD in both men and women. Women had a higher SC/SR ratio than men regardless of the presence or absence of NAFLD, and the SC/SR ratio in women decreased with exacerbation of hepatic steatosis, whereas the SC/SR ratio in men did not change despite exacerbation of hepatic steatosis. After adjusting for confounding factors, the likelihood of NAFLD among participants in the highest quartile of SC/SR ratio decreased by two-thirds compared with participants in the lowest quartile (adjusted odds ratio, 0.64; 95% confidence interval, 0.21–1.92; $P = 0.041$). The SC/SR ratio was positively correlated with serum high-density lipoprotein cholesterol level, and negatively correlated with serum triacylglycerol level.

Conclusions: The SC/SR ratio was lower in NAFLD with sex differences, and was associated with the severity of hepatic steatosis and lipid profile. Future studies are needed to expand on these findings.

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Introduction

Non-alcoholic fatty liver disease (NAFLD) is one of the most common liver disorders worldwide, and includes a spectrum of clinical features from simple steatosis to non-alcoholic steatohepatitis (NASH), which can progress to cirrhosis, liver failure, and hepatocellular carcinoma with poor health-related quality of life [1,2]. Despite these facts, knowledge of dietary nutritional factors to prevent NAFLD remains incomplete.

Vitamins A, C, and E, and carotenoids are known as dietary antioxidants [3]. Based on the fact that oxidative stress plays a key role in the progression of NAFLD [4,5], the beneficial effect of vitamin E on NAFLD has been reported; however, its long-term outcomes are unclear [6,7]. On the other hand, high doses of β -carotene and vitamins A and E are associated with increased risk for cancers and all-cause mortality [8,9]. Thus, there are still challenges to establishing therapeutic strategies with dietary antioxidants in NAFLD.

More than 90% of vitamin A is stored in the liver, especially in the hepatic stellate cells (HSCs) [10]. Carotenoids can be metabolized to vitamin A, and the six most abundant carotenoids account for >95% of carotenoids found in the blood: lutein, zeaxanthin, β -cryptoxanthin, α -carotene, β -carotene, and lycopene. β -carotene, which is the most abundant carotenoid in the liver, has the highest provitamin activity [11] and pronounced antioxidative effects [12]. Recent experimental studies have shown the preventive and protective effects of β -carotene on hepatic steatosis, fibrosis, oxidative stress, inflammation, and apoptosis [13–15]. In clinical studies, lower serum β -carotene levels were associated with NAFLD development [16], and in contrast, higher levels of serum β -carotene were associated with NAFLD improvement [17,18]. Additionally, the bioconversion rate of β -carotene to retinol was inversely related to the level of body store and circulating retinol concentration [19]. Therefore, the ratio of β -carotene to retinol (SC/SR) is considered appropriate as an indicator of the status of retinol and β -carotene metabolism [16,20].

Clinical studies on metabolism of retinol and carotenoids in NAFLD are limited [16–18,21]. We previously reported clinical studies of NAFLD with adipokines and microbiota [22,23]. Therefore, we investigated the association between SC/SR ratio and NAFLD diagnosed by ultrasonography in a Japanese community study.

Materials and methods

Study design and participants

The Iwaki Health Promotion Project is an ongoing community-based health promotion study of Japanese people ≥ 20 y of age designed to prevent lifestyle-related diseases and prolong their life span. This program has been carried out annually since 2005, although the number of participants has varied depending on the year (814–1,167 participants), with about 1000 participants in the Iwaki region

of Hirosaki City in Aomori Prefecture located in northern Japan [22–24]. All those in the study participated voluntarily in response to a public announcement, and ~600 data points were collected from each participant, including their demographic characteristics, medical history, lifestyle data, and microbiota and blood chemical analysis data. Our research on the association between NAFLD and natural antioxidants including retinol and carotenoids is one part of this project. In 2016, 1148 individuals were enrolled in this project. Of these, we excluded all individuals who did not have complete clinical data, those who were positive for hepatitis B surface antigen or anti-hepatitis C virus antibody, and/or those who had excessive alcohol consumption (daily alcohol intake >30 g/d for men and 20 g/d for women), and/or took dietary nutritional supplements (Fig. 1). Ultimately, 606 individuals (208 men and 398 women) were included in the present study. This study was approved by the Ethics Committee of the Hirosaki University School of Medicine, and written informed consent was obtained from all participants.

Dietary assessment

Dietary habits were assessed by using a brief-type self-administered diet history questionnaire (BDHQ) that included questions on the consumption frequency of 56 foods and beverages and nine dishes commonly consumed in the general Japanese population [25,26]. For each food item, the participants indicated their mean frequency of consumption of the food over the previous month. The mean daily consumption of nutrients was calculated using an ad hoc computer program for BDHQ, which was based on the Standard Tables of Food Composition in Japan [27]. Retinol equivalent and β -carotene equivalent were calculated from its content of retinol and carotenoids based on the following formula: [28].

$$\begin{aligned} \text{retinol equivalent} &= \text{retinol}(\text{mg}) + \beta\text{-carotene equivalent}(\text{mg})/12, \beta \\ &\quad \text{-carotene equivalent} \\ &= \beta\text{-carotene}(\text{mg}) + \alpha\text{-carotene}(\text{mg})/2 \\ &\quad + \text{cryptoxanthin}(\text{mg})/2 \end{aligned}$$

Clinical and laboratory assessment

The following clinical characteristics were measured: height, body weight, and body composition. Body mass index (BMI) was calculated as body weight divided by height squared and expressed in kg/m². Body fat percentage (BFP) was measured with a body composition analyzer (MC-190; Tanita Corp., Tokyo, Japan). Alcohol intake, smoking, and exercise habits were determined from a questionnaire. Diabetes was defined as fasting serum glucose ≥ 126 mg/dL, glycated hemoglobin (HbA1c) $\geq 6.5\%$, use of diabetes medication, or a prior known diabetes diagnosis. Dyslipidemia was defined as total cholesterol (TC) ≥ 220 mg/dL, triacylglycerols (TGs) ≥ 150 mg/dL, or use of antihyperlipidemic medication. Hypertension was defined as blood pressure $\geq 140/90$ mm Hg or use of antihypertensive medication.

Whole blood samples were obtained after overnight fast, and laboratory tests included platelet count, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ -glutamyl-transpeptidase (GGT), total bilirubin, glucose, HbA1c, insulin, TC, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), TGs, and free fatty acids (FFAs). The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the following formula:

$$[\text{fasting plasma glucose}(\text{mg/dL}) \times \text{fasting plasma insulin}(\text{mU/mL})]/405.$$

The AST-to-platelet ratio index (APRI) was calculated using the following formula:

$$[\text{AST level/upper normal limit for AST}]/\text{platelet counts}(10^9/\text{L}) \times 100.$$

The concentration of blood antioxidants was measured using a Kagome kit (Nagoya, Japan) according to the manufacturer's instructions. Serum concentrations of carotenoids (lutein, zeaxanthin, β -cryptoxanthin, α -carotene, β -carotene, and lycopene) and retinol were measured by using high-performance liquid chromatography analysis [29,30].

Assessment of NAFLD

NAFLD was diagnosed based on abdominal ultrasound findings using a Pro-sound F37 (Hitachi Aloka Medical Ltd., Tokyo, Japan), as in previous studies [22,23]. Based on observation on B-mode ultrasonography, the severity of echogenicity was graded as follows: normal, normal echogenicity; mild, slight diffuse increase in fine echoes in liver parenchyma with normal visualization of diaphragm and intrahepatic vessel borders; moderate, moderate diffuse increase in fine echoes with slightly impaired visualization of hepatic vessel border and diaphragm; severe, marked increase in fine echoes with poor or non-visualization of the intrahepatic vessel borders, diaphragm, and posterior right lobe of the liver [31].

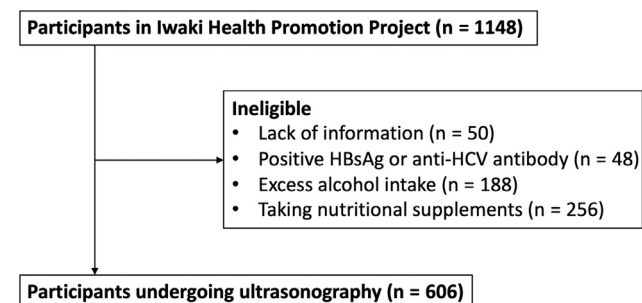


Fig. 1. Study enrollment flowchart. HBsAg, hepatitis B virus surface antigen; HCV, hepatitis C virus.

Table 1

Baseline characteristics of study participants: Demographics, anthropometry, and dietary survey

	Total (N = 606)	Normal (n = 478, 78.9%)			NAFLD (n = 128, 21.1%)			P value*	P value [†]
		Men (n = 150)	Women (n = 328)	P value	Men (n = 58)	Women (n = 70)	P value		
Demographics									
Age (y)	53 (16.4)	50.9 (17.7)	53.4 (16.8)	0.116	52 (14.5)	56.7 (11.9)	0.050	0.550	0.199
Women	398 (65.6)		328 (68.6)			70 (54.6)			0.003
Clinical									
BMI (kg/m ²)	22.8 (3.5)	22.8 (2.6)	21.6 (3.1)	< 0.001	26.3 (3.4)	25.6 (3.8)	0.144	< 0.001	< 0.001
BFP (%)	26.9 (8.3)	19 (5.6)	28.9 (6.8)	< 0.001	25.1 (4.9)	36.5 (6.4)	< 0.001	< 0.001	< 0.001
Diabetes [‡]	54 (8.9)	13 (8.7)	19 (5.8)	0.243	10 (17.2)	12 (17.1)	0.988	0.077	0.001
Dyslipidemia	248 (40.9)	46 (30.7)	129 (39.3)	0.068	34 (58.6)	39 (55.7)	0.741	< 0.001	0.012
Hypertension [§]	210 (34.7)	50 (33.3)	97 (29.6)	0.408	27 (46.6)	36 (51.4)	0.583	0.077	< 0.001
Current smoker	98 (16.2)	43 (28.7)	31 (9.5)	< 0.001	15 (25.9)	9 (12.9)	0.061	0.686	0.390
Habitual exerciser	175 (28.9)	46 (30.7)	94 (28.7)	0.654	16 (27.6)	19 (27.1)	0.955	0.663	0.798
Daily retinol and carotenoids intake (μg/1000 kcal)									
Energy (kcal)	1796.7 (570)	2025.5 (618.7)	1669.2 (520.9)	< 0.001	2082 (548.6)	1666.8 (459)	< 0.001	0.520	0.737
Retinol	186.6 (135)	199.1 (162.8)	187.7 (127.4)	0.737	176.4 (141.8)	163.2 (89.6)	0.610	0.435	0.436
Retinol equivalent	320.4 (167.7)	302.4 (179.9)	337.3 (166.9)	0.004	279.5 (158.8)	313.4 (142.7)	0.053	0.394	0.356
α-carotene	180.2 (138)	130.3 (98)	207.9 (151.8)	< 0.001	129.4 (98.9)	199.5 (131.6)	0.001	0.971	0.958
β-carotene	1446.1 (917.5)	1116.7 (675.7)	1620.4 (955.9)	< 0.001	1102.3 (733.5)	1620.1 (1053.3)	0.002	0.745	0.748
β-carotene equivalent	1586.2 (987.1)	1221.8 (726.7)	1775.4 (1025.7)	< 0.001	1220.3 (793)	1783.6 (1134.6)	0.001	0.874	0.775
Cryptoxanthin	95.9 (100)	75.6 (77.5)	98.4 (93.3)	0.003	100.9 (124.8)	123.3 (137.2)	0.141	0.476	0.370
Alcohol (g)	4.2 (7.1)	8.6 (9.4)	2.2 (4.5)	< 0.001	7.4 (9.3)	1.5 (3.8)	< 0.001	0.255	0.169

ALT, alanine aminotransferase; APRI, aspartate aminotransferase-to-platelet ratio index; AST, aspartate aminotransferase; BFP, body fat percentage; BMI, body mass index; FFA, free fatty acid; GGT, γ-glutamyl-transpeptidase; HbA1c, glycated hemoglobin; HOMA-IR, homeostasis model assessment as an index of insulin resistance; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Data are n (%) or means (±SD). Boldface type indicates $P < 0.05$.

*P value for comparison between men with or without NAFLD.

†P value for comparison between women with or without NAFLD.

‡Diabetes was defined as fasting serum glucose ≥ 126 mg/dL, HbA1c $\geq 6.5\%$, use of diabetes medication, or a prior known diabetes diagnosis.

§Hypertension was defined as blood pressure $\geq 140/90$ mm Hg or use of antihypertensive medication.

||Retinol equivalent = retinol (μg) + β-carotene equivalent (μg)/12, β-carotene equivalent = β-carotene (μg)/2 + cryptoxanthin (μg)/2. Dyslipidemia was defined as total cholesterol ≥ 220 mg/dL, triacylglycerides ≥ 150 mg/dL, or use of antihyperlipidemic medication.

Statistical analysis

All statistical analyses of collected data were performed using the Excel statistical software package for Macintosh (Ekuseru-Toukei 2016; Esumi Co., Ltd., Tokyo, Japan). Categorical variables were compared using the χ^2 test. Characteristics were compared between individuals with or without NAFLD using the Mann–Whitney U test. Sex differences in SC/SR ratio and serum levels of β-carotene and retinol were compared using the Kruskal–Wallis test. Spearman's correlation coefficient was used to calculate the correlation between the correlation between hepatic steatosis and retinol, β-carotene, or SC/SR ratio; and the correlation between SC/SR ratio and lipid profile. Logistic regression models were used to estimate odds ratio (OR) and 95% confidence interval (CI) to determine the association between quartiles of SC/SR ratio and the hepatic steatosis of NAFLD after adjusting for factors including age, sex, diabetes, dyslipidemia, hypertension, BMI, BFP, current smoking, habitual exercise, energy intake, alcohol consumption, and daily intake of retinol and carotenoids. Differences were considered to be significant with $P < 0.05$.

Results

Characteristics of the participants

The characteristics of the study participants with or without NAFLD by sex are shown in Tables 1 and 2. Of the 606 participants included, 128 (21.1%) had NAFLD. The average age of the participants was 53 y (±16.4). The male-to-female ratio was lower in the participants with NAFLD compared with the participants without NAFLD (54.6 vs 68.6%, $P = 0.003$). None of the participants had chronic liver disease or liver cirrhosis evaluated by ultrasonography. Regardless of the presence or absence of NAFLD, women had a greater BFP, showed lower daily intake of energy and alcohol, and exhibited higher daily intake of α-carotene and β-carotene (Table 1). In both men and women, the participants with NAFLD had a higher BMI and greater BFP, and showed a higher prevalence

of dyslipidemia than those without NAFLD. There were no differences in age, percentage of current smokers and habitual exercisers, and mean daily retinol and carotenoid intake between participants with and without NAFLD for either men or women (Table 1).

Severity of hepatic steatosis did not differ between sexes (Table 2). Both male and female participants with NAFLD presented higher levels of AST, ALT, GGT, glucose, HbA1c, insulin, HOMA-IR, TC, and TG, and a lower level of HDL-C compared with participants without NAFLD. Regardless of the presence or absence of NAFLD, men showed higher levels of AST, ALT, GGT, TG, and APRI, and a lower level of HDL-C than women. Men showed a higher serum level of retinol, and lower serum levels of β-cryptoxanthin, α-carotene, and β-carotene, than women, regardless of the presence or absence of NAFLD. There were no differences in serum and carotenoid levels between male participants with and without NAFLD. Only serum β-carotene level was lower in the female participants with NAFLD compared with those without NAFLD. The SC/SR ratio was higher in women than men regardless of the presence or absence of NAFLD, and in women, the participants with NAFLD showed a lower SC/SR ratio than the participants without NAFLD. On the other hand, here was no differences in the SC/SR ratio between men with or without NAFLD (Table 2).

Relationship between SC/SR and steatosis in NAFLD

There was no evidence of associations across quartiles of serum levels of retinol, lutein, α-carotene, and β-carotene and likelihood of NAFLD (highest versus lowest quartile, retinol adjusted OR, 2.27; 95% CI, 0.95–5.44, $P_{\text{trend}} = 0.184$; lutein-adjusted OR, 0.63; 95% CI, 0.26–1.40, $P_{\text{trend}} = 0.455$; α-carotene-adjusted OR, 0.92; 95% CI, 0.44–1.96, $P_{\text{trend}} = 0.566$; β-carotene-adjusted OR, 0.56;

Table 2

Baseline characteristics of study participants: Ultrasonography and laboratory data.

	Total (N = 606)	Normal (n = 478, 78.9%)			NAFLD (n = 128, 21.1%)			P value*	P value [†]
		Men (n = 150)	Women (n = 328)	P value	Men (n = 58)	Women (n = 70)	P value		
Hepatic steatosis									
Normal, n (%)	478 (78.9)	150 (100)	328 (100)						
Mild, n (%)	71 (11.7)				34 (58.6)	37 (52.9)	0.514		
Moderate, n (%)	45 (7.4)				17 (29.3)	28 (40)	0.207		
Severe, n (%)	12 (2)				7 (12.1)	5 (7.1)	0.341		
Biochemical profile									
Platelet count (10 ⁴ /μL)	24.6 (5.6)	23.9 (5.2)	24.3 (5.6)	0.606	24.9 (5.5)	26.7 (6.1)	0.056	0.199	<0.001
Albumin (g/dL)	4.49 (0.30)	4.58 (0.28)	4.43 (0.32)	<0.001	4.59 (0.21)	4.46 (0.28)	0.001	0.663	0.709
AST (U/L)	22.0 (8.2)	23.1 (7)	20.1 (6.8)	<0.001	27.3 (12)	24.5 (9.7)	0.038	0.004	<0.001
ALT (U/L)	21.2 (15.2)	23.2 (15.9)	15.8 (8)	<0.001	39.6 (21.1)	27.4 (18.5)	<0.001	<0.001	<0.001
GGT (U/L)	27.3 (26)	34.1 (35.1)	19.7 (11.7)	<0.001	51.3 (41.7)	28.8 (17.9)	<0.001	<0.001	<0.001
Total bilirubin (mg/dL)	0.77 (0.28)	0.80 (0.27)	0.78 (0.28)	0.466	0.76 (0.29)	0.70 (0.25)	0.248	0.276	0.008
Glucose (mg/dL)	90.9 (21.2)	90.9 (13.6)	88.7 (23.9)	0.002	95.5 (15.4)	97.5 (23.2)	0.896	0.025	<0.001
HbA1c (%)	5.85 (0.62)	5.76 (0.41)	5.79 (0.55)	0.476	6.06 (0.76)	6.19 (0.94)	0.470	<0.001	<0.001
Insulin (μU/mL)	5.69 (5.09)	4.77 (2.57)	4.99 (2.68)	0.251	9.27 (13.3)	7.94 (3.28)	0.331	<0.001	<0.001
HOMA-IR	1.32 (1.44)	1.08 (0.63)	1.13 (0.82)	0.776	2.29 (3.72)	1.98 (1.11)	0.330	<0.001	<0.001
TC (mg/dL)	203.7 (36.6)	195.2 (31.1)	204.4 (39.2)	0.021	205.4 (32.6)	216.9 (33.8)	0.111	0.042	0.008
HDL-C (mg/dL)	63.2 (16.5)	57.6 (12.8)	69.3 (15.7)	<0.001	49.1 (18.9)	58.2 (11.9)	<0.001	<0.001	<0.001
LDL-C (mg/dL)	117.6 (31.1)	114.7 (27.6)	115.5 (33)	0.790	123.5 (27)	128.4 (29.6)	0.482	0.040	0.001
TG (mg/dL)	94.7 (65.1)	98 (55.1)	76 (40.7)	<0.001	150.7 (74.2)	129 (112.7)	0.002	<0.001	<0.001
FFA (mEq/L)	0.66 (0.25)	0.58 (0.24)	0.68 (0.23)	<0.001	0.69 (0.28)	0.74 (0.28)	0.283	0.009	0.094
APRI	0.23 (0.11)	0.25 (0.10)	0.22 (0.09)	<0.001	0.29 (0.16)	0.24 (0.13)	0.019	0.178	0.308
Serum retinol and carotenoids levels (μg/mL)									
Retinol	0.587 (0.154)	0.662 (0.159)	0.540 (0.135)	<0.001	0.676 (0.152)	0.577 (0.136)	<0.001	0.554	0.025
Lutein	0.304 (0.147)	0.269 (0.114)	0.331 (0.164)	<0.001	0.251 (0.101)	0.296 (0.127)	0.056	0.451	0.101
Zeaxanthin	0.061 (0.023)	0.059 (0.021)	0.063 (0.025)	0.278	0.058 (0.017)	0.058 (0.019)	0.764	0.840	0.150
β-cryptoxanthin	0.140 (0.081)	0.108 (0.052)	0.159 (0.093)	<0.001	0.105 (0.048)	0.152 (0.067)	<0.001	0.910	0.897
α-carotene	0.148 (0.124)	0.128 (0.119)	0.165 (0.136)	<0.001	0.108 (0.080)	0.139 (0.084)	0.002	0.282	0.066
β-carotene	0.441 (0.319)	0.312 (0.243)	0.539 (0.349)	<0.001	0.242 (0.140)	0.422 (0.232)	<0.001	0.193	0.014
Lycopene	0.283 (0.166)	0.269 (0.166)	0.297 (0.173)	0.084	0.250 (0.129)	0.276 (0.157)	0.610	0.747	0.367
β-carotene/retinol ratio	0.793 (0.587)	0.497 (0.372)	1.012 (0.625)	<0.001	0.369 (0.206)	0.752 (0.422)	<0.001	0.184	0.001

ALT, alanine aminotransferase; APRI, aspartate aminotransferase-to-platelet ratio index; AST, aspartate aminotransferase; FFA, free fatty acid; GGT, γ-glutamyl-transpeptidase; HbA1c, glycated hemoglobin; HOMA-IR, homeostasis model assessment as an index of insulin resistance; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NAFLD, non-alcoholic fatty liver disease; TC, total cholesterol; TG, triacylglycerol

Data are n (%) or means (±SD). Boldface type indicates $P < 0.05$

*P value for comparison between men with or without NAFLD.

[†]P value for comparison between women with or without NAFLD.

Table 3

Serum levels of retinol and β-carotene, and β-carotene/retinol ratio by quartile and likelihood of NAFLD

	Quartiles of antioxidant vitamin and carotenoid levels				P _{trend}
	Quartile 1 (lowest)	Quartile 2	Quartile 3	Quartile 4 (highest)	
Retinol					
NAFLD/Normal, n	21/151	29/152	36/152	42/151	
Median (range), (μg/mL)	0.422 (0.244–0.480)	0.528 (0.480–0.572)	0.621 (0.573–0.669)	0.759 (0.669–1.176)	
Adjusted OR* (95% CI)	1.00	1.72 (0.79–3.71)	1.12 (0.50–2.52)	2.27 (0.95–5.44)	0.184
Lutein					
NAFLD/Normal, n	41/151	30/152	34/152	23/151	
Median (range), (μg/mL)	0.164 (0.062–0.209)	0.242 (0.209–0.278)	0.315 (0.278–0.363)	0.453 (0.364–1.325)	
Adjusted OR* (95% CI)	1.00	0.82 (0.42–1.57)	1.24 (0.61–2.48)	0.63 (0.26–1.40)	0.455
α-carotene					
NAFLD/Normal, n	44/151	33/152	24/152	27/151	
Median (range), (μg/mL)	0.059 (0.016–0.077)	0.094 (0.077–0.113)	0.124 (0.115–0.177)	0.233 (0.178–1.534)	
Adjusted OR* (95% CI)	1.00	0.66 (0.34–1.27)	0.59 (0.29–1.22)	0.92 (0.44–1.96)	0.566
β-carotene					
NAFLD/Normal, n	39/151	42/152	32/152	15/151	
Median (range), (μg/mL)	0.161 (0.023–0.213)	0.281 (0.215–0.345)	0.437 (0.346–0.576)	0.814 (0.580–3.019)	
Adjusted OR* (95% CI)	1.00	1.95 (0.95–4.02)	1.32 (0.64–2.72)	0.56 (0.19–1.67)	0.162
β-carotene/Retinol ratio					
NAFLD/Normal, n	43/151	42/152	31/152	12/151	
Median (range), (μg/mL)	0.245 (0.045–0.379)	0.514 (0.380–0.626)	0.798 (0.630–1.068)	1.464 (1.069–5.099)	
Adjusted OR* (95% CI)	1.00	1.69 (0.83–3.43)	1.10 (0.52–2.35)	0.64 (0.21–1.92)	0.041

NAFLD, non-alcoholic fatty liver disease.

Boldface type indicates $P < 0.05$.

*Adjusted OR was adjusted for age, sex, diabetes, dyslipidemia, hypertension, body mass index, body fat percentage, current smoking, habitual exercise, energy intake, alcohol consumption, and each daily intake of retinol and carotenoids.

Table 4

Logistic regression analysis of factors associated with NAFLD

Variables	Adjusted OR (95% CI)	P value
Dyslipidemia	2.48 (1.54–3.99)	<0.001
BMI (kg/m ²)	1.24 (1.06–1.44)	0.006
β-carotene/retinol ratio	0.76 (0.59–0.99)	0.041

BFP, body fat percentage; BMI, body mass index; NAFLD, nonalcoholic fatty liver disease

Adjusted factors: age, sex, diabetes, dyslipidemia, hypertension, BMI, BFP, current smoking, habitual exercise, energy intake, alcohol consumption, and daily intake of retinol equivalent and β-carotene equivalent.

Boldface type indicates $P < 0.05$.

95% CI, 0.19–1.67, $P_{\text{trend}} = 0.162$). On the other hand, the highest quartile of SC/SR ratios had reduced likelihood of NAFLD by two-thirds of those in the lowest quartile (adjusted OR, 0.64; 95% CI, 0.21–1.92; $P_{\text{trend}} = 0.041$; Table 3). Dyslipidemia, BMI, and SC/SR ratio were independently associated with NAFLD (Table 4).

The SC/SR ratio decreased with increasing severity of steatosis in women, but not in men (Fig. 2A). The SC/SR ratio was higher in women than men regardless of the presence (0.36 ± 0.20 versus 0.75 ± 0.42 , $P < 0.001$) or absence (0.54 ± 0.42 versus 1.01 ± 0.62 , $P < 0.001$) of NAFLD, and the SC/SR ratio in participants with NAFLD was lower in women than in those without NAFLD ($P = 0.003$; Fig. 2B). Similarly, serum β-carotene levels were higher in women than men regardless of the presence (0.24 ± 0.14 versus 0.42 ± 0.23 μg/mL, $P < 0.001$) or absence (0.31 ± 0.24 versus 0.53 ± 0.34 μg/mL, $P < 0.001$, Fig. 2C) of NAFLD. On the other hand, serum retinol levels were lower in women than men regardless of the presence (0.67 ± 0.15 versus 0.57 ± 0.13 , $P < 0.001$) or absence (0.66 ± 0.15 versus 0.54 ± 0.13 μg/mL, $P = 0.001$, Fig. 2D) of NAFLD. The SC/SR ratio showed positive correlations with age and BFP ($r = 0.204$, $P < 0.001$, Fig. 2E; $r = 0.146$, $P < 0.001$, Fig. 2G), whereas the SC/SR ratio showed a negative correlation with BMI ($r = -0.251$, $P < 0.001$, Fig. 2F).

Correlations between SC/SR and lipid profile and APRI

The SC/SR ratio was positively correlated best with serum HDL-C levels ($r = 0.354$, $P < 0.001$, Fig. 3B), and was very weakly correlated with levels of serum TC ($r = 0.176$, $P < 0.001$, Fig. 3A), LDL-C ($r = 0.120$, $P < 0.001$, Fig. 3C), and FFA ($r = 0.079$, $P = 0.020$, Fig. 2E).

On the other hand, the SC/SR ratio was negatively correlated with levels of serum TGs ($r = -0.301$, $P < 0.001$, Fig. 3D). There was no correlation between APRI and SC/SR ratio ($r = 0.016$, $P = 0.691$, Fig. 3F).

Discussion

The present study investigated the relationship between the SC/SR ratio and severity of steatosis in NAFLD in an unselected, community-dwelling population using a cross-sectional study design. The SC/SR ratio was significantly associated with NAFLD. Women, in particular, had a higher SC/SR ratio than men, and the SC/SR ratio in women decreased with exacerbation of hepatic steatosis.

β-carotene, the most abundant carotenoid in the liver [11], is metabolized into retinol [32]. Previous studies have shown that a higher serum retinol level and lower serum levels of retinoic acid and carotenoids with altering hepatic gene expression are involved in retinol metabolism in individuals with NAFLD [16,21,33]. In the present study, women showed higher serum levels of β-cryptoxanthin, α-carotene, and β-carotene; lower serum levels of retinol; and a higher SC/SR ratio than men, regardless of the presence or absence of NAFLD. Furthermore, only in women, were the serum levels of β-carotene and SC/SR ratio lower in the participants with NAFLD than in those without NAFLD. Sex differences have been reported as factors affecting the serum levels of β-carotene and retinol [34]. Indeed, the present study showed sex differences in the SC/SR ratio and serum levels of β-carotene and retinol regardless of the presence or absence of NAFLD. After all, sex differences may be considered an important factor for the evaluation of β-carotene and retinol metabolism.

Furthermore, we observed that the decline in the SC/SR ratio significantly depended on exacerbation of steatosis in women. Therefore, retinol and β-carotene might be most involved in the pathogenesis of NAFLD, especially in women. The differences in daily carotenoid intake might cause a sex difference in the SC/SR ratio. Women had higher daily intake of α-carotene and β-carotene, but in both men and women, there were no differences in mean daily retinol and carotenoid intake between participants with or without NAFLD. However, by adjusting for confounding factors including carotenoid intake, only the SC/SR ratio was associated with hepatic steatosis. Additionally, previous studies reported that gut microbiota [35], genetic variations [36], and dietary patterns [37] affected bioavailability and absorption of carotenoids, and serum retinol level via alteration of β-carotene

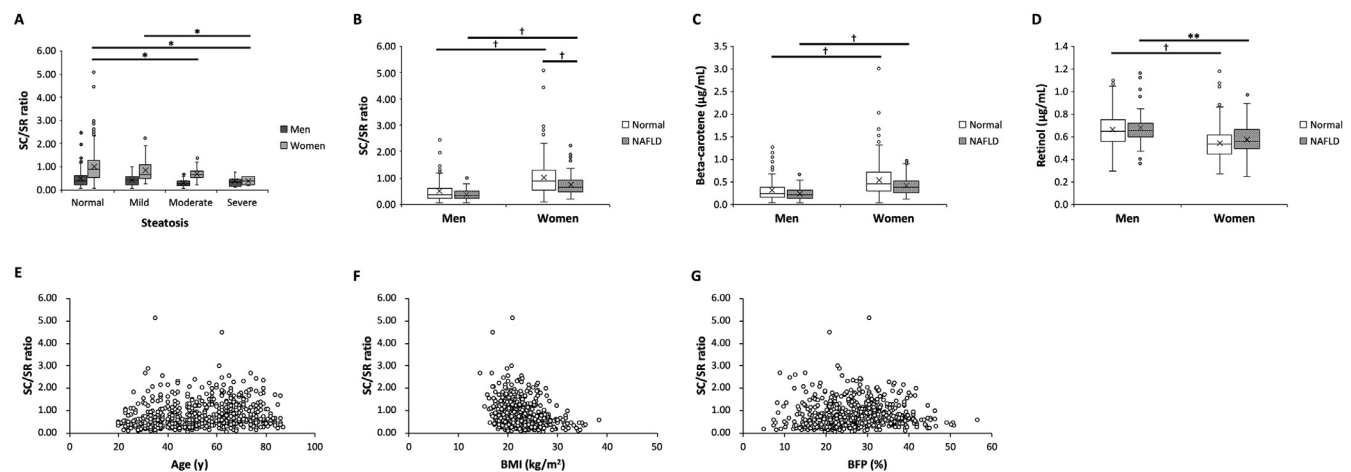


Fig. 2. Relationship of the SC/SR ratio to severity of hepatic steatosis and clinical characteristics in NAFLD. (A) Correlation between hepatic steatosis and the SC/SR ratio by sex. Sex differences with or without NAFLD in SC/SR ratio (B), serum β-carotene level (C), and serum retinol level (D). Correlation between the SC/SR ratio and age (E), BMI (F), and BFP (G). * $P < 0.05$; † $P < 0.001$; ‡ $P < 0.01$. BFP, body fat percentage; BMI, body mass index; NAFLD, non-alcoholic fatty liver disease; SC/SR, serum β-carotene-to-retinol.

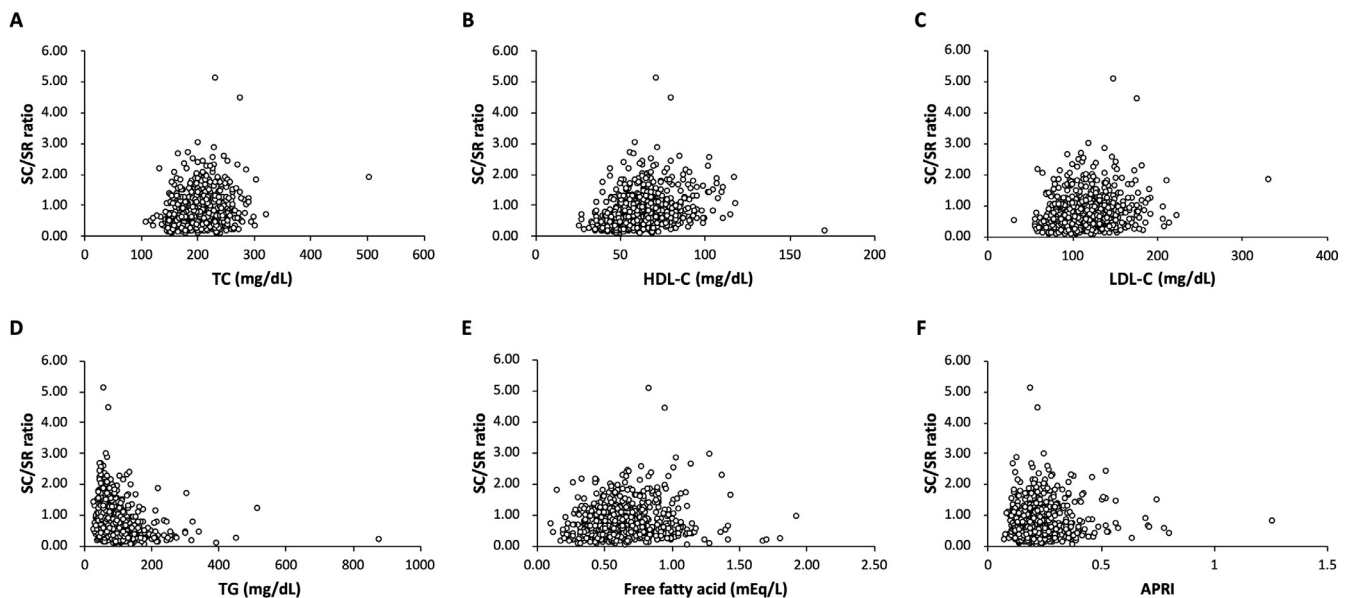


Fig. 3. Correlation between the SC/SR ratio and lipid profile and APRI. (A) TC; (B) HDL-C; (C) LDL-C; (D) TG; (E) FFA. (F) APRI. APRI, aspartate aminotransferase to platelet ratio index; FFA, free fatty acid; HDL-C, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; SC/SR, serum β -carotene-to-retinol; TC, total cholesterol; TG, triacylglycerol.

15,15'-monooxygenase (BCMO) activity. Unfortunately, we did not have enough data to analyze these items in this study. Regarding dietary assessment, we used the BDHQ in this study as in previous studies [22,38]. Recently, the Dietary Approaches to Stop Hypertension (DASH) dietary pattern analysis has been used for analysis of the association between dietary pattern and the risk for NAFLD [39–41]. The DASH score is calculated based on energy-adjusted intakes of food and nutrients emphasized or minimized in the DASH diet [42]. This method uses higher adherence to assess the dietary pattern and health outcomes [43,44]. Thus, we consider that DASH dietary pattern analysis will be necessary for our future longitudinal study of NAFLD.

From the perspective of retinol metabolism, alteration of the SC/SR ratio may indicate changes in activity and expression in hepatic metabolizing enzymes such as BCMO, aldo-keto reductase, and alcohol dehydrogenase, which are involved in the conversion between β -carotene and retinol [33]. Regarding the correlation between the SC/SR ratio and lipid profile, the SC/SR ratio was positively correlated with HDL-C level, while the SC/SR ratio was negatively correlated with TG in our study. However, a previous study reported that women had higher HDL-C levels and lower TG levels than men [45]. On the other hand, the SC/SR ratio was correlated positively with age and negatively with BMI; whereas in our study, the SC/SR ratio was not correlated with APRI, which indicates liver fibrosis and cirrhosis. The pathogenesis of NAFLD is multifactorial including lifestyle, nutrition, and genetic predisposition. Although dyslipidemia, BMI, and the SC/SR ratio were independently associated with NAFLD in the present study, further analysis is needed to clarify the relationship between the SC/SR ratio and each factor.

As shown in epidemiologic studies, certain carotenoids play protective roles in the development of NAFLD, and the protective mechanisms of carotenoids in NAFLD are based on their antioxidant, lipid-lowering, and insulin-sensitizing actions [18,46,47]. In the present study, however, NAFLD was associated with the SC/SR ratio rather than serum carotenoid levels. Based on these results, it is deduced that not only serum carotenoid levels alone but also the interaction between carotenoids and retinol might be an important factor for the pathogenesis of NAFLD. However, the causal

relationship between the SC/SR ratio and NAFLD was unclear, because this was a cross-sectional study.

On the other hand, from the viewpoint of basic research, HSCs play a key role in hepatic fibrosis, and they store the most vitamin A in the whole body through retinyl ester-filled lipid droplets [48]. In the process of hepatic fibrosis, quiescent HSCs transform to activated HSCs while losing the vitamin A-containing lipid droplets [10]. In fact, the SC/SR ratio is lower in patients with liver fibrosis than in those without liver fibrosis in NAFLD [16]. Moreover, a recent study has shown that serum levels of retinoid and carotenoid are associated with the risk for developing hepatocellular carcinoma [49]. Therefore, the SC/SR ratio might also reflect the fibrogenic risk in NAFLD, especially NASH, and hepatocarcinogenesis. Therefore, further studies with longitudinal observations are needed to clarify the causal relationship of the SC/SR with NAFLD.

There were several limitations to the present study. First, because this study was limited by its cross-sectional design, we cannot determine whether alteration of retinol and carotenoid status is a risk factor for future onset of NAFLD. Thus, longitudinal studies must be considered in the future to investigate the association between NAFLD and retinol and carotenoids. Second, the diagnosis of NAFLD was made based on ultrasonography examination without liver biopsy due to the invasive nature of biopsy. Instead, a common ultrasonographic definition of fatty liver was established and used as a noninvasive modality [50]. Although the sensitivity to detect hepatic steatosis by ultrasonography is low [51], ultrasonography is easily available and cheap [52]. Third, the present study was limited to Japanese individuals, and hence possible ethnic differences were not considered. Fourth, we could not analyze the medications being used in detail due to a lack of interview information; thus, the influence of medications was not completely eliminated from the evaluation of the serum levels of β -carotene and retinol in the present study. Medications have been reported as factors affecting the serum levels of β -carotene and retinol [53,54]. Despite these limitations, this general population-based study showed an association between the SC/SR ratio and NAFLD.

Conclusion

We found that the SC/SR ratio was lower in NAFLD evaluated by ultrasonography, and it was associated with the severity of hepatic steatosis in NAFLD with sex differences in a Japanese community population. Additionally, the SC/SR ratio was also correlated with HDL-C positively, and with TG negatively, in NAFLD. Further prospective studies are needed to confirm the association between the SC/SR ratio and NAFLD.

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