Public Health Nutrition: ***27****(e168), 1–9* doi:10.1017/S1368980024001502

Association between dietary intake of carotenoids and metabolic dysfunction-associated fatty liver disease in US adults: National Health and Nutrition Examination Survey 2017–March 2020

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Submitted 12 October 2023: Final revision received 14 June 2024: Accepted 15 July 2024

Abstract

Objective: To assess the relationship between dietary intake of α-carotene, β-carotene, β-cryptoxanthin, lycopene and lutein+zeaxanthin (LZ) and occurrence of metabolic dysfunction-associated fatty liver disease (MAFLD).

Design: Cross-sectional study design. The MAFLD diagnosis was based on hepatic steatosis and metabolic dysregulation. Carotenoid intake was adjusted for using an energy-adjusted model. Logistic regression and restricted cubic spline (RCS) analyses were used to assess the relationships, with sensitivity analysis to validate the findings. Weighted quantile sum regression (WQS) was used to explore the combined effect of these carotenoids on MAFLD. Subgroup analyses were conducted to identify population-specific associations.

Setting: National Health and Nutrition Examination Survey (NHANES) 2017–March 2020.

Participants: This study included 5098 individuals aged 18 years and older.

Results: After adjusting for potential confounders, a weak inverse association was observed between α-carotene and β-carotene intakes and MAFLD occurrence (all P value <0·05). The highest quartile of β-carotene intake showed a significantly lower occurrence of MAFLD compared with the lowest quartile (OR = 0·65; 95 % CI: 0·44, 0·97). RCS analysis showed that a significantly lower occurrence of MAFLD was associated with a higher intake of the four carotenoids, excluding lycopene. Furthermore, the WQS analysis revealed a negative relationship between combined carotenoid intake and MAFLD occurrence (OR = 0·95, 95 % CI: 0·90, 1·00, P = 0·037). Subgroup analyses showed dietary carotenoid intake was associated with reduced MAFLD occurrence in populations aged 50–69 years, females, physically active individuals and non-drinkers.

Conclusion: Higher dietary intake of carotenoids is associated with lower MAFLD occurrence. However, this relationship varies among individuals of different ages, sexes and lifestyles.

Keywords
Dietary intake
Carotenoids
Metabolic dysfunction-associated fatty liver disease
National Health and Nutrition Examination Survey

Metabolic Dysfunction-Associated Fatty Liver Disease (MAFLD) is characterised by the co-occurrence of ≥5 % hepatic steatosis and metabolic disorders. This new term supersedes nonalcoholic fatty liver disease, as proposed by an international expert panel in early 2020, emphasising the crucial role of metabolic risk factors in liver steatosis[1]. Besides liver disease, individuals with MAFLD are prone to extrahepatic diseases such as CVD[2], type 2 diabetes mellitus, chronic kidney disease[3] and cognitive impairment[4]. Among patients with MAFLD, the primary cause of death is CVD[5], with a 65 % increased risk of adverse cardiovascular events and a higher all-cause mortality rate[6].

The prevalence of MAFLD has escalated, affecting nearly one-quarter of adults globally and causing significant clinical and economic burdens. Unhealthy lifestyles, such as inadequate physical activity (PA), excessive caloric intake and unbalanced nutrient intake, primarily contribute

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https://doi.org/10.1017/S1368980024001502 Published online by Cambridge University Press

to this disease[1,7]. Targeting the improvement of metabolic disorders rather than organ-specific therapy can enhance the effectiveness of treatments. Because there are currently no approved drugs for MAFLD[7], dietary modifications and increased PA are the initial treatment strategies[8]. Some dietary nutrients that influence fat metabolism, such as sulforaphane and naringin, have gained attention[9,10]. Therefore, exploring dietary protective factors for MAFLD and improving lifestyle habits to reduce MAFLD occurrence are critical.

Although B-scan ultrasonography and CT are widely used in clinical imaging diagnostics, their sensitivity and specificity for diagnosing fatty liver disease are limited. Although magnetic resonance spectroscopy offers an accurate detection of hepatic steatosis, its high cost is a barrier to its widespread use. Fibroscan (Echosens, Paris, France), which uses vibration-controlled transient elastography to obtain controlled attenuation parameters (CAP) liver stiffness measurements, permits non-invasive and quantitative assessments of hepatic steatosis and fibrosis, offering superior sensitivity and specificity to B-ultrasound and CT[11].

Carotenoids, which are phytochemicals of polyphenols abundantly found in various green leafy vegetables, colourful fruits, fungi, algae and bacteria, are primarily composed of β-carotene, α-carotene, lutein, zeaxanthin, β-cryptoxanthin and lycopene[12]. These compounds offer several health benefits, including eye health, cognitive function, cardiovascular system health, bone health, immune function and cancer prevention[13]. As potent antioxidants and anti-inflammatory nutrients, carotenoids decrease malondialdehyde, tumor necrosis factor-α, IL-6 and IL-12 levels while increasing glutathione peroxidase and catalase activity to prevent liver steatosis and hepatocyte apoptosis[14]. Epidemiological studies have shown that individuals consuming higher carotenoid levels, including β-carotene, α-carotene, lutein, zeaxanthin and β-cryptoxanthin, were significantly associated with a decreased risk of nonalcoholic fatty liver disease[15]. However, the beneficial effects of carotenoids on MAFLD prevalence and liver fibrosis require further investigation.

This study utilised a large population from the National Health and Nutrition Examination Survey (NHANES) 2017–March 2020 to explore the association between the five types of carotenoids and MAFLD, as well as liver fibrosis evaluated by vibration-controlled transient elastography. The investigation also explored a dose–response relationship.

Methods

Study population

The study population was obtained from the NHANES, conducted from January 2017 to March 2020, which is a research program designed to assess the health and nutritional status of adults and children in the USA[16]. The survey cycle yielded 15 560 participants. Participants aged 18 years were excluded (n =5867). Individuals whose dietary recall status in the two interviews was reliable and met the minimum criteria were eligible for further analysis, with 2732 participants being excluded. Among the remaining subjects, 6173 completed the liver elastography examination. One participant with missing median CAP values was excluded from this study. Subsequently, participants with missing values for education level, house income, smoking status and PA were excluded (n =992). Furthermore, eighty-two participants whose MAFLD diagnoses were inconclusive were excluded. The final sample size comprised 5098 participants.

Definition of metabolic dysfunction-associated fatty liver disease

MAFLD was diagnosed based on the presence of hepatic steatosis coupled with one or more of the following criteria: overweight/obesity, type 2 diabetes mellitus or metabolic dysregulation[1]. Metabolic dysregulation was defined as meeting at least two of the following criteria. (1) waist circumference ≥102 cm in men and ≥88 cm in women, (2) blood pressure ≥130/85 mmHg or specific drug treatment, (3) plasma TAG ≥1·70 mmol/l or specific drug treatment, (4) plasma HDL-cholesterol <1·0 mmol/l for men and <1·3 mmol/l for women or specific drug treatment, (5) prediabetes (i.e. fasting glucose level 5·6–6·9 mmol/l, or HbA1c 5·7 % to 6·4 %), (6) homeostasis model assessment-insulin resistance score ≥2·5 and (7) plasma high-sensitivity C-reactive protein level >2 mg/l. Liver steatosis was defined as a CAP score ≥248 dB/m, while liver fibrosis was confirmed with liver stiffness measurements ≥ 6·3 kPa[17]. CAP and liver stiffness measurements were obtained using a FibroScan® model 502 V2 Touch.

Intake of dietary carotenoids

Detailed dietary intake data were obtained from the NHANES participants through two 24-h dietary recall interviews to estimate the consumption of energy, various nutrients and beverages over the preceding day. The first interview was conducted face-to-face at the NHANES mobile examination center, and the second was conducted via telephone 3–10 d later. The average of the two dietary surveys was used for the subsequent analysis. The complex survey design weight from the second dietary interview was applied to all the analyses in this study. Dietary carotenoids, including α-carotene, lycopene, β-carotene, β-cryptoxanthin and lutein+zeaxanthin (LZ), along with total energy and alcohol intake, were extracted from the dietary interviews. The intake of energy and carotenoids underwent logarithmic transformation. The dietary intake of carotenoid was adjusted for total energy intake using an energy-adjusted model[18,19].

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Definition of covariate

The NHANES provides extensive participant data through standardised questionnaires and laboratory tests, including demographic details, lifestyle factors and biochemical indicators. The selected covariates for our analysis included the healthy eating index-2015 (HEI-2015) scores, age, gender, race, education level, house income, PA, smoking and alcohol consumption. HEI-2015 scores were used to assess the alignment of individual dietary intake with the Dietary Guidelines of Americans, thus reflecting the dietary quality of the participants. Detailed methods for calculating HEI-2015 scores have been documented previously[20,21]. In summary, data on food pattern equivalents were derived from the United States Department of Agriculture Food Patterns Equivalents Database, and HEI-2015 scores were calculated using the SAS code provided by the National Cancer Institute (https://epi.grants.cancer.gov/hei/sas-code.html). Age was categorised into four groups: 18–29, 30–49, 50–69 and ≥70 years. Race was subdivided into Mexican American, Other Hispanic, Non-Hispanic White, Non-Hispanic Black and other races. Education level was categorised as less than high school, high school and more than high school. House income was assessed by poverty income ratio (PIR) and classified as low income (PIR < 1·30), middle income (1·3 ≤ PIR < 3·5) and high income (PIR ≥ 3·5)[17]. Smoking status was divided into never smokers (smoked less than 100 cigarettes in life), former smokers (smoked more than 100 cigarettes but did not smoke currently) and current smokers (smoked more than 100 cigarettes and smoke currently)[22]. Alcohol consumption was divided into non-drinkers (0 g/d), drinkers (> 0– < 70 g/d in males, > 0–< 56 g/d in females) and heavy drinkers (≥ 70 g/d in males, ≥ 56 g/d in females). PA was classified as low PA (<1MET-h/week), moderate PA (1-48 MET-h/week) and high PA (>48 MET-h/week)[23].

Statistical analysis

All statistical analyses were performed using R software (4·2·2). The 'survey' and 'rms' packages facilitated adjustments for the complex survey design to ensure national representativeness of the results. Continuous variables were presented as weighted mean ± SE and weighted median (P_{25}, P_{75}) and categorical variables as unweighted frequencies (weighted percentages). To discern the basic characteristic differences between subjects with and without MAFLD, weighted student's *t*-tests or χ^2 tests were employed. The logistic regression model examined the association between MAFLD and each of the five carotenoids (*β*-cryptoxanthin, *β*-carotene, *α*-carotene, LZ and lycopene). The first model was unadjusted; the second model was adjusted for age, gender and HEI-2015 scores and the third model was further adjusted for education level, race, smoking status, drinking status, PA and PIR. Carotenoid intake was also categorised based on quartile cutoffs and analyzed using logistic regression. OR and 95 % CI were calculated. Non-linear relationships were explored using a restricted cubic spline (RCS) with three (10th, 50th and 90th percentiles) or four knots (5th, 35th, 65th and 95th percentiles), with knot selection guided by the minimisation of Akaike's Information Criterion unless specified otherwise[24]. RCS analyses were executed by 'svyglm' and 'rcs' functions in the fully adjusted model, determining $P_{\text{for overall}}$ and $P_{\text{for non-linear}}$ via Wald tests and calculating 95 % CI using bootstrap methods. Statistical significance was defined as a two-tailed *P* value of <0·05.

Several sensitivity analyses were performed to assess the robustness of the proposed model. Initially, the diagnostic criterion for hepatic steatosis was modified to a CAP score ≥274, and the analysis was performed again. The combined effect of the five carotenoids on MAFLD was evaluated using weighted quantile sum regression (WQS), with the contribution of each carotenoid to the overall effect assessed. Carotenoid weights were obtained by bootstrapping 500 times in a training dataset and were tested using a validation dataset. The WQS index was calculated for all participants based on the weights of the five carotenoids. We primarily focused on the negative direction in the WQS analysis to avoid the reversal paradox[25]. We then performed RCS analysis adjusted for age, gender, education level, race, smoking status, drinking status, PA, PIR and HEI-2015, using the WQS index and MAFLD as the basis, while incorporating NHANES weights. Finally, subgroup analyses accounting for age, gender, alcohol consumption, smoking status and PA, but not considering interaction, were conducted to assess whether dietary carotenoids exerted specific effects on certain population subgroups.

Results

Basic characteristics of the study participants

This study included 5098 participants, of which 55·5 % were diagnosed with MAFLD based on the baseline characteristics outlined in Table 1. Subjects without MAFLD exhibited higher HEI-2015 scores, *α*-carotene, *β*-carotene and LZ intakes and significantly lower energy consumption. Additionally, participants with MAFLD were more likely to be older, male, smokers, less physically active and have lower education levels. Finally, the subjects with MAFLD were more susceptible to liver fibrosis.

Association between the five carotenoids and metabolic dysfunction-associated fatty liver disease

Logistic regression analysis was used to assess the relationship between these five carotenoids and MAFLD (Table 2). In the unadjusted model, *β*-carotene, *α*-carotene and LZ were inversely associated with the occurrence of MAFLD. After adjusting for factors including age, gender, education

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Table 1 Baseline characteristics of participants included in study according to the MAFLD

	Non-MAFLD		MAFLD		
Characteristic	*n* 2173	% (44·5 %)*	*n* 2925	% (55·5 %)*	*P*†
Age, %					<0·001
18–29	474	26·4	285	11·6	
30–49	744	36·6	894	33·0	
50–69	661	26·9	1262	40·6	
>=70	294	10·1	484	14·8	
Gender, %					<0·001
Male	973	42·9	1482	53·3	
Female	1200	57·1	1443	46·7	
Race, %					<0·001
Mexican American	161	5·8	409	9·6	
Other Hispanic	187	6·6	295	7·5	
Non-Hispanic White	775	65·3	1109	63·8	
Non-Hispanic Black	690	13·0	701	9·4	
Other Race	360	9·3	411	9·7	
Education level, %					0·006
<High School	297	7·5	477	10·0	
High School	474	23·5	708	28·7	
>High School	1402	68·9	1740	61·3	
Poverty income ratio, %					0·128
Low	600	18·1	773	18·2	
Middle	789	31·4	1169	34·6	
High	784	50·5	983	47·2	
Smoking status, %					<0·001
Current smoker	436	16·4	457	15·2	
Former smoker	420	21·0	833	29·1	
Never smoking	1317	62·6	1635	55·7	
PA level, %					<0·001
High	857	42·0	1025	39·3	
Moderate	889	43·7	1139	38·7	
Low	427	14·3	761	22·0	
Drink status, %					<0·001
Non-drinkers	1517	65·4	2137	69·1	
Drinkers	611	32·6	690	25·6	
Heavy drinkers	45	2·0	98	5·2	
Liver fibrosis, %					<0·001
No	1866	87·5	2021	72·8	
Yes	307	12·5	904	27·2	
HEI-2015	54·18	0·75	51·72	0·44	<0·001
	53·30	43·70, 64·06	51·58	42·24, 60·50	
Log α-carotene, mcg	6·45	0·13	6·06	0·09	0·003
	6·27	4·44, 8·92	5·95	4·25, 8·31	
Log β-carotene, mcg	10·26	0·08	9·96	0·06	<0·001
	10·28	8·86, 11·79	9·99	8·65, 11·22	
Log LZ, mcg	9·95	0·05	9·73	0·04	<0·001
	9·80	9·05, 10·84	9·72	8·87, 10·54	
Log Lycopene, mcg	10·10	0·17	9·93	0·13	0·413
	11·19	9·44, 12·64	11·07	9·46, 12·33	
Log β-cryptoxanthin, mcg	5·19	0·08	5·06	0·06	0·107
	5·14	3·97, 6·45	5·15	3·86, 6·32	
Log Energy, kcal	10·88	0·02	10·95	0·02	0·008
	10·87	10·51, 11·24	10·99	10·63, 11·34	

MAFLD, metabolic dysfunction-associated fatty liver disease; PA, physical activity; HEI-2015, health eating index-2015; LZ, lutein+zeaxanthin.
*Data are represented as *n* (unweighted) (weighted proportions %) for categorical variables and as weighted mean (SE) and weighted median (P_{25}, P_{75}) for continuous variables.
†Weighted *t* test and weighted χ^2 test were used to explore the difference between groups.

level, race, smoking status, drinking status, PA, PIR and HEI-2015, only β-carotene (OR = 0·94, 95 % CI: 0·89, 0·99) and α-carotene (OR = 0·96, 95 % CI: 0·91, 1·00) maintained a significant association with MAFLD occurrence, indicating that per 1 log mcg/d increase in β-carotene and α-carotene was associated with 6 % and 4 % lower risk for MAFLD occurrence, respectively, although the associations were weak. Subsequent analysis utilising quartile divisions of the dietary intake of the five carotenoids revealed that being in the highest quartile of carotenoid intake was associated with a 35 % lower risk for MAFLD for β-carotene (β-carotene: OR = 0·65, 95 % CI: 0·44, 0·97).

These results suggest a non-linear association between carotenoid intake and the risk of MAFLD. Hence, to

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Table 2 Logistic regression results for the association between dietary carotenoids and MAFLD

	Model 1*		Model 2†		Model 3‡	
	OR	95 % CI	OR	95 % CI	OR	95 % CI
β-cryptoxanthin						
per 1 log mcg/d increase	0·96	0·92, 1·01	0·98	0·93, 1·03	0·97	0·92, 1·03
Q1 (<3·899)		Reference		Reference		Reference
Q2 (≥3·899)	0·85	0·69, 1·05	0·91	0·74, 1·11	0·88	0·67, 1·17
Q3 (≥5·174)	1·01	0·83, 1·23	1·06	0·82, 1·36	1·07	0·73, 1·56
Q4 (≥6·397)	0·88	0·74, 1·05	0·97	0·77, 1·22	0·90	0·65, 1·25
β-carotene						
per 1 log mcg/d increase	0·92	0·89, 0·95	0·94	0·90, 0·98	0·94	0·89, 0·99
Q1 (<8·758)		Reference		Reference		Reference
Q2 (≥8·758)	0·86	0·64, 1·17	0·87	0·64, 1·18	0·84	0·52, 1·34
Q3 (≥10·129)	1·05	0·87, 1·27	1·04	0·83, 1·32	0·99	0·69, 1·41
Q4 (≥11·479)	0·60	0·48, 0·74	0·64	0·50, 0·81	0·65	0·44, 0·97
α-carotene						
per 1 log mcg/d increase	0·95	0·93, 0·98	0·96	0·93, 0·99	0·96	0·91, 1·00
Q1 (<4·346)		Reference		Reference		Reference
Q2 (≥4·346)	0·94	0·69, 1·29	1·00	0·70, 1·42	0·98	0·57, 1·68
Q3 (≥6·068)	0·93	0·72, 1·21	0·93	0·72, 1·20	0·87	0·60, 1·26
Q4 (≥8·581)	0·76	0·58, 1·00	0·83	0·62, 1·11	0·83	0·51, 1·33
LZ						
per 1 log mcg/d increase	0·90	0·86, 0·94	0·94	0·88, 1·00	0·94	0·87, 1·02
Q1 (<8·945)		Reference		Reference		Reference
Q2 (≥8·945)	0·69	0·55, 0·87	0·86	0·67, 1·10	0·86	0·58, 1·28
Q3 (≥9·763)	0·95	0·78, 1·16	0·94	0·74, 1·20	0·91	0·62, 1·34
Q4 (≥10·650)	0·86	0·69, 1·08	0·82	0·58, 1·15	0·86	0·49, 1·51
Lycopene						
per 1 log mcg/d increase	0·99	0·96, 1·02	0·99	0·97, 1·02	0·99	0·96, 1·03
Q1 (<9·461)		Reference		Reference		Reference
Q2 (≥9·461)	1·08	0·83, 1·40	1·15	0·88, 1·49	1·15	0·77, 1·73
Q3 (≥11·122)	1·13	0·81, 1·56	1·11	0·77, 1·60	1·08	0·62, 1·88
Q4 (≥12·463)	0·84	0·64, 1·09	0·93	0·71, 1·21	0·90	0·58, 1·39

MAFLD, metabolic dysfunction-associated fatty liver disease; LZ, lutein+zeaxanthin; OR, OR; 95 % CI, 95 % CI; HEI-2015, health eating index-2015.
*Model 1 was unadjusted model.
†Model 2 adjusted for age, gender and HEI-2015.
‡Model 3 was adjusted for age, gender, race, education level, smoking status, drinking status, physical activity, poverty income ratio and HEI-2015.

examine the dose–response relationship, RCS was incorporated into the fully adjusted logistic regression model. The results, displayed in Fig. 1, indicated a decrease in the MAFLD occurrence corresponded to dietary intakes of *α*-carotene ($P_{overall} = 0{\cdot}030$ and $P_{non\text{-}linear} = 0{\cdot}390$, Fig. 1(a)), *β*-carotene ($P_{overall} = 0{\cdot}008$ and $P_{non\text{-}linear} = 0{\cdot}050$, Fig. 1(b)), *β*-cryptoxanthin ($P_{overall} = 0{\cdot}013$ and $P_{non\text{-}linear} = 0{\cdot}007$, Fig. 1(c)), lycopene ($P_{overall} = 0{\cdot}178$ and $P_{non\text{-}linear} = 0{\cdot}101$, Fig. 1(d)) and LZ ($P_{overall} = 0{\cdot}013$ and $P_{non\text{-}linear} = 0{\cdot}080$, Fig. 1(e)) that surpassed approximately 0.29 mg, 1·05 mg, 0.15 mg, 0.83 mg and 1·91 mg, respectively.

Association between the five carotenoids and fibrosis

On the basis of the fully adjusted logistic regression model, no linear associations were identified between the five carotenoids and liver fibrosis (see online supplementary material, Supplemental Table S1). However, an interesting non-linear dose–response relationship was observed between *β*-cryptoxanthin and MAFLD (see online supplementary material, Supplemental Fig. S1), in which the occurrence of liver fibrosis rapidly increased when dietary *β*-cryptoxanthin intake exceeded 0·04 mg.

Sensitivity analysis

We re-evaluated the association between carotenoids and MAFLD using a CAP ≥ 274 diagnostic criterion for fatty liver disease, as suggested by prior studies. No carotenoids were associated with the occurrence of MAFLD in the fully adjusted model (see online supplementary material, Supplemental Table S2). However, RCS analysis suggested that the dietary intake of *β*-carotene ($P_{overall} = 0{\cdot}030$ and $P_{non\text{-}linear} = 0{\cdot}209$) and lycopene ($P_{overall} = 0{\cdot}015$ and $P_{non\text{-}linear} = 0{\cdot}005$) had a significant dose–response relationship with MAFLD occurrence (see online supplementary material, Supplemental Fig. S2).

The WQS index, representing the combined effect of the five carotenoids calculated using WQS regression, was negatively correlated with MAFLD development (OR = 0·95, 95 % CI: 0·90, 1·00, $P = 0{\cdot}037$). The non-linear relationship between this index and the risk of MAFLD was explored by RCS (see online supplementary material, Supplemental Fig. S3(a)), with *β*-carotene identified as the largest contributor to this combined effect (see online supplementary material, Supplemental Fig. S3(b)).

Subgroup analyses were conducted to investigate the effects of carotenoids on different population groups,

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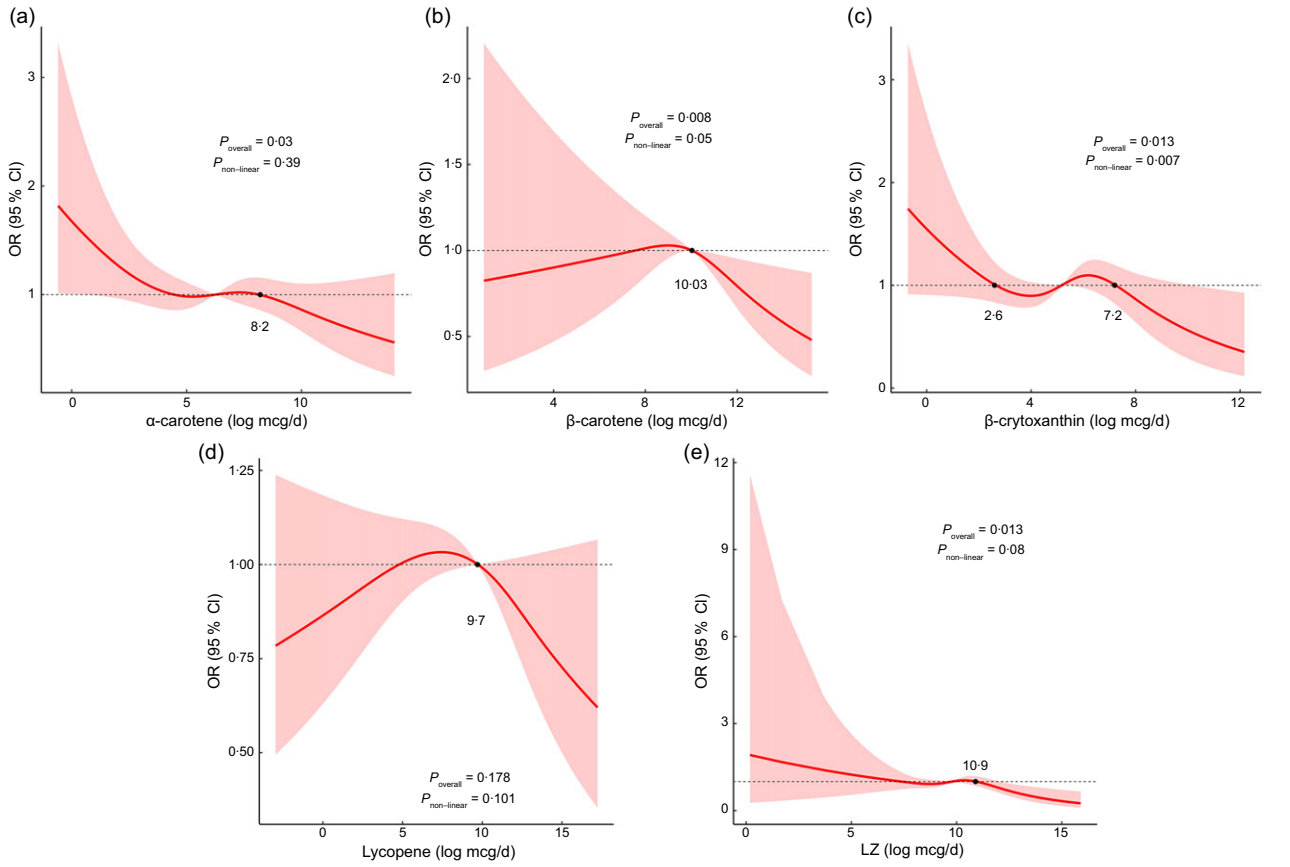

Fig. 1 The dose–response relationships between the dietary intake of five carotenoids and MAFLD using restricted cubic spline regression model. The model was adjusted for age, gender, race, education level, smoking status, drinking status, physical activity, poverty income ratio and HEI-2015 scores. The intake of carotenoids was log-transformed. HEI-2015, health eating index-2015; LZ, lutein+zeaxanthin; MAFLD, metabolic dysfunction-associated fatty liver disease.

revealing a predominantly negative relationship between carotenoids and MAFLD occurrence in the 50–69 age group (see online supplementary material, Supplemental Table S3, Fig. S4). An increase in the dietary intake of carotenoids was more likely to be associated with a decreased MAFLD occurrence in females (see online supplementary material, Supplemental Table S4, Fig. S5). Sex-specific carotenoids, *β*-carotene ($P_{overall} = 0{\cdot}027$ and $P_{non\text{-}linear} = 0{\cdot}036$), lycopene ($P_{overall} = 0{\cdot}003$ and $P_{non\text{-}linear} = 0{\cdot}001$) and LZ ($P_{overall} < 0{\cdot}001$ and $P_{non\text{-}linear} = 0{\cdot}011$), were identified, which only had a dose–response relationship with MAFLD in females. In the smoking status stratified analysis, only an increase in *α*-carotene ($P_{overall} < 0{\cdot}001$ and $P_{non\text{-}linear} = 0{\cdot}022$) intake was associated with a decreased occurrence of MAFLD in current smokers, whereas LZ potentially acted as a risk factor (see online supplementary material, Supplemental Table S5, Fig. S6). Moreover, when participants were divided into drinkers and non-drinkers, carotenoids exhibited no association with the occurrence of MAFLD in either group according to logistic regression (see online supplementary material, Supplemental Table S6). However, RCS analysis revealed that increases in *β*-carotene ($P_{overall} = 0{\cdot}015$ and $P_{non\text{-}linear} = 0{\cdot}086$), *β*-cryptoxanthin ($P_{overall} = 0{\cdot}039$ and $P_{non\text{-}linear} = 0{\cdot}021$) and LZ ($P_{overall} < 0{\cdot}001$ and $P_{non\text{-}linear} < 0{\cdot}001$) were significantly associated with reduced occurrence of MAFLD in non-drinkers (see online supplementary material, Supplemental Fig. S7). Finally, subgroup analysis revealed that carotenoids were not significantly associated with MAFLD occurrence in individuals with low or moderate PA (see online supplementary material, Supplemental Table S7, Fig. S8).

Discussion

MAFLD is characterised by liver lesions accompanied by one or more metabolic disorders. MAFLD has become the most common liver disease worldwide, and its development is mainly due to unhealthy lifestyles and dietary habits, posing a serious threat to public health[26]. Previous studies have indicated that polyphenols can mitigate MAFLD through the regulation of the gut microbiota[27,28]. In this study, we investigated the correlation between the five types of carotenoids and MAFLD using cross-sectional data from the NHANES 2017–March 2020. The results

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showed a weak association between β-carotene and MAFLD. After categorising the dietary intake of carotenoids into quantiles, higher quantile intakes of β-carotene and α-carotene were significantly associated with a reduced occurrence of MAFLD in the unadjusted model, with only β-carotene maintaining this association in the fully adjusted model. We also uncovered a dose–response relationship between carotenoid intake and the occurrence of MAFLD. In conclusion, our results suggest a protective effect of a higher intake of carotenoids against MAFLD.

RCS results indicated that the risk of MAFLD occurrence hardly changed or even increased as carotenoid intake increased below a certain threshold. However, when intake exceeded this value, these carotenoids became protective factors. These findings might be partially explained by the anti-inflammatory and anti-oxidant properties of carotenoids[29,30]. Certain provitamin As, such as β-cryptoxanthin, β-carotene and α-carotene, can be converted to vitamin A in the body, thereby improving liver fat accumulation and metabolic disorders[31,32]. Moreover, apocarotenoids, especially non-retinoid cleavage products of carotenoids, can also interact with retinoic acid receptors[33] and exert beneficial effects on the prevention of abdominal adiposity[34]. Finally, carotenoids, as polyphenols, might also regulate the gut microbiota to improve the MAFLD phenotype[27,28]. Consistent with our results, epidemiological data have revealed that higher serum carotenoids are inversely associated with the prevalence and progression of nonalcoholic fatty liver disease[35].

The RCS results also revealed that higher intake of β-cryptoxanthin and lycopene was positively and negatively associated with the occurrence of liver fibrosis, respectively. However, some researchers have reported that only a higher dietary intake of α-carotene among the five types of carotenoids is positively associated with liver fibrosis[36]. This discrepancy could be attributed to differences in the study populations, as our study was based on a larger sample size. In addition, many studies have demonstrated the hepatoprotective properties of lycopene[37,38], consistent with our findings.

Retinol metabolism occurs in the hepatic microsomes of cytochrome P450s, which metabolise alcohol, drugs and xenobiotic pollutants[39]. Consequently, the toxicity of vitamin A and pro-vitamin A carotenoids can be intensified in individuals simultaneously exposed to alcohol, drugs and some pollutants, potentially leading to hepatotoxicity. Furthermore, an increase in hepatic retinol levels may be positively associated with liver fibrosis[40]. These findings could partially explain why vitamin A exacerbated liver fibrosis during CCl4 treatment in a previous rat model study[41] and why β-cryptoxanthin increased the occurrence of liver fibrosis in our analysis.

Evidence suggests that smoking can induce a shift in carotenoids towards pro-oxidants and affect the cellular response to smoking[42]. Two large epidemiological studies have also reported that β-carotene supplementation was associated with a higher risk of lung cancer among smokers[43,44]. These studies showed that the interaction between smoking and carotenoids could diminish or even eliminate their beneficial effects, which aligns with our findings.

The stratified analysis in our study also suggested that PA might influence the effects of carotenoids. PA is known to enhance blood antioxidant capacity, and several studies have found a positive association between higher intake of carotenoids and higher levels of PA[45–48]. We hypothesised that PA might amplify the effects of carotenoids and increase their utilisation, implying that higher carotenoid intake is positively associated with a lower occurrence of MAFLD in populations with more PA.

This study had several notable strengths. The dietary intake of carotenoids was adjusted for dietary energy intake according to the energy-adjusted model. Additionally, the combined effect of the five carotenoids was considered using WQS regression. Finally, the data were sourced from the NHANES to ensure accuracy and reliability.

However, our study also had several limitations. First, the causal relationship explored in a cross-sectional study might not be confirmed, necessitating further cohort studies and clinical trials. Second, some potential confounders might not have been considered. Third, our study population was from the USA, which might limit the applicability of our findings to other populations. Finally, although vibration-controlled transient elastography possesses the merits of high efficiency and a non-invasive approach to determining liver steatosis and fibrosis, liver biopsy remains the gold standard and the cut-off value of CAP and liver stiffness measurements a subject of controversy.

Conclusion

This study suggests that a higher dietary intake of carotenoid is associated with a lower occurrence of MAFLD. However, the association between certain carotenoids and the occurrence of MAFLD may vary among individuals with different age, gender and lifestyles. These findings suggest that dietary carotenoid intake may play an important role in preventing MAFLD. Further prospective studies are needed to validate these findings.

Acknowledgements

We thank the participants in the NHANES study.

Financial support

This research received no external funding.

https://doi.org/10.1017/S1368980024001502 Published online by Cambridge University Press

Conflict of interest

None.

Authorship

J.Y. and P.G. conceived and advised on all aspects of the study. J.Y. and P.G. analyzed data and wrote the manuscript. P.G. and J.Y. supervised all aspects of the study. All authors discussed and commented on the manuscript.

Ethics of human subject participation

All data were obtained from secondary sources and available publicly. No protocol approval was necessary. The datasets analyzed for present study can be found in the NHANES database https://www.cdc.gov/nchs/nhanes.

Supplementary material

For supplementary material accompanying this paper visit https://doi.org/10.1017/S1368980024001502

References

1. Eslam M, Newsome PN, Sarin SK *et al.* (2020) A new definition for metabolic dysfunction-associated fatty liver disease: an international expert consensus statement. *J Hepatol* **73**, 202–209.
2. Lee H, Lee YH, Kim SU *et al.* (2021) Metabolic dysfunction-associated fatty liver disease and incident cardiovascular disease risk: a nationwide cohort study. *Clin Gastroenterol Hepatol* **19**, 2138–2147 e2110.
3. Wang TY, Wang RF, Bu ZY *et al.* (2022) Association of metabolic dysfunction-associated fatty liver disease with kidney disease. *Nat Rev Nephrol* **18**, 259–268.
4. Yu Q, He R, Jiang H *et al.* (2022) Association between metabolic dysfunction-associated fatty liver disease and cognitive impairment. *J Clin Transl Hepatol* **10**, 1034–1041.
5. Ramandi A, George J, Merat S *et al.* (2023) Polypill protects MAFLD patients from cardiovascular events and mortality: a prospective trial. *Hepatol Int* **17**, 882–888.
6. Kim D, Konyn P, Sandhu KK *et al.* (2021) Metabolic dysfunction-associated fatty liver disease is associated with increased all-cause mortality in the United States. *J Hepatol* **75**, 1284–1291.
7. Eslam M, Sanyal AJ, George J *et al.* (2020) MAFLD: a consensus-driven proposed nomenclature for metabolic associated fatty liver disease. *Gastroenterology* **158**, 1999–2014 e1991.
8. Shi Y & Fan J (2022) Therapeutic developments in metabolic dysfunction-associated fatty liver disease. *Chin Med J* **135**, 1009–1018.
9. Li J, Xie S & Teng W (2021) Sulforaphane attenuates nonalcoholic fatty liver disease by inhibiting hepatic steatosis and apoptosis. *Nutrients* **14**, 16.
10. Guo P, Wang P, Liu L *et al.* (2023) Naringin alleviates glucose-induced aging by reducing fat accumulation and promoting autophagy in caenorhabditis elegans. *Nutrients* **15**, 907.
11. Castera L, Friedrich-Rust M & Loomba R (2019) Noninvasive assessment of liver disease in patients with nonalcoholic fatty liver disease. *Gastroenterology* **156**, 1264–1281 e1264.
12. Melendez-Martinez AJ, Mandic AI, Bantis F *et al.* (2022) A comprehensive review on carotenoids in foods and feeds: status quo, applications, patents, and research needs. *Crit Rev Food Sci Nutr* **62**, 1999–2049.
13. Eggersdorfer M & Wyss A (2018) Carotenoids in human nutrition and health. *Arch Biochem Biophys* **652**, 18–26.
14. Yilmaz B, Sahin K, Bilen H *et al.* (2015) Carotenoids and non-alcoholic fatty liver disease. *Hepatobil Surg Nutr* **4**, 161–171.
15. Christensen K, Lawler T & Mares J (2019) Dietary carotenoids and non-alcoholic fatty liver disease among US Adults, NHANES 2003–2014. *Nutrients* **11**, 1101.
16. Ahluwalia N, Dwyer J, Terry A *et al.* (2016) Update on NHANES dietary data: focus on collection, release, analytical considerations, and uses to inform public policy. *Adv Nutr* **7**, 121–134.
17. Tian T, Zhang J, Xie W *et al.* (2022) Dietary quality and relationships with metabolic dysfunction-associated fatty liver disease (MAFLD) among United States adults, results from NHANES 2017–2018. *Nutrients* **14**, 4505.
18. Willett WC, Howe GR & Kushi LH (1997) Adjustment for total energy intake in epidemiologic studies. *Am J Clin Nutr* **65**, 1220S–1228S.
19. Kan B, Guo D, Yuan B *et al.* (2021) Dietary carotenoid intake and osteoporosis: the national health and nutrition examination survey, 2005–2018. *Arch Osteoporos* **17**, 2.
20. Zhu Y & Wang Z (2024) Association between joint physical activity and healthy dietary patterns and hypertension in US adults: cross-sectional NHANES study. *BMC Public Health* **24**, 855.
21. Shea MK, Barger K, Rogers GT *et al.* (2024) Dietary intakes of community-dwelling adults in the United States across older adulthood: national health and nutrition examination survey 2015-March 2020. *J Nutr* **154**, 691–696.
22. Zhou J, Meng X, Deng L *et al.* (2022) Non-linear associations between metabolic syndrome and four typical heavy metals: data from NHANES 2011–2018. *Chemosphere* **291**, 132953.
23. Tian X, Xue B, Wang B *et al.* (2022) Physical activity reduces the role of blood cadmium on depression: a cross-sectional analysis with NHANES data. *Environ Pollut* **304**, 119211.
24. Wei X, Min Y, Song G *et al.* (2024) Association between triglyceride-glucose related indices with the all-cause and cause-specific mortality among the population with metabolic syndrome. *Cardiovasc Diabetol* **23**, 134.
25. Li W, Xiao H, Wu H *et al.* (2022) Analysis of environmental chemical mixtures and nonalcoholic fatty liver disease: NHANES 1999–2014. *Environ Pollut* **311**, 119915.
26. Fu Y, Wang Z & Qin H (2024) Examining the pathogenesis of MAFLD and the medicinal properties of natural products from a metabolic perspective. *Metabolites* **14**, 4.
27. Tang W, Yuan M, Li Z *et al.* (2022) Polyphenol-rich liupao tea extract prevents high-fat diet-induced MAFLD by modulating the gut microbiota. *Nutrients* **14**, 4930.
28. Tan J, Hu R, Gong J *et al.* (2023) Protection against metabolic associated fatty liver disease by protocatechuic acid. *Gut Microbes* **15**, 2238959.
29. Arruda SF, Souza EM & Siqueira E (2005) Carotenoids from malanga (Xanthosoma sagittifolium) leaves protect cells against oxidative stress in rats. *Int J Vitam Nutr Res* **75**, 161–168.

https://doi.org/10.1017/S1368980024001502 Published online by Cambridge University Press

30. de Souza Mesquita LM, Casagrande BP, Santamarina AB *et al.* (2021) Carotenoids obtained from an ionic liquid-mediated process display anti-inflammatory response in the adipose tissue-liver axis. *Food Funct* **12**, 8478–8491.
31. Blaner WS (2019) Vitamin A signaling and homeostasis in obesity, diabetes, and metabolic disorders. *Pharmacol Ther* **197**, 153–178.
32. Clugston RD (2020) Carotenoids and fatty liver disease: current knowledge and research gaps. *Biochim Biophys Acta Mol Cell Biol Lipids* **1865**, 158597.
33. Harrison EH, dela Sena C, Eroglu A *et al.* (2012) The formation, occurrence, and function of beta-apocarotenoids: beta-carotene metabolites that may modulate nuclear receptor signaling. *Am J Clin Nutr* **96**, 1189S–1192S.
34. Harrison EH & Quadro L (2018) Apocarotenoids: emerging roles in mammals. *Annu Rev Nutr* **38**, 153–172.
35. Xiao ML, Chen GD, Zeng FF *et al.* (2019) Higher serum carotenoids associated with improvement of non-alcoholic fatty liver disease in adults: a prospective study. *Eur J Nutr* **58**, 721–730.
36. Liu X, Shen H, Chen M *et al.* (2021) Clinical relevance of vitamins and carotenoids with liver steatosis and fibrosis detected by transient elastography in adults. *Front Nutr* **8**, 760985.
37. Huang HC, Hsu SJ, Chang CC *et al.* (2022) Lycopene treatment improves intrahepatic fibrosis and attenuates pathological angiogenesis in biliary cirrhotic rats. *J Chin Med Assoc* **85**, 414–420.
38. Ibrahim IM, Althagafy HS, Abd-Alhameed EK *et al.* (2022) Promising hepatoprotective effects of lycopene in different liver diseases. *Life Sci* **310**, 121131.
39. Leo MA & Lieber CS (1999) Alcohol, vitamin A, and beta-carotene: adverse interactions, including hepatotoxicity and carcinogenicity. *Am J Clin Nutr* **69**, 1071–1085.
40. Yamane M, Tanaka Y, Marumo F *et al.* (1993) Role of hepatic vitamin A and lipocyte distribution in experimental hepatic fibrosis. *Liver* **13**, 282–287.
41. Knook DL, Bosma A & Seifert WF (1995) Role of vitamin A in liver fibrosis. *J Gastroenterol Hepatol* **10**, S47–49.
42. Palozza P, Simone R & Mele MC (2008) Interplay of carotenoids with cigarette smoking: implications in lung cancer. *Curr Med Chem* **15**, 844–854.
43. Blumberg J & Block G (1994) The alpha-tocopherol, beta-carotene cancer prevention study in Finland. *Nutr Rev* **52**, 242–245.
44. Hennekens CH, Buring JE, Manson JE *et al.* (1996) Lack of effect of long-term supplementation with beta carotene on the incidence of malignant neoplasms and cardiovascular disease. *N Engl J Med* **334**, 1145–1149.
45. Rousseau AS, Margaritis I, Arnaud J *et al.* (2006) Physical activity alters antioxidant status in exercising elderly subjects. *J Nutr Biochem* **17**, 463–470.
46. Jeong D, Park S, Kim H *et al.* (2020) Association of carotenoids concentration in blood with physical performance in Korean adolescents: the 2018 national fitness award project. *Nutrients* **12**, 1821.
47. Crichton G, Elias M, Alkerwi A *et al.* (2015) Intake of lutein-rich vegetables is associated with higher levels of physical activity. *Nutrients* **7**, 8058–8071.
48. Carlsohn A, Rohn S, Mayer F *et al.* (2010) Physical activity, antioxidant status, and protein modification in adolescent athletes. *Med Sci Sports Exerc* **42**, 1131–1139.

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