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Higher Dietary Choline Intake Is Associated with Lower Risk of Nonalcoholic Fatty Liver in Normal-Weight Chinese Women[1,2]

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Abstract

Background: Choline deficiency has been shown to induce liver fat accumulation in both rodent and human studies. However, it is unclear whether dietary choline intake is related to fatty liver in the general population.

Objective: We examined the association between choline intake and nonalcoholic fatty liver.

Methods: Participants included 56,195 Chinese women and men, 40–75 y of age, with no or negligible alcohol consumption and with no history of hepatitis, cardiovascular disease, or cancer. All participants reported undergoing liver ultrasonography. Fatty liver was defined by self-report of a physician diagnosis. Habitual dietary intakes were assessed via validated food-frequency questionnaires.

Results: The average total choline intakes were 289 ± 85 mg/d in women and 318 ± 92 mg/d in men. Major food sources were eggs, soy foods, red meat, fish, and vegetables. A higher choline intake was associated with lower risk of fatty liver; after adjustment for sociodemographic characteristics, lifestyle factors, and other dietary intakes, the ORs (95% CIs) for the highest vs. the lowest quintiles of choline intake were 0.68 (0.59, 0.79) in women and 0.75 (0.60, 0.93) in men (both P-trend < 0.01). The inverse association was attenuated after further adjustment for history of metabolic disease and, in particular, BMI. The corresponding ORs (95% CIs) were 0.88 (0.75, 1.03) in women (P-trend = 0.05) and 0.85 (0.68, 1.06) in men (P-trend = 0.09). Stratified analyses suggested a potential effect modification by obesity status in women; the OR (95% CI) across extreme quintiles was 0.72 (0.57, 0.91) in normal-weight women vs. 1.05 (0.84, 1.31) in overweight or obese women (P-trend = 0.007 vs. 0.99, P-interaction < 0.0001).

Conclusion: Higher dietary choline intake may be associated with lower risk of nonalcoholic fatty liver only in normal-weight Chinese women. *J Nutr* 2014;144:2034–40.

Keywords: choline, diet, nonalcoholic fatty liver, obesity, population-based study

Introduction

Ectopic fat deposition in the liver is a manifestation of nonalcoholic fatty liver disease (NAFLD)[5], which is closely associated with insulin resistance and dyslipidemia and is a strong risk factor for cardiovascular disease, some cancers, and liver-related morbidity and mortality (1–3). As the most common chronic liver disease in developed countries, NAFLD affects 20–30% of the general population, 50–70% of diabetic patients, and ~90% of morbidly obese individuals (4). In developing countries, NAFLD has increased rapidly in parallel with the epidemic of obesity and metabolic syndrome (5). By using biennial ultrasonography data, Fan (6) reported an increasing prevalence of fatty liver from 4% to 14% in just 6 y from 1995 to 2000 in Shanghai, China. Obesity and the associated metabolic syndrome are well-established risk factors for fatty liver. Weight loss via exercise and/or diet modification has been shown to reduce hepatic steatosis (7). Few epidemiologic studies have directly examined the potential role of diet in the development of fatty liver. Several dietary factors, including intakes of saturated fat, polyunsaturated fat, fructose, protein, and choline, have been linked to fatty liver, although the evidence is limited and inconsistent (8).

Choline is necessary to maintain normal liver function. It is involved in hepatic secretion of VLDL cholesterol, host–gut microbiota interactions, and one-carbon metabolism, all of which underlie the pathogenesis of NAFLD (9–12). Choline is an

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[5] Abbreviations used: NAFLD, nonalcoholic fatty liver disease; SMHS, Shanghai Men's Health Study; SWHS, Shanghai Women's Health Study.
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essential nutrient because its endogenous biosynthesis is generally not sufficient to meet human requirements, especially for men and postmenopausal women (13). A choline-deficient diet (e.g., <50 mg/d) could lead to accumulated liver fat and elevated liver enzymes in healthy adults in a few days to a few weeks (14). Choline deficiency has been used to induce fatty liver in experimental studies, but very few epidemiologic studies examined habitual choline intake in relation to the risk of fatty liver. One cross-sectional study in 664 patients with NAFLD reported that choline intake was associated with the progression of steatosis to fibrosis in postmenopausal women, but not the degree of steatosis (15).

In the Shanghai Women's Health Study (SWHS) and the Shanghai Men's Health Study (SMHS), we estimated the association between dietary choline intake and self-reported physician diagnosis of fatty liver in 56,195 middle-aged and older Chinese adults who drank little or no alcohol; who had no history of hepatitis, cardiovascular disease, or cancer; and who had undergone liver ultrasound examinations.

Methods

Study population. The SWHS and SMHS are 2 population-based prospective cohort studies conducted in urban communities of Shanghai, China. Detailed study designs and methods were reported previously (16, 17). Briefly, we recruited 74,941 women (aged 40–70 y) during 1997–2000 with a participation rate of 92.7% and 61,482 men (aged 40–75 y) during 2002–2006 with a participation rate of 74.1%. All participants completed an in-person baseline survey conducted by trained interviewers. Information on sociodemographic characteristics, diet and lifestyle habits, and medical history and anthropometric measurements were collected according to standardized protocols. All participants provided written informed consent, and the study protocol was approved by the institutional review boards of Vanderbilt University and the Shanghai Cancer Institute.

Participants were followed up by home visits every 2–3 y and annual records linkage to the Shanghai Vital Statistics Registry. Overall follow-up rates by the end of 2011 were 92.3% in the SWHS and 93.8% in the SMHS. During each follow-up, we updated medical history and measured body weight. Questions on fatty liver and ultrasound examination were added at the third follow-up of the SWHS (2004–2007; n = 69,582) and the second follow-up of the SMHS (2008–2011; n = 55,187), referred to hereafter as the "fatty liver survey." Participants were asked the following questions: "Have you ever been diagnosed with fatty liver by a physician?"; "If yes, when was your first diagnosis?"; "Have you ever had an ultrasound examination of the liver?"; "If yes, when was your most recent ultrasound examination?" To minimize misclassification from self-reported diagnosis, we included only participants who had at least 1 liver ultrasonography in the present analysis (46,120 women and 31,122 men). Participants with an ultrasound had higher socioeconomic status and higher prevalence of metabolic diseases than did those without an ultrasound, but their choline intake and other dietary intakes were similar.

Outcome assessment. For the present study, the presence of nonalcoholic fatty liver was considered if participants reported *1*) diagnosis of fatty liver by a physician during follow-up, *2*) had at least 1 ultrasound examination of the liver during follow-up, and *3*) had no or negligible alcohol consumption (≤1 drink/d for women and ≤2 drinks/d for men; 1 drink = 14 g ethanol) at baseline. These alcohol consumption thresholds were based on the *Dietary Guidelines for Americans* (18), because there is a paucity of data supporting specific cutoffs for defining excessive alcohol consumption in the assessment of NAFLD (19).

Dietary intake assessment. Habitual dietary intakes were assessed by using validated FFQs at baseline in a face-to-face interview. The FFQs used in the SWHS and SMHS contained similar food items, which covered ~80 commonly consumed foods in Shanghai. Both FFQs were validated in comparison with 24-h dietary recalls recorded once or twice a month for 1 y (17, 20). The correlation coefficients were 0.59–0.66 for macronutrients and 0.41–0.66 for major food groups in the SWHS and 0.38–0.64 for macronutrients and 0.41–0.72 for major food groups in the SMHS.

Daily intakes of energy and nutrients, except for choline, were calculated on the basis of the Chinese Food Composition Table, 2002 (21). Food choline contents were obtained from the 2008 USDA Database for the Choline Content of Common Foods, release 2 (22). We further searched the USDA National Nutrient Database for Standard Reference, release 25, as a complementary database (23). For a few food items (e.g., rice) choline value was only available in cooked form, so we used a factor to adjust different water content in raw vs. cooked foods. We also calculated dietary betaine and methionine intakes by using the USDA database. The validity of using the USDA database in our FFQs was shown in a previous study of SWHS, which indicated that the correlation coefficients were >0.90 for several vitamin intakes between Chinese and U.S. food databases (24).

Habitual alcohol intake was also assessed at baseline. Participants were asked if they had ever consumed alcohol on a regular basis (≥3 times/wk for ≥6 consecutive months). Those who answered "yes" were further asked about the age when they started or quit (former drinkers) drinking regularly. For current regular drinkers, detailed information was collected on the frequency of drinking (times per week, on average, in the past 12 mo), type of alcoholic beverages (rice wine, beer, spirits, or grape wine), and amount of consumption each time. Alcohol intake in grams per day was estimated by summing the amount consumed per day multiplied by the average ethanol content of each type of alcoholic beverage. Moderate alcohol consumption was defined as ≤14 g ethanol (1 drink)/d for women and ≤28 g ethanol (2 drinks)/d for men, and heavy consumption was defined as >14 g ethanol/d for women and >28 g ethanol/d for men.

Statistical analysis. For the present analysis, we excluded participants who had existing hepatitis, cardiovascular disease, or cancer at the time of the fatty liver survey (7070 women and 7914 men). We also excluded participants with extreme energy intakes (<500 or >3500 kcal/d for women, n = 53; <800 or >4200 kcal/d for men, n = 57), heavy alcohol consumption (139 women and 2696 men), and diagnosis of fatty liver at or before baseline (1426 women and 1677 men). The final analysis included 37,432 women and 18,763 men.

Dietary intakes were adjusted for total energy by using the residual method (25). Participants' characteristics were compared by ANOVA for continuous variables and by using χ^2 tests for categorical variables. ORs and 95% CIs were estimated by using multivariate logistic regression. Several models were applied in the analyses. Model 1 was adjusted for age and covariates collected at baseline, including education (4 levels), income (4 levels), physical activity (quartiles of metabolic equivalents score; h/wk) (26), cigarette smoking (women: never or ever; men: never; past; or current: 1–10, 11–20, or >20 cigarettes/d), alcohol drinking (never, former, or current moderate consumption), and intakes of total energy, protein, saturated fat, and polyunsaturated fat. Model 2 was further adjusted for covariates updated until the fatty liver survey, including menopausal status (in women only); presence of hypertension, diabetes, gallstones, and dyslipidemia; and BMI (continuous). P-trend was obtained by treating the median value of each quintile as a continuous variable. Stratified analyses were conducted according to potential risk factors for fatty liver, especially by obese status [BMI (kg/m^2) of <25 or ≥25] and alcohol drinking status (no or light alcohol consumption). Effect modification was examined via likelihood ratio test by comparing the model with and without the interaction term (choline intake quintile × stratified variable). A 2-sided $P < 0.05$ was considered significant. All analyses were conducted by using SAS 9.3 (SAS Institute).

Results

Mean ± SD intakes of total choline were 289 ± 85 mg/d in women and 318 ± 92 mg/d in men. Approximately 60% of choline was

phosphatidylcholine, with mean intakes of 164 ± 60 mg/d in women and 177 ± 63 mg/d in men. The top 5 food sources of choline were eggs (22%), soy foods (20%), red meat (14%), fish and shellfish (12%), and vegetables (11%) in both sexes.

Table 1 shows the age-adjusted characteristics by quintiles of total choline intake. In both cohorts, subjects with a higher choline intake had higher education and income, exercised more, smoked less, and had higher intakes of protein, saturated fat, and polyunsaturated fat. At the time of the fatty liver survey, women who consumed more choline had a lower BMI and lower prevalence of hypertension, dyslipidemia, and gallstones, but a higher prevalence of diabetes. In men, total choline intake was not associated with BMI but was inversely associated with hypertension and gallstones and positively associated with dyslipidemia and diabetes.

TABLE 1 Age-adjusted characteristics by total choline intake in the Shanghai Women's and Men's Health Studies[1]

	Quintile of total choline intake				
Characteristics	1	2	3	4	5
Women (n = 37,432)					
Choline intake, mg/d	179	242	284	327	412
Age, y	54.0	51.6	50.8	50.5	50.1
High education,[2] %	10.0	16.6	18.5	20.6	20.6
High income,[3] %	14.8	19.7	21.6	22.5	24.1
Exercise regularly, %	31.1	32.6	33.9	37.4	39.3
Cigarette smoking, %	3.3	2.3	1.7	1.4	1.9
Alcohol consumption ≤1 drink/d, %	1.4	1.4	1.8	1.8	2.7
Total energy intake, kcal/d	1647	1695	1713	1705	1636
Protein intake, g/d	54	61	66	70	79
Saturated fat intake, g/d	6	7	9	9	11
Polyunsaturated fat intake, g/d	5	7	7	8	10
Postmenopausal, %	81.1	75.6	72.9	71.6	68.6
Hypertension, %	34.6	31.8	28.5	27.1	26.6
Dyslipidemia, %	16.8	16.1	15.8	16.0	14.6
Gallstones, %	28.9	21.6	19.5	17.6	16.2
Diabetes, %	6.3	5.4	5.1	5.3	7.8
BMI, kg/m^2	24.2	23.9	23.7	23.5	23.6
Men (n = 18,763)					
Choline intake, mg/d	199	266	312	361	452
Age, y	53.4	53.5	53.7	53.9	53.8
High education,[2] %	21.1	28.2	33.8	36.9	40.2
High income,[3] %	8.3	11.2	13.0	15.0	19.1
Exercise regularly, %	27.4	32.2	35.8	38.2	42.1
Cigarette smoking, %					
Never	31.6	33.9	35.9	36.6	36.4
Past	8.5	8.9	9.0	9.3	9.5
Current	59.9	57.3	55.2	54.0	54.2
Alcohol consumption ≤2 drinks/d, %	15.1	19.2	22.9	26.9	34.9
Total energy intake, kcal/d	1918	1944	1959	1930	1923
Protein intake, g/d	63	71	76	81	92
Saturated fat intake, g/d	7	9	10	11	13
Polyunsaturated fat intake, g/d	6	7	8	9	11
Hypertension, %	30.1	29.3	30.6	28.8	28.0
Dyslipidemia, %	12.6	12.5	13.9	14.7	14.2
Gallstones, %	13.5	13.6	12.3	10.8	9.8
Diabetes, %	5.8	6.2	6.1	8.3	9.7
BMI, kg/m^2	23.9	23.7	23.7	23.7	23.8

[1] Values are age-adjusted means or percentages. Information on sociodemographic characteristics, lifestyle factors, and habitual diets was collected at baseline. Information on diagnoses of chronic diseases, BMI, and postmenopausal status (in women only) was updated in follow-up visits.

[2] High education was defined as professional education, college, or higher.

[3] High income was defined as household income ≥30,000 yuan/y for women and a personal income ≥24,000 yuan/y for men.

Among participants who had ultrasound examinations, 14.7% women and 16.2% men reported being diagnosed with fatty liver by a physician after the baseline survey. Obesity was the strongest risk factor for fatty liver in both sexes. Compared with normal-weight subjects, the odds were ~3-fold in overweight adults and 5-fold in obese adults (**Table 2**). After mutual adjustment, higher education, postmenopausal status, and presence of hypertension, diabetes, gallstones, and dyslipidemia were associated with higher risks of fatty liver. In contrast, higher physical activity was associated with a lower risk. The consumption of alcohol, protein, saturated fat, and mono-/polyunsaturated fat showed null associations (data not shown).

After adjustment for sociodemographic, lifestyle, and dietary factors (**Table 3**; model 1), women in the highest quintile of choline intake had a 32% lower risk of fatty liver than those in the lowest quintile (95% CI: 21%, 41%). Similarly, men in the highest quintile showed a 25% lower risk (95% CI: 7%, 40%) (both P-trend < 0.0001). However, after further adjustment for metabolic disease status and BMI (model 2), the associations were attenuated, with ORs (95% CIs) comparing the extreme quintiles of 0.88 (0.75, 1.03) in women and 0.85 (0.68, 1.06) in men (P-trend = 0.05 and 0.09, respectively). Betaine and methionine intakes were not associated with risk of fatty liver, and adjustment for them did not influence the association between choline intake and fatty liver.

Stratified analyses (Table 3) indicated that choline intake was associated with fatty liver in normal-weight women but not in overweight or obese women (P-interaction < 0.0001). However, such an effect modification was not observed in men. No effect modification by alcohol consumption was observed for either men or women.

In the SMHS, the choline–fatty liver association seemed more evident in men with saturated fat intakes of less than median (9.7 g/d) than in men with a higher saturated fat intake (ORs across extreme quintiles: 0.68 vs. 0.82), but the interaction test was not significant. No significant interactions were found for age, education, physical activity, protein intake, and chronic disease status in either of the sexes and menopausal status in women (data not shown).

Discussion

To the best of our knowledge, this is the first report on habitual dietary choline intake in relation to NAFLD in a large population–based study. Among ~56,000 Chinese adults, we found that higher choline intake, mainly from eggs and soy foods, was associated with lower risk of fatty liver independent of socioeconomic status, lifestyle factors, and related nutrient intakes. However, the association was attenuated after adjustment for other metabolic diseases and BMI and remained significant only in normal-weight women.

Choline was recognized as an essential nutrient in 1998, with recommended intakes of 425 mg/d for women and 550 mg/d for men (13). However, little was known about dietary choline intake in general populations until the food composition data became available (21). The average total choline intake in the present study population was 290–320 mg/d, which is comparable to intakes in previous reports from Western or Chinese populations: 313 mg/d in the Framingham Offspring Study (27), 290–330 mg/d in the Atherosclerosis Risk in Communities Study

TABLE 2 Risk of nonalcoholic fatty liver by sociodemographic characteristics, lifestyle factors, and presence of metabolic diseases in the Shanghai Women's and Men's Health Studies[1]

	Cases/participants (*n*/*n*)	OR (95% CI)	*P*
Women	5501/37,432		
Education			<0.0001
Elementary school or less	639/6073	1 (ref)	
Middle school	1976/13,266	1.93 (1.71, 2.18)	
High school	1756/11,637	2.24 (1.98, 2.53)	
Professional education or college or higher	1130/6456	2.62 (2.30, 2.99)	
Household income (yuan/y)			<0.0001
<10,000	575/4910	1 (ref)	
10,000–19,999	1866/13,371	1.13 (1.01, 1.26)	
20,000–29,999	1753/11,476	1.26 (1.12, 1.41)	
≥30,000	1307/7675	1.29 (1.15, 1.46)	
Smoking			0.01
Never	5416/36,644	1 (ref)	
Ever	85/788	0.73 (0.57, 0.94)	
Physical activity (median MET-h/wk)			0.03
Quartile 1: 58.8	1514/9358	1 (ref)	
Quartile 2: 86.4	1354/9345	0.87 (0.80, 0.95)	
Quartile 3: 112.3	1325/9374	0.88 (0.81, 0.96)	
Quartile 4: 155.8	1308/9355	0.88 (0.80, 0.96)	
Postmenopausal			<0.0001
No	1092/9750	1 (ref)	
Yes	4409/27,682	1.56 (1.43, 1.71)	
Hypertension			<0.0001
No	3131/26,317	1 (ref)	
Yes	2370/11,115	1.31 (1.23, 1.40)	
Diabetes			<0.0001
No	5010/35,197	1 (ref)	
Yes	491/2235	1.37 (1.21, 1.54)	
Gallstones			<0.0001
No	3935/29,670	1 (ref)	
Yes	1566/7762	1.46 (1.36, 1.57)	
Dyslipidemia			<0.0001
No	3394/31,499	1 (ref)	
Yes	2107/5933	3.82 (3.57, 4.10)	
BMI (kg/m^2)			<0.0001
<25.0; median 22.3	2371/25,609	1 (ref)	
≥25.0–29.9; median 26.6	2635/10,400	3.32 (3.11, 3.55)	
≥30.0; median 31.4	495/1423	5.43 (4.78, 6.18)	
Per 1-kg/m^2 increase	—	1.25 (1.24, 1.27)	<0.0001
Men	3036/18,763		
Education			<0.0001
Elementary school or less	42/637	1 (ref)	
Middle school	847/5274	2.42 (1.72, 3.40)	
High school	1123/6838	2.48 (1.76, 3.50)	
Professional education or college or higher	1024/6014	2.88 (2.04, 4.06)	
Personal income (yuan/y)			0.15
<6000	285/1759	1 (ref)	
6,000–11,999	1101/7041	0.99 (0.85, 1.15)	
12,000–23,999	1237/7463	0.97 (0.83, 1.14)	
≥24,000	413/2500	0.88 (0.73, 1.06)	
Smoking			0.36
Never	1033/6543	1 (ref)	
Former	262/1699	0.99 (0.84, 1.16)	
Current (cigarettes/d)			
≤10	749/4393	1.04 (0.93, 1.17)	
≤11–20	844/5331	0.91 (0.81, 1.02)	
>20	148/803	1.00 (0.81, 1.23)	

(Continued)

TABLE 2 *Continued*

	Cases/participants (*n*/*n*)	OR (95% CI)	*P*
Physical activity (median MET-h/wk)			0.23
Quartile 1: 23.3	866/4690	1 (ref)	
Quartile 2: 42.5	753/4690	0.92 (0.82, 1.03)	
Quartile 3: 62.6	742/4692	0.96 (0.85, 1.07)	
Quartile 4: 94.3	675/4691	0.91 (0.80, 1.03)	
Hypertension			<0.0001
No	1862/13,253	1 (ref)	
Yes	1174/5510	1.27 (1.16, 1.39)	
Diabetes			0.20
No	2785/17,409	1 (ref)	
Yes	251/1354	1.12 (0.96, 1.31)	
Gallstones			<0.0001
No	2592/16,512	1 (ref)	
Yes	444/2251	1.29 (1.14, 1.45)	
Dyslipidemia			<0.0001
No	2223/16,216	1 (ref)	
Yes	813/2547	2.36 (2.13, 2.61)	
BMI (kg/m^2)			<0.0001
<25.0; median 22.6	1371/12,752	1 (ref)	
≥25.0–29.9; median 26.4	1512/5640	2.77 (2.55, 3.01)	
≥30.0; median 31.0	153/371	5.36 (4.28, 6.72)	
Per 1-kg/m^2 increase	—	1.28 (1.26, 1.30)	<0.0001

[1] MET-h, metabolic equivalent task hours; ref, reference.
[2] Adjusted for age, education, income, smoking, alcohol drinking, physical activity, postmenopausal status (in women only), hypertension, diabetes, gallstones, dyslipidemia, and BMI.

(28), and 325 mg/d in a Taiwan survey (29). Although the amount of choline consumption was similar, the food sources appeared to differ in Western and Chinese populations. The top contributors of total choline in U.S. populations were red meat (14–22%), poultry (~13%), milk (10–18%), and eggs (8–11%) (27, 28, 30). In Shanghai and Taiwan, eggs contributed 22–25% of total choline (29). Notably, 20% of choline was from soy foods and 11% was from vegetables in our cohorts. More plant-based food sources of choline reflect a prudent dietary pattern in our population, which may be important when interpreting current findings.

Choline exerts several biological functions that may protect against liver fat accumulation. First, as a predominant component of phospholipids in VLDL cholesterol, choline, especially phosphatidylcholine, is necessary for exporting TGs from the liver (10). Reduced availability of choline, because of either low dietary intake or impaired endogenous biosynthesis, could increase intrahepatic lipids. In animal models, choline-deficient diets and deletion of choline biosynthetic genes were used as dietary and genetic approaches to induce fatty liver (11, 31, 32). In human studies, single nucleotide polymorphisms in the genes of choline biosynthesis were linked to higher susceptibility to fatty liver (33, 34). Second, choline is a methyl-donor nutrient involved in one-carbon metabolism and epigenetic regulation (12). The Framingham Offspring Study and Nurses' Health Study both found that lower choline and betaine intakes were associated with higher plasma total homocysteine (27, 30). Genetic defects in choline and one-carbon metabolism were associated with more severe hepatic steatosis (12, 35). Third, accumulated evidence has highlighted the importance of gut microbiota and their interactions with choline metabolism in liver diseases (9, 36, 37). Gut bacteria can degrade dietary choline and thereby reduce choline bioavailability to the host (38) and may also trigger proinflammatory pathways via producing trimethylamines in the liver (39). Conversely, gut microbiota could be altered by changes in choline intake and other dietary factors. For example, studies suggested that high dietary fat intake may induce gut bacterial dysbiosis (37). We speculate that healthier gut microbiota associated with a lower fat intake might be 1 possible explanation for the relatively stronger association of choline with fatty liver observed in men with lower saturated fat intakes in our stratified analysis. The inter- and intraindividual variations in bacteria composition after a choline challenge, together with the host's genotypes related to choline biosynthesis, predicted the risk of developing fatty liver under choline deficiency (40). As we observed, the favorable association between choline and fatty liver seemed more evident in men with low saturated fat intakes than in men with high intakes, which might indicate the influence of varied gut microbiota in the choline–fatty liver association.

We also observed that choline intake was inversely associated with fatty liver in normal-weight but not in overweight or obese women. Liver fat content is a balance between lipids-in (FA uptake from circulation and de novo lipogenesis) and lipids-out (FA oxidation and VLDL secretion) (3). It is possible that obesity-related metabolic disorders, such as elevated adipose tissue lipolysis and insulin resistance, significantly increase the abundance of hepatic lipids, which cannot be offset by lipid oxidation and secretion enhanced by choline (41). Also, obesity-related gut microbiota may metabolize dietary choline into proinflammatory molecules (9, 37). Therefore, the inverse choline–fatty liver association found in the present study may not be generalizable to populations having Western dietary patterns and a high obesity prevalence.

We acknowledge several limitations. First, we did not ask questions about fatty liver diagnosis at baseline. Although the information on fatty liver was collected after the dietary survey and participants with fatty liver diagnosed at or before baseline

TABLE 3 Risk of nonalcoholic fatty liver by total choline intake in the Shanghai Women's and Men's Health Studies[1]

	Quintile of intake					
	1	2	3	4	5	*P*-trend
Women						
All women (*n* = 37,432)						
Median intake, mg/d	185	242	284	326	394	
Cases, *n*	1109	1192	1087	1088	1025	
Model 1	1 (ref)	0.96 (0.87, 1.06)	0.82 (0.73, 0.91)	0.78 (0.69, 0.88)	0.68 (0.59, 0.79)	<0.0001
Model 2	1 (ref)	1.06 (0.96, 1.18)	0.96 (0.86, 1.08)	0.96 (0.84, 1.09)	0.88 (0.75, 1.03)	0.05
Normal weight (*n* = 25,609)	1 (ref)	0.94 (0.81, 1.09)	0.80 (0.67, 0.94)	0.85 (0.70, 1.02)	0.72 (0.57, 0.91)	0.007
Overweight or obese (*n* = 11,823)	1 (ref)	1.16 (1.01, 1.34)	1.12 (0.95, 1.31)	1.07 (0.89, 1.28)	1.05 (0.84, 1.31)	0.99
No alcohol consumption (*n* = 36,747)	1 (ref)	1.07 (0.96, 1.19)	0.96 (0.85, 1.08)	0.96 (0.84, 1.09)	0.89 (0.75, 1.04)	0.06
Light alcohol consumption (*n* = 685)	1 (ref)	0.67 (0.23, 1.96)	1.21 (0.46, 3.16)	1.03 (0.37, 2.88)	0.57 (0.17, 1.91)	0.45
Men						
All men (*n* = 18,763)						
Median intake, mg/d	205	266	312	360	435	
Cases, *n*	582	613	609	606	626	
Model 1	1 (ref)	0.95 (0.84, 1.09)	0.88 (0.76, 1.02)	0.82 (0.70, 0.97)	0.75 (0.60, 0.93)	0.004
Model 2	1 (ref)	1.04 (0.90, 1.19)	0.96 (0.82, 1.12)	0.91 (0.77, 1.09)	0.85 (0.68, 1.06)	0.09
Normal weight (*n* = 12,752)	1 (ref)	1.01 (0.83, 1.23)	0.91 (0.73, 1.14)	0.92 (0.71, 1.18)	0.82 (0.59, 1.13)	0.19
Overweight or obese (*n* = 6010)	1 (ref)	1.05 (0.86, 1.28)	1.00 (0.81, 1.25)	0.91 (0.71, 1.17)	0.90 (0.66, 1.24)	0.36
No alcohol consumption (*n* = 14,295)	1 (ref)	1.10 (0.94, 1.29)	1.02 (0.85, 1.22)	0.95 (0.77, 1.16)	0.87 (0.67, 1.14)	0.17
Light alcohol consumption (*n* = 4467)	1 (ref)	0.76 (0.54, 1.06)	0.71 (0.50, 0.99)	0.74 (0.52, 1.06)	0.71 (0.46, 1.10)	0.24

[1] All values are ORs (95% CIs) unless indicated otherwise. Model 1 adjusted for age, total energy intake, education, income, physical activity, smoking, alcohol consumption, and dietary intakes of protein, saturated fat, and polyunsaturated fat. Model 2 further adjusted for menopause (in women only), history of hypertension, diabetes, gallstones, or dyslipidemia, and BMI. All categories are adjusted as per model 2 unless indicated otherwise. Normal weight was defined as BMI <25 kg/m^2, overweight/obese was defined as BMI ≥25 kg/m^2. *P*-interaction between choline quintile and obesity status was < 0.0001 in women and 0.70 in men. Light alcohol consumption was defined as 0–14 g ethanol/d for women and 0–28 g ethanol/d for men. *P*-interaction between choline quintile and alcohol drinking was 0.52 in women and 0.26 in men. ref, reference.

were excluded, we could not establish the temporal relation between choline intake and the development of fatty liver, which limited a causal inference. Second, misclassification of cases may have occurred because of self-reported diagnoses and the largely asymptomatic nature of nonalcoholic fatty liver. Therefore, we restricted our analyses to participants who had at least one liver ultrasound examination (62% of all participants in the SWHS and SMHS). The prevalence of fatty liver in the present cohorts is similar to that reported in another population-based study conducted in Shanghai that used repeated ultrasound examination data (6). Ultrasound may be acceptable as a screening tool for the detection of hepatic steatosis in large population–based surveys, but it is limited by its inability to provide more precise and detailed information on the severity and stage of the disease. Liver biopsy is the gold standard to evaluate hepatic steatosis. Hepatic enzymes are also used as surrogate markers for liver disease, but their accuracy for assessing hepatic steatosis is limited. Another limitation is that potential selection bias may be introduced by excluding participants without ultrasound examinations. We found no differences in intakes of choline or other dietary factors between those with and without ultrasound, suggesting that selection bias is unlikely to seriously affect our results. However, it may limit the generalizability of our results. Third, dietary measurement errors could come from FFQ assessment and calculation with the use of food composition databases. Although both FFQs were validated and show fairly high accuracy and reproducibility for macronutrients and major food groups, the validity for choline intake has not been assessed. Other studies in non-Caucasian populations that used the USDA database to estimate total dietary choline found intake amounts similar to ours (29, 42). Nevertheless, inevitable measurement errors, correlations between choline and other nutrients, and uncontrolled or unknown variables (e.g., gut microbial metabolism of dietary choline) may affect the estimate of real choline–fatty liver associations, which will most likely result in an underestimation.

In conclusion, we found an inverse association between dietary choline intake and risk of nonalcoholic fatty liver in middle-aged and older Chinese women with a normal body weight but not in overweight/obese women or in men. Further prospective studies are needed to confirm or refute these findings and to evaluate the potential interactions of choline with obesity and gut microbiota in the development of fatty liver.

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