Association between dietary raw garlic intake and newly diagnosed nonalcoholic fatty liver disease: a population-based study

Shunming Zhang^{1,*}, Yeqing Gu^{1,*}, Liu Wang¹, Qing Zhang², Li Liu², Min Lu³, Ge Meng¹, Zhanxin Yao^{1,4}, Hongmei Wu¹, Yang Xia¹, Xue Bao¹, Honglei Wang¹, Hongbin Shi², Shaomei Sun², Xing Wang², Ming Zhou², Qiyu Jia², Kun Song², Huiling Xiang³ and Kaijun Niu^{1,2,5,6}

¹Nutritional Epidemiology Institute and School of Public Health, Tianjin Medical University, Tianjin, China, ²Health Management Centre, Tianjin Medical University General Hospital, Tianjin, China, ³Department of Gastroenterology, Tianjin Third Central Hospital, Tianjin, China, ⁴Tianjin Institute of Health and Environmental Medicine, Tianjin, China, ⁵Tianjin Key Laboratory of Environment, Nutrition and Public Health, Tianjin, China, and ⁶Center for International Collaborative Research on Environment, Nutrition and Public Health, Tianjin, China

*(S Zhang and Y Gu contributed equally to this work)

Correspondence should be addressed to K Niu
Email
nkj0809@gmail.com

Abstract

Background and Aims: The protective effect of garlic against nonalcoholic fatty liver disease (NAFLD) has been reported in animal studies. However, in humans, the association between garlic consumption and NAFLD is unclear. The study sought to explore the association between habitual raw garlic intake and newly diagnosed NAFLD among Chinese adults.

Methods: We performed a study of 11,326 men and 12,780 women aged 20–90 years. Habitual food intake was assessed using a validated and standardized 100-item food frequency questionnaire. Diagnosis of NAFLD was based on the liver ultrasonography and self-reported alcohol intake. Multiple logistic regression was used to evaluate the association of raw garlic intake with newly diagnosed NAFLD.

Results: The prevalence of newly diagnosed NAFLD was 28.9% in men and 10.1% in women, respectively. In men, the fully adjusted odds ratios (95% confidence interval) of having NAFLD across increasing frequency of raw garlic intake were 1.00 (reference) for <1 time/week, 0.81 (0.73, 0.90) for 1–3 times/week, 0.66 (0.54, 0.80) for 4–6 times/week, and 0.71 (0.55, 0.90) for ≥7 times/week (*P* for trend <0.0001). The odds ratio for NAFLD associated with each 1 g of raw garlic/1000 kcal was 0.93 (0.90, 0.97) in men. In women, no significant association between raw garlic intake and NAFLD was identified. These associations between raw garlic intake and NAFLD were consistent in several sensitivity analyses.

Conclusions: Frequent consumption of raw garlic is inversely associated with NAFLD in Chinese men. Further investigations are needed to confirm this finding.

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Introduction

Nonalcoholic fatty liver disease (NAFLD) refers to a wide spectrum of liver disorders, ranging from steatosis to nonalcoholic steatohepatitis, fibrosis, and cirrhosis (1). NAFLD is the predominant cause of chronic liver diseases and it is strongly associated with cardiovascular disease

(CVD) and mortality (2). The prevalence of NAFLD has been estimated to be around 20–30% in Western countries and 15–30% in China (3). In the absence of curative treatment, lifestyle interventions remain the mainstay in the prevention and control of NAFLD (4).

Dietary modifications are beneficial in the prevention of NAFLD. Garlic (*Allium sativum*) is widely consumed in many countries and cultures for centuries. It contains many organosulfur compounds such as S-allylcysteine, allicin, diallyl sulfide, diallyl disulfide and diallyl trisulfide (5). Animal studies have shown that garlic essential oil and its major organosulfur component (diallyl disulfide) could protect against NAFLD through by ameliorating lipid metabolic disorders and oxidative stress (6). Moreover, allicin is the principal sulfur-containing compounds obtained from raw garlic (5). Many studies have suggested that allicin has beneficial effects on oxidative damage (7, 8) and inflammation (9), both of which play an important role in the development of NAFLD (10, 11). Furthermore, human studies and animal models have shown that garlic has hypolipidemic effects (12, 13) and can improve insulin sensitivity (14), thereby playing a direct role in NAFLD prevention (11). Finally, intestinal microflora plays an important role in the pathogenesis of NAFLD (15, 16). According to a recent study, garlic polysaccharides can change the microbiota equilibrium (17). Therefore, we speculated that dietary raw garlic intake may have a beneficial effect on the prevention of NAFLD.

Although a randomized clinical trial (18) and several animal studies (6, 19, 20, 21, 22, 23) have suggested that garlic preparations may have a protective effect against NAFLD, potential effects of raw garlic intake on NAFLD have not been evaluated in population-based studies. On the other hand, the consumption of fresh and raw garlic in China is high, which provides an exceptional opportunity to investigate the association between raw garlic consumption and NAFLD. In the present study, therefore, we assessed the association between dietary raw garlic intake and newly diagnosed NAFLD in a large population of Chinese adults.

Subjects and methods

Study participants

We used data from the Tianjin Chronic Low-grade Systemic Inflammation and Health (TCLSIH) cohort study. The details of the TCLSIH study have been previously described (24). The protocol of this study was approved by the Institutional Review Board of the Tianjin Medical University and all participants gave their informed consent to participate in this study.

This cross-sectional study included 31,834 participants who had received health examinations during 2013–2016.

Subjects who were newly diagnosed with NAFLD were identified by the results of annual medical examinations and/or self-reported history of the disease. Participants diagnosed with alcoholic fatty liver disease and other liver diseases (including chronic hepatitis B or C, operations on the liver, autoimmune liver diseases, cirrhotic or liver cancer, etc.) were identified by the results of their annual medical examinations and/or self-reported history of diseases. We excluded those participants who were previously diagnosed with NAFLD based on the results of their annual medical examination ($n=4949$) and participants with missing dietary data ($n=196$). Moreover, those with a history of CVD ($n=1113$) or cancer ($n=255$) were excluded because of important dietary changes after the diagnosis (25). Those who had a history of other liver diseases ($n=134$) or alcoholic fatty liver disease ($n=1081$) were also excluded. After these exclusions, a total of 24,106 subjects (mean (standard deviation) age: 40.6 (11.9) years; men, 47.0%) were included in the current analysis. The flowchart of study participants is given in Fig. 1.

Definition of NAFLD

All participants underwent real-time ultrasonography examination as part of the health examinations. Fatty liver was diagnosed by abdominal ultrasonography

Figure 1

Selection of subjects for the study. CVD, cardiovascular disease; NAFLD, nonalcoholic fatty liver disease.

using standardized criteria performed by experienced sonographers. Diagnosis of fatty liver disease required the presence of at least two of the following three abnormal findings (26): hyperechogenicity of liver tissue ('bright liver') when compared to the hypoechogenicity of the kidneys, vascular blurring, and deep attenuation of ultrasound signal. Participants with sonographic fatty liver disease and the weekly alcohol intake of <140g for men and <70g for women were defined as having NAFLD (27). Inter-observer variations for NAFLD status (yes or no) were evaluated in a subsample of 200 participants from the TCLSIH study. The Kappa coefficient was 0.90, and the total agreement was 96.4%. Furthermore, as a confirmatory analysis to the ultrasound-established NAFLD, participants who had NAFLD and elevated serum alanine aminotransferase (ALT) levels (ALT >30U/L for men and >19U/L for women) were defined as NAFLD with elevated ALT (28, 29).

Assessment of dietary intake

Dietary intake was assessed using a valid and reliable food frequency questionnaire (FFQ), which included 100 items of foods with specified serving sizes. The FFQ assessed consumption frequency and portion sizes of various foods over the past month by checking one of seven frequency categories ranging from 'almost never' to '≥2 times/day' for foods (including total onions, including both raw and cooked, as well as raw garlic) and eight frequency categories ranging from 'almost never' to '≥4 times/day' for beverages (including soft drinks). The FFQ was validated against 4-day weighed dietary records (WDRs) including 3 weekdays and 1 weekend day and the data from two FFQs collected approximately 3 months apart using a random sampling of 150 participants from the TCLSIH study (30). The participants were randomly selected from different subgroups (age: 20–30, 30–40, 40–50, 50–60, 60–70, and >70 years), and at least ten men and ten women were included in each subgroup. The characteristics of the 150 participants were similar to that of the entire study population. Spearman correlation coefficients between the WDRs and the FFQ results were 0.49 for energy intake, 0.35–0.54 for nutrients (n-3 fatty acid, fat, and carbohydrate), and 0.69 for raw garlic (0.70 for men and 0.68 for women). Spearman's rank correlation coefficients between two FFQs were 0.68 for energy intake, 0.62–0.79 for food items (fruits, vegetables, sweet foods, and beverages), and 0.78 for raw garlic. The mean daily intakes of energy and nutrients were calculated using an *ad hoc* computer program for the FFQ, which was based on

the 2009 Chinese Food Composition Table. In the present study, to assess the potential confounding effect of overall diet quality on the association between raw garlic intake and NAFLD, three major dietary patterns (sweet pattern, healthy pattern, and animal food pattern) were derived by factor analysis after removing the raw garlic and total onions (31). Some unhealthy foods such as red meat or processed meat, preserved egg, and soft drinks were included in the animal food pattern.

Participants reported their frequency of raw garlic and total onions consumption over the previous month by selecting the following options: almost never, <1 time/week, 1 time/week, 2–3 times/week, 4–6 times/week, 1 time/day, and ≥2 times/day. Based on the frequency distribution of responses, the categories of raw garlic consumption were divided into four groups: <1 time/week, 1–3 times/week, 4–6 times/week, and ≥7 times/week.

Assessment of other variables

The anthropometric indices (height, weight, and waist circumference (WC)) were measured by well-trained investigators using a standard protocol. Body mass index (BMI) was calculated as the body weight (kg) divided by the square of the body height (m). The sociodemographic characteristics, including age, sex, household income, educational level, and occupation, were self-reported. Smoking status, drinking status, individual and family history of disease (including CVD, hypertension, hyperlipidemia, and diabetes) were also assessed by a questionnaire survey.

The blood samples were drawn 12 mL from fasting subjects. Serum total cholesterol (TC) and triglycerides (TGs) were measured by enzymatic methods, low-density lipoprotein cholesterol (LDL) was measured by the polyvinyl sulfuric acid precipitation method, and high-density lipoprotein cholesterol (HDL) was measured by the chemical precipitation method. Fasting blood glucose (FBG) was measured using the glucose oxidase method. Serum ALT was measured by the International Federation of Clinical Chemistry method. These measurements were performed by using reagents from Roche diagnostics on an automatic biochemistry analyzer (Roche Cobas 8000 modular analyzer).

According to the criteria of the JNC 7, hypertension was defined as systolic blood pressure (SBP) of ≥140 mmHg and/or diastolic blood pressure (DBP) of ≥90 mmHg or having a history of hypertension. PA was assessed using the short form of the International Physical Activity

Questionnaire (32). PA was estimated as metabolic equivalents in hours per week (MET-hour/week).

Statistical analysis

All analyses were stratified by sex because the interaction between sex and raw garlic intake (continuous; g/day per 1000kcal (33)) in the multivariate model was statistically significant (P for the interaction was <0.0001). Analysis of covariance for continuous variables and logistic regression analysis for categorical variables were used to compare participant characteristics among different categories of raw garlic consumption or status of NAFLD. The characteristics of the participants were presented as geometric mean (95% confidence interval, CI) for continuous variables, or as percentage for categorical variables. Multiple logistic regression models were used to assess the association between raw garlic intake and NAFLD (or NAFLD with elevated ALT). Raw garlic intake was assessed as both categorical and continuous variables; in the categorical analyses, the odds ratio (OR) and corresponding 95% CI were calculated using the <1 time/week group as the reference, whereas in the continuous analyses, the OR (95% CI) was calculated as 1g/day per 1000kcal. In model 1, we adjusted for age and BMI. In model 2, we additionally adjusted for educational level, occupation, household income, PA, and total energy intake. In a final model, we further adjusted for all variables in model 2 in addition to three major dietary patterns (raw garlic and total onions were not included in the calculation) and total onion intake. We constructed a directed acyclic graph (DAG) to justify the inclusion of these confounders (which were associated with both exposures and the outcomes in this study) (34). Figure 2 shows the DAG was derived from previous literature (35, 36) and expert knowledge. To identify the minimally sufficient adjustment set, we used the program, DAGitty (37).

We tested the interactions of raw garlic intake (as a continuous variable (33)) with all covariates by the inclusion of cross-product terms in the fully adjusted model. Moreover, variance inflation factors (VIFs) were used to detect multicollinearity among covariates in the final model. VIFs exceeding 10 were a sign of multicollinearity. To minimize the potential reverse causation, we did several sensitivity analyses: (1) excluded 11,274 participants who had hypertension, hyperlipidemia, and/or diabetes from the analyses; (2) excluded 4,915 participants who have changed their lifestyles in the past 5 years, with changes in dietary intake, drinking status, smoking status, PA, and sleeping; (3) excluded 1,775 participants with long-

term medication use. Furthermore, we used the E-value methodology of VanderWeele and Ding to quantify residual confounding (38, 39).

The P values for linear trends were calculated by using the categories of raw garlic consumption (<1 time/week: 1; 1–3 times/week: 2; 4–6 times/week: 3; and ≥ 7 times/week: 4) as an ordinal variable. Bonferroni-corrected P values were used in multiple tests. SAS version 9.4 (SAS Institute, Inc.) was used for all statistical analyses. Two-sided $P < 0.05$ was considered statistically significant.

Results

Study participant characteristics

In total, 11,326 men (age: 41.0 ± 12.2 years) and 12,780 women (age: 40.3 ± 11.6 years) participated in this study. Table 1 shows the age-adjusted participant characteristics according to their categories of raw garlic consumption. In both sexes, participants with higher raw garlic intake were more likely to be older, had higher BMI, consumed total energy and onions, and more adherence to three major dietary patterns (sweet pattern, healthy pattern, and animal food pattern). In men, those with higher raw garlic intake tended to be physically active, were more likely to smoke, had a higher proportion of everyday drinkers, but had a lower proportion of ex drinkers. In addition, the proportion of non-drinkers had U-shaped association across raw garlic intake categories in men. In women, the proportion of sometime drinkers had reverse U-shaped association, whereas the proportion of non-drinkers had U-shaped association across raw garlic intake categories.

Table 2 describes participants' characteristics in relation to NAFLD. Compared with men and women without NAFLD, those with NAFLD had higher age, BMI, WC, TC, TG, LDL, SBP, DBP, FBG, ALT, and lower HDL and total energy intake. However, there were also some differences between men and women. Men with NAFLD had lower sweet pattern scores, animal food pattern scores, PA, and a lower proportion of everyday drinkers, but had a higher proportion of current smokers, non-smokers, ex-drinkers, and non-drinkers; they were less likely to be employed as managers, had lower education level. Women with NAFLD had a higher proportion of ex-drinkers and a lower proportion of everyday drinkers, sometime drinkers, were more likely to be employed not as managers or professionals and had lower education level and household income.

Figure 2

Directed acyclic graph (DAG) derived from previous literature and expert knowledge. Arrows represent causal associations. Raw garlic is exposure, and NAFLD is the outcome. BMI, body mass index; NAFLD, nonalcoholic fatty liver disease; SES, socioeconomic status (including educational level, occupation, and household income).

Association between raw garlic intake and NAFLD

Table 3 presents the associations between raw garlic consumption categories and newly diagnosed NAFLD. Adjusting for age and BMI, the ORs (95% CIs) for NAFLD across categories of raw garlic consumption were 1.00 (reference) for <1 time/week, 0.78 (0.71, 0.87) for 1–3 times/week, 0.62 (0.52, 0.75) for 4–6 times/week, and 0.62 (0.50, 0.78) for ≥ 7 times/week (P for trend <0.0001) in men. However, the association was not statistically significant in women (P for trend=0.14). After additional adjustment for sociodemographic status, lifestyle factors, total energy intake, and dietary intake, these associations were not altered. The fully adjusted ORs (95% CIs) for NAFLD across categories of raw garlic consumption were 1.00 (reference) for <1 time/week, 0.81 (0.73, 0.90) for 1–3 times/week, 0.66 (0.54, 0.80) for 4–6 times/week, and 0.71 (0.55, 0.90) for ≥ 7 times/week (P for trend <0.0001) in men; the corresponding ORs (95% CIs) were 1.00 (reference), 1.12 (0.96, 1.30), 1.17 (0.88, 1.55), and 1.17 (0.86, 1.58) (P for trend=0.12) in women. An increase of 1 g/1000 kcal per day of raw garlic was inversely associated

with NAFLD in men (OR=0.93; 95% CI: 0.90, 0.97), but no in women (OR=1.05; 95% CI: 0.98, 1.11). In both sexes, we did not observe any significant interactions between raw garlic intake and covariates in the final model. For multicollinearity, all VIF values ranged from 1.01 to 1.56. Furthermore, in sensitivity analyses to minimize the potential reverse causation, similar results were also observed (Supplementary Tables 1, 2 and 3, see section on [supplementary data](#) given at the end of this article). The sensitivity analysis using E-value methodology to assess the potential effect of unmeasured confounding showed that the E-value for an OR of 0.71 (95% CI: 0.55, 0.90) was 1.66 for point estimate and 1.29 for the upper confidence limit.

Association between raw garlic intake and NAFLD with elevated ALT

As a confirmatory analysis to the ultrasound-established NAFLD, similar associations were observed in NAFLD with elevated ALT (Table 4). The fully adjusted ORs (95% CIs)

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Table 1 Age-adjusted participant characteristics according to categories of raw garlic consumption ($n = 24,106$)*.

Characteristics	Categories of raw garlic consumption (Men, $n = 11,326$)				P for trend†
	<1 time/week	1–3 times/week	4–6 times/week	≥7 times/week	
No. of subjects	4653	5165	918	590	–
Age (years)	37.9 (37.6, 38.2)	39.8 (39.4, 40.1)	41.0 (40.2, 41.7)	45.3 (44.3, 46.4)	<0.0001
BMI (kg/m^2)	25.0 (24.9, 25.1)	25.2 (25.2, 25.3)	25.3 (25.1, 25.5)	25.4 (25.1, 25.7)	0.02
WC (cm)	87.7 (87.5, 88.0)	88.3 (88.1, 88.6)	88.3 (87.8, 88.9)	88.5 (87.8, 89.2)	0.06
TC (mmol/L)	4.65 (4.62, 4.67)	4.67 (4.64, 4.69)	4.64 (4.59, 4.70)	4.63 (4.57, 4.70)	0.94
TG (mmol/L)	1.27 (1.25, 1.29)	1.29 (1.27, 1.31)	1.30 (1.25, 1.35)	1.30 (1.25, 1.36)	0.17
LDL (mmol/L)	2.74 (2.72, 2.76)	2.74 (2.72, 2.76)	2.70 (2.65, 2.75)	2.73 (2.67, 2.80)	0.20
HDL (mmol/L)	1.20 (1.19, 1.21)	1.21 (1.20, 1.21)	1.20 (1.18, 1.22)	1.18 (1.15, 1.20)	0.72
SBP (mmHg)	122.7 (122.2, 123.1)	123.0 (122.6, 123.4)	123.0 (122.0, 123.9)	123.7 (122.5, 124.8)	0.56
DBP (mmHg)	77.5 (77.2, 77.8)	78.1 (77.8, 78.4)	78.2 (77.5, 78.9)	78.4 (77.6, 79.3)	0.051
FBG (mmol/L)	5.16 (5.14, 5.19)	5.18 (5.16, 5.20)	5.18 (5.14, 5.23)	5.25 (5.19, 5.32)	0.44
ALT (U/L)	21.6 (21.2, 21.9)	21.5 (21.2, 21.9)	21.5 (20.8, 22.3)	22.2 (21.2, 23.2)	0.93
Physical activity (MET-h/week)	9.91 (9.51, 10.3)	11.2 (10.7, 11.6)	13.3 (12.1, 14.6)	11.7 (10.1, 12.8)	<0.0001
Total energy intake (kcal/day)	1985.4 (1970.3, 2000.6)	2096.7 (2081.5, 2111.9)	2183.8 (2146.7, 2221.7)	2310.7 (2261.5, 2361.1)	<0.0001
'Sweets' dietary pattern score	−0.11 (−0.13, −0.08)	0.06 (0.04, 0.09)	0.49 (0.43, 0.55)	1.26 (1.18, 1.34)	<0.0001
'Vegetables' dietary pattern score	−0.21 (−0.24, −0.18)	−0.05 (−0.08, −0.02)	0.13 (0.07, 0.20)	0.24 (0.16, 0.32)	<0.0001
'Animal foods' dietary pattern score	0.10 (0.07, 0.13)	0.29 (0.26, 0.32)	0.37 (0.30, 0.44)	0.42 (0.33, 0.50)	<0.0001
Total onions intake (g/day)	3.17 (3.07, 3.28)	5.23 (5.08, 5.38)	7.05 (6.61, 7.52)	7.57 (6.98, 8.20)	<0.0001
Smoking status (%)					
Current smoker	33.4	37.2	37.9	40.0	<0.01
Ex-smoker	8.05	8.85	9.98	14.0	0.051
Non-smoker	58.6	54.0	52.1	46.0	<0.0001
Drinking status (%)					
Everyday drinker	4.64	7.26	9.64	10.7	<0.0001
Sometime drinker	69.6	73.8	72.6	68.3	0.10
Ex-drinker	12.6	9.52	9.31	7.98	<0.0001
Non-drinker	13.1	9.40	8.43	13.1	<0.0001
Education level (≥College graduate, %)	69.4	69.1	63.2	56.5	0.45
Occupation (%)					
Managers	41.2	43.9	42.5	41.1	0.22
Professionals	21.1	21.4	19.5	20.8	0.43
Other	37.7	34.7	38.0	38.2	0.06
Household income (≥10,000 Yuan, %)	35.2	35.8	34.9	34.7	0.57

for NAFLD with elevated ALT across the categories of raw garlic consumption were 1.00 (reference) for <1 time/week, 0.89 (0.78, 1.02) for 1–3 times/week, 0.74 (0.57, 0.94) for 4–6 times/week, and 0.71 (0.51, 0.98) for ≥7 times/week (P for trend <0.01) in men; the fully adjusted ORs (95% CIs) were 1.00 (reference), 1.11 (0.90, 1.37), 1.34 (0.92, 1.90), and 1.16 (0.79, 1.69) (P for trend=0.16).

Discussion

The results of this study showed that dietary raw garlic intake was significantly associated with a lower prevalence

of NAFLD among Chinese men, but not women. To our knowledge, this was the first study to assess the association between raw garlic consumption and NAFLD in such a large general population.

In this study, we adjusted for confounding factors identified using the DAG program based on prior knowledge, which can avoid collider bias and overadjustment bias (34, 35). The minimal sufficient adjustment sets for estimating the effect of raw garlic intake on NAFLD included the following confounders: age, sex, BMI, PA, educational level, occupation, household income, total energy intake, dietary patterns, and onions. First, we made an adjustment for age and BMI. Second, we made further adjustments

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Categories of raw garlic consumption (Women, <i>n</i> = 12,780)					<i>P</i> for trend [†]
<1 time/week	1–3 times/week	4–6 times/week	≥7 times/week		
7146	4272	745	617	–	
37.3 (37.0, 37.5)	40.0 (39.7, 40.3)	40.8 (40.0, 41.6)	44.8 (43.9, 45.8)	<0.0001	
22.7 (22.6, 22.8)	23.0 (22.9, 23.1)	23.0 (22.8, 23.2)	23.1 (22.8, 23.3)	0.02	
76.6 (76.4, 76.7)	77.2 (76.9, 77.4)	77.1 (76.5, 77.6)	77.1 (76.5, 77.8)	0.10	
4.61 (4.59, 4.63)	4.63 (4.60, 4.65)	4.58 (4.52, 4.63)	4.59 (4.53, 4.65)	0.22	
0.90 (0.89, 0.91)	0.91 (0.90, 0.92)	0.89 (0.86, 0.92)	0.91 (0.88, 0.94)	0.49	
2.56 (2.54, 2.57)	2.59 (2.57, 2.61)	2.54 (2.49, 2.59)	2.56 (2.51, 2.62)	0.49	
1.54 (1.53, 1.54)	1.52 (1.51, 1.53)	1.51 (1.49, 1.54)	1.51 (1.48, 1.54)	0.14	
114.4 (114.1, 114.7)	114.8 (114.4, 115.2)	114.9 (113.9, 115.9)	114.9 (113.9, 116.0)	0.32	
71.3 (71.0, 71.5)	71.6 (71.3, 71.8)	71.7 (71.0, 72.4)	71.8 (71.0, 72.5)	0.24	
4.95 (4.93, 4.96)	4.97 (4.95, 4.98)	4.97 (4.93, 5.01)	4.95 (4.90, 4.99)	0.30	
12.9 (12.7, 13.0)	13.1 (12.9, 13.3)	13.1 (12.6, 13.5)	13.4 (12.9, 14.0)	0.41	
7.59 (7.33, 7.86)	7.96 (7.61, 8.32)	8.02 (7.21, 8.92)	8.47 (7.53, 9.50)	0.33	
1846.2 (1833.3, 1859.2)	1972.6 (1954.9, 1990.5)	2094.2 (2049.4, 2139.9)	2246.9 (2193.9, 2301.2)	<0.0001	
–0.22 (–0.24, –0.19)	–0.08 (–0.11, –0.05)	0.30 (0.23, 0.37)	1.02 (0.94, 1.09)	<0.0001	
–0.04 (–0.07, –0.02)	0.13 (0.10, 0.16)	0.34 (0.27, 0.41)	0.72 (0.64, 0.79)	<0.0001	
–0.29 (–0.31, –0.27)	–0.11 (–0.13, –0.08)	–0.03 (–0.09, 0.04)	0.02 (–0.05, 0.09)	<0.0001	
2.61 (2.54, 2.69)	4.50 (4.35, 4.65)	6.02 (5.58, 6.49)	7.58 (6.99, 8.21)	<0.0001	
1.36	1.66	1.46	2.53	0.30	
0.75	0.65	0.73	0.72	0.60	
97.9	97.7	97.8	96.8	0.57	
0.59	0.87	0.68	0.99	0.46	
38.0	42.2	44.2	38.7	0.001	
10.2	8.52	10.3	11.1	0.30	
51.2	48.4	44.8	49.3	<0.0001	
67.2	62.6	62.7	47.4	0.19	
41.3	41.3	40.1	36.6	0.74	
13.1	12.5	14.0	11.1	0.84	
45.6	46.2	45.9	52.3	0.87	
34.7	33.9	33.0	30.5	0.38	

*Values are geometric mean (95% confidence interval) or percentage unless otherwise indicated. ALT, alanine aminotransferase; BMI, body mass index; DBP, diastolic blood pressure; FBG, fasting blood glucose; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; MET, metabolic equivalent; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides; WC, waist circumference.

[†]Analysis of covariance or logistic regression analysis. *P* for trend was calculated using the categories of raw garlic consumption ordinal as the predictor variable.

for educational level, occupation, household income, PA, and total energy intake. However, adjustments for these confounding factors did not change the observed associations. Third, to test for the potential influence of nutritional quality of diets on the association between raw garlic intake and NAFLD, we additionally adjusted for three major dietary patterns (removing the raw garlic and total onions). In addition, because onions have a similar composition to garlic (40), we also adjusted for total intake of onions. However, the adjustment for these dietary factors did not change the associations between raw garlic consumption and NAFLD. Furthermore, animal studies have shown that garlic+onion have a better and

significant effect on NAFLD (21). However, the interaction between raw garlic intake and total onions intake for NAFLD was not statistically significant (*P*=0.95 for men and *P*=0.75 for women). This could be due to the fact that people in Tianjin, China hardly eat raw onions, whereas cooking decreases the main bioactive compound, sulfur compounds, contained in onions (41). Finally, our results remained robust in several sensitivity analyses.

Many previous studies that investigated the association between garlic intake and NAFLD were performed in animal models (6, 19, 20, 21, 22, 23), and therefore, unable to determine whether garlic intake has also an effect on NAFLD in humans. Only one clinical trial showed that

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Table 2 Age-adjusted participant characteristics by NAFLD status ($n = 24,106$)*.

Characteristics	NAFLD status (men, $n = 11,326$)		P value [†]	NAFLD status (women, $n = 12,780$)		P value [†]
	No	Yes		No	Yes	
No. of subjects	8048	3278	–	11,485	1295	–
Age (years)	39.0 (38.8, 39.2)	40.3 (39.9, 40.7)	<0.0001	37.9 (37.7, 38.1)	46.8 (46.1, 47.5)	<0.0001
BMI (kg/m^2)	24.1 (24.1, 24.2)	27.9 (27.8, 28.0)	<0.0001	22.4 (22.4, 22.5)	26.8 (26.6, 27.0)	<0.0001
WC (cm)	85.5 (85.3, 85.7)	94.7 (94.4, 95.0)	<0.0001	75.9 (75.7, 76.0)	85.7 (85.2, 86.2)	<0.0001
TC (mmol/L)	4.57 (4.55, 4.59)	4.86 (4.83, 4.89)	<0.0001	4.59 (4.58, 4.61)	4.81 (4.76, 4.85)	<0.0001
TG (mmol/L)	1.12 (1.11, 1.13)	1.76 (1.73, 1.79)	<0.0001	0.86 (0.85, 0.87)	1.37 (1.34, 1.40)	<0.0001
LDL (mmol/L)	2.67 (2.66, 2.69)	2.88 (2.86, 2.91)	<0.0001	2.54 (2.53, 2.55)	2.80 (2.76, 2.84)	<0.0001
HDL (mmol/L)	1.27 (1.26, 1.28)	1.06 (1.05, 1.06)	<0.0001	1.57 (1.56, 1.57)	1.25 (1.24, 1.27)	<0.0001
SBP (mmHg)	120.8 (120.5, 121.1)	128.2 (127.6, 128.7)	<0.0001	113.5 (113.2, 113.7)	124.7 (123.9, 125.5)	<0.0001
DBP (mmHg)	76.3 (76.0, 76.5)	81.9 (81.6, 82.3)	<0.0001	70.8 (70.6, 70.9)	77.3 (76.7, 77.9)	<0.0001
FBG (mmol/L)	5.09 (5.07, 5.10)	5.41 (5.38, 5.44)	<0.0001	4.91 (4.90, 4.92)	5.39 (5.35, 5.42)	<0.0001
ALT (U/L)	18.7 (18.5, 18.9)	30.6 (30.1, 31.2)	<0.0001	12.5 (12.4, 12.6)	18.5 (18.1, 19.0)	<0.0001
Physical activity (MET-h/week)	12.0 (11.6, 12.3)	11.3 (10.8, 11.8)	0.02	8.75 (8.54, 8.96)	9.05 (8.41, 9.73)	0.40
Total energy intake (kcal/day)	2080.8 (2068.6, 2093.0)	2035.0 (2016.4, 2053.8)	<0.0001	1924.1 (1913.4, 1934.9)	1879.8 (1848.0, 1912.1)	0.01
'Sweets' dietary pattern score	0.03 (0.00, 0.05)	−0.06 (−0.10, −0.03)	<0.0001	0.00 (−0.02, 0.02)	−0.01 (−0.07, 0.05)	0.71
'Vegetables' dietary pattern score	−0.01 (−0.03, 0.02)	0.01 (−0.02, 0.05)	0.39	0.00 (−0.01, 0.02)	−0.04 (−0.1, 0.01)	0.11
'Animal foods' dietary pattern score	0.01 (−0.01, 0.04)	−0.03 (−0.07, 0.00)	0.02	0.00 (−0.01, 0.02)	−0.04 (−0.09, 0.02)	0.17
Total onions intake (g/day)	4.53 (4.43, 4.65)	4.37 (4.20, 4.54)	0.11	3.52 (3.44, 3.60)	3.41 (3.18, 3.64)	0.38
Smoking status (%)						
Current smoker	34.9	38.2	<0.01	8.85	8.96	0.57
Ex-smoker	56.3	52.9	0.98	1.45	2.23	0.27
Non-smoker	0.68	1.07	0.01	97.9	96.7	0.28
Drinking status (%)						
Everyday drinker	8.10	2.76	<0.0001	0.75	0.40	0.03
Sometime drinker	71.4	72.4	0.28	40.3	35.8	<0.01
Ex-drinker	10.0	12.4	<0.001	9.61	10.2	<0.01
Non-drinker	10.5	12.4	<0.01	49.4	53.6	0.12
Education level (≥College graduate, %)	69.6	64.3	<0.0001	67.0	41.3	<0.0001
Occupation (%)						
Managers	43.6	39.9	<0.001	41.9	33.6	<0.01
Professionals	21.5	20.2	0.25	13.2	9.60	<0.01
Other	34.9	39.9	<0.0001	45.0	56.8	<0.0001
Household income (≥10,000 Yuan, %)	35.2	35.9	0.37	34.8	28.0	<0.01

*Values are geometric mean (95% confidence interval) or percentage unless otherwise indicated.

ALT, alanine aminotransferase; BMI, body mass index; DBP, diastolic blood pressure; FBG, fasting blood glucose; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; MET, metabolic equivalent; NAFLD, nonalcoholic fatty liver disease; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides; WC, waist circumference.

[†]Analysis of covariance or logistic regression analysis.

15 weeks garlic powder supplementation could reduce body weight and fat mass among NAFLD subjects, whose consisted of 98 volunteers (including men and women) aged 20–70 years (18). In addition, a 12-week double-blind, randomized, placebo-controlled trial in 75 adults, aged 20–75 years, found that fermented garlic extract may be beneficial in improving for hepatic dysfunction (42). Our findings, obtained in men, were consistent with prior results suggesting that garlic intake has a profoundly beneficial effect on NAFLD. Therefore, this study indicated that raw garlic intake could be part of a preventive strategy

to combat NAFLD in men. However, previous studies also showed that high dose of garlic extract could elicit pro-oxidant conditions or induce morphological changes in the liver and kidneys (43, 44). Notably, these extracts, which is not the same as actual garlic, tend to be highly concentrated. Whether a high raw garlic intake would have a harmful effect on human health is unclear. Therefore, further population studies examining a safe dose range for raw garlic intake are warranted.

In China, Chinese meals and side dishes often include fresh and raw garlic, which is generally chopped

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Table 3 Associations between raw garlic consumption and NAFLD ($n = 24,106$)*.

Logistic regression models	Categories of raw garlic consumption				P for trend [†]	1 g/1000 kcal increase
	<1 time/week	1–3 times/week	4–6 times/week	≥7 times/week		
Men (<i>n</i> = 11,326)						
Total number of participants	4653	5165	918	590	–	11,326
NAFLD, <i>n</i> (%)	1424 (30.6)	1458 (28.2)	237 (25.8)	159 (26.9)	–	3278 (28.9)
Model 1 [‡]	1.00 (reference)	0.78 (0.71, 0.87) [§]	0.62 (0.52, 0.75)	0.62 (0.50, 0.78)	<0.0001	0.93 (0.89, 0.97)
Model 2	1.00 (reference)	0.79 (0.71, 0.88)	0.62 (0.51, 0.75)	0.63 (0.50, 0.79)	<0.0001	0.92 (0.88, 0.95)
Model 3 [¶]	1.00 (reference)	0.81 (0.73, 0.90)	0.66 (0.54, 0.80)	0.71 (0.55, 0.90)	<0.0001	0.93 (0.90, 0.97)
Women (<i>n</i> = 12,780)						
Total number of participants	7,146	4,272	745	617	–	12,780
NAFLD, <i>n</i> (%)	613 (8.58)	496 (11.6)	89 (11.9)	97 (15.7)	–	1295 (10.1)
Model 1 [‡]	1.00 (reference)	1.14 (0.98, 1.32) [§]	1.11 (0.83, 1.46)	1.16 (0.87, 1.53)	0.14	1.07 (1.01, 1.14)
Model 2	1.00 (reference)	1.12 (0.96, 1.30)	1.15 (0.86, 1.52)	1.14 (0.85, 1.51)	0.16	1.04 (0.99, 1.10)
Model 3 [¶]	1.00 (reference)	1.12 (0.96, 1.30)	1.17 (0.88, 1.55)	1.17 (0.86, 1.58)	0.12	1.05 (0.98, 1.11)

*NAFLD, nonalcoholic fatty liver disease.

[†]Obtained by using multiple logistic regression analysis. The P values for trend were calculated by using the categories of raw garlic consumption (<1 time/week: 1; 1–3 times/week: 2; 4–6 times/week: 3; and ≥7 times/week: 4) as an ordinal variable.[‡]Adjusted for age (continuous; years) and BMI (continuous; kg/m²).[§]Odds ratio (95% confidence interval) (all such values).^{||}Additionally adjusted for educational level (categorical: < or ≥ college graduate), occupation (categorical; managers, professionals, and other), household income (categorical: ≤ or > 10,000 Yuan), physical activity (continuous; MET-hour/week), and total energy intake (continuous; kcal/day).[¶]Further adjusted for total onions intake (continuous; g/day per 1000 kcal) and three major dietary pattern scores (raw garlic and total onions were not included in the calculation).

or crushed. Additionally, some Chinese people chew raw fresh garlic cloves when they are eating noodles. Because chopping, crushing or chewing raw garlic releases more allicin than by preparing and consuming cooked garlic (45), individuals can ingest a high amount of allicin from dietary raw garlic intake. Accumulated evidence

suggested that allicin has the strong antioxidant activity and antiinflammatory effects (7, 8, 46), which have preventive effects against NAFLD. On the other hand, raw garlic intake can decrease insulin resistance (14), which is a key pathogenic factor of NAFLD (47). Furthermore, an important cause of NAFLD is dyslipidemia (47).

Table 4 Associations between raw garlic consumption and NAFLD with elevated ALT ($n = 24,106$)*.

Logistic regression models	Categories of raw garlic consumption				P for trend [†]	1 g/1000 kcal increase
	<1 time/week	1–3 times/week	4–6 times/week	≥7 times/week		
Men (<i>n</i> = 11,326)						
Total number of participants	4653	5165	918	590	–	11,326
NAFLD with elevated ALT, <i>n</i> (%)	682 (14.7)	698 (13.5)	109 (11.9)	62 (10.5)	–	1551 (13.7)
Model 1 [‡]	1.00 (reference)	0.90 (0.79, 1.02) [§]	0.73 (0.57, 0.93)	0.67 (0.49, 0.90)	<0.001	0.93 (0.89, 0.98)
Model 2	1.00 (reference)	0.88 (0.78, 1.00)	0.70 (0.54, 0.88)	0.66 (0.48, 0.89)	<0.001	0.94 (0.89, 0.98)
Model 3 [¶]	1.00 (reference)	0.89 (0.78, 1.02)	0.74 (0.57, 0.94)	0.71 (0.51, 0.98)	<0.01	0.95 (0.90, 0.99)
Women (<i>n</i> = 12,780)						
Total number of participants	7146	4272	745	617	–	12,780
NAFLD with elevated ALT, <i>n</i> (%)	278 (3.89)	224 (5.24)	45 (6.04)	46 (7.46)	–	593 (4.64)
Model 1 [‡]	1.00 (reference)	1.14 (0.94, 1.40) [§]	1.29 (0.89, 1.83)	1.25 (0.86, 1.78)	0.08	1.07 (0.99, 1.15)
Model 2	1.00 (reference)	1.13 (0.92, 1.38)	1.34 (0.94, 1.89)	1.20 (0.83, 1.70)	0.10	1.07 (1.00, 1.14)
Model 3 [¶]	1.00 (reference)	1.11 (0.90, 1.37)	1.34 (0.92, 1.90)	1.16 (0.79, 1.69)	0.16	1.06 (0.98, 1.14)

*ALT, alanine aminotransferase; NAFLD, nonalcoholic fatty liver disease.

[†]Obtained by using multiple logistic regression analysis. The P values for trend were calculated by using the categories of raw garlic consumption (<1 time/week: 1; 1–3 times/week: 2; 4–6 times/week: 3; and ≥7 times/week: 4) as an ordinal variable.[‡]Adjusted for age (continuous; years) and BMI (continuous; kg/m²).[§]Odds ratio (95% confidence interval) (all such values).^{||}Additionally adjusted for educational level (categorical: < or ≥ college graduate), occupation (categorical; managers, professionals, and other), household income (categorical: ≤ or > 10,000 Yuan), physical activity (continuous; MET-hour/week), and total energy intake (continuous; kcal/day).[¶]Further adjusted for total onions intake (continuous; g/day per 1000 kcal) and three major dietary pattern scores (raw garlic and total onions were not included in the calculation).

Many animal studies have demonstrated that garlic extracts can lower serum lipids, lipoprotein profiles, and cholesterol by controlling biosynthesis of hepatic fatty acid and cholesterol (48, 49). Therefore, the inverse association between raw garlic consumption and NAFLD is biologically reasonable.

Interestingly, no association was found between raw garlic intake and NAFLD in women. A possible explanation for the sex differences is that estrogen signaling pathway might be mediators for NAFLD in women. Evidence has shown that estrogens have a protective effect against the development of NAFLD in women (50). However, animal studies found that sulfides in garlic inhibit estrogen receptor α (51). Meanwhile, studies demonstrated that estrogen receptor α is the primary mediator for estrogen signaling to protect against fatty liver (52). Therefore, the possible explanation for the lack of association between raw garlic intake and NAFLD may be that sulfides in garlic diminish the responsiveness of hormone-receptive tissue by inhibiting estrogen receptor α . Further study is needed to determine the molecular mechanisms responsible for the lack of association between raw garlic consumption and NAFLD in women.

The major strengths of this study include adjustments for confounding factors identified by the DAG, large sample size, detailed measurements of lifestyles, and use of a validated FFQ for assessment of dietary intake. Moreover, we only included newly diagnosed NAFLD, which further reduced the potential effects of reverse causation in the present study. Meanwhile, this study has several limitations worth considering. First, this was a cross-sectional study, restricting causal inference. However, we performed several sensitivity analyses to minimize the potential reverse causation; results from these sensitivity analyses support our findings. More importantly, the inverse association between raw garlic intake and NAFLD is biologically reasonable, which strengthens the biological plausibility of our findings. Nevertheless, we admit that further longitudinal studies and trials are needed to confirm our findings. Second, although we adjusted for many confounding factors underlying causal assumptions, residual confounding factors could persist. However, the sensitivity analysis using E-value methodology showed that the observed OR of 0.71 could be explained away by an unmeasured confounder that was associated with both raw garlic intake and NAFLD by an OR of 1.66-fold each, above and beyond the measured confounders, but weaker confounding could not do so; the CI could be moved to include the null by

an unmeasured confounder that was associated with both raw garlic intake and NAFLD by an OR of 1.29-fold each, above and beyond the measured confounders, but again, weaker confounding could not do so. Since an OR of 1.66 was much greater than any observed confounders in our study, it is unlikely that an unmeasured confounder would be able to overcome the effects of raw garlic intake on NAFLD in the current study. Nevertheless, the drawbacks of a cross-sectional study cannot be remedied by statistical methods. Thus, future prospective studies are warranted. Third, owing to the observational nature of this study, imprecision in the measurement of the included confounding factors cannot be excluded. Finally, we were unable to collect data on intake of cooked garlic or garlic products. Therefore, further studies are still required to determine the association between intake of cooked garlic or garlic products and NAFLD.

Conclusion

In conclusion, the higher intake of raw garlic was associated with a decreased prevalence of NAFLD in men, but not women. These results suggest that raw garlic intake may have a protective effect on NAFLD. Further prospective studies or clinical trials are needed to confirm the causality of the associations.

Supplementary data

This is linked to the online version of the paper at <https://doi.org/10.1530/EJE-19-0179>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

S Z and Y G analyzed data and wrote the paper. L W, Q Z, L L, M L, G M, Z Y, H W, Y X, X B, H W, H S, S S, X W, M Z, Q J, K S, and H X conducted research. K N designed research and had primary responsibility for final content. All authors read and approved the final manuscript.

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