Choline intake in a large cohort of patients with nonalcoholic fatty liver disease[1–3]

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ABSTRACT

Background: There is significant histologic and biochemical overlap between nonalcoholic fatty liver disease (NAFLD) and steatohepatitis associated with choline deficiency.

Objective: We sought to determine whether subjects with biopsy-proven NAFLD and evidence of an inadequate intake of choline had more severe histologic features.

Design: We performed a cross-sectional analysis of 664 subjects enrolled in the multicenter, prospective Nonalcoholic Steatohepatitis Clinical Research Network (NASH CRN) with baseline data on diet composition (from a recall-based food-frequency questionnaire) within 6 mo of a liver biopsy. Food questionnaires were analyzed with proprietary software to estimate daily intakes of choline. Liver biopsies were centrally read, and consensus was scored with the NASH CRN–developed scoring system. Because choline needs vary by age, sex, and menopausal status, participants were segregated into corresponding categories (children 9–13 y old, males $\geq$14 y old, premenopausal women $\geq$19 y old, and postmenopausal women) on the basis of the Institute of Medicine's definition of adequate intake (AI) for choline. Deficient intake was defined as $<$50% AI.

Results: Postmenopausal women with deficient choline intake had worse fibrosis (P = 0.002) once factors associated with NAFLD (age, race-ethnicity, obesity, elevated triglycerides, diabetes, alcohol use, and steroid use) were considered in multiple ordinal logistic regression models. Choline intake was not identified as a contributor to disease severity in children, men, or premenopausal women.

Conclusion: Decreased choline intake is significantly associated with increased fibrosis in postmenopausal women with NAFLD. The Pioglitazone vs Vitamin E vs Placebo for Treatment of Non-Diabetic Patients With Nonalcoholic Steatohepatitis trial was registered at clinicaltrials.gov as NCT00063622, and the Treatment of Nonalcoholic Fatty Liver Disease in Children trial was registered at clinicaltrials.gov as NCT00063635. *Am J Clin Nutr* 2012; 95:892–900.

INTRODUCTION

The spectrum of NAFLD[4] includes NASH, which is an inflammatory process with accompanying hepatocellular necrosis and subsequent fibrosis. NASH can progress to cirrhosis and liver failure and is associated with an increased risk of development of hepatocellular carcinoma (1–3). Current estimates place the prevalence of NAFLD in the general US population at 1 in 3 people, whereas 1 in 20 people have NASH (2, 3). Progression to steatohepatitis is not imminent or absolute in all patients with NAFLD. A second insult may be necessary for the activation of proinflammatory pathways that results in the development of a chronic inflammatory reaction in a liver rendered vulnerable by the accumulation of fat (4–6).

In one animal model of steatohepatitis, rodents that consumed a high-fat, methionine and choline–deficient diet developed steatosis with inflammation and hepatic necrosis with the eventual development of hepatocellular carcinoma. Some strains of mice also manifest hepatic fibrosis. Hepatic fibrosis also developed in rodents that consumed a choline and B-12–deficient diet as well as a choline-deficient diet alone, although histologic changes developed more slowly. Although the liver histology of rodents on a methionine and choline–deficient diet may be similar to that with NASH, the overall phenotype of these mice is significantly different because they tend to lose weight on this diet and are not insulin resistant (7–12).

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[4] Abbreviations used: AI, adequate intake; Hb A_{1c}, hemoglobin A_{1c}; HRT, hormone replacement therapy; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NASH CRN, Nonalcoholic Steatohepatitis Clinical Research Network.

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Choline is a quaternary amine present in all mammalian tissues. Foods high in choline include dairy, liver, eggs, legumes, nuts, beef, leafy greens, seed oils, and grain germs (13). Choline is an essential component of cell membranes and is required for the synthesis of phospholipids. Choline is a precursor for the neurotransmitter acetylcholine and serves as a methyl group donor (14). Despite endogenous pathways for choline synthesis (15), humans on a low-choline diet will develop choline deficiency (16). Furthermore, humans on long-term parenteral nutrition that lacks adequate choline will show a reversal of hepatic steatosis and a decrease in serum aminotransferases with choline supplementation. The pathologic changes return with the cessation of choline supplementation (17, 18).

In addition to age- and sex-dependent differences in choline requirements (14), there is also a differential susceptibility to choline deficiency that is based on menopausal status. Premenopausal women are less likely to develop hepatic steatosis or an elevation of their aspartate aminotransferase or alanine aminotransferase than either men or postmenopausal women (19). This differential response has been hypothesized to arise from an estrogen-response element in the upstream region of the *PEMT* gene, which is an enzyme in one of the choline biosynthetic pathways (20). Genome-association studies have demonstrated susceptibility to choline deficiency with single nucleotide polymorphisms in this gene (20–22). It has also been shown that *PEMT* gene expression and enzyme activity are inducible on estrogen exposure in mouse and human hepatocytes (23).

Given the histologic overlap between choline deficiency–associated steatohepatitis and NASH, we hypothesized that individuals with a limited intake of choline may demonstrate more severe histologic changes.

SUBJECTS AND METHODS

Subjects

Subjects were enrolled in one of following 3 studies of the NASH CRN (24): *1*) the NAFLD Database (observational cohort) (25), *2*) the randomized, placebo control trial for Pioglitazone vs Vitamin E vs Placebo for Treatment of Non-Diabetic Patients With Nonalcoholic Steatohepatitis (www.clinicaltrials.gov; NCT00063622) (26, 27), and *3*) the Treatment of Nonalcoholic Fatty Liver Disease in Children (www.clinicaltrials.gov; clinical trial number NCT00063635) (28, 29). *See* Table 1 under "Supplemental data" in the online issue for a summary of patient selection and inclusion and exclusion criteria. The NASH CRN consists of 8 clinical centers and a central data coordinating center. Laboratory studies were drawn within 6 mo of screening, and biopsies were obtained within 1 y of screening. Patients enrolled with baseline data on diet composition (detailed in "Dietary intake" below) within 180 d of a liver biopsy were included. Subjects with significant alcohol consumption (>20 g/d for women and >30 g/d for men, either currently or for a period of >3 consecutive months in the 5 y before screening) or who were unable to reliably quantify alcohol intake were excluded.

Ethics

All study protocols were approved by the institutional review boards of the participating centers. All participants provided written informed consent and assent, if applicable.

Patient baseline data and histology

Baseline data regarding demographics, anthropometric measures, and laboratory data were obtained as previously described (30). Postmenopausal patients were self-identified as part of the entry questionnaire. Liver biopsies were centrally read, and consensus was scored with the NASH CRN scoring system (31). Enrollment liver biopsies from patients enrolled in the Pioglitazone vs Vitamin E vs Placebo for Treatment of Non-Diabetic Patients With Nonalcoholic Steatohepatitis and Treatment of Nonalcoholic Fatty Liver Disease in Children trials were used (pretreatment).

Dietary intake

Dietary data were collected with the Block Food Questionnaire (adults) or Brief Food Questionnaire (children) (NutritionQuest) (32–36), which are recall-based food inventories regarding eating habits over the past year. The questionnaires were completed at enrollment and analyzed with NutritionQuest proprietary software (NutritionQuest) to provide daily intakes of choline and betaine in milligrams per day. Choline and betaine contents of specific foods that were used to score the food-frequency questionnaire were obtained from the USDA Database for the Choline Content of Common Foods, Release 2 (37). An additional analysis was performed to provide daily intake of calories, carbohydrates, protein, fat, vitamin B-12, and folate.

Statistics

Participants were divided into categories corresponding to the Institute of Medicine's definition of AI for choline (**Table 1**). Deficient intake was defined as intake less than one-half of the AI (14) for each group as follows: children 9–13 y old (<188 mg/d), males ≥14 y old (<275 mg/d), adolescent females 14–18 y old (<200 mg/d), and women ≥19 y old (<212 mg/d). Children <9 y old (n = 13) and adolescent females 14–18 y old (n = 18) were excluded because of insufficient numbers. Because postmenopausal women are more likely to develop fatty liver with choline deprivation (19), adult women were subdivided into premenopausal and postmenopausal categories. For analyses, patients were classified as Hispanic (any race), white (non-Hispanic), or other. Correlations for measured variables were calculated by Spearman's rank correlation. P values were calculated by using the Kruskal-Wallis test for categorical variables and the t test for Spearman's rank correlation for measured variables. Multiple ordinal logistic regression models in which known contributors to NAFLD and NASH were controlled for were constructed to compare the calculated daily choline intake to the steatosis grade and fibrosis stage. Steatosis was categorized as <34%, 34–66%, >66% of low- to medium-power

TABLE 1
Adequate intake for choline as defined in reference 14[1]

Age	Males	Females
	mg choline/d	
9–13 y	375	375
14–18 y	550	400
≥19 y	550	425

[1] Table includes ages for subjects in the current study only.

evaluation of parenchymal involvement by steatosis, consistent with the NAFLD histology scoring system. Fibrosis was categorized into 4 groups on the basis of NAFLD histology scoring as follows: stage 0; stages 1a, 1b, and 1c combined; stage 2; and stages 3 and 4 combined. For children, age, race (white, Hispanic, or other), BMI *z* score, triglyceride concentration (split at the median), Hb A_{1c} (split at the median), daily caloric intake (split at the median), and HOMA-IR >3.5 ($mg \cdot dL^{-1} \cdot \mu U \cdot mL^{-1} \cdot 405^{-1}$) (yes compared with no) were adjusted for in regression analyses. For all other groups, age, race (white, Hispanic, or other), BMI (in kg/m^2), waist circumference (cm), triglyceride concentration (split at the median), Hb A_{1c} (split at the median), daily caloric intake (split at the median), HOMA-IR >3.5 ($mg \cdot dL^{-1} \cdot \mu U \cdot mL^{-1} \cdot 405^{-1}$) (yes compared with no), alcohol use, and steroid use were adjusted for in regression analyses. Total caloric intake was also adjusted for in both models for decreased overall intake. The proportional odds assumption for multiple ordinal logistic regression models was assessed by using the score test. Because the score test showed a violation of the assumption in one model, additional analyses were conducted by using the Brant test to assess the proportional odds for individual variables. The primary variable of interest (ie, daily choline intake) was shown to meet the assumption.

Because choline deficiency may be unmasked in a folate-sufficient/B-12–deficient state because of interactions between the pathways for choline, vitamin B-12, and folate metabolism (38, 39), reported B-12 and folate intakes calculated from the dietary questionnaire were included as a separate variable in the multiple logistic regression along with an interaction variable between the included vitamins and choline. For postmenopausal women, a second model with HRT adjusted for was performed in addition to the previously mentioned parameters.

All statistical analyses were performed with SAS for Windows (version 9.1; SAS Institute Inc) and STATA (version 10; StataCorp).

RESULTS

Baseline characteristics

Six hundred sixty-four patients were included in this study. Baseline characteristics are listed in **Table 2**. Whites composed the greatest percentage of each group (66% males ≥14 y old; 70% premenopausal women ≥19 y old; 83% postmenopausal women), except for children ≤13 y old, of whom the majority were self-classified as Hispanic (59%). The proportion of subjects in the steatosis and fibrosis categories was analyzed by race-ethnicity, and there was no difference in the distribution by using Fisher's exact test except in the fibrosis categories for males ≥14 y old. The median BMI for all adult and adolescent groups was classified as obese, whereas for children, the median BMI *z* score was >2 SDs above the mean. The median choline intake was below AI concentrations for all groups as follows: 292 mg/d for children ≤13 y old (compared with 375 mg/d AI), 308 mg/d for males ≥14 y old (compared with 550 mg/d AI), 257 mg/d for premenopausal women ≥19 y old (compared with 425 mg/d AI), and 262 mg/d for postmenopausal women

TABLE 2
Characteristics of subjects[1]

	Children 9–13 y old (*n* = 114)	Males ≥14 y old (*n* = 240)	Premenopausal women ≥19 y old (*n* = 116)	Postmenopausal women (*n* = 194)
Demographic characteristics				
Age (y)	12 (11, 13)[2]	39 (26.5, 52)	41 (32, 46)	57 (52, 61)
Race [*n* (%)]				
White	39 (34.5)	158 (66.1)	81 (69.8)	161 (83.0)
Black	1 (0.9)	2 (0.8)	1 (0.9)	6 (3.1)
Hispanic	67 (59.3)	50 (20.9)	30 (25.9)	15 (7.7)
Asian/Pacific Islander	1 (0.9)	20 (8.4)	1 (0.9)	5 (2.6)
American Indian/Alaska Native	2 (1.8)	1 (0.4)	1 (0.9)	1 (0.5)
More than one race	3 (2.7)	8 (3.4)	2 (1.7)	6 (3.1)
Laboratory/measured values				
ALT (U/L)	83 (63, 138)	77 (57, 109)	59 (37, 105)	56.5 (39, 88)
AST (U/L)	53 (39, 76)	44 (34, 64)	46 (32, 73)	47 (32, 70)
Triglycerides (mg/dL)	115 (90, 160)	152 (108, 218)	150 (97, 193)	141 (105, 201)
BMI (kg/m^2)	NA	33.4 (29.8, 36.8)	36.6 (31.3, 42.0)	33.6 (29.3, 38.4)
BMI *z* score	2.4 (2.1, 2.5)	NA	NA	NA
History of corticosteroid use [*n* (%)]	4 (3.5)	19 (7.9)	12 (10.3)	24 (12.4)
Estimated daily intakes				
Calories	1789 (1258, 2370)	1903 (1466, 2670)	1576 (1182, 2158)	1562 (1054, 2130)
Protein (g)	71.9 (50.4, 96.3)	75.8 (52.8, 98.4)	61.7 (41.3, 84.2)	58.5 (41.5, 83.4)
Carbohydrate (g)	234 (153, 314)	227 (172, 317)	193 (124, 259)	187 (134, 253)
Fat (g)	66 (43.8, 98.3)	80.4 (55.2, 118)	65.3 (49.4, 90.1)	66 (41.3, 91.8)
Choline (mg)	292 (218, 402)	308 (221, 407)	257 (189, 325)	262 (176, 373)
Vitamin B-12 (μg)	4.5 (2.9, 7.5)	5.0 (3.2, 9.0)	4.9 (2.5, 8.0)	6.4 (2.9, 9.8)
Folate (μg)	415.9 (274, 564)	420 (292, 686)	387 (260, 663)	450 (312, 777)
Any alcohol use [*n* (%)]	0 (0.0)	121 (50.4)	65 (56.0)	91 (46.9)

[1] ALT, alanine aminotransferase; AST, aspartate aminotransferase; NA, not applicable.
[2] Median; 25th, 75th percentiles in parentheses (all such values).

(compared with 425 mg/d AI). The median reported intakes of vitamin B-12 and folate were greater than the Recommended Daily Allowance for all groups. The percentage of participants who had any alcohol intake (but less than the cutoff for study eligibility, as previously described) was highest in premenopausal women ≥19 y old (56%), followed by in males ≥14 y old (50%), postmenopausal women (47%), and children ≤13 y old (0%). The daily caloric intake was lowest in postmenopausal women (1562 calories), followed by in premenopausal women (1576 calories), children (1789 calories), and males (1903 calories), with daily intakes of protein that followed a similarly ranked order as follows: postmenopausal women: 58.5 g; premenopausal women: 61.7 g; children: 71.9 g; and males: 75.8 g. Carbohydrate intake followed a different pattern, with the most carbohydrates consumed by children (234 g) followed by males (227 g), premenopausal women (193 g), and postmenopausal women (187 g). The daily fat intake was highest in males (80 g), whereas children and pre- and postmenopausal women all consumed approximately equal amounts of daily fat (~66 g).

Details of biopsy histologies of participants are shown in **Table 3**. The greatest percentage of high-grade (>66%) steatosis was seen in children (42%) compared with in only 26% of males ≥14 y old, 32% of premenopausal women ≥19 y old, and 24% of postmenopausal women. Most participants showed little lobular inflammation, but fibrosis was common; 79% of postmenopausal women had fibrosis as did 76% of children ≤13 y old, 67% of males ≥14 y old, and 70% of premenopausal women ≥19 y old. Cirrhosis was shown in 12% of postmenopausal women, whereas only 0.9% of children ≤13 y old, 5% of males ≥14 y old, and 1.7% of premenopausal women ≥19 y old had cirrhosis.

Correlation with reported choline intake

Associations of baseline data and histology with choline intake are detailed in **Tables 4** and **5**. Choline intake, which was unadjusted for other variables, was not significantly associated with the NAFLD activity score, steatosis, lobular inflammation, ballooning, or fibrosis in any group. Choline intake differed by race-ethnicity for children and males ≥14 y old. For males ≥14 y old, whites had the highest choline intake (327 mg/d) followed by subjects who were self-classified of more than one race (297 mg/d), Hispanic (277 mg/d), and other (230 mg/d) ($P = 0.001$). In children, subjects of more than one race consumed the most choline (716 mg/d), followed by Hispanic (311 mg/d), other (309 mg/d), and white (274 mg/d) races ($P = 0.03$). Choline intake for premenopausal women increased with BMI ($P = 0.008$; Spearman's rank correlation coefficient = 0.25). There was a significant correlation between choline intake and calories,

TABLE 3
Characteristics of biopsy specimens

	Children 9–13 y old (n = 114)	Males ≥14 y old (n = 240)	Premenopausal women ≥19 y old (n = 116)	Postmenopausal women (n = 194)
Steatosis grade [n (%)]				
<5%	2 (1.8)	10 (4.2)	4 (3.5)	9 (4.6)
5–33%	26 (22.8)	85 (35.4)	36 (31.0)	78 (40.2)
>33–66%	38 (33.3)	83 (34.6)	39 (33.6)	61 (31.4)
>66%	48 (42.1)	62 (25.8)	37 (31.9)	46 (23.7)
Lobular inflammation [n (%)]				
<2, <20× magnification	58 (50.9)	132 (55.0)	54 (46.6)	94 (48.5)
2–4, <20× magnification	53 (46.5)	93 (38.8)	43 (37.1)	77 (39.7)
>4, <20× magnification	3 (2.6)	15 (6.3)	19 (16.4)	23 (11.9)
Ballooning [n (%)]				
None	51 (44.7)	93 (38.8)	33 (28.5)	52 (26.8)
Few	39 (34.2)	83 (34.6)	32 (27.6)	45 (23.2)
Many	24 (21.1)	64 (26.7)	51 (44.0)	97 (50.0)
NAFLD[1] activity score [n (%)]				
1	1 (0.9)	4 (1.7)	1 (0.9)	3 (1.6)
2	7 (6.1)	32 (13.3)	12 (10.3)	23 (11.9)
3	23 (20.2)	47 (19.6)	21 (18.1)	22 (11.3)
4	29 (25.4)	56 (23.3)	16 (13.8)	41 (21.1)
5	27 (23.7)	52 (21.7)	24 (20.7)	45 (23.2)
6	18 (15.8)	28 (11.7)	17 (14.7)	36 (18.6)
7	9 (7.9)	18 (7.5)	18 (15.5)	18 (9.3)
8	0 (0.0)	3 (1.3)	7 (6.0)	6 (3.1)
Fibrosis stage [n (%)]				
0 (none)	27 (23.9)	78 (32.5)	34 (29.6)	40 (20.7)
1a (mild, zone 3, perisinusoidal)	7 (6.2)	42 (17.5)	19 (16.5)	20 (10.4)
1b (moderate, zone 3, perisinusoidal)	1 (0.9)	26 (10.8)	18 (15.7)	20 (10.4)
1c (portal/periportal only)	38 (33.6)	9 (3.8)	3 (2.6)	5 (2.6)
2 (zone 3 and periportal, any combination)	22 (19.5)	45 (18.0.8)	26 (22.6)	38 (19.7)
3 (bridging)	17 (15.0)	28 (11.7)	13 (11.3)	46 (23.8)
4 (cirrhosis)	1 (0.9)	12 (5.0)	2 (1.7)	24 (12.4)

[1] NAFLD, nonalcoholic fatty liver disease.

TABLE 4
Distribution of choline intake by selected categorical variables[1]

	Choline intake							
	Children 9–13 y old	*P*	Males ≥14 y old	*P*	Premenopausal women ≥19 y old	*P*	Postmenopausal women	*P*
	mg/d		*mg/d*		*mg/d*		*mg/d*	
Demographic characteristics								
Race		0.03		0.001		0.13		0.34
White	274 (195, 402)		327 (259, 425)		262 (207, 338)		259 (178, 373)	
Hispanic	311 (236, 386)		277 (178, 338)		214 (141, 300)		266 (146, 319)	
Other	309 (158, 454)		230 (192, 349)		213 (145, 263)		282 (178, 342)	
More than one race	716 (589, 1026)		297 (245, 430)		239 (139, 338)		482 (223, 512)	
Histology								
Steatosis (grade)		0.25		0.83		0.52		0.26
<5%	293 (214, 371)		294 (212, 495)		199 (180, 223)		361 (273, 420)	
5–33%	355 (263, 420)		314 (226, 483)		260 (188, 341)		258 (172, 344)	
>33–66%	292 (198, 406)		305 (211, 388)		243 (195, 324)		249 (178, 338)	
>66%	286 (195, 392)		307 (223, 389)		273 (174, 338)		277 (223, 396)	
Lobular inflammation		0.27		0.66		0.18		0.20
<2, <20× magnification	286 (198, 380)		308 (229, 447)		266 (207, 326)		278 (203, 404)	
2–4, <20× magnification	328 (235, 415)		308 (204, 387)		261 (174, 413)		249 (163, 347)	
>4, <20× magnification	354 (273, 577)		325 (205, 381)		206 (143, 308)		248 (175, 334)	
Ballooning		0.41		0.32		0.74		0.50
None	291 (212, 383)		313 (227, 446)		261 (206, 322)		255 (186, 341)	
Few	288 (214, 420)		309 (243, 409)		260 (191, 372)		272 (147, 347)	
Many	358 (239, 467)		299 (205, 373)		239 (172, 310)		270 (183, 393)	
NAFLD activity score		0.66		0.14		0.60		0.74
1–2	326 (244, 384)		365 (281, 519)		236 (211, 312)		260 (208, 372)	
3	291 (238, 383)		313 (227, 387)		275 (203, 357)		252 (152, 340)	
4	275 (173, 380)		283 (204, 404)		270 (201, 427)		286 (205, 404)	
5	362 (222, 469)		296 (202, 364)		241 (183, 297)		268 (163, 329)	
6	299 (228, 398)		345 (263, 410)		285 (239, 320)		268 (179, 417)	
7	273 (195, 402)		333 (285, 373)		227 (146, 338)		252 (153, 388)	
8	NA		306 (205, 389)		203 (124, 373)		250 (234, 277)	
Fibrosis stage		0.38		0.66		0.34		0.50
None	306 (182, 371)		308 (204, 448)		274 (205, 393)		260 (207, 360)	
Stage 1a, 1b, or 1c	286 (212, 406)		305 (243, 373)		276 (201, 330)		273 (178, 404)	
Zone 3 and periportal, any combination	346 (280, 502)		353 (226, 459)		231 (146, 324)		281 (208, 393)	
Bridging or cirrhosis	274 (230, 446)		306 (208, 368)		235 (179, 308)		249 (163, 357)	

[1] All values are medians; 25th, 75th percentiles in parentheses. *P* values were calculated by using the Kruskal-Wallis test for categorical variables. NA, not applicable; NAFLD, nonalcoholic fatty liver disease.

protein, fat, and carbohydrates ($P < 0.0001$ for all comparisons in all groups).

Ordinal logistic regression

The breakdown of subjects in each of the age and menopausal status groups into fibrosis and steatosis categories by reported choline-intake status is detailed in **Table 6**. These data were analyzed by using ordinal logistic regression models constructed to account for known contributors to the development of NASH/NAFLD including age, race-ethnicity, obesity (especially central obesity) hypertriglyceridemia, diabetes and insulin resistance, daily caloric intake, alcohol use, and steroid use. Results are summarized in **Table 7**. Subjects with deficient reported choline intakes did not have worse steatosis in any analysis group. The analysis of fibrosis stage showed that postmenopausal women with a reported choline intake less than one-half the defined AI had more significant fibrosis after known contributors to NASH/NAFLD were controlled for ($P = 0.002$). To further investigate the association between reported choline intakes and fibrosis stages in postmenopausal women, additional ordinal logistic regression models were constructed that contained only the reported choline intake and one other term. The model that used Hb A_{1c} had the most significant *P* value ($P = 0.008$). Other terms leading to significant *P* values were BMI ($P = 0.03$), HOMA-IR ($P = 0.03$), and waist circumference ($P = 0.03$).

Additional ordinal logistic regression models were constructed with adjustment for vitamin B-12 and folate intakes. These models included an interaction term for choline and either vitamin B-12 or folate. No significant change in any association was shown by including these additional vitamins, and none of the interaction terms reached significance. Similarly, given the association with estrogen status and susceptibility to choline deficiency, an additional model that incorporated HRT was constructed for the postmenopausal group. The number of postmenopausal women who took any kind of HRT was 27% (53 of 194 women). The association of worse fibrosis with decreased choline intake was not affected by the inclusion of this term.

TABLE 5
Correlation of measured variables with choline intake (mg/d)[1]

	Children 9–13 y old		Males ≥14 y old		Premenopausal women ≥19 y old		Postmenopausal women	
	ρ	*P*	ρ	*P*	ρ	P	ρ	*P*
ALT (U/L)	0.05	0.60	0.02	0.60	−0.01	0.60	0.08	0.60
AST (U/L)	0.03	0.75	0.02	0.78	−0.11	0.25	0.03	0.71
BMI *z* score	0.12	0.21	NA	—	NA	—	NA	—
BMI (kg/m^2)	NA	—	0.12	0.07	0.25	0.008	0.11	0.12
Total (calories/d)	0.87	<0.0001	0.86	<0.0001	0.84	<0.0001	0.87	<0.0001
Protein (g/d)	0.94	<0.0001	0.91	<0.0001	0.93	<0.0001	0.94	<0.0001
Carbohydrates (g/d)	0.78	<0.0001	0.68	<0.0001	0.70	<0.0001	0.78	<0.0001
Fat (g/d)	0.81	<0.0001	0.86	<0.0001	0.79	<0.0001	0.78	<0.0001

[1] *P* values calculated using the *t* test for Spearman's rank correlation. ALT, alanine aminotransferase; AST, aspartate aminotransferase; NA, not applicable; ρ, Spearman's rank correlation coefficient.

DISCUSSION

NASH is the major cause of cryptogenic cirrhosis (40). The process is likely multifactorial but has known associations with obesity (41–45), insulin resistance (26, 46–48), and hyperlipidemia (49–51). Given these associations, a nutritional contribution to the development of NAFLD and NASH has attracted significant attention with multiple micronutrients that showed an association (52, 53). We sought to determine whether an association exists between choline intake and histologic severity of NAFLD or NASH. Remarkably, we showed that postmenopausal women with NASH and a daily calculated choline intake less than one-half the defined AI exhibited worse fibrosis. Choline was previously thought to be nonessential; however, it is now known that healthy humans are unable to synthesize enough choline de novo to prevent deficiency (16). In addition, patients on long-term parenteral nutrition with steatohepatitis who are choline deficient show reversal of steatohepatitis with supplementation of choline (17, 18). Also, individuals with specific polymorphisms in genes involved in the choline pathway have altered susceptibility to choline deficiency (20, 54); subjects with V175M substitution in the PEMT protein, which is an enzyme in one of the choline biosynthetic pathways, show increased susceptibility to develop NAFLD, commonly without obesity (55). Of note, the promoter region of the *PEMT* gene contains a polymorphism that increased susceptibility to choline deficiency in postmenopausal women (20) who, then, were vulnerable to develop fatty liver when placed on a severely choline deficient diet (<10% AI) in clinical trials (19). The current study considered intakes <50% AI deficient. Although it did not reach statistical significance, we showed the same trend in both premenopausal women and men. These findings, coupled with the results of clinical trials, may suggest that men have additional mechanisms of upregulating genes in the choline synthesis pathway that are able to compensate for moderate deficiencies but not severe deficiencies. Other genes, including *PNPLA3*, also appear to play an important role in the development of NAFLD and have been shown to be associated with both increased hepatic fat amounts and fibrosis (56, 57).

Although we expected to see an increase in steatosis with decreased choline intakes, the relative lack of steatosis suggested that other contributing factors outweigh choline deficiency for deposition of fat, the progression to fibrosis is faster in a choline-

TABLE 6
Steatosis grade and fibrosis stage by reported dietary choline intake in different age and menopausal status groups[1]

		Steatosis grade				Fibrosis stage					
Choline intake	*n*	<34%	34–66%	>66%	*P*	*n*[2]	0	1a, 1b, or 1c	2	3 or 4	*P*
		n (%)					*n* (%)				
Children 9–13 y old					0.05						0.44
<188 mg/d	20	1 (5.0)	9 (45.0)	10 (50.0)		20	7 (35.0)	9 (45.0)	2 (10.0)	2 (10.0)	
≥188 mg/d	94	27 (28.7)	29 (30.9)	38 (40.4)		93	20 (21.5)	37 (39.8)	20 (21.5)	16 (17.2)	
Males ≥14 y old					0.74						0.92
<275 mg/d	87	35 (40.2)	32 (36.8)	20 (23.0)		87	29 (33.3)	27 (31.0)	15 (17.2)	16 (18.4)	
≥275 mg/d	153	60 (39.2)	51 (33.3)	42 (27.5)		153	49 (32.0)	50 (32.7)	30 (19.6)	24 (15.7)	
Premenopausal women ≥19 y old					0.94						0.49
<212 mg/d	39	13 (33.3)	14 (35.9)	12 (30.8)		38	9 (23.7)	12 (31.6)	10 (26.3)	7 (18.4)	
≥212 mg/d	77	27 (35.1)	25 (32.5)	25 (32.5)		77	25 (32.5)	28 (36.4)	16 (20.8)	8 (10.4)	
Postmenopausal women					0.25						0.17
<212 mg/d	63	28 (44.4)	24 (38.1)	11 (17.5)		63	11 (17.5)	12 (19.1)	10 (15.9)	30 (47.6)	
≥212 mg/d	131	59 (45.0)	37 (28.2)	35 (26.7)		130	29 (22.3)	33 (25.4)	28 (21.5)	40 (30.8)	

[1] *P* values were calculated by using the Fisher's exact test.
[2] Three subjects did not have a fibrosis score recorded.

TABLE 7
Relation between dietary choline and levels of steatosis and fibrosis[1]

	Steatosis[2]		Fibrosis[3]	
	Values	*P*	Values	*P*
Children 9–13 y old[4]	1.20 (0.37, 3.90)	0.76	0.64 (0.20, 2.04)	0.45
Males ≥14 y old[5]	0.68 (0.33, 1.38)	0.28	1.89 (0.94, 3.79)	0.07
Premenopausal women ≥19 y old[5]	1.57 (0.61, 4.06)	0.35	2.55 (1.00, 6.48)	0.05
Postmenopausal women[5]	0.88 (0.42, 1.86)	0.74	3.37 (1.58, 7.19)	0.002

[1] All values are cumulative ORs; 95% CIs in parentheses. The cumulative OR of worse steatosis or fibrosis associated with a deficient daily choline intake (less than one-half the defined ADI) was assessed by using an ordinal logistic regression model with known contributors to NAFLD and NASH controlled for. ORs and *P* values were derived from multiple ordinal logistic regression models. Dietary choline was analyzed as a dichotomous variable, whereby choline values less than the deficient intake concentration were compared with values greater than or equal to deficient intake. Values of deficient choline intake are specific to each group. ADI, adequate dietary intake; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis.

[2] Defined as <34%, 34–66%, and >66%.

[3] Defined as stage 0; stages 1a, 1b, and 1c combined; stage 2; and stages 3 and 4 combined.

[4] Age, race (white, Hispanic, or other), BMI *z* score, triglyceride concentration, hemoglobin A_{1c}, and daily caloric intake split at the median, and HOMA-IR >3.5 ($mg \cdot dL^{-1} \cdot \mu U \cdot mL^{-1} \cdot 405^{-1}$) (yes compared with no) were controlled for.

[5] Age, race (white, Hispanic, or other), BMI (kg/m^2), waist circumference (cm), triglyceride concentration, hemoglobin A_{1c} and daily caloric intake split at the median, HOMA-IR >3.5 ($mg \cdot dL^{-1} \cdot \mu U \cdot mL^{-1} \cdot 405^{-1}$) (yes compared with no), alcohol use (yes compared with no), and steroid use (yes compared with no) were controlled for.

deficient state (and, thus, the stage that shows increased steatosis is missed by the biopsy protocol used in these studies), or dietary choline deficiency only plays a role in advanced stages of liver disease accompanied by an impaired ability to synthesize choline, because cirrhotic patients have decreased ability to synthesize choline de novo (58). A fourth possibility is that patients enrolled in the studies already had histologic evidence of NAFLD or NASH, or, at the very least, a strong suspicion of either. In this population, the disease stage at which steatosis is hypothesized to be hastened by choline deficiency may have already passed, or alternatively, some threshold level of choline deficiency may cause steatosis, but more severe deficiency may not cause additional increases in steatosis.

Our study was limited by its cross-sectional nature. We obtained a dietary history only at enrollment in the study, and dietary choline intake was not validated by plasma concentrations. The dietary history was recall based and required participants to select from a predefined list of foods with an estimated frequency of intake. The recall-based nature may have led to an underestimation of intakes. For example, the total caloric intake for adults in the study averaged <2000 calories/d, whereas BMI was >33. Furthermore, the preprinted list of foods may not have captured the dietary subtleties of ethnic diets of some subjects. Last, we did not genotypes these subjects. The variation in genes known to affect the choline synthesis pathway may more specifically identify individuals susceptible to choline deficiency or for whom choline supplementation may have a beneficial effect (20–22, 54–57).

A previous study in 47 patients did not find a correlation between plasma choline concentration and the severity of liver damage in NASH (59). Because NASH is the presumed end result of multiple pathways with behavioral, environmental, and genetic contributions, choline deficiency may play a role in only a subset of patients with NAFLD or NASH. Pure choline deficiency may only become apparent in an appropriately selected phenotypic subset of NASH patients, such as in postmenopausal women who may be unable to upregulate genes in the choline synthesis pathway that have estrogen-response elements in their promoters (23).

In conclusion, decreased choline intake is associated with worse fibrosis in a subset of patients with NASH. Additional study is needed to determine whether a reported low choline intake is associated with low plasma choline concentrations and if low choline concentrations are associated with the initiation or progression of NAFLD or NASH.

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