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Folate intake and non-alcoholic fatty liver disease in US adults

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ABSTRACT

Background and Objectives: The relationship between dietary folate intake and Non-alcoholic fatty liver disease (NAFLD) is controversial. This study aimed to investigate the relationship between dietary folate equivalent (DFE) intake and NAFLD in U.S. adults. **Methods and Study Design:** Data from the National Health and Nutrition Examination Survey (NHANES) 2007-2014 were used. NAFLD was defined as a US fatty liver index (FLI) value ≥ 30 . DFE intake was assessed by two 24-hour dietary recall interviews. Multivariable logistic regression models and restricted cubic spline models were used to investigate the association between DFE intake and NAFLD risk. **Results:** A total of 6,603 adult participants were included in this study. After adjusting for multiple confounding factors, the odds ratios and 95% confidence intervals of NAFLD for the highest quartile versus lowest quartile of DFE intake was 0.77(0.59-0.99). In stratified analyses by sex, age, and body mass index (BMI), there were statistically significant negative associations between DFE intake and NAFLD risk in women and participants with BMI ≥ 25 . Dose-response analysis indicated a negative linear correlation between DFE intake and NAFLD risk. **Conclusions:** Dietary folate equivalent intake is negatively associated with NAFLD risk in the general U.S. adult population.

Key Words: Non-alcoholic fatty liver disease, dietary folate equivalent intake, dose-response, National Health and Nutrition Examination Survey, U.S. adults

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is one of the most common chronic liver diseases around the world.¹ NAFLD is a common chronic liver disease characterized by hepatic fat accumulation on imaging or histology.² NAFLD is a group of liver diseases, ranging from hepatic steatosis to non-alcoholic steatohepatitis (NASH) and fibrosis, which can even develop into cirrhosis or liver cancer in severe cases.²⁻⁴ The prevalence of NAFLD is about 15 %-30% worldwide.^{4,5} In the USA, this figure is about 24%.⁶ There is growing evidence that NAFLD may lead to high risk of metabolic diseases, such as diabetes, hypertension, and hyperlipidemia.⁶⁻⁹ Nowadays, effective treatments for NAFLD are limited.⁶ Therefore, it is necessary to seek the potential regulatory factors to reduce the incidence of NAFLD.

Some dietary factors are associated with NAFLD. According to previous literature, high-energy diets, refined sugars, fructose rich foods (such as soft drinks), processed foods rich in saturated fats and fried foods may increase the risk of NAFLD,¹⁰⁻¹³ whereas fish oils, dairy

products, nuts, coffee, dietary fiber, and plant-based foods may be linked with a decreased risk of NAFLD.¹⁴⁻¹⁷ In addition to folic acid, it is reported that the dietary intake of sugar, saturated fatty acids and selenium is positively associated with the risk of NAFLD,^{18,19} whereas dietary intake of fiber (soluble and insoluble), n-3 and n-6 PUFA, vitamin D, B1, B2, B12, B3 and zinc is negatively correlated with the risk of NAFLD.^{17,19-21} Folate, a water-soluble B vitamin, plays a crucial role in biosynthesis and metabolism. Folic acid is a wholly oxidized and synthetic form of the folate, with a higher bioavailability than its natural counterpart.²² Considering the difference in bioavailability between natural folate and folic acid, dietary folate equivalent (DFE) is often used to indicate folate intake.²³ Studies have shown that dietary folate is closely related to some metabolic diseases or chronic diseases, such as the metabolic syndrome and hyperhomocysteinemia.²⁴⁻²⁹

Nevertheless, the relationship between folate intake and NAFLD risk is controversial. A case-control study based on an Isfahan City sample showed that patients with NAFLD consumed less folate than controls.¹⁹ Another cross-sectional study of Korean adults showed that folate intake was inversely associated with NAFLD in Korean adults.³⁰ Mahmud Mahamid's study shown that low level of folate (B9) could be regarded as an independent predictor of nonalcoholic steatohepatitis (NASH), an extreme form of NAFLD.³¹ However, a nested case-control study in US found no significant association between folate intake and NAFLD.³² An experimental study by Sara Youssry confirmed that folate, improving liver enzyme function and the reducing immune mediators, played a therapeutic role in NAFLD.³³ An open-label pilot study claimed that therapy with folate didn't lead to a significant improvement in patients with NASH.²⁹

In summary, the relationship between DFE and NAFLD risk remains controversial. In particular, no study has investigated the dose-response relationship between DFE and NAFLD. In view of the limitation and conflicting outcomes of previous studies, the present study examined the association between DFE and the risk of NAFLD in the U.S. adults using data from the National Health and Nutrition Examination Survey (NHANES) 2007 to 2014.

MATERIALS AND METHODS

Study population

The NHANES 2007-2014 included 40,617 participants, and our analyses were limited to 23,482 participants aged 20 years or older. Participants with missing information to calculate the United States fatty liver index (USFLI) were excluded (n=13,728). Participants who were positive for hepatitis B surface antigen and hepatitis C virus antibody (n=200) or those with

elevated alcohol intake ≥ 10 g/day for women or ≥ 20 g/day for men ($n=1,535$) were excluded. Pregnant women ($n=94$) were counted out. Participants with unreliable or incomplete dietary recall ($n=1,224$) or missing weight data ($n=8$), or those with average energy intake $>$ mean $+3SD$ (4,261 kcal) or $<$ mean $-3SD$ (0 kcal) ($n=80$) were also excluded. In the end, individuals with lack of BMI information were excluded ($n=10$). Finally, 6,603 people (3,060 men and 3,543 women) were included in our analysis (Figure 1).

NAFLD Measurement

Referring to previous studies, we use US FLI to define NAFLD.^{34,35} Age, race, waist circumference, GGT activity, and fasting insulin and fasting glucose levels were used to calculate US FLI. A US FLI ≥ 30 was considered to indicate NAFLD.³⁶

Dietary Folate Equivalent Intake

Data of DFE intake was obtained from two 24-h dietary recall interviews. The first interview was performed in a mobile examination center, and the second interview was conducted by phone three to ten days later. Nutrient intakes were calculated based upon the United States Department of Agriculture Food and Nutrient Database for Dietary Studies (FNDDS 2007-2008, 2009–2010, 2011–2012 and 2013–2014).³⁷ Referring to our previous studies, average DFE intakes from the two 24-h recalls were used for those who completed both recalls, whereas single recall data was used for those who completed only one recall.^{17,38} The final DFE intake was adjusted to daily energy intake (ng/kcal). Daily energy intake was calculated in the same way as DFE.

Study covariates

In order to improve the accuracy of the experiment, several relevant factors were included in the regression analysis model to control for potential confounding. The relevant variables were as follows: age (20–44 y, 45–59 y, 60–74 y, and ≥ 75 y), sex (men, women), race (Mexican American, other Hispanic, non-Hispanic White, non-Hispanic Black, and other races), education background (below high school, high school diploma, above high school), economic condition (annual income $< \$20,000$, $\$20,000$ – $\$44,999$, $\$45,000$ – $\$74,999$, and $\geq \$75,000$), smoking status (smoking at least 100 cigarettes in life or not), vigorous recreational activity (yes or no), diabetes status (yes or no), hypertension status (yes or no), serum uric acid (UA) level, total cholesterol (TC) level and body mass index (BMI). Hypertension was defined as mean systolic blood pressure ≥ 130 mmHg, or mean diastolic

blood pressure ≥ 80 mmHg, or use of antihypertensive drugs or self-reported hypertension diagnosis.³⁹ Diabetes was defined as fasting plasma glucose ≥ 7.0 mmol/L, or glycohemoglobin (HbA1c) $\geq 6.5\%$, or the 2-h plasma glucose level (OGTT) ≥ 11.1 mmol/L, or use of insulin or antidiabetic drugs, or self-reported diabetes diagnosis.

Statistical analysis

Statistical analyses were carried out with the Stata 15.0. Proper sampling weights, primary sampling units, and strata information were considered in our analysis to make our results more representative. 2-year sample weights for NHANES 2007-2008, 2009-2010, 2011-2012 and 2013-2014 could be obtained from public-use files.⁴⁰ To combine these cycles to produce 8-year estimates, a new 8-year weights should be calculated by taking one-quarter of the 2-year dietary weights according to the NHANES analytical guidelines. Descriptive statistics were presented as median (interquartile ranges) for continuous variables or frequencies (percentage) for categorical variables. For comparing the differences in average value of continuous variables between the NAFLD group and non-NAFLD group, Student's t-test (for normally distributed data) or non-parametric test (for non-normal distribution) was applied. Differences in categorical variables were evaluated by chi² tests. DFE intake was classified according to quartiles. Using the lowest quartile as a reference category, the relationships between DFE intake and NAFLD were investigated via multivariable logistic regression models. Model 1 was adjusted for age and sex. Model 2 was further adjusted for BMI, race, education background, economic condition, smoking status, vigorous recreational activity, diabetes, hypertension, UA and TC levels. Then, stratified analyses were conducted by sex, age and BMI to determine the associations between DFE intake and NAFLD risk. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated from multivariable logistic regression analyses. Dose-response relationship was evaluated using a restricted cubic spline function with three knots located at the 5, 50, and 95th percentiles of the exposure distribution in Model 2. Two-sided $p \leq 0.05$ indicated statistical significance for above-mentioned analyses.

RESULTS

Table 1 provides a comparison of baseline characteristics by sex between NAFLD and non-NAFLD groups. Among 6,603 participants, the overall prevalence of NAFLD was 35.1% (40.6% in men and 30.4% in women). Compared with the non-NAFLD group, men or women with NAFLD tended to be older, Mexican-American, and smokers. In addition, participants in the NAFLD group tended to have higher levels of BMI, serum UA, and have higher

prevalence of hypertension or diabetes, whereas the education level, DFE intake and physical activity were lower in the NAFLD group ($p<0.05$). We further compared the levels of folic acid intake from food and supplements between NAFLD and control groups (Supplementary table 1). Folic acid intake from both food and supplements were lower in patients with NAFLD than that in non-NAFLD ($p<0.05$). According to the Food and Nutrient Database, Department of Agriculture, US, the main dietary sources of folic acid in the US are grain (64.2%), meat (10.2%), vegetables (7.39%) and nut (4.92%) (Supplementa table 2). There is no data of folic acid fortification in the Food and Nutrient Database.

The weighted ORs (95%CI) for NAFLD were given based on the quartiles of DFE intake (Table 2). Univariate logistic regression analysis revealed that DFE intake was negatively associated with the risk of NAFLD. The crude OR (95%CI) of NAFLD in the highest quartile of DFE intake was 0.65 (0.53-0.79) compared with the lowest quartile. After adjusting for age and sex (Model 1), the OR (95%CI) was 0.61 (0.50-0.75). After further adjustment for race, education background, economic condition, smoking status, vigorous recreational activity, diabetes, hypertension, UA level, TC level and BMI (Model 2), DFE intake remained negatively associated with NAFLD risk (0.77 (0.59–0.99)).

Associations of DFE intake with NAFLD in stratified analyses by sex, age and BMI were displayed in Table 3 (by sex), Table 4 (by age) and Table 5 (by BMI) respectively. In the stratified analyses by sex, as for women, compared to the lowest quartile, the OR (95% CI) of NAFLD for the highest quartile was 0.63 (0.44-0.90) in Model 2. Nonetheless, when it came to men participants, the correlated result was not statistically significant. In the stratified analyses by age, after adjustment for multiple covariates, in the younger group (<45 years), compared with quartile 1, the ORs of NAFLD for quartile 2, quartile 3 and quartile 4 of DFE intake were 0.96 (0.58-1.59), 0.63 (0.42-0.96) and 0.82 (0.52-1.31), respectively. For the participants aged 45 years or older, there was no statistically significant associations between DFE intake and NAFLD in model 2. In the stratified analyses by BMI, in Model 2, an inverse association between DFE intake and NAFLD was only observed in participants with BMI ≥ 25 (OR: 0.73; 95% CI: 0.57-0.93).

Figure 2 depicts the relationship between DFE intake and NAFLD. In the restricted cubic spline model, folate intake was linear negatively correlated with NAFLD (for nonlinearity, $p=0.128$). With the increase of DFE intake, OR of NAFLD diminished. When DFE intake reached 438 ng/kcal/day, DFE had a significant protective effect on NAFLD (OR, 0.69; 95%CI, 0.47-0.99).

DISCUSSION

In this large, nationally representative cross-sectional study, we examined the association between folate intake and NAFLD risk. After adjusting for multiple potential confounders, including age, sex, BMI, race, education level, economic condition, smoking status, physical activity level, diabetes, hypertension, levels of UA and TC, DFE intake was inversely associated with NAFLD risk in US. adults. In stratified analyses by age, sex and BMI, the negative association between DFE intake and NAFLD remained statistically significant in women and participants with BMI ≥ 25 . We also investigated the dose-response relationship between DFE intake and NAFLD and found a linear inverse association between DFE intake and the risk of NAFLD.

So far, studies on the relationship between dietary folate intake and NAFLD risk are limited and conflicting. Based on data from Imam Hossain Hospital in Shahroud, Vahedi H et al showed that serum folate level in healthy subjects was significantly higher than that in patients with fatty liver.⁴¹ A case-control study conducted in Isfahan city also indicated that the mean of dietary intake of folate in patients with NAFLD was lower than that of healthy individuals.¹⁹ Our finding of the negative association between DFE intake and NAFLD was consistent with the aforementioned studies. However, A Korean study among 348 adults (20y-69y) demonstrated that after adjusting for multiple confounders, dietary folate intake was not significantly associated with NAFLD.³⁰ In addition, a nested case-control study demonstrated that folate intake was not significantly associated with NAFLD.³² The inconsistent results of association between folate intake and NAFLD in different populations may be partly explained by differences in dietary pattern, food culture and/or the method to obtain dietary information.

Although the potential mechanisms of the association between folate intake and NAFLD have not been fully elucidated, several theories have been proposed. Folate deficiency can affect folate-dependent sulfur amino acid metabolism and lead to hyperhomocysteinemia which promotes hepatic fat accumulation.^{4,31,42,43} Folate deficiency can lead to high expression of lipid synthesis genes, while increased intake of folate can activate AMPK and then improve the metabolism of glucose and lipid.^{44,45} AMPK pathway regulates lipid synthesis, fatty acid degradation, and fatty acid oxidation.⁴⁶ It was reported that folic acid can restore the relative expression levels of phospho-LKB1 which is the upstream kinase of AMPK pathway.⁴⁷ In addition, animal experimental research demonstrated that folate can prevent hepatic oxidative injury via transcriptional regulation of NADPH oxidase.⁴⁸

There are some advantages in our study. First, we used data from a large national survey (n=6603), which enhanced the reliability of our study. Besides, DFE intake was still significantly negatively associated with NAFLD risk after correcting potential confounders, which authenticated the association. Third, we assessed the dose-response relationship between DFE intake and NAFLD risk for the first time.

However, our study also has some limitations. First of all, due to the cross-sectional design of this study, it is difficult to determine a causal inference and further prospective studies are needed to investigate the causal relationship between dietary folate intake and NAFLD risk. Secondly, dietary data was obtained from two 24-hour recall interviews, which may result in recall bias. Moreover, in this study, NAFLD was defined by US FLI, which is adequate but not the perfect proxy for NAFLD diagnosis using liver biopsy.

Conclusion

In brief, DFE intake is negatively correlated with NAFLD risk in the U.S. adult population. Increasing dietary folate intake may be a promising method in the prevention of NAFLD.

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AUTHOR DISCLOSURE

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Table 1. Baseline characteristics of the participants by NAFLD, U.S. adult aged ≥ 20 years, NHANES 2007-2014

Characteristic	NAFLD (total)			NAFLD (men)			NAFLD (women)		
	No	Yes	<i>p</i> -value	No	Yes	<i>p</i> -value	No	Yes	<i>p</i> -value
Number of participants (%)	4183 (64.9%)	2420 (35.1%)		1776 (59.4%)	1284 (40.6%)		2407 (69.6%)	1136 (30.4%)	
Age group (n, %)			<0.001			<0.001			<0.001
20-44 years	1890 (73.6%)	709 (26.4%)		805 (68.6%)	388 (31.4%)		1085 (78.1%)	321 (21.9%)	
45-59 years	988 (61.1%)	655 (38.9%)		427 (55.6%)	328 (44.4%)		561 (65.9%)	327 (34.1%)	
60-74 years	860 (54.5%)	739 (45.5%)		345 (46.7%)	388 (53.3%)		515 (60.8%)	351 (39.2%)	
≥ 75 years	445 (58.3%)	317 (41.7%)		199 (52.6%)	180 (47.4%)		246 (62.6%)	137 (37.4%)	
Race (n, %)			<0.001			<0.001			<0.001
Mexican American	488 (49.4%)	552 (50.6%)		204 (43.5%)	281 (56.6%)		284 (55.3%)	271 (44.7%)	
Other Hispanic	443 (64.1%)	294 (35.9%)		175 (61.4%)	144 (38.6%)		268 (66.5%)	150 (33.5%)	
Non-Hispanic White	1840 (63.9%)	1153 (36.1%)		784 (58.0%)	649 (42.0%)		1056 (69.0%)	504 (31.0%)	
Non-Hispanic Black	931 (79.1%)	268 (20.9%)		394 (79.3%)	120 (20.7%)		537 (79.0%)	148 (21.1%)	
Other Race	481 (75.4%)	153 (24.6%)		219 (69.9%)	90 (30.1%)		262 (80.3%)	63 (19.7%)	
Annual household income			<0.001			<0.001			<0.001
<\$20,000	777 (61.3%)	559 (38.7%)		263 (58.8%)	234 (41.2%)		514 (62.6%)	325 (37.4%)	
\$20,000-\$44,999	1,352 (59.0%)	929 (41.0%)		549 (51.2%)	485 (48.9%)		803 (64.9%)	444 (35.1%)	
\$45,000-\$74,999	781 (64.4%)	409 (35.6%)		340 (57.3%)	237 (42.7%)		441 (71.0%)	172 (29.1%)	
\geq \$75,000	1,095 (72.3%)	423 (27.7%)		533 (66.7%)	277 (33.3%)		562 (78.6%)	146 (21.4%)	
Educational Background (n, %)			<0.001			0.004			<0.001
< High school	899 (54.7%)	802 (45.3%)		395 (52.1%)	396 (47.9%)		504 (57.0%)	406 (43.1%)	
High school	952 (64.1%)	543 (35.9%)		417 (59.8%)	298 (40.2%)		536 (67.9%)	245 (32.1%)	
>High school	2,328 (68.3%)	1,071 (31.8%)		963 (61.4%)	588 (38.6%)		1,365 (74.1%)	483 (25.9%)	
Smoking status (n, %)			<0.001			<0.001			0.010
Yes	1,573 (59.9%)	1,128 (40.2%)		837 (54.5%)	704 (45.6%)		736 (66.3%)	426 (33.7%)	
No	2,608 (68.3%)	1,292 (31.7%)		938 (63.8%)	580 (36.2%)		1,670 (71.4%)	712 (28.6%)	
Vigorous recreational activity (n, %)			<0.001			<0.001			<0.001
Yes	1,031 (81.6%)	270 (18.4%)		571 (77.1%)	192 (23.0%)		460 (87.8%)	78 (12.2%)	
No	3,152 (59.9%)	2,150 (40.1%)		1,205 (52.2%)	1,092 (47.8%)		1,947 (65.6%)	1,058 (34.4%)	
Diabetes (n, %)			<0.001			<0.001			<0.001
Yes	504 (32.5%)	908 (67.5%)		228 (30.3%)	460 (69.7%)		276 (34.5%)	448 (65.6%)	
No	3,679 (71.3%)	1,512 (28.7%)		1,548 (65.4%)	824 (34.6%)		2,131 (76.4%)	689 (23.6%)	
Hypertension (n, %)			<0.001			<0.001			<0.001
Yes	1,801 (51.2%)	1,635 (48.8%)		806 (47.1%)	863 (52.9%)		995 (55.1%)	772 (44.9%)	
No	2,382 (77.2%)	785 (22.8%)		970 (71.6%)	421 (28.5%)		1,412 (81.7%)	364 (18.3%)	
BMI (kg/m ²)	26.0 (23.2, 29.1)	33.1 (29.6, 37.8)	<0.001	26.0 (23.6, 28.5)	31.9 (28.8, 35.5)	<0.001	26.0 (22.7, 29.6)	35.0 (30.8, 40.2)	<0.001
UA (mg/dL)	5.00 (4.20, 5.90)	6.00 (5.20, 6.90)	<0.001	5.70 (5.10, 6.40)	6.40 (5.70, 7.30)	<0.001	4.50 (3.80, 5.20)	5.60 (4.80, 6.30)	<0.001

BMI: body mass index; UA: uric acid; TC: total cholesterol; DFE: dietary folate equivalent.

Data are presented as median (25th, 75th) for continuous variables or participants (percentage) for categorical variables.

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

Characteristic	NAFLD (total)			NAFLD (men)			NAFLD (women)		
	No	Yes	<i>p</i> -value	No	Yes	<i>p</i> -value	No	Yes	<i>p</i> -value
TC (mg/dL)	189 (165, 215)	192 (166, 220)	0.020	184 (161, 208)	189 (162, 217)	0.002	194 (168, 221)	195 (171, 223)	0.232
Average energy intake (kcal/day)	1,870 (1,464, 2,387)	1,942 (1,479, 2,464)	0.566	2,226 (1,782, 2,762)	2,179 (1,719, 2,710)	0.058	1,639 (1,337, 2,037)	1,667 (1,336, 2,112)	0.142
DFE intake (ng/kcal/day)	252 (191, 336)	235 (183, 307)	<0.001	249 (189, 333)	235 (181, 310)	0.041	254 (195, 342)	236 (185, 301)	0.003

BMI: body mass index; UA: uric acid; TC: total cholesterol; DFE: dietary folate equivalent.

Data are presented as median (25th, 75th) for continuous variables or participants (percentage) for categorical variables.

Table 2. Weighted ORs and 95% CIs for NAFLD according to the quartiles of DFE intake

	Crude OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)
Folate intake (ng /kcal /day)			
<188	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
188 to <245	0.93 (0.76-1.14)	0.91 (0.74-1.11)	0.99 (0.74-1.33)
245 to <328	0.80 (0.67-0.97)*	0.79 (0.65-0.96)*	0.93 (0.73-1.17)
≥328	0.65 (0.53-0.79)**	0.61 (0.50-0.75)**	0.77 (0.59-0.99)*

DFE: dietary folate equivalent.

Model 1 adjusted for age and sex. Model 2 adjusted for age, sex, race, education background, economic condition, smoking status, physical activity, diabetes, hypertension, BMI, UA and TC. The lowest quartile of DFE intake 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 DFE intake, stratified by sex

DFE intake (ng /kcal/day)	Crude OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)
Men			
<185	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
185 to <243	0.93 (0.67-1.28)	0.90 (0.65-1.25)	1.15 (0.74-1.79)
243 to <325	0.80 (0.60-1.08)	0.77 (0.56-1.04)**	1.03 (0.71-1.51)
≥325	0.72 (0.53-0.99)*	0.68 (0.49-0.94)*	1.00 (0.65-1.53)
Women			
<191	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
191 to <248	0.95 (0.70-1.28)	0.91 (0.67-1.23)	0.95 (0.68-1.32)
248 to <329	0.81 (0.62-1.06)	0.78 (0.60-1.03)	0.85 (0.64-1.12)
≥329	0.59 (0.44-0.79)**	0.55 (0.40-0.74)**	0.63 (0.44-0.90)*

DFE: dietary folate equivalent.

Model 1 adjusted for age. Model 2 adjusted for age, race, education background, economic condition, smoking status, physical activity, diabetes, hypertension, BMI, UA and TC. The lowest quartile of DFE intake 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 DFE intake, stratified by age

DFE intake (ng /kcal/day)	Crude OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)
<45 years			
<184	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
184 to <240	0.82 (0.61-1.10)	0.83 (0.61-1.12)	0.96 (0.58-1.59)
240 to <320	0.57 (0.42-0.77)**	0.58 (0.43-0.79)**	0.63 (0.42-0.96)*
≥320	0.54 (0.39-0.75)**	0.55 (0.40-0.75)**	0.82 (0.52-1.31)
≥45 years			
<184	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
184 to <240	0.98 (0.70-1.28)	1.01 (0.74-1.38)	1.07 (0.71-1.61)
240 to <320	0.87 (0.67-1.14)	0.89 (0.68-1.16)	1.06 (0.79-1.42)
≥320	0.66 (0.51-0.85)**	0.67 (0.51-0.87)**	0.77 (0.55-1.06)

DFE: dietary folate equivalent.

Model 1 adjusted for sex. Model 2 adjusted for sex, race, education background, economic condition, smoking status, physical activity, diabetes, hypertension, BMI, UA and TC. The lowest quartile of DFE intake was used as the reference group. Results are survey-weighted.

* $p < 0.05$; ** $p < 0.01$.

Table 5. Weighted ORs and 95% CIs for NAFLD according to the quartiles of DFE intake, stratified by BMI

DFE intake (ng /kcal/day)	Crude OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)
BMI <25			
<201	1.00 (ref.)	1.00(ref.)	1.00 (ref.)
201 to <258	1.33 (0.65-2.72)	1.27 (0.62-2.61)	1.30 (0.66-2.57)
258 to <340	0.76 (0.37-1.58)	0.67 (0.32-1.42)	0.73 (0.30-1.76)
≥340	0.88 (0.46-1.68)	0.78 (0.40-1.52)	0.89 (0.43-1.83)
BMI ≥25			
<185	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
185 to <241	1.04 (0.83-1.29)	1.01 (0.81-1.27)	1.03 (0.80-1.33)
241 to <322	0.92 (0.74-1.14)	0.89 (0.70-1.12)	0.95 (0.72-1.25)
≥322	0.75 (0.61-0.93)**	0.70 (0.56-0.86)**	0.71 (0.56-0.90)**

BMI: body mass index; DFE: dietary folate equivalent.

Model 1 adjusted for age and sex. Model 2 adjusted for age, sex, race, education background, economic condition, smoking status, physical activity, diabetes, hypertension, BMI, UA and TC. The lowest quartile of DFE intake was used as the reference group. Results are survey-weighted.

* $p < 0.05$; ** $p < 0.01$.

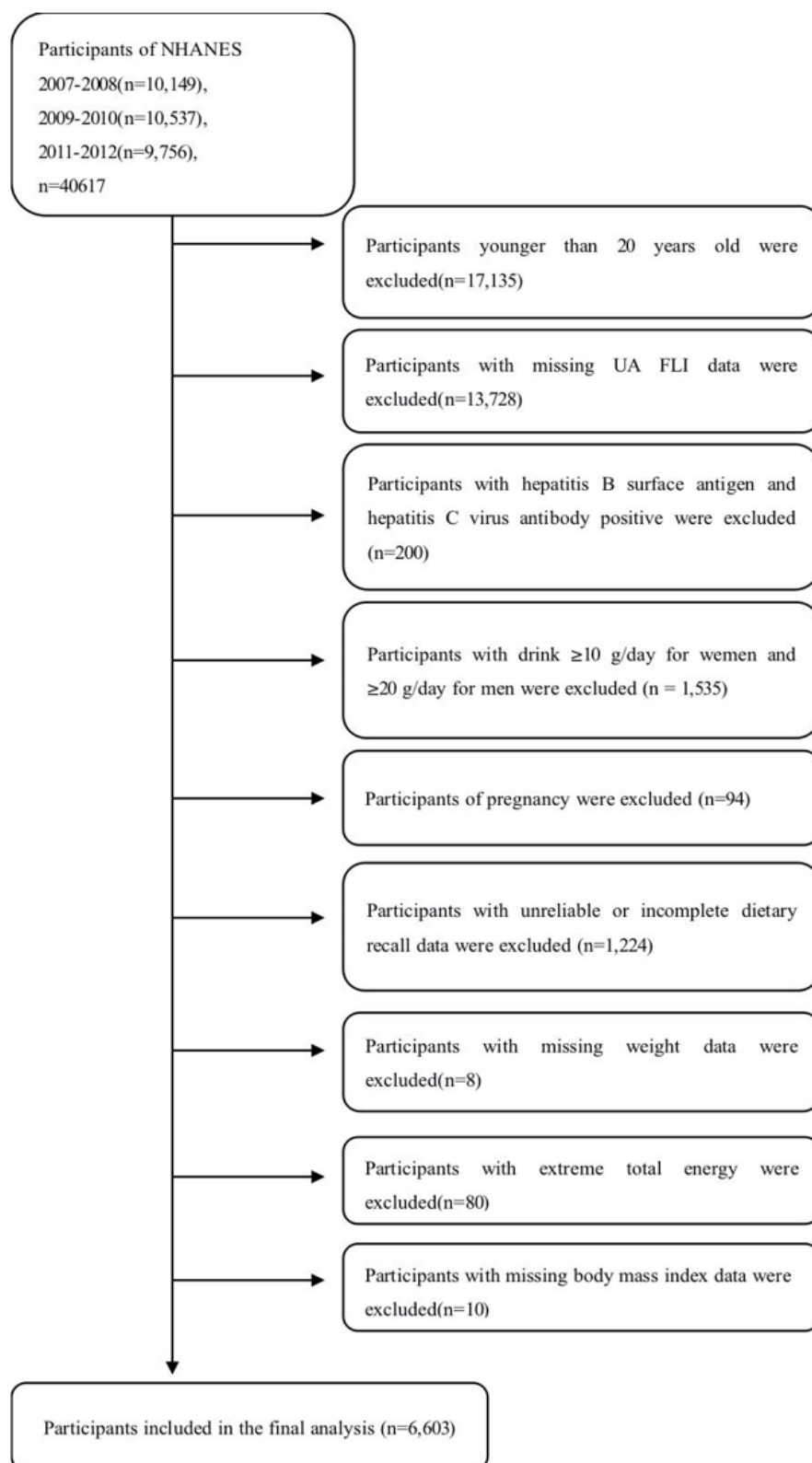


Figure 1. Flow chart of the selection process. NHANES, National Health and Nutrition Examination Survey.

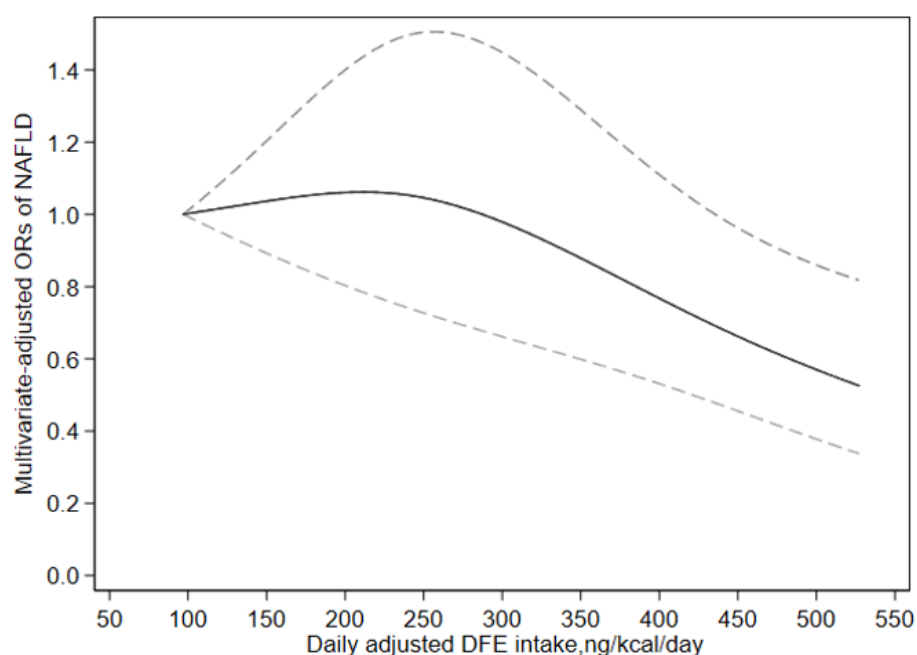
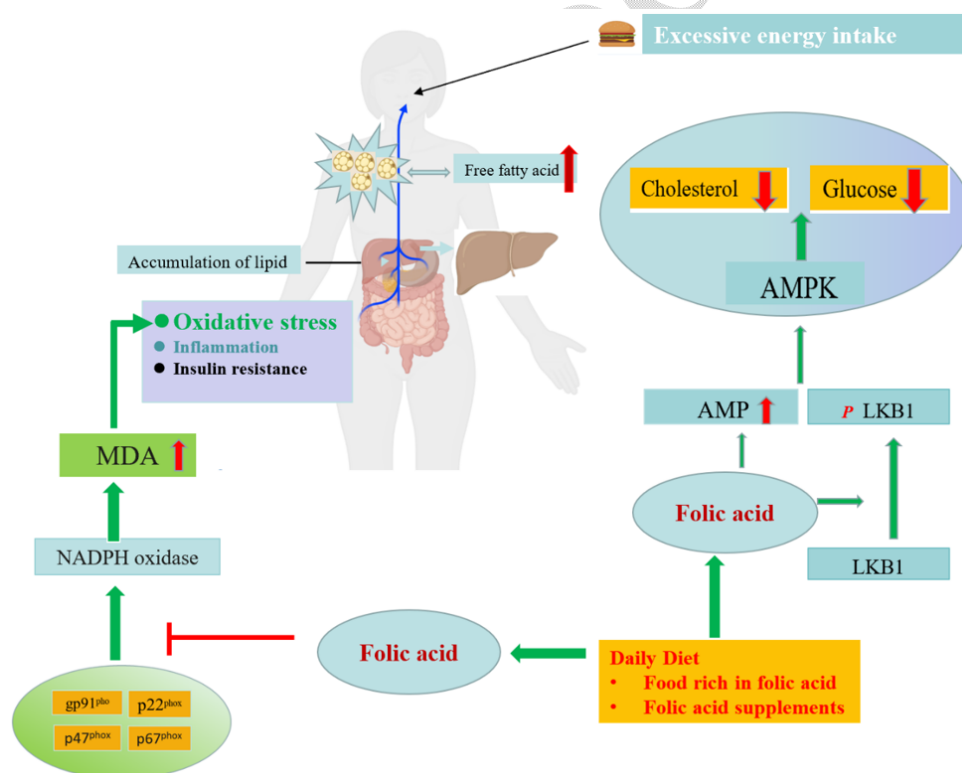


Figure 2. The restricted cubic spline model showed a dose-response relationship between DFE intake per kilocalorie (kcal) of energy intake per day and NAFLD. The lowest level of folate intake (97ng/kcal/day) was used as the reference group. Adjustments were made according to age, sex, race, education background, economic condition, smoking status, diabetes, hypertension, physical activity, BMI, UA and TC. The solid line and the dotted line represent the estimated OR and the corresponding 95%CI, respectively. OR odds ratio.



Graphical abstract.

Supplementary table 1. Dietary sources of folic acid, folic acid supplements in the participants distribution

	NAFLD (total)		<i>p</i> -value	NAFLD (men)		<i>p</i> -value	NAFLD (women)		<i>p</i> -value
	No	Yes		No	Yes		No	Yes	
DFE intake (ng/day)	522±292	502±287	<0.01	594±327	563±317	<0.01	468±250	433±250	<0.01
Supplements (ng/day)	182±144	177±143	<0.01	211±161	203±159	0.182	161±125	148±116	<0.01
DFE from food (ng/day)	212±128	201±107	0.15	236±149	218±119	<0.01	194±106	182±88.6	<0.01

DFE (ug) = Dietary folic acid (ug) + 1.7× Folic acid supplement (ug)

Supplementary table 2. Food sources of folic acid

Food Group	Provide folic acid(ng/d)	Percentage (%)
Grain	303	64.2
Meat	48.0	10.2
Vegetables	34.8	7.39
Nut	23.2	4.92
Milk	13.7	4.14
Egg	8.36	2.91

Supplementary table 3. Association between folic acid and risk of NAFLD In different populations

Countries	Type of Research	Number of participants	Folic acid concentration	Conclusion
Iran (Isfahan city)	Case-control study	Case group:159, control group:158	Dietary intake: case group: 366.3±20.3 ug, control group:411.6±199.5 ug, $p=0.04$	Low intake of folic acid appears to be associated with the development of NAFLD. ¹⁹
Korean	Case-control study	348 subjects, Case group:169, control group:179	Dietary intake: case group: men: 426.93±18.83 µg, women: 417.88±18.64 µg; control group: men: 486.76±19.71 µg , women: 446.15±15.72 µg	The intake of folate (OR: 3.37; p -trend=0.077) had a tendency to decrease the NAFLD risk in male subjects but did not remain statistically significant after the multivariate adjustment. ³⁰
United States(southeastern)	nested case-control study	Case group:1201, control group:4533	Dietary intake: case group: 411 (287–594) ug/day, control group: 422 (298–597) ug/day, $p>0.05$	Folate intake was not significantly associated with NAFLD. ³²
Italy	Case-control study	Case group:124 patients affected by NAFLD and 162 HCV; control group:2326 apparently healthy blood donors	Dietary intake: case group: 400.2±153.9 control group: 342±101.5	Noticed a statistically relevant increase in the consumption of folic acid in our NASH patients. ⁴⁹

DFE (ug) = Dietary folic acid (ug) + 1.7× Folic acid supplement (ug).