Original Article

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

Bo Zhang MSc[1†], Jun-chuan Cao MB[2†], Fu-rong Liu MB[3], Zhuo Deng MB[4], Chuan-jing Chen MSc[1], Yong-ye Sun PhD[1]

[1]*Department of Nutrition and Food Hygiene, School of Public Health, Qingdao University, Qingdao, China*
[2]*School of Stomatology Qingdao University, Qingdao, China*
[3]*Nursing Department, Women and Children's Hospital, Qingdao University, Qingdao, China*
[4]*Department of Medicine, Medical imaging, Qingdao University, Qingdao, China*
[†]*Both authors contributed equally to this manuscript*

**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

**Corresponding Author:** Dr Yong-ye Sun, Department of Nutrition and Food Hygiene, School of Public Health, Qingdao University, Qingdao 266021, China.
Tel: +86-138-6398-0712; Fax: 0532-83812434
Email: yongye.sun@126.com
Manuscript received 27 November 2022. Initial review completed 08 January 2022. Revision accepted 16 May 2022.
doi: 10.6133/apjcn.202303_32(1).0019

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.

## 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 ≥$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) ≥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 $chi^2$ 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

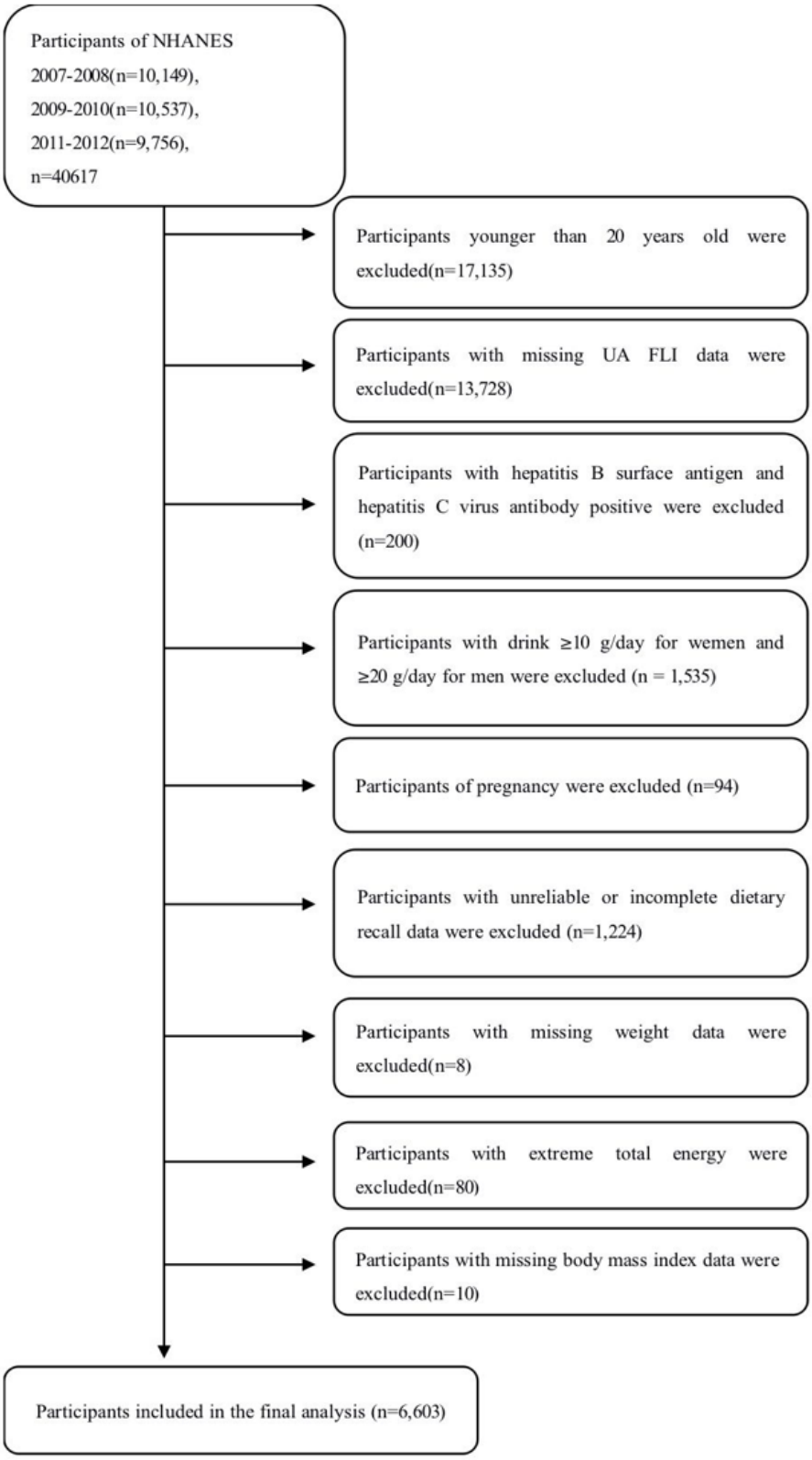



**Figure 1.** Flow chart of the selection process. NHANES, National Health and Nutrition Examination Survey.

$p \leq 0.05$ 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

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

| Characteristic | NAFLD (total) | | *p*-value | NAFLD (men) | | *p*-value | NAFLD (women) | | *p*-value |
|---|---|---|---|---|---|---|---|---|---|
| | No | Yes | | No | Yes | | No | Yes | |
| 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%) | |
| ≥$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/m²) | 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 |
| 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.

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-

**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.

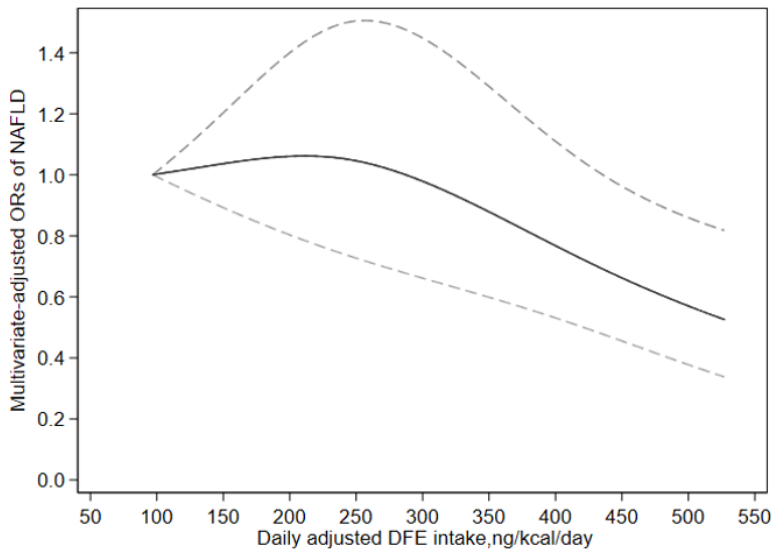
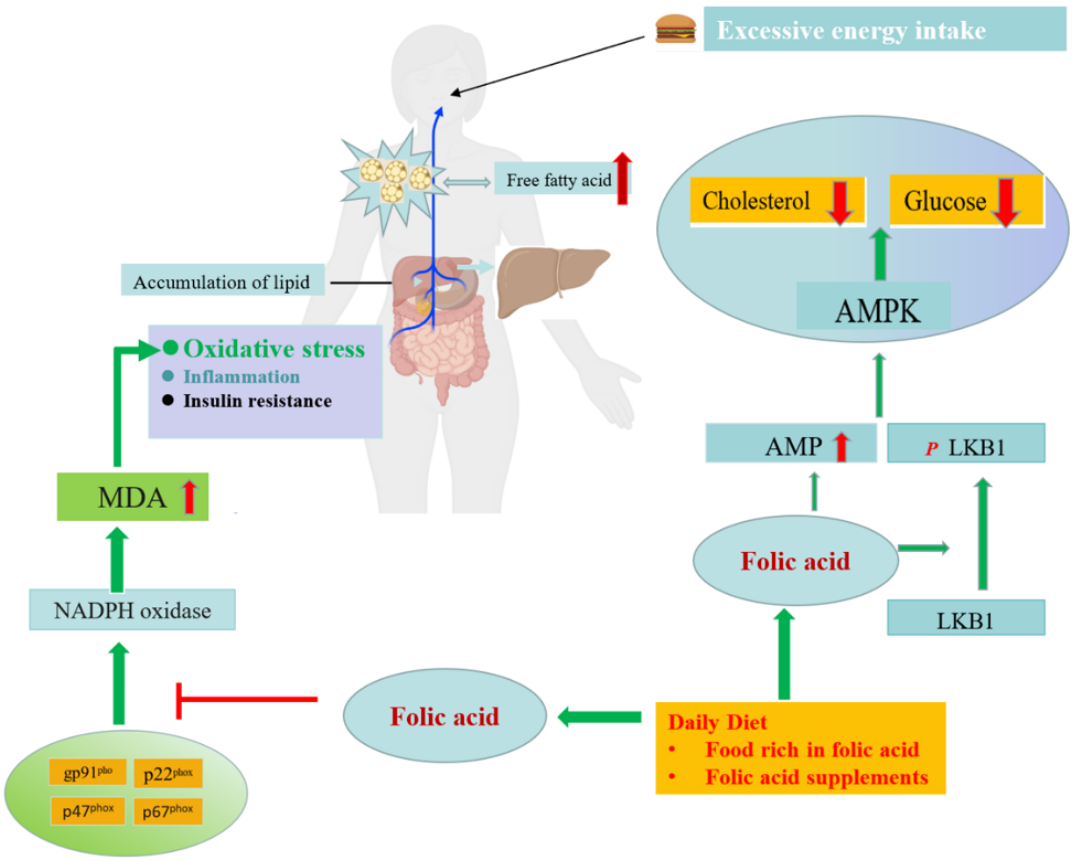



**Figure 2.** The restricted cubic spline model showed a dose-response relationship between DFE intake per kilocalorie (kcal) of energy intake per day and NAFLD. The lowest level of folate intake (97ng/kcal/day) was used as the reference group. Adjustments were made according to age, sex, race, education 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.

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,




**Graphical abstract.**

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 NALFD 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.

**ACKNOWLEDGEMENTS**

The authors thank all of the people who subserved this study.

**AUTHOR DISCLOSURES**

The author has no financial or proprietary interest in any of the materials discussed in this article.

No funding was received for conducting this study.

**REFERENCES**


1. Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, Harrison SA, Brunt EM, Sanyal AJ. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. Hepatology. 2018;67:328-57. doi: 10.1002/hep.29367.
2. Friedman SL, Neuschwander-Tetri BA, Rinella M, Sanyal AJ. Mechanisms of NAFLD development and therapeutic strategies. Nat Med. 2018;24:908-22. doi: 10.1038/s41591-018-0104-9.
3. Buzzetti E, Pinzani M, Tsochatzis EA. The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). Metabolism. 2016;65:1038-48. doi: 10.1016/j.metabol.2015.12.012.
4. Sid V, Siow YL, O K. Role of folate in nonalcoholic fatty liver disease. Can J Physiol Pharmacol. 2017;95:1141-8. doi: 10.1139/cjpp-2016-0681.
5. Younossi Z, Anstee QM, Marietti M, Hardy T, Henry L, Eslam M, George J, Bugianesi E. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. Nat Rev Gastroenterol Hepatol. 2018;15:11-20. doi: 10.1038/nrgastro.2017.109.
6. Vancells Lujan P, Viñas Esmel E, Sacanella Meseguer E. Overview of non-alcoholic fatty liver disease (NAFLD) and the role of sugary food consumption and other dietary components in its development. Nutrients. 2021;13:1442. doi: 10.3390/nu13051442.
7. Byrne CD, Targher G. NAFLD: a multisystem disease. J Hepatol. 2015;62:S47-64. doi: 10.1016/j.jhep.2014.12.012.
8. Nilsson E, Matte A, Perfilyev A, de Mello VD, Käkelä P, Pihlajamäki J, Ling C. Epigenetic alterations in human liver from subjects with type 2 diabetes in parallel with reduced folate levels. J Clin Endocrinol Metab. 2015;100:E1491-501. doi: 10.1210/jc.2015-3204.
9. Ryoo JH, Suh YJ, Shin HC, Cho YK, Choi JM, Park SK. Clinical association between non-alcoholic fatty liver disease and the development of hypertension. J Gastroenterol Hepatol. 2014;29:1926-31. doi: 10.1111/jgh. 12643.
10. Worm N. Beyond body weight-loss: Dietary strategies targeting intrahepatic fat in NAFLD. Nutrients. 2020;12:1316. doi: 10.3390/nu12051316.
11. Jensen T, Abdelmalek MF, Sullivan S, Nadeau KJ, Green M, Roncal C et al. Fructose and sugar: A major mediator of non-alcoholic fatty liver disease. J Hepatol. 2018;68:1063-75. doi: 10.1016/j.jhep.2018.01.019.
12. Mollard RC, Sénéchal M, MacIntosh AC, Hay J, Wicklow BA, Wittmeier KD et al. Dietary determinants of hepatic steatosis and visceral adiposity in overweight and obese youth at risk of type 2 diabetes. Am J Clin Nutr. 2014;99:804-12. doi: 10.3945/ajcn.113.079277.
13. Chen H, Wang J, Li Z, Lam CWK, Xiao Y, Wu Q, Zhang W. Consumption of sugar-sweetened beverages has a dose-dependent effect on the risk of non-alcoholic fatty liver disease: An updated systematic review and dose-response meta-analysis. Int J Environ Res Public Health. 2019;16. doi: 10.3390/ijerph16122192.
14. Perdomo CM, Frühbeck G, Escalada J. Impact of nutritional changes on nonalcoholic fatty liver disease. Nutrients. 2019;11:677. doi: 10.3390/nu11030677.
15. Saab S, Mallam D, Cox GA, 2nd, Tong MJ. Impact of coffee on liver diseases: a systematic review. Liver Int. 2014;34:495-504. doi: 10.1111/liv.12304.
16. Leoni S, Tovoli F, Napoli L, Serio I, Ferri S, Bolondi L. Current guidelines for the management of non-alcoholic fatty liver disease: A systematic review with comparative analysis. World J Gastroenterol. 2018;24:3361-73. doi: 10.3748/wjg.v24.i30.3361.
17. Zhao H, Yang A, Mao L, Quan Y, Cui J, Sun Y. Association between dietary fiber intake and non-alcoholic fatty liver disease in adults. Front Nutr. 2020;7:593735. doi: 10.3389/fnut.2020.593735.
18. Wu J, Zeng C, Yang Z, Li X, Lei G, Xie D, Wang Y, Wei J, Yang T. Association between dietary selenium intake and the prevalence of nonalcoholic fatty liver disease: A cross-sectional study. J Am Coll Nutr. 2020;39:103-11. doi: 10.1080/07315724.2019.1613271.
19. Zolfaghari H, Askari G, Siassi F, Feizi A, Sotoudeh G. Intake of nutrients, fiber, and sugar in patients with nonalcoholic fatty liver disease in comparison to healthy individuals. Int J Prev Med. 2016;7:98. doi: 10.4103/2008-7802.188083.
20. Cui J, Li L, Ren L, Sun J, Zhao H, Sun Y. Dietary n-3 and n-6 fatty acid intakes and NAFLD: A cross-sectional study in the United States. Asia Pac J Clin Nutr. 2021;30:87-98. doi: 10.6133/apjcn.202103_30(1).0011.
21. Vahid F, Hekmatdoost A, Mirmajidi S, Doaei S, Rahmani D, Faghfoori Z. Association between index of nutritional quality and nonalcoholic fatty liver disease: The role of vitamin D and B group. Am J Med Sci. 2019;358:212-8. doi: 10.1016/j.amjms.2019.06.008.
22. Selhub J, Rosenberg IH. Excessive folic acid intake and relation to adverse health outcome. Biochimie. 2016;126:71-8. doi: 10.1016/j.biochi.2016.04.010.
23. Zhu J, Chen C, Lu L, Yang K, Reis J, He K. Intakes of folate, vitamin B(6), and vitamin B(12) in relation to diabetes incidence among American young adults: A 30-year follow-up study. Diabetes Care. 2020;43:2426-34. doi: 10.2337/dc20-0828.
24. Hirsch S, Poniachick J, Avendaño M, Csendes A, Burdiles P, Smok G, Diaz JC, de la Maza MP. Serum folate and homocysteine levels in obese females with non-alcoholic fatty liver. Nutrition. 2005;21:137-41. doi: 10.1016/j.nut.2004.03.022.
25. Mahabir S, Ettinger S, Johnson L, Baer DJ, Clevidence BA, Hartman TJ, Taylor PR. Measures of adiposity and body fat distribution in relation to serum folate levels in postmenopausal women in a feeding study. Eur J Clin Nutr. 2008;62:644-50. doi: 10.1038/sj.ejcn.1602771.
26. Bailey RL, Dodd KW, Gahche JJ, Dwyer JT, McDowell MA, Yetley EA et al. Total folate and folic acid intake from foods and dietary supplements in the United States: 2003-2006. Am J Clin Nutr. 2010;91:231-7. doi: 10.3945/ajcn.2009.28427.
27. Ashok T, Puttam H, Tarnate VCA, Jhaveri S, Avanthika C, Trejo Treviño AG, Sl S, Ahmed NT. Role of vitamin B12 and folate in metabolic syndrome. Cureus. 2021;13:e18521. doi: 10.7759/cureus.18521.
28. Werge MP, McCann A, Galsgaard ED, Holst D, Bugge A, Albrechtsen NJW, Gluud LL. The role of the transsulfuration pathway in non-alcoholic fatty liver disease. J Clin Med. 2021;10:1081. doi: 10.3390/jcm10051081.
29. Bird JK, Ronnenberg AG, Choi SW, Du F, Mason JB, Liu Z. Obesity is associated with increased red blood cell folate despite lower dietary intakes and serum concentrations. J Nutr. 2015;145:79-86. doi: 10.3945/jn.114.199117.
30. Han JM, Jo AN, Lee SM, Bae HS, Jun DW, Cho YK et al. Associations between intakes of individual nutrients or whole food groups and non-alcoholic fatty liver disease among Korean adults. J Gastroenterol Hepatol. 2014;29:1265-72. doi: 10.1111/jgh.12520.

31. Charatcharoenwitthaya P, Levy C, Angulo P, Keach J, Jorgensen R, Lindor KD. Open-label pilot study of folic acid in patients with nonalcoholic steatohepatitis. Liver Int. 2007;27:220-6. doi: 10.1111/j.1478-3231.2006.01404.x.
32. Sarkar S, Lipworth L, Kabagambe EK, Bian A, Stewart TG, Blot WJ, Ikizler TA, Hung AM. A description of risk factors for non-alcoholic fatty liver disease in the Southern Community Cohort study: A nested case-control study. Front Nutr. 2020;7:71. doi: 10.3389/fnut.2020.00071.
33. Youssry S, Kamel MA. Effect of folate supplementation on immunological and autophagy markers in experimental non-alcoholic fatty liver disease. Eur Cytokine Netw. 2019; 30:135-43. doi: 10.1684/ecn.2019.0437.
34. Kim D, Kim W, Adejumo AC, Cholankeril G, Tighe SP, Wong RJ et al. Race/ethnicity-based temporal changes in prevalence of NAFLD-related advanced fibrosis in the United States, 2005-2016. Hepatol Int. 2019;13:205-13. doi: 10.1007/s12072-018-09926-z.
35. Kim D, Yoo ER, Li AA, Tighe SP, Cholankeril G, Harrison SA, Ahmed A. Depression is associated with non-alcoholic fatty liver disease among adults in the United States. Aliment Pharmacol Ther. 2019;50:590-8. doi: 10.1111/apt. 15395.
36. Ruhl CE, Everhart JE. Fatty liver indices in the multiethnic United States National Health and Nutrition Examination Survey. Aliment Pharmacol Ther. 2015;41:65-76. doi: 10.1111/apt.13012.
37. Ahuja JK, Moshfegh AJ, Holden JM, Harris E. USDA food and nutrient databases provide the infrastructure for food and nutrition research, policy, and practice. J Nutr. 2013;143: 241s-9s. doi: 10.3945/jn.112.170043.
38. Sun Y, Sun J, Zhang P, Zhong F, Cai J, Ma A. Association of dietary fiber intake with hyperuricemia in U.S. adults. Food Funct. 2019;10:4932-40. doi: 10.1039/c8fo01917g.
39. Whelton PK, Carey RM, Aronow WS, Casey DE, Jr., Collins KJ, Dennison Himmelfarb C et al. 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. J Am Coll Cardiol. 2018;71: e127-e248. doi: 10.1016/j.jacc.2017.11.006.
40. Chen TC, Parker JD, Clark J, Shin HC, Rammon JR, Burt VL. National Health and Nutrition Examination Survey: Estimation procedures, 2011-2014. Vital Health Stat 2. 2018:1-26.
41. Hv A, Nb B, Pz C, My D, Mbs E. Association between serum folate levels and fatty liver disease - ScienceDirect. Clinical Nutrition Experimental. 2020;29:30-5.
42. Werstuck GH, Lentz SR, Dayal S, Hossain GS, Sood SK, Shi YY et al. Homocysteine-induced endoplasmic reticulum stress causes dysregulation of the cholesterol and triglyceride biosynthetic pathways. J Clin Invest. 2001;107: 1263-73. doi: 10.1172/jci11596.
43. Raza S, Tewari A, Rajak S, Sinha RA. Vitamins and non-alcoholic fatty liver disease: A Molecular Insight⋆. Liver Res. 2021;5:62-71. doi: 10.1016/j.livres.2021.03.004.
44. Sid V, Wu N, Sarna LK, Siow YL, House JD, O K. Folic acid supplementation during high-fat diet feeding restores AMPK activation via an AMP-LKB1-dependent mechanism. Am J Physiol Regul Integr Comp Physiol. 2015;309:R1215-25. doi: 10.1152/ajpregu.00260.2015.
45. Champier J, Claustrat F, Nazaret N, Fèvre Montange M, Claustrat B. Folate depletion changes gene expression of fatty acid metabolism, DNA synthesis, and circadian cycle in male mice. Nutr Res. 2012;32:124-32. doi: 10.1016/j. nutres.2011.12.012.
46. Carling D. AMPK signalling in health and disease. Curr Opin Cell Biol. 2017;45:31-7. doi: 10.1016/j.ceb.2017.01. 005.
47. Kim H, Min H. Folic acid supplementation prevents high fructose-induced non-alcoholic fatty liver disease by activating the AMPK and LKB1 signaling pathways. Nutr Res Pract. 2020;14:309-21. doi: 10.4162/nrp.2020.14.4.309.
48. Sarna LK, Wu N, Wang P, Hwang SY, Siow YL, O K. Folic acid supplementation attenuates high fat diet induced hepatic oxidative stress via regulation of NADPH oxidase. Can J Physiol Pharmacol. 2012;90:155-65. doi: 10.1139/ y11-124.
49. Federico A, Dallio M, Caprio GG, Granvina AG, Picascia D, Masarone M, Persico M, Loguercio C. Qualitative and quantitative evaluation of dietary intake in patients with non-alcoholic steatohepatitis. Nutrients. 2017;9:1074. doi: 10.3390/nu9101074.

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

| | NAFLD (total) | | *p*-value | NAFLD (men) | | *p*-value | NAFLD (women) | | *p*-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).