Original Article

Dietary n-3 and n-6 fatty acid intakes and NAFLD: A cross-sectional study in the United States

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Background and Objectives: PUFAs play critical roles in the development of nonalcoholic fatty liver disease (NAFLD). This study examined the associations between dietary n-3 and n-6 PUFA intake and NAFLD risk in a US population. **Methods and Study Design:** Data from the National Health and Nutrition Examination Survey (NHANES) 2007–2014 was used in this cross-sectional study. Data on dietary n-3 and n-6 PUFAs were extracted through two 24-h dietary recall interviews, and the dietary n-3 and n-6 PUFA intakes were adjusted by weight. NAFLD was defined based on the US fatty liver index (FLI) value ≥30. Multivariable logistic regression models and restricted cubic spline models were applied to investigate the associations between dietary n-3 and n-6 PUFA intakes and NAFLD risk. **Results:** Dietary n-3 and n-6 PUFA intakes were inversely associated with NAFLD risk. The multivariable-adjusted OR (95% CI) of NAFLD for the highest versus lowest quartile of dietary n-3 and n-6 PUFA intakes was 0.24 (0.17–0.35) and 0.18 (0.13–0.26), respectively. In stratified analyses by sex and age, the negative associations between dietary n-3 and n-6 PUFA intakes and NAFLD risk were significant in men, women, and individuals younger and older than 45 years. Dose–response analyses indicated that NAFLD risk was associated with dietary n-3 and n-6 PUFA intakes in a nonlinear manner. **Conclusions:** Dietary n-3 and n-6 PUFA intakes were inversely associated with NAFLD risk in US adults.

Key Words: nonalcoholic fatty liver disease, dietary n-3 polyunsaturated fatty acids, dietary n-6 polyunsaturated fatty acids, dose-response

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is the consequence of excessive lipid infiltration in the liver without significant alcohol intake. NAFLD includes simple steatosis and nonalcoholic steatohepatitis (NASH), which may progress to liver cirrhosis and hepatocellular carcinoma.[1] NAFLD is a multisystem disease[2,3] and can increase the risk of some chronic diseases, such as cardiovascular disease, type 2 diabetes mellitus, and chronic kidney disease.[4,5] It does not have any widely accepted medical treatment,[6] and therefore, lifestyle modifications such as dietary changes are critical to prevent its development.

Although the pathophysiology of NAFLD is complicated, oxidative stress, metabolic disorders, and inflammation play key roles in its progression.[7] PUFAs, which are commonly classified as omega-3 (n-3) and omega-6 (n-6) with the first of the double bonds, respectively, starting from the third and sixth carbon atom in the cis configuration,[8] can affect lipid metabolism, inflammatory processes, and oxidative stress.[8,9] Limited observational studies on associations between dietary n-3 and n-6 PUFA intake and NAFLD risk have revealed inconsistent results. Two cross-sectional studies conducted in Israeli and Chinese populations observed a negative association between dietary n-3 PUFA intake and NAFLD,[10,11] but a Japanese cross-sectional study reported no significant difference in dietary n-3 PUFA intake between NAFLD and healthy participants.[12] A case–control study by Cortez-Pinto et al. reported that n-6 PUFA intake in patients with NASH was higher than in controls,[13] but another case–control study involving Asian Indians revealed no significant difference in dietary n-6 PUFA intake between controls and patients with NAFLD.[14]

In the past decades, the prevalence of NAFLD in the US has significantly increased from 20% to 31.9%,[15] leading to an increase in NAFLD-related diseases[16] and liver deaths.[17] Moreover, no study has investigated the associations between dietary PUFA intake and NAFLD in the US general population, along with the dose–response relationship. Therefore, this cross-sectional study investigated the associations of dietary n-3 and n-6 PUFA intake with NAFLD in US adults using data from the National

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Health and Nutrition Examination Survey (NHANES) from 2007 to 2014.

METHODS

Data source and study population

The NHANES, a successive survey every 2 years, adopted a stratified multistage probabilistic sampling design to select a representative sample of the civilian noninstitutionalized US population. The National Center for Health Statistics Research Ethics Review Board approved the NHANES protocol, and informed consent was obtained from every participant.

This cross-sectional study used publicly available data from NHANES 2007–2008, 2009–2010, 2011–2012, and 2013–2014. The 2007–2014 NHANES data included 40,617 individuals, and 23,482 individuals aged ≥20 years were included. The individuals with missing information required to calculate the US fatty liver index (FLI) (race/ethnicity, age, waist circumference, gamma-glutamyl transferase level, insulin level, and glucose level) (n=13,728); positive hepatitis surface B antigen and hepatitis C antibody (n=200); missing or elevated alcohol intake (≥10 g/day for women or 20 g/day for men) (n=1988); pregnancy (n=90); unreliable 24-h recall data (n=775); and missing weight data (n=8) were excluded. Finally, 6693 individuals (3140 men and 3553 women) were included in this study (Figure 1).

Dietary n-3 and n-6 PUFA intakes

The information of dietary n-3 and n-6 PUFA intakes was obtained from two 24-h dietary recall interviews. One was collected in person in the mobile examination center, and the other was collected by telephone 3–10 days later. Linolenic acid includes primarily alpha-linolenic acid (n-3) and lesser amounts of gamma-linolenic acid (n-6); because NHANES did not include a detailed classification of linolenic acid, it was categorized into n-3 PUFAs.[18] As a result, in this study, n-3 PUFAs included linolenic acid (18:3), stearidonic acid (18:4), eicosapentaenoic acid (20:5), clupanodonic acid (22:5), and docosahexaenoic acid (22:6), and n-6 PUFAs included linoleic acid (18:2) and arachidonic acid (20:4). The average daily dietary total n-3 and n-6 PUFAs were calculated if an individual completed two 24-h recalls; otherwise, the single dietary recall data were applied. Dietary n-3 and n-6 PUFAs were adjusted to the body weight and then divided into quartiles.

US FLI

The definition of NAFLD is based on the US FLI[19] because of the absence of ultrasound data for most NHANES survey cycles. Age, race/ethnicity, waist circumference, gamma-glutamyl transferase (GGT) level, insulin level, and glucose level were included in the FLI calculation. The US FLI has been validated and correlates well with the presence of NAFLD diagnosed through ultrasound in the multiethnic US general population (area

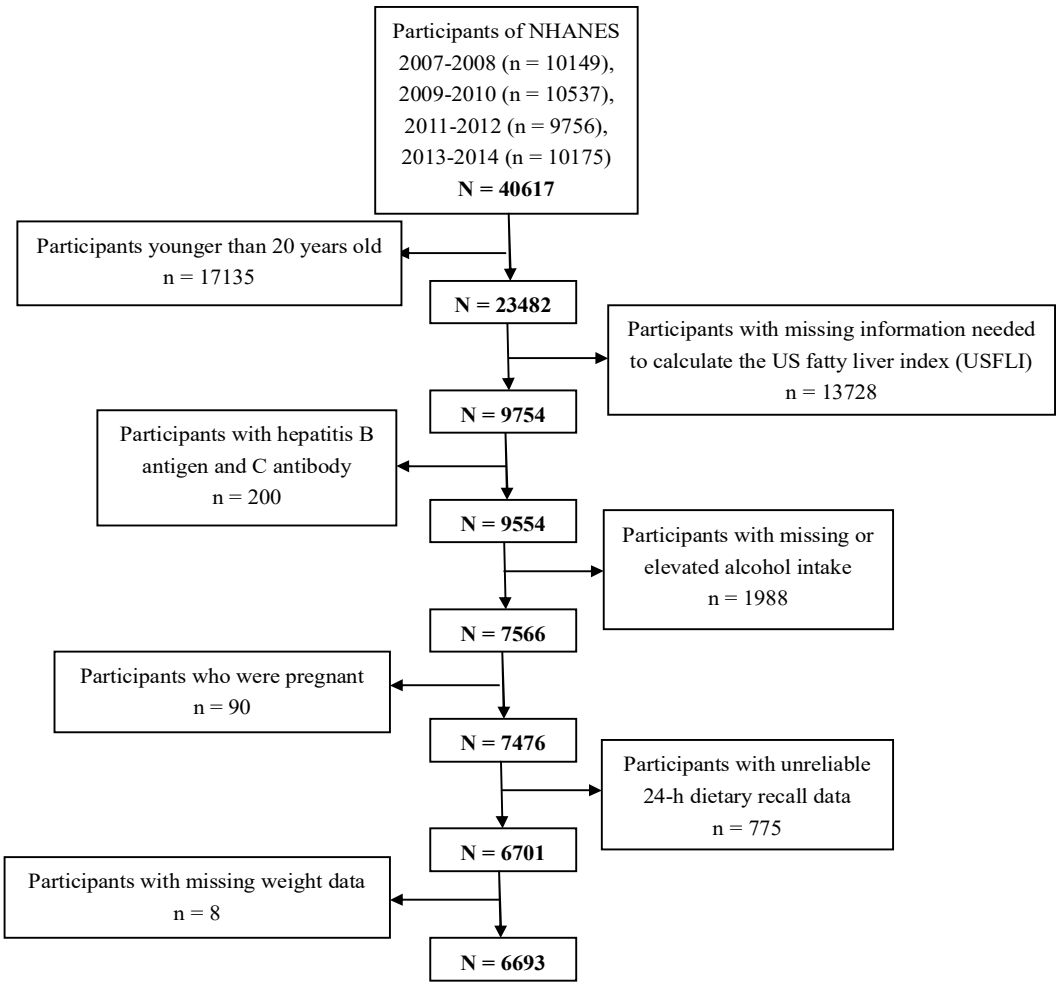

Figure 1. Flow chart of selecting eligible participants from NHANES 2007-2014.

under the receiver operating characteristic curve=0.80; 95% CI=0.77–0.83).[19] A US FLI ≥30 was considered to indicate NAFLD, as suggested by the authors.

Covariates

Demographic characteristics included age, sex, race/ethnicity (Mexican American, Other Hispanic, Non-Hispanic White, Non-Hispanic Black, and Other race), and educational level (lower than high school, high school diploma, and higher than high school). Other covariates included smoking (smoking ≥100 cigarettes in life or not), vigorous recreational activity (Yes or No), hypertension status (Yes or No), diabetes status (Yes or No), total daily energy intake, total cholesterol (TC) levels, triglyceride (TG) levels, and HDL levels. Hypertension was defined as mean systolic blood pressure ≥130 mm Hg, mean diastolic blood pressure ≥80 mm Hg, taking prescribed medicine for hypertension, or self-reported hypertension diagnosis.[20] Diabetes was defined as fasting plasma glucose level ≥7.1 mmol/L, 2-h plasma glucose level (OGTT) ≥11.1 mmol/L, taking anti-diabetes medication or insulin, or self-reported diabetes diagnosis.[21,22]

Statistical analysis

All statistical analyses were performed with Stata 15.0.[23] To conduct a nationally representative estimate, appropriate sampling weights, primary sampling units, and strata information were considered in all analyses. In this study, four 2-year survey cycles of the continuous NHANES (2007–2008, 2009–2010, 2011–2012, and 2013–2014) were combined, and therefore, a new special 8-year dietary weight was calculated by taking one-quarter of the 2-year dietary weights according to the NHANES analytical guidelines.

Continuous variables are presented as mean ± standard deviation, and categorical variables are presented as frequency (percentage). The Student's t test and the chi-square test were used to compare the differences in continuous and categorical variables, respectively, between NAFLD and non-NAFLD groups. All dietary PUFA intakes were categorized based on quartiles (quartile 1: <25th percentile, quartile 2: 25th–50th percentile, quartile 3: 50th–75th percentile, quartile 4: ≥75th percentile), and quartile 1 was used as the reference category. Binary logistic regression analyses were used to examine the relationship between dietary PUFA intake and NAFLD. Model 1 was adjusted for age and sex. Model 2 was adjusted for age, sex, race, educational level, smoking status, vigorous recreational activity, hypertension, diabetes, daily total energy intake, TC, TG, and HDL. Next, stratified analysis by sex and age was performed to evaluate the associations between dietary PUFA intake and NAFLD. ORs with 95% CIs were calculated using logistic regression analyses. To explore the dose–response relationship between dietary PUFA intake and NAFLD, a restricted cubic spline was used with three knots located at the 5th, 50th, and 95th percentiles of the exposure distribution in the fully adjusted model. A two-sided $p<0.05$ indicated statistical significance.

RESULTS

Table 1 compares the baseline characteristics of participants with or without NAFLD stratified by sex. Of the 6693 participants, 46.9% were men. The prevalence of NAFLD was 35.1% (40.5% in men and 30.3% in women), with a significant difference in NAFLD prevalence for men among the four survey cycles. Moreover, a decreasing trend in NAFLD prevalence (Supplementary figure 1) and increasing intake of PUFAs (Supplementary table 1) for men over the four survey cycles were observed. Compared with the controls, the participants with NAFLD tended to be older; Mexican American; obese; more likely to smoke ≥100 cigarettes in their life; have lower educational level, lower vigorous recreational activity, hypertension, and diabetes; have higher waist circumference and higher levels of insulin, glucose, TG, and GGT; and have lower HDL levels and less dietary n-3 and n-6 PUFA intake in both sexes. Men with NAFLD were more likely to have higher TC levels (all $p<0.05$).

The weighted ORs (95% CI) of NAFLD based on quartiles of dietary n-3 and n-6 PUFA intakes are presented in Table 2. In binary logistic regression analyses, the crude ORs (95% CI) of NAFLD suggested that dietary n-3 and n-6 PUFA intakes were inversely associated with NAFLD. Model 1 results were similar to the crude ORs (95% CIs). Model 2 results revealed that for the highest quartile versus the lowest quartile, the ORs (95% CI) of NAFLD were 0.24 (0.17–0.35) and 0.18 (0.13–0.26) for dietary n-3 and n-6 PUFA intake, respectively. Moreover, significant negative associations between dietary n-3 and n-6 PUFA intakes and NAFLD were observed in all models for each survey cycle (Supplementary table 2).

The associations of dietary n-3 and n-6 PUFA intake with NAFLD in stratified analyses by sex and age are presented in Tables 3 and 4, respectively. In both men and women, significant inverse relationships between n-3 and n-6 PUFA intakes and NAFLD were found with or without adjustment for confounders. Similar results were also observed when participants were stratified by age (younger or older than 45 years). In model 2, for the highest versus lowest quartile, the OR (95% CI) of NAFLD was 0.19 (0.12–0.30) and 0.13 (0.07–0.23) for dietary n-3 and n-6 PUFA intake, respectively, for participants younger than 45 years and 0.26 (0.17–0.39) and 0.22 (0.14–0.33), respectively, for participants older than 45 years.

In the restricted cubic spline model, dietary n-3 PUFA intake was negatively associated with NAFLD risk in a nonlinear manner (p for nonlinearity = 0.088). NAFLD risk decreased with an increased intake of dietary n-3 PUFAs, reaching a plateau at 30 mg/kg/day (OR: 0.20; 95% CI: 0.13–0.30) (Figure 2). A nonlinear inverse association was noted between dietary n-6 PUFA intake and NAFLD risk (p for nonlinearity=0.161), and NAFLD risk did not decrease significantly beyond 220 mg/kg/day (OR: 0.20; 95% CI: 0.13–0.29) (Figure 3).

DISCUSSION

This study explored the associations between dietary PUFA intake and NAFLD risk. After adjustment for multiple potential confounders, dietary n-3 and n-6 PUFA intakes were inversely related to NAFLD risk in general US adults. When stratified by sex and age, similar findings were found in men, women, and individuals younger

Table 1. Baseline characteristics of the participants by NAFLD, US adults aged ≥20 years, NHANES 2007-2014

Characteristics	NAFLD (total)		*p* value	NAFLD (men)		*p* value	NAFLD (women)		*p* value
	No	Yes		No	Yes		No	Yes	
Participants	4244 (64.9%)	2449 (35.1%)		1830 (59.5%)	1310 (40.5%)		2414 (69.7%)	1139 (30.3%)	
Survey cycle			0.322			0.007			0.470
2007-2008	1063 (65.5 %)	630 (34.5%)		455 (57.4%)	345 (42.6%)		608 (72.8%)	285 (27.2%)	
2009-2010	1091 (62.8%)	734 (37.2%)		431 (54.1%)	402 (45.9%)		660 (70.2%)	332 (29.8%)	
2011-2012	1006 (64.3%)	543 (35.7%)		464 (61.2%)	283 (38.9%)		542 (67.1%)	260 (32.9%)	
2013-2014	1084 (67.1%)	542 (32.9%)		480 (65.3%)	280 (34.7%)		604 (68.6%)	262 (31.4%)	
Race/Ethnicity (n, %)			<0.001			<0.001			<0.001
Mexican American	494 (49.5%)	558 (50.5%)		210 (43.8%)	287 (56.2%)		284 (55.3%)	271 (44.7%)	
Other Hispanic	446 (64.2%)	295 (35.8%)		177 (61.5%)	145 (38.5%)		269 (66.5%)	150 (33.5%)	
Non-Hispanic White	1872 (63.9%)	1169 (36.1%)		814 (58.1%)	663 (41.9%)		1058 (69.1%)	506 (30.9%)	
Non-Hispanic Black	949 (79.3%)	272 (20.7%)		409 (79.6%)	123 (20.4%)		540 (79.0%)	149 (21.00%)	
Other Race	483 (75.3%)	155 (24.7%)		220 (69.7%)	92 (30.3%)		263 (80.4%)	63 (19.6%)	
BMI group (n, %)			<0.001			<0.001			<0.001
Under weight (<18.5)	98 (98.8%)	2 (1.20%)		28 (98.1%)	1 (1.90%)		70 (99.1%)	1 (0.90%)	
Normal (18.5-24.9)	1628 (94.8%)	104 (5.20%)		723 (92.3%)	65 (7.70%)		905 (96.7%)	39 (3.30%)	
Overweight (25-29.9)	1596 (74.6%)	635 (25.4%)		803 (69.5%)	414 (30.5%)		793 (80.7%)	221 (19.3%)	
Obese (≥30)	916 (34.0%)	1704 (64.0%)		271 (25.2%)	828 (74.8%)		645 (41.00%)	876 (59.0%)	
Educational level (n, %)			<0.001			<0.001			<0.001
<high school	349 (48.7%)	398 (51.3%)		162 (43.8%)	195 (56.2%)		187 (53.6%)	203 (46.4%)	
High school	565 (57.9%)	410 (42.1%)		247 (56.9%)	207 (43.1%)		318 (58.7%)	203 (41.3%)	
>high school	3330 (67.1%)	1641 (32.9%)		1421 (61.2%)	908 (38.8%)		1909 (72.4%)	733 (27.6%)	
Smoke status (n, %)			<0.001			0.0003			0.010
Yes	1605 (59.9%)	1145 (40.1%)		866 (54.8%)	718 (45.2%)		739 (66.3%)	427 (33.7%)	
No	2637 (68.3%)	1304 (31.7%)		963 (63.8%)	592 (36.2%)		1674 (71.4%)	712 (28.6%)	
Vigorous recreational activity (n, %)			<0.001			<0.001			<0.001
Yes	1054 (81.5%)	274 (18.5%)		594 (77.2%)	196 (22.8%)		460 (87.8%)	78 (12.2%)	
No	3190 (59.9%)	2175 (40.1%)		1236 (52.3%)	1114 (47.7%)		1954 (65.7%)	1061 (34.3%)	
Hypertension (n, %)			<0.001			<0.001			<0.001
Yes	1591 (50.6%)	1504 (49.4%)		726 (46.9%)	799 (53.1%)		865 (54.1%)	705 (45.9%)	
No	2653 (75.2%)	945 (24.8%)		1104 (69.3%)	511 (30.7%)		1549 (80.1%)	434 (19.9%)	
Diabetes (n, %)			<0.001			<0.001			<0.001
Yes	471 (31.5%)	888 (68.5%)		223 (30.5%)	453 (69.5%)		248 (32.5%)	435 (67.5%)	
No	3773 (71.1%)	1561 (28.9%)		1607 (65.2%)	857 (34.8%)		2166 (76.2%)	704 (23.8%)	
Age (years)	46.5 (16.9)	52.3 (16.3)	<0.001	45.2 (16.7)	51.6 (16.0)	<0.001	47.6 (16.9)	53.2 (16.6)	<0.001
Waist circumference (cm)	92.2 (11.8)	114 (14.8)	<0.001	94.5 (10.8)	114 (14.3)	<0.001	90.5 (12.2)	113 (15.5)	<0.001
BMI (kg m^{-2})	26.4 (4.80)	34.3 (7.00)	<0.001	26.2 (3.80)	32.9 (6.10)	<0.001	26.6 (5.40)	36.0 (7.70)	<0.001
Insulin (pmol L^{-1})	49.3 (24.3)	144 (144)	<0.001	47.2 (23.2)	145 (179)	0..004	50.8 (25.0)	142 (86.0)	<0.001
Glucose, Plasma (mg dL^{-1})	98.8 (20.9)	120 (42.5)	<0.001	102 (24.1)	119 (41.4)	<0.001	96.5 (17.8)	121 (43.8)	<0.001

BMI: body mass index; TC: total cholesterol; TG: triglycerides; LDL: low density lipoprotein cholesterol; HDL: high density lipoprotein cholesterol; GGT: gamma glutamyl transferase; PUFA: polyunsaturated fatty acid.

Data are presented as mean ± standard deviation (SD) for continuous variables or participants (percentage) for categorical variables.

Table 1. Baseline characteristics of the participants by NAFLD, US adults aged ≥20 years, NHANES 2007-2014 (cont.)

Characteristics	NAFLD (total)		*p* value	NAFLD (men)		*p* value	NAFLD (women)		*p* value
	No	Yes		No	Yes		No	Yes	
Participants	4244 (64.9%)	2449 (35.1%)		1830 (59.5%)	1310 (40.5%)		2414 (69.7%)	1139 (30.3%)	
TC (mg dL^{-1})	192 (40.3)	195 (42.2)	0.065	186 (37.7)	192 (42.4)	0.016	197 (41.6)	199 (41.6)	0.226
TG (mg dL^{-1})	104 (78.4)	176 (121)	<0.001	109 (96.1)	183 (134)	<0.001	100 (61.6)	168 (103)	<0.001
LDL (mg dL^{-1})	115 (34.4)	116 (36.3)	0.289	114 (33.7)	115 (36.2)	0.621	116 (34.9)	118 (36.3)	0.198
HDL (mg dL^{-1})	56.2 (14.7)	45.4 (11.7)	<0.001	50.2 (12.8)	42.0 (9.8)	<0.001	60.7 (14.5)	49.4 (12.4)	<0.001
GGT (U L^{-1})	19.0 (14.0)	37.1 (38.1)	<0.001	21.4 (15.0)	37.3 (33.2)	<0.001	17.1 (12.9)	36.7 (43.2)	<0.001
Daily total energy intake (kcal d^{-1})	2008 (767)	2049 (804)	0.103	2369 (817)	2311 (840)	0.124	1736 (600)	1740 (622)	0.894
Total adjusted n-3 PUFA intake (mg kg^{-1} day^{-1})	24.3 (15.2)	18.4 (10.10)	<0.001	25.8 (16.3)	19.2 (11.00)	<0.001	23.2 (14.1)	17.5 (10.9)	<0.001
Total adjusted n-6 PUFA intake (mg kg^{-1} day^{-1})	216 (118)	168 (92.6)	<0.001	230 (123)	178 (94.9)	<0.001	205 (114)	157 (88.3)	<0.001

BMI: body mass index; TC: total cholesterol; TG: triglycerides; LDL: low density lipoprotein cholesterol; HDL: high density lipoprotein cholesterol; GGT: gamma glutamyl transferase; PUFA: polyunsaturated fatty acid.
Data are presented as mean±standard deviation (SD) for continuous variables or participants (percentage) for categorical variables.

Table 2. Weighted ORs and 95% CIs for NAFLD according to the quartiles of adjusted dietary n-3, n-6 PUFA intakes

NAFLD	Crude	Model 1	Model2
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Adjusted n-3 (mg kg^{-1} day^{-1})			
<12.8	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
12.8 to <19.1	0.88 (0.73-1.06)	0.84 (0.70-1.01)	0.76 (0.59-0.99)*
19.1 to <27.9	0.53 (0.44-0.63)**	0.50 (0.41-0.60)**	0.43 (0.33-0.55)**
≥27.9	0.32 (0.26-0.39)**	0.30 (0.24-0.37)**	0.24 (0.17-0.35)**
Adjusted n-6 (mg kg^{-1} day^{-1})			
<121.3	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
121.3 to <178.2	0.84 (0.72-0.98)*	0.81 (0.69-0.94)**	0.73 (0.58-0.91)**
178.2 to <250.4	0.50 (0.42-0.60)**	0.48 (0.40-0.58)**	0.38 (0.29-0.50)**
≥250.4	0.31 (0.26-0.37)**	0.30 (0.25-0.35)**	0.18 (0.13-0.26)**

CI: confidence interval; OR: odds ratio. Model 1 adjusted for age and sex. Model 2 adjusted for age: sex: race/ethnicity: educational level: smoking status: vigorous recreational activity: hypertension: diabetes: daily total energy intake: total cholesterol: triglycerides and high density lipoprotein cholesterol.
The lowest quartile of adjusted dietary n-3: n-6 PUFA intakes separately was used as the reference group. Results are survey-weighted.
*p<0.05; **p<0.01.

Table 3. Weighted ORs and 95% CIs for NAFLD according to the quartiles of adjusted dietary n-3, n-6 PUFA intakes, stratified by sex

NAFLD	Crude	Model 1	Model2
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Men			
Adjusted n-3 (mg kg^{-1} day^{-1})			
<13.7	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
13.7 to <20.2	0.75 (0.58-0.98)*	0.76 (0.59-0.99)*	0.69 (0.47-0.99)*
20.2 to <29.0	0.50 (0.38-0.65)**	0.50 (0.38-0.66)**	0.42 (0.29-0.59)**
≥29.0	0.27 (0.20-0.37)**	0.28 (0.20-0.38)**	0.20 (0.14-0.29)**
Adjusted n-6 (mg kg^{-1} day^{-1})			
<129	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
129 to <190	0.73 (0.58-0.91)**	0.75 (0.60-0.94)*	0.60 (0.46-0.78)**
190 to <259	0.47 (0.35-0.62)**	0.48 (0.36-0.64)**	0.32 (0.22-0.45)**
≥259	0.28 (0.21-0.37)**	0.30 (0.22-0.39)**	0.17 (0.11-0.16)**
Women			
Adjusted n-3 (mg kg^{-1} day^{-1})			
<12.2	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
12.2 to <18.3	0.89 (0.66-1.19)	0.86 (0.65-1.13)	0.82 (0.57-1.18)
18.3 to <27.0	0.52 (0.39-0.70)**	0.52 (0.39-0.69)**	0.46 (0.33-0.65)**
≥27.0	0.30 (0.23-0.40)**	0.30 (0.23-0.40)**	0.26 (0.16-0.42)**
Adjusted n-6 (mg kg^{-1} day^{-1})			
<113	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
113 to <171	0.85 (0.67-1.07)	0.83 (0.67-1.03)	0.75 (0.55-1.03)
171 to <240	0.52 (0.41-0.66)**	0.53 (0.42-0.67)**	0.47 (0.35-0.65)**
≥240	0.29 (0.22-0.38)**	0.30 (0.22-0.39)**	0.18 (0.11-0.29)**

OR: odds ratio; CI: confidence interval.
Model 1 adjusted for age. Model 2 adjusted for age, race/ethnicity, educational level, smoking status, vigorous recreational activity, hypertension, diabetes, daily total energy intake, total cholesterol, triglycerides and high density lipoprotein cholesterol. The lowest quartile of adjusted dietary n-3, n-6 PUFA intakes separately was used as the reference group. Results are survey-weighted.
$^{*}p<0.05$; $^{**}p<0.01$.

Table 4. Weighted ORs and 95% CIs for NAFLD according to the quartiles of adjusted dietary n-3, n-6 PUFA intakes, stratified by age

NAFLD	Crude	Model 1	Model2
	OR (95% CI)	OR (95% CI)	OR (95% CI)
<45years			
Adjusted n-3 (mg kg^{-1} day^{-1})			
<13.2	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
13.2 to <19.7	0.88 (0.69-1.14)	0.85 (0.66-1.10)	0.68 (0.49-0.94)*
19.7 to <28.6	0.54 (0.41-0.71)**	0.51 (0.39-0.67)**	0.40 (0.28-0.55)**
≥28.6	0.31 (0.22-0.44)**	0.29 (0.20-0.41)**	0.19 (0.12-0.30)**
Adjusted n-6 (mg kg^{-1} day^{-1})			
<126	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
126 to <185	0.83 (0.65-1.08)	0.79 (0.62-1.02)	0.59 (0.44-0.80)**
185 to <262	0.55 (0.42-0.72)**	0.51 (0.39-0.67)**	0.33 (0.22-0.48)**
≥262	0.31 (0.23-0.42)**	0.29 (0.21-0.39)**	0.13 (0.07-0.23)**
≥ 45 years			
Adjusted n-3 (mg kg^{-1} day^{-1})			
<12.6	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
12.6 to <18.7	0.82 (0.63-1.07)	0.80 (0.60-1.06)	0.78 (0.56-1.08)
18.7 to <27.5	0.51 (0.41-0.63)**	0.48 (0.38-0.59)**	0.45 (0.32-0.62)**
≥27.5	0.31 (0.24-0.40)**	0.29 (0.22-0.37)**	0.26 (0.17-0.39)**
Adjusted n-6 (mg kg^{-1} day^{-1})			
<119	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
119 to <173	0.75 (0.60-0.93)*	0.71 (0.57-0.89)**	0.67 (0.51-0.88)**
173 to <242	0.50 (0.40-0.63)**	0.47 (0.38-0.59)**	0.42 (0.31-0.56)**
≥242	0.30 (0.23-0.40)**	0.28 (0.21-0.36)**	0.22 (0.14-0.33)**

OR: odds ratio; CI: confidence interval.
Model 1 adjusted for sex. Model 2 adjusted for sex, race/ethnicity, educational level, smoking status, vigorous recreational activity, hypertension, diabetes, daily total energy intake, total cholesterol, triglycerides and high density lipoprotein cholesterol. The lowest quartile of adjusted dietary n-3, n-6 PUFA intakes separately was used as the reference group. Results are survey-weighted.
$^{*}p<0.05$; $^{**}p<0.01$.

or older than 45 years. A nonlinear negative association between dietary n-3 and n-6 PUFA intakes and NAFLD risk was also found. Furthermore, NAFLD prevalence exhibited a decreasing trend for men across the four survey cycles, perhaps partly because of the increasing PUFA intake by men. However, the number of survey

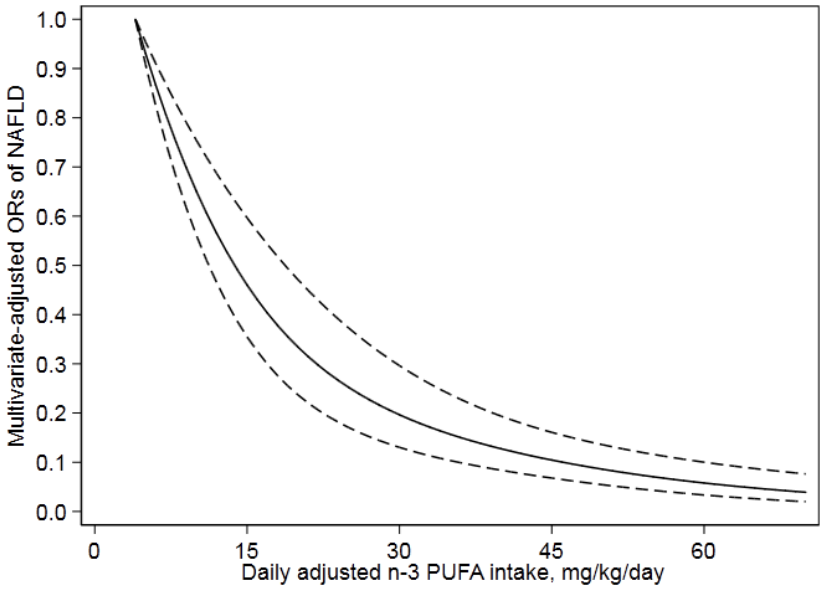

Figure 2. Dose-response relationship between dietary n-3 PUFA intake and NAFLD. The association was adjusted for age, sex, race/ethnicity, educational level, smoking status, vigorous recreational activity, hypertension, diabetes, daily total energy intake, total cholesterol, triglycerides and high density lipoprotein cholesterol. The solid line and dashed line represent the estimated ORs and the corresponding 95% confidence intervals, respectively. OR: odds ratio.

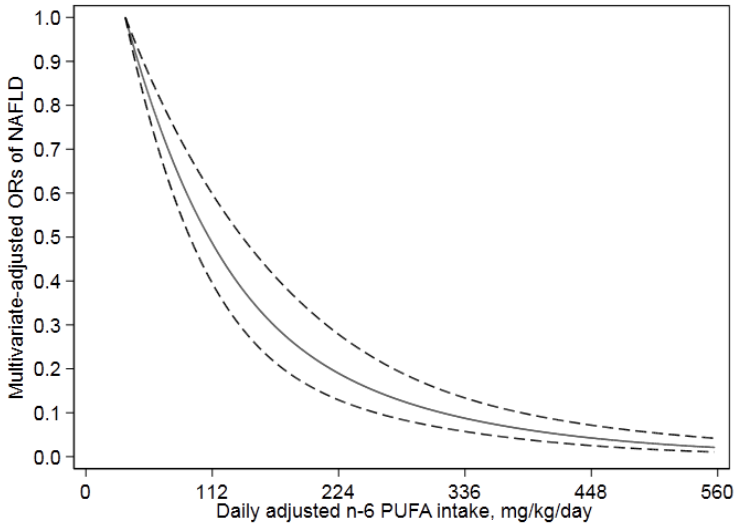

Figure 3. Dose-response relationship between dietary n-6 PUFA intake and NAFLD. The association was adjusted for age, sex, race/ethnicity, educational level, smoking status, vigorous recreational activity, hypertension, diabetes, daily total energy intake, total cholesterol, triglycerides and high density lipoprotein cholesterol. The solid line and dashed line represent the estimated ORs and the corresponding 95% confidence intervals, respectively. OR: odds ratio.

cycles is limited, and more cycles should be analyzed to explore the exact correlations.

Several studies have investigated the associations between n-3 PUFA intake and NAFLD risk. Similar to the results of this present study, Israeli and Chinese cross-sectional studies concluded that dietary n-3 PUFA intake was inversely associated with NAFLD.[10,11] By contrast, a Japanese cross-sectional study observed no significant association between the two,[12] likely due to the high intake of fish rich in n-3 PUFAs in the general Japanese population. In addition, several randomized controlled trials (RCTs) and meta-analyses of RCTs have suggested a protective role of n-3 PUFA supplementation on NAFLD by, for example, reducing liver fat[24] or decreasing alanine aminotransferase (ALT), TC, and TG levels and increasing HDL levels.[25] The weighted mean dosages of n-3 PUFA supplementation applied in these RCTs (4622 and 3551 mg/day) were much higher than the recommended dietary intake (500 mg/day).[26] Based on the key roles of hepatic lipid metabolism, insulin sensitivity, oxidative stress, and inflammation in NAFLD pathophysiology, some mechanisms underlying the inverse relationship between n-3 PUFAs and NAFLD have been suggested.[27-29] First, n-3 PUFAs have beneficial effects on lipid metabolism, inflammation, and oxidative stress by activating the peroxisome proliferator-activated receptor (PPARα), which regulates many metabolic processes.[9] In addition, the two main components of n-3 PUFAs—eicosatetraenoic acid and docosahexaenoic acid—also have beneficial effects on inflammation and oxidative

stress.[30] Moreover, n-3 PUFAs can improve insulin resistance by upregulating the genes involved in insulin sensitivity (PPARγ), insulin receptor signaling (IRS-1/IRS-2), and glucose transport (GLUT-2/GLUT-4).[31]

Few studies have investigated the associations of dietary n-6 PUFA intake with NAFLD. In the present study, n-6 PUFA intake was inversely associated with NAFLD risk. One RCT concluded that dietary n-6 PUFAs could reduce liver fat, even when participants gained weight and adipose mass.[32] By contrast, a case–control study of 147 participants revealed no significant difference in dietary n-6 PUFA intake between controls and patients with NAFLD in Asian Indians.[14] Although original studies on the associations of dietary n-6 PUFA intake with NAFLD are limited, some reviews have indicated that increased dietary n-6 PUFA intake may increase NAFLD risk.[33,34] To date, the roles of dietary n-6 PUFA intake on NAFLD remain poorly established. Linoleic acid, the major dietary n-6 PUFAs,[26] has positive effects on blood lipids, and n-6 PUFA intake may reduce inflammation and increase insulin sensitivity,[32,35,36] which may partly explain the inverse correlation between dietary n-6 PUFA intake and NAFLD. Nevertheless, further studies are required to clarify the precise mechanisms underlying this association.

All the aforementioned studies on the associations between dietary PUFAs and NAFLD in populations with different ethnicities and dietary backgrounds are tabulated in Supplementary Table 3. In addition, Supplementary Table 4 presents the food pattern and sources of n-3 and n-6 PUFAs in the American diet.

This study has several strengths. First, multiple years of data from a large and nationally representative sample were used, thus increasing the statistical power and reliability of the results. Second, the inverse association between dietary PUFA intake and NAFLD remained statistically significant even after adjustment for multiple potential confounders. Third, the dose–response relationships between dietary n-3 and n-6 PUFA intakes and NAFLD risk were assessed.

However, this study also has some limitations. First, it was a cross-sectional study, thus precluding the determination of causality. Second, the dietary data were calculated using two 24-h recall interviews, and therefore, the influence of recall bias could not be avoided. Third, the possible confounding factors may not have been adjusted completely. Fourth, NHANES data do not provide a detailed classification of linolenic acid; it was categorized under n-3 PUFAs, which might influence the results. Fifth, NAFLD was defined based on the US FLI, which is adequate but not the perfect proxy for NAFLD diagnosis using liver biopsy or other invasive methods. Finally, the use of US FLI precluded adjustment for potential critical covariates, including markers of insulin sensitivity and body composition.

In conclusion, dietary n-3 and n-6 PUFA intakes were inversely associated with NAFLD risk in US adults. These findings add to the limited data on the association between dietary PUFA intake and NAFLD. Further studies must verify these findings and investigate the underlying mechanisms.

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AUTHOR DISCLOSURES

Conflict of interest: None.

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Supplementary table 1. Comparisons of dietary n-3, n-6 PUFA among four survey cycles in men

Survey cycles	2007-2008	2009-2010	2011-2012	2013-2014	*p* value
Dietary n-3 PUFA (mg kg^{-1}day^{-1})	20.6 (13.4)	21.2 (12.7)	23.9 (13.8)	23.8 (15.5)	<0.001
Dietary n-6 PUFA (mg kg^{-1}day^{-1})	189 (110)	189 (107)	210 (111)	207 (117)	<0.001

Data are presented as mean±standard deviation (SD) for continuous variables.

Supplementary table 2. Weighted ORs and 95% CIs for NAFLD according to the quartiles of adjusted dietary n-3, n-6 PUFA intakes in each survey cycle

NAFLD	Crude	Model 1	Model2
	OR (95% CI)	OR (95% CI)	OR (95% CI)
2007-2008			
Adjusted n-3 (mg kg^{-1} day^{-1})			
<11.0	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
11.0 to <16.5	0.81 (0.59-1.11)	0.78 (0.55-1.09)	0.74 (0.49-1.10)
16.5 to <24.7	0.68 (0.53-0.87)**	0.63 (0.49-0.82)**	0.53 (0.37-0.76)**
≥24.7	0.40 (0.29-0.55)**	0.37 (0.25-0.56)**	0.30 (0.19-0.49)**
Adjusted n-6 (mg kg^{-1} day^{-1})			
<105	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
105 to <155	0.80 (0.60-1.07)	0.76 (0.57-1.00)	0.78 (0.51-1.18)
155 to <226	0.60 (0.49-0.73)**	0.58 (0.47-0.73)**	0.59 (0.42-0.84)**
≥226	0.47 (0.35-0.63)**	0.43 (0.32-0.58)**	0.35 (0.18-0.66)**
2009-2010			
Adjusted n-3 (mg kg^{-1} day^{-1})			
<11.8	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
11.8 to <17.5	0.84 (0.61-1.18)	0.73 (0.52-1.03)	0.69 (0.44-1.09)
17.5 to <25.8	0.66 (0.44-1.00)	0.59 (0.41-0.86)**	0.47 (0.31-0.72)**
≥25.8	0.38 (0.25-0.57)**	0.33 (0.22-0.48)**	0.24 (0.14-0.41)**
Adjusted n-6 (mg kg^{-1} day^{-1})			
<110	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
110 to <162	0.80 (0.60-1.08)	0.70 (0.53-0.92)*	0.54 (0.36-0.80)**
162 to <230	0.55 (0.39-0.77)**	0.52 (0.38-0.72)**	0.35 (0.23-0.54)**
≥230	0.34 (0.25-0.47)**	0.31 (0.23-0.42)**	0.17 (0.11-0.26)**
2011-2012			
Adjusted n-3 (mg kg^{-1} day^{-1})			
<13.5	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
13.5 to <20.0	0.99 (0.67-1.45)	0.97 (0.67-1.40)	0.79 (0.41-1.50)
20.0 to <29.1	0.58 (0.37-0.91)*	0.58 (0.36-0.92)*	0.42 (0.23-0.76)**
≥29.1	0.28 (0.17-0.48)**	0.28 (0.16-0.47)**	0.14 (0.05-0.40)**
Adjusted n-6 (mg kg^{-1} day^{-1})			
<122	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
122 to <181	0.82 (0.55-1.22)	0.79 (0.54-1.16)	0.66 (0.39-1.13)
181 to <253	0.56 (0.37-0.87)*	0.53 (0.33-0.83)**	0.35 (0.17-0.71)**
≥253	0.29 (0.18-0.46)**	0.28 (0.18-0.44)**	0.13 (0.06-0.27)**
2013-2014			
Adjusted n-3 (mg kg^{-1} day^{-1})			
<13.3	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
13.3 to <20.5	0.73 (0.57-0.94)*	0.71 (0.55-0.92)*	0.67 (0.45-1.00)
20.5 to <29.2	0.31 (0.21-0.46)**	0.30 (0.20-0.45)**	0.30 (0.15-0.62)**
≥29.2	0.20 (0.12-0.32)**	0.19 (0.12-0.31)**	0.21 (0.09-0.51)**
Adjusted n-6 (mg kg^{-1} day^{-1})			
<122	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
122 to <180	0.70 (0.47-1.05)	0.71 (0.48-1.06)	0.71 (0.38-1.35)
180 to <259	0.38 (0.24-0.60)**	0.38 (0.24-0.60)**	0.37 (0.16-0.85)*
≥259	0.19 (0.12-0.30)**	0.19 (0.12-0.30)**	0.18 (0.06-0.56)**

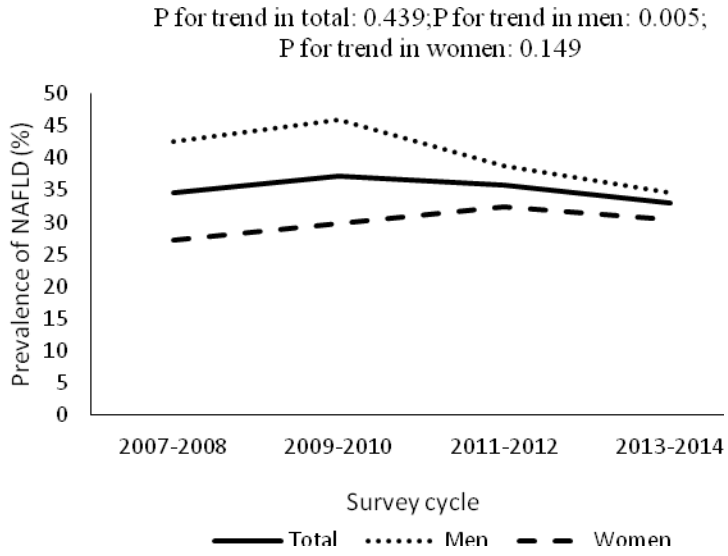
OR: odds ratio; CI: confidence interval.
Model 1 adjusted for age and sex. Model 2 adjusted for age, sex, race/ethnicity, educational level, smoking status, vigorous recreational activity, hypertension, diabetes, daily total energy intake, total cholesterol, triglycerides and high density lipoprotein cholesterol. The lowest quartile of adjusted dietary n-3, n-6 PUFA intakes separately was used as the reference group. Results are survey-weighted.
*p<0.05; **p<0.01.

Supplementary table 3. A tabulated summary of other published work

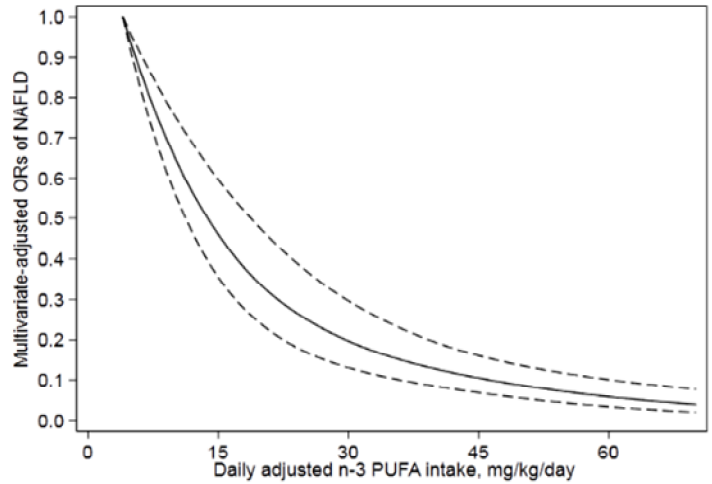
Authors, year	Research type	Population	Background diet	Findings
Zelber-Sagi S et al., (2007)[10]	A cross-sectional study	Israeli population	Mediterranean diet	NAFLD patients had a lower intake of fish rich in n-3 PUFA
Chen ZY et al., (2020)[11]	A cross-sectional study	Chinese population	Plant-based diet	Dietary n-3 PUFAs were inversely associated with NAFLD
Oya J et al., (2010)[12]	A cross-sectional study	Japanese population	Japanese diet	No significant difference in dietary n-3 PUFAs intake between NAFLD and healthy subjects was found
Vernekar M et al., (2018)[14]	A case-control study	Asian Indians	Plant-based diet	No significant difference for dietary n-3 and n-6 PUFAs intake between controls and NAFLD patients was observed
Cortez-Pinto H et al., (2006)[13]	A case-control study	Portuguese Population	Mediterranean diet	Intake of n-6 PUFAs in NASH patients was higher than in controls
Bjermo H et al., (2012)[32]	A randomized controlled trial	Swedish population	Mediterranean diet	Dietary n-6 PUFAs could reduce liver fat without weight loss

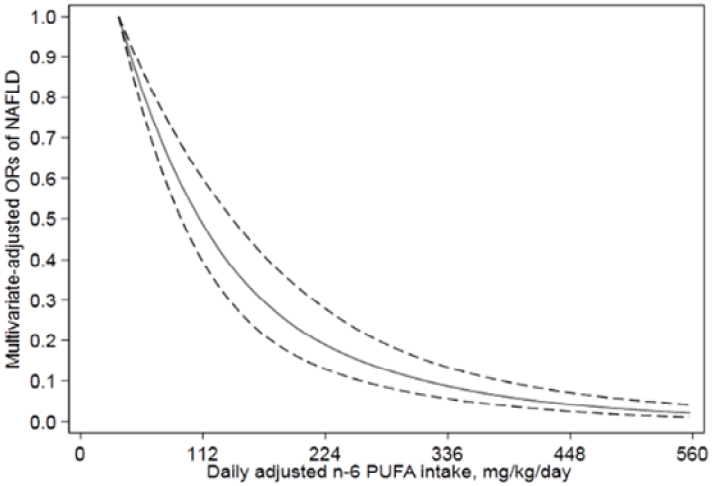
Supplementary table 4. N-3, n-6 polyunsaturated fatty acids in the American diet.[26]

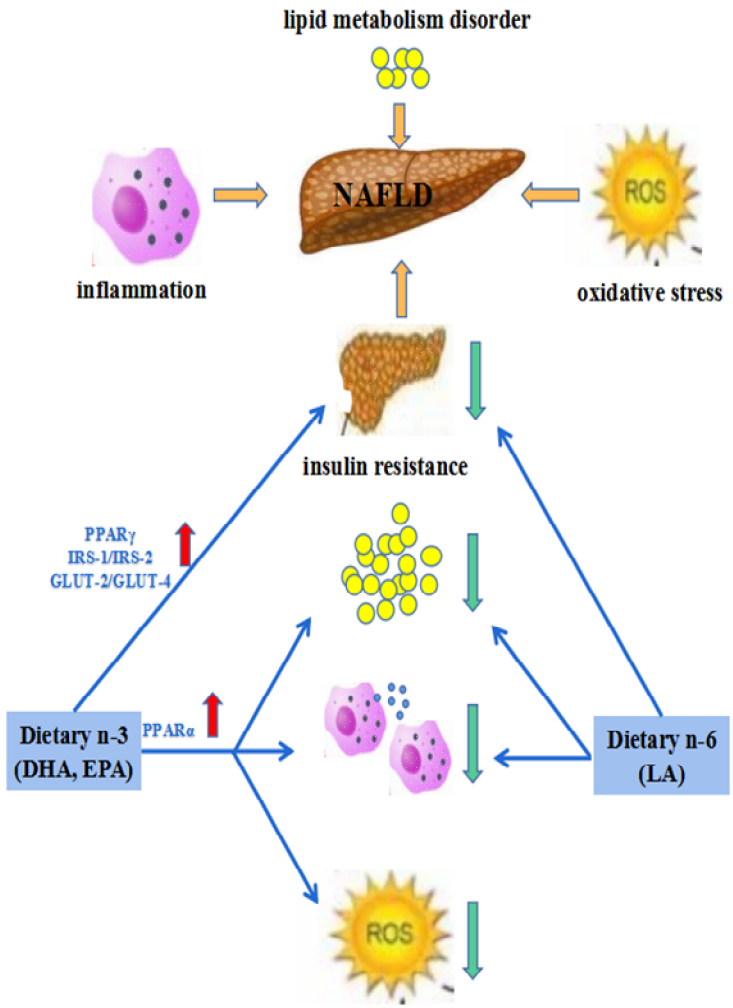
Food patterns	Polyunsaturated fatty acids	Common food sources
Animal-based diet	n-3 fatty acids	Flaxseed, canola oil, soybean oil, walnuts, fish oil, algae
	n-6 fatty acids	Liquid vegetable oils, nuts, seeds, meat, poultry, fish, eggs



Supplementary figure 1. Trends in the prevalence of NAFLD, NHANES 2007-2014.







Supplementary figure 2. Graphical abstract.