BMJ Open

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Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-028961
Article Type:	Research
Date Submitted by the Author:	07-Jan-2019
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Keywords:	EPIDEMIOLOGY, NUTRITION & DIETETICS, Lipid disorders < DIABETES & ENDOCRINOLOGY

Association between nut intake and NAFLD risk: a case-control study in a sample of Chinese Han adults

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Running title：Nut intake and non-alcoholic fatty liver disease

Word count：2622

Abstract

Background/Objectives: Nut consumption has been associated with a lower risk of type 2 diabetes, metabolic syndrome, and insulin resistance. However, its effect on the risk of non-alcoholic fatty liver disease (NAFLD) is unknown. Therefore, we investigated the relationship between nut consumption and NAFLD risk.

Subjects/Methods: A case-control study was conducted among 534 patients diagnosed with NAFLD and 534 controls matched by sex and age (± 5) from the Affiliated Nanping First Hospital of Fujian Medical University in China**.** Information on dietary intake was collected using a semi-quantitative food frequency questionnaires and nut consumption was calculated. Nut consumption was categorized using quartiles based on the distribution of daily nut intake of the controls. Binary logistic regression models were used to estimate odds ratio (ORs) and the 95% confidence intervals (CIs) for the association between nut consumption and NAFLD risk.

Results: After adjusting for potential confounding variables, nut consumption was not associated with NAFLD risk in the overall sample. When the fully adjusted model was stratified by sex, a significant inverse association was found between high nut consumption and NAFLD only among the men in the highest quartile (OR = 0.45; 95% CI: 0.27–0.76; P_{trend} = 0.01). The inverse association of nut consumption with NAFLD risk in men remained significant after controlling for other known or suspected risk factors for NAFLD.

Conclusions: Diets with a higher intake of nuts may be associated with a decreased risk of NAFLD, particularly in men.

Keywords:NAFLD ; Nut consumption ; case-control study

Introduction

Non-alcoholic fatty liver disease (NAFLD) is defined as macrovesicular steatosis in ≥5% of hepatocytes, in the absence of a secondary cause, such as alcohol or drug use. NAFLD has become a leading cause of chronic liver disease, with a 25% prevalence worldwide [1].Furthermore, a fatty liver is more prevalent in men compared with women[2]. The prevalence of NAFLD among the adults in China's general population is approximately 15% (6.3–27.0%), depending on the population studied [3]. Many studies suggest that diet and lifestyle may significantly influence the risk of NAFLD [4, 5]. These studies indicate that type 2 diabetes (T2D) [6], metabolic syndrome (MetS), obesity [7], physical activity, and a high-fat diet [4] are associated with an increased risk for the development of NAFLD.

Nuts are nutrient-dense foods with complex matrices rich in unsaturated fatty and other bioactive compounds (e.g., high-quality vegetable protein, fiber, minerals, tocopherols, phytosterols, and phenolic compounds) [8]. The global intake of nuts has increased by 59% over the past decade[9]. China's per capita intake of nuts was 2.2 g/day in 1982, and increased to 3.8g/day in 2012 [10]. Although they are high in fat and energy dense, a high intake of nuts has been associated with several health benefits, including reduced risk of cardiovascular disease [11], T2D [12], MetS[13], and insulin resistance[14, 15].Moreover, nuts have antioxidative effects by decreasing lipid peroxidation and protecting against oxidative DNA damage [16]. Beneficial health effects have been attributed to the macronutrient and micronutrient profiles of nuts [17].

NAFLD is regarded as the "hepatic manifestation of MetS." Few studies have assessed the effects of nut intake on NAFLD, despite previous findings of an inverse correlation between high nut consumption and the risk of T2D and cardiovascular disease. In this study, we analyzed the association between nut intake and NAFLD

risk and the interactions between nut intake and other established NAFLD risk factors in a large case-control study with a sample of Han adults in China.

Patient and Public Involvement

This study is a case-control design focused on a Chinese Han population between 18 and 70 years old. Subjects were recruited from a health examination center of Nanping First Affiliated Hospital of Fujian Medical University from October 2015 to September 2017. All subjects underwent abdominal ultrasound and blood biochemical tests. Once cases and controls have been linked to the NAFLD, a letter of invitation and information about the study will be sent to each potential case and control to obtain consent. Eligible subjects will be interviewed face-to-face by investigators to collect data .The study was were approved by the local ethics committees of Fujian Medical University (ethics number 2014096).In addition, all methods were performed in accordance with the relevant guidelines and regulations.

Subjects and Methods

Participants and study design

We conducted the case-control study in a health examination center at the Affiliated Nanping First Hospital of Fujian Medical University from April 2015 to August 2017. Patients newly diagnosed with NAFLD using ultrasonography in accordance with the "Guidelines for the diagnosis and treatment of nonalcoholic fatty liver disease in China" were included in the study. All participants were of Chinese Han ethnicity. The exclusion criteria were as follows: (a) daily alcohol intake of >40 g (men) and >20 g (women), (b) a history of other liver diseases including drug-induced liver disease, viral hepatitis, autoimmune hepatitis, total parenteral nutrition, and hepatolenticular degeneration, (c) taking hypolipidemic or weight reduction drugs, (d) age <18 or >70 years, (e) non-resident of Nanping, or (f) not of Han ethnicity. Adults

who reported extremely abnormal levels of energy intake (2 511.60 kJ [600 kcal] or 17 581.20 kJ [4 200kcal] per day for men; 2 093 kJ [500 kcal] or 14,651.00 kJ [3500 kcal] per day for women), and those who did not answer 25 or more food-related items or questions about nut intake on the questionnaire, were excluded from the study.

The controls were randomly selected from the same center during the study period. Their eligibility criteria were identical to those of the cases, except for the requirement of a diagnosis of liver steatosis; they were frequency-matched with cases by age (within 5-yr intervals), gender, ethnicity, and region of origin. The study was approved by the local ethics committee of Fujian Medical University. All procedures were performed in accordance with relevant guidelines and regulations, and all participants provided written informed consent.

Dietary Assessment

Trained interviewers performed a comprehensive medical history on each participant that included eliciting information about their demographic and socio-economic characteristics, and lifestyle habits (e.g., smoking, drinking (alcohol), tea drinking, and physical activity). The data were obtained from participants using structured questionnaires during face-to-face interviews. Information about participants' typical food consumption was collected using a semi-quantitative food frequency questionnaire, which was developed and validated in a sample from southern China [18]. Participants were asked to estimate the average frequency of consumption of selected foods using the following response options: rarely, <once/month, 1–3 times/month, 1–2 times/week, 3–4 times/week, 5–6 times/week, once/day, twice/day, and >twice/day. Nut intake was defined as the consumption of "peanuts, walnuts, seeds, or other nuts." Data from a semi-quantitative food frequency

questionnaire were used to calculate daily nut and energy intake. Nut consumption was converted to grams/day by multiplying the food-intake frequency by fixed portion sizes. Nutrient intake, such as mono-unsaturated fatty acids (MUFAs) and poly-unsaturated fatty acids (PUFAs) were calculated by multiplying the intake frequency of each food by the nutrient content of the specified portion, and summing the products of all the food items.

Statistical analysis

Participants' characteristics were analyzed using Pearson's Chi-square test for categorical variables, and Wilcoxon's rank-sum test and ANOVA for continuous variables. Nut consumption was categorized by quartiles based on the distribution of daily nut intake by the controls (Q1-Q4). Binary logistic regression models were used to estimate the odds ratios (ORs) and their 95% confidence intervals (CIs) for the associations between nut consumption and NAFLD risk. To account for the energy content of the nut, our initial model adjusted for total energy intake by using the residuals method. The final model adjusted for potential confounders, including age, income, smoking status, educational level, and tea-drinking status, occupational status, marital status, body mass index (BMI), physical activity(The intensity of physical activity was defined in terms of Metabolic Equivalent of Energy(MET). According to a standard reference, each kind of activity was assigned a specific MET value: light physical activities were defined as <3METs, moderate activities defined as 3~6 METs and severe activities defined as >6METs). The total dose of physical activity equals the sum of the doses for each specific activity., and history of diabetes, hypertension, and hyperlipidemia. The final model also adjusted for MUFA and PUFA intake to control for their effects. The selection of covariates for the final model was based on clinical significance, results of previous studies, and strength of the correlation with

exposure. We tested for linear trends across categories of nut intake by assigning each participant the median value for each category and modeling this value as a continuous variable.

We evaluated the influence of nut consumption across strata of other potential predictors and confounders, comparing participants in the highest nut-consumption category to the lowest nut-consumption category(reference). We also analyzed the interactions of nut consumption with age, sex, BMI, smoking status, educational level, tea-drinking status, and other risk factors. We used the medians of the continuous variables to categorize them and evaluate the interactions. The criteria for statistical significance of the likelihood-ratio test of interaction effects was $P < 0.05$. Statistical analyses were performed using SPSS version 22 (IBM, Armonk, NY, US). All

P-values were 2-tailed, and $P < 0.05$ was considered statistically significant.

Table 1 General characteristics of cases and controls stratified by sex,*n*(%)

Variables	Men (*n*=728)			Women(*n*=340)		
	Cases	Controls(*n*=364)	*P*-value[a]	Cases (*n*=170)	Controls(*n*=170)	*P*-value[a]
Age			0.66			0.99
＜40	118(32.42)	116(31.87)		25(14.71)	22(12.94)	
40~60	217(59.61)	212(58.24)		123(72.35)	122(71.76)	
⩾60	29(7.97)	36(9.89)		22(12.94)	26(15.30)	
Educational level			0.04			0.75
primary school and less than	10(2.75)	23(6.32)		30(17.65)	26(15.29)	
junior middle and high school	147(40.38)	128(35.16)		76(44.70)	74(43.53)	
junior college or above	207(56.87)	213(58.52)		64(37.65)	70(41.18)	
Occupational status			0.39			0.43
mental labour	108(29.67)	104(28.57)		50(29.41)	48(28.24)	
physical labor	86(23.63)	102(28.02)		25(14.71)	34(20.00)	
other	170(46.70)	158(43.41)		95(55.88)	88(51.76)	
Income(yuan/month)			0.35			0.85
＜2000	17(4.67)	17(4.67)		15(8.82)	18(10.59)	
2000~3000	87(23.90)	104(28.57)		70(41.18)	70(41.18)	
⩾3000	260(71.43)	243(66.76)		85(50.00)	82(48.23)	
Marital status			0.24			0.52
single	45(12.36)	56(15.38)		4(2.35)	6(3.53)	
married or other	319(87.64)	308(84.62)		166(97.65)	164(96.47)	
Smoking status			0.30			0.16
never smoker	199(54.67)	213(58.52)		168(98.82)	170(100)	
smoker	165(45.33)	151(41.48)		2(1.18)	0(0.00)	
Tea-drinking status			0.30			0.01
yes	258(70.88)	245(63.31)		77(45.29)	55(32.35)	
no	106(29.12)	119(32.69)		93(54.71)	115(67.55)	
Physical activity			0.02			0.88
light	175(48.08)	137(37.64)		19(11.18)	19(11.18)	
moderate	100(27.47)	121(33.24)		47(27.64)	43(25.29)	
severe	89(24.45)	106(29.12)		104(61.18)	108(63.53)	
BMI(kg/m^2)			0.00			0.00
＜18.5	2(0.55)	11(3.02)		1(0.59)	9(5.29)	
18.5~24.0	107(29.40)	257(70.61)		72(42.35)	125(73.53)	
⩾24.0	255(70.05)	96(26.37)		97(57.06)	36(21.18)	
History of diabetes			0.67			0.18
yes	12(3.30)	10(2.75)		10(5.88)	5(2.94)	
no	352(96.70)	354(97.25)		160(94.12)	165(94.06)	
History of hypertension			0.85			0.63
yes	15(4.12)	16(4.40)		8(4.71)	10(5.88)	
no	349(95.88)	348(95.60)		162(95.29)	160(94.12)	
History of hyperlipidemia			0.01			0.00
yes	108(29.67)	77(21.15)		51(30.00)	21(12.35)	
no	256(70.33)	287(78.85)		119(70.00)	149(87.65)	
MUFA intake,g/d[b]	34.10(28.49,	31.39(26.55,36.79)	0.00	29.99(26.37,36.37)	28.05(22.40,31.39)	0.00
PUFA intake,g/d[b]	26.25(24.04,	23.90(22.01,26.02)	0.00	24.52(22.11,27.12)	21.21(18.83,24.53)	0.00
Energy intake,kJ/d[c]	10395.64±24	9890.97±2348.76	0.01	7509.73±1650.71	7323.78±1852.97	0.04

[a]*P*-values were calculated by using the Chi-square test for categorical variables and Wilcoxon rank sum test and ANOVA test for continues variables.

[b]Medians (IQRs).

[c]Mean(s.d).

Results

Baseline Characteristics

Table 1 presents health-related and demographic characteristics of the NAFLD cases and matched controls. Compared with the controls, the NAFLD cases tended to have a higher BMI, higher total intake of energy, higher MUFA and PUFA consumption, and a higher number of participants with a history of hyperlipidemia among both men and women. Patients with NAFLD had a lower educational level and the men engaged in less physical activity than the controls. No statistically significant associations were found for age, occupational status, income, marital status, smoking status, history of diabetes, or hypertension between the cases and the controls.

The associations of dietary nut intake with NAFLD

The participants' nut consumption is summarized in Table 2. Among the women, those in the NAFLD group consumed a significantly higher amount of nuts (6.80 g/d vs. 2.50 g/d; P = 0.02) than those in the control group. No statistically significant differences were found between the cases and controls in the the sample or among the male participants. In order to adjust for potential confounding factors, quartile distributions of dietary nut consumption among the controls were used to categorize the nut intake of all the participants; the results are shown in Table 3. After adjusting for potential confounders, nut consumption was not associated with NAFLD risk among the participants in the sample. When the fully adjusted model was stratified by sex, a significant inverse association was found between nut consumption and NAFLD, but only among the men in the highest quartile (OR = 0.45; 95% CI: 0.27–0.76; P_{trend} = 0.01).

Table 2 Comparison of nut daily intake between the case and the control

Nut intake (g/d)	Case		Control		*P*-value[a]	*P*-value[b]
	Median	Quartile	Median	Quartile		
Total population	3.15	1.46-8.80	2.86	1.22-8.98	0.35	0.18
Men	2.68	1.15-8.43	2.86	1.22-8.98	0.36	0.94
Women	6.80	1.75-8.86	2.50	1.07-7.84	0.01	0.02

[a]*P*-values:calculated by using Wilcoxon rank sum test;before adjusting for energy.

[b]*P*-values :calculated by using Wilcoxon rank sum test; after adjusting for energy by using the residuals methods.

Table 3 Odds ratios (OR) and 95% confidence intervals (Cl) for NAFLD in relation to nut intake

Nut intake	Case *n*	Control *n*	Crude OR(95%CI)	Model 1[a] OR(95%CI)	Model 2[a] OR(95%CI)
Total population[b]	534	534			
Q1	125	133	1	1	1
Q2	112	134	1.30(0.93-1.81)	0.89(0.63-1.26)	0.98(0.65-1.47)
Q3	159	134	1.77(1.29-2.44)	1.26(0.90-1.77)	1.16(0.78-1.72)
Q4	138	133	1.17(0.81-1.68)	1.10(0.78-1.55)	0.85(0.57-1.27)
P for trend*			0.46	0.45	0.07
Men	364	364			
Q1	106	102	1	1	1
Q2	91	79	1.31(0.88-1.94)	0.90(0.60-1.35)	0.92(0.56-1.49)
Q3	74	123	1.81(1.24-2.65)	1.73(1.16-2.57)	1.36(0.85-2.18)
Q4	93	60	1.04(0.66-1.63)	0.67(0.44-1.02)	0.45(0.27-0.76)
P for trend*			0.81	0.04	0.01
Women	170	170			
Q1	23	27	1	1	1
Q2	33	43	1.28(0.69-2.37)	0.90(0.44-1.85)	0.86(0.37-2.02)
Q3	36	60	1.71(0.95-3.08)	0.70(0.35-1.41)	0.56(0.25-1.28)
Q4	78	40	1.41(0.76-2.64)	2.29(1.17-4.49)	1.34(0.59-3.04)
P for trend*			0.36	0.00	0.12

[a]Model 1:Adjusted for total energy intake.

[a]Model 2:Adjusted further for age,income,smoking status,educational level, teaing status, occupationanl status,marital status,body mass index,physical activity,the history of diabetes,hypertension and hyperlipidemia,MUFA and PUFA intake.

[b]:further adiusted for sex.

*P for trend: Trend across intake levels with the categorical median.

Stratified Analyses

In the stratified analysis, the inverse association between total-nut consumption and NAFLD risk was consistent across age, sex, BMI, educational level, income, physical activity, smoking, tea drinking, and history of diabetes, hypertension, and hyperlipidemia. In addition to the association between nut consumption and NAFLD in men, we also found the highest nut-consumption category was associated with a lower risk of developing NAFLD than the reference (OR = 0.53; 95% CI: 0.31–0.93) among the participants who engaged in light and moderate physical activity. No significant interactions of total nut consumption and the potentially modifying effects of NAFLD risk factors were identified (Figure 1).

Discussion

In this case-control study, we found a significant inverse relationship between high nut consumption and NAFLD risk among the males, but found either no or unclear associations among the females and the overall sample. Moreover, the associations seemed to be independent of other predictors, including diet and lifestyle factors. The effect of nut consumption remained among men after controlling for other known or suspected risk factors for developing NAFLD. Furthermore, no significant interactions between nut consumption and the potential modifying effects of NAFLD risk factors were identified. To the best of our knowledge, this was the first study to assess the association between nut consumption and the risk of NAFLD in a Chinese sample.

Our results are consistent with a previous study conducted in Korea, which found that a low intake of nuts and seeds (OR = 3.66; $P\text{-}_{trend} = 0.007$) was associated with a significantly higher risk of developing NAFLD among male participants, but not among females [19]. Although another case–control study found an association between

a high intake of nuts with a lower likelihood of NAFLD, after adjusting for the confounders age, sex, waist circumference, and the values of the homeostasis model assessment of insulin resistance (OR = 0.61; 95% CI: 0.38–0.98), the association disappeared after further adjustment for adiponectin and TNF-α (OR = 0.72; 95% CI: 0.41–1.25) [20]. However, the study did not examine nut intake separately; therefore, the results cannot be directly compared with those of our study. Although the results of our study suggest nuts may play a protective role in the development of NAFLD in males, a significant association between nut intake and the risk of NAFLD was not found in females. NAFLD has been regarded as the "hepatic manifestation of MetS"; therefore, our findings are indirectly supported by several studies showing an inverse correlation between high nut intake and the risk of T2D and cardiovascular disease, which share common metabolic parameters with NAFLD[21-23]. Nevertheless, the relationship between nut intake and NAFLD warrants further exploration, and additional studies are needed to examine gender differences in the association between nut consumption and NAFLD, and its' possible mechanisms should be explored.

Several biological pathways have been proposed to explain the association between nut intake and NAFLD risk. Ellagic acid, a natural antioxidant polyphenol found in nuts, can suppress oxidative stress and inflammation [24] and improve hepatic insulin sensitivity and lipid metabolism [25]. Vitamin E and selenium in almonds and walnuts, as well as folic acid and resveratrol in pine nuts, have been reported to have beneficial effects on NAFLD. Numerous trials testing the therapeutic value of vitamin E in NAFLD prevention found that Vitamin E significantly improved liver function and histologic changes by significantly reducing aspartate aminotransferase, alanine aminotransferase, steatosis, and inflammation in patients with NAFLD/non-alcoholic

steatohepatitis (NASH) [26]. Selenium is an antioxidant agent, and selenium deficiency is associated with insulin resistance in patients with chronic liver disease [27]. Folate plays an essential role in lipid metabolism and folic acid supplementation can attenuate steatosis and improve oxidative stress in rodent models of NAFLD. Folate can also blunt the increase of inflammatory cytokines secreted by immune cells [28]. Resveratrol has antioxidant, anti-apoptotic, and anti-inflammatory properties in NAFLD patients [29]. Nuts, which are known to be rich in unsaturated fatty acids, are especially rich in n-3 PUFAs, a source of alpha-linolenic acid, which lowers LDL cholesterol [30], and has been found to have beneficial effects on NAFLD [31]. Each type of nut has many nutrients and phytochemicals that may be beneficial to health, and it is likely that unknown salubrious effects of nuts may be related to NAFLD prevention. Moreover, many studies have shown a beneficial association between high nut intake and decreased risk of obesity [32], T2D [33], and MetS [34], which are risk factors for NAFLD.

Our study has both strengths and limitations. The first strength is the large sample size, which may have reduced type II errors. Second, the collection of data using face-to-face interviews and a semi-quantitative food frequency questionnaire, which elicited extensive information on anthropometrics and lifestyle factors, allowed us to adjust for confounding factors. Finally, the study had sufficient power to investigate interactions between nut intake and other risk factors, for which a biologically plausible mechanism may exist. Nonetheless, several limitations should be considered when interpreting the study's findings. First, although we accounted for a wide range of socioeconomic characteristics and lifestyle factors, we cannot exclude the effects of unknown or poorly measured confounding variables or residual confounding attributable to other dietary/lifestyle factors, which might have influenced the

observed associations. However, the associations persisted even after controlling for known and suspected predictors of NAFLD. Second, the intake of nuts was assessed by asking about using one question; therefore, we could not investigate the effects of different types of nuts, such as peanuts and walnuts, which contain different amounts of energy, fat content, and other nutrients[35]. Third, we did not consider manufacturing methods (e.g., raw, roasted, or boiled) or extra ingredients (e.g., sugar, salt, seasoning). Different preparation methods before and after roasting, the amount of time roasted, and the temperature used can affect the nutrient composition and ingredients in the nuts[36, 37]. Fourth, because we used a case-control study design, recall bias is an inherent limitation. People who are health-conscious may over-report or under-report some food items. However, the protective effect of nuts on NAFLD was not generally known at the time of the survey, and thus, should be unrelated to recall bias. Finally, due to the small number of participants in the highest nut-intake category, we cannot rule out the possibility that some of our results are due to chance. However, the associations were consistent in the analyses stratified by some of the other factors, which reduces the likelihood of chance findings. In these stratified analyses, the statistical power needed to detect differences was limited by the sample size, and such analyses should be considered exploratory.

Our study had a retrospective design, which is lower on the evidence hierarchy than prospective studies. Nevertheless, case-control studies can provide evidence supporting the general relationship between diet and NAFLD, as there currently are no available prospective studies.

In conclusion, this case-control study indicated that high nut intake was associated with a significantly reduced risk of NAFLD among Han men in China. However, no relationship was found between total nut intake and NAFLD risk among the Han

women. Our data contribute to the growing evidence showing that a relatively simple prevention strategy of incorporating a modest amount of nuts in the diet may contribute to maintaining good health at both the individual and population levels.

Acknowledgements

We thank all study participants for their cooperation. We also thank our staff for recruiting participants and for their technical assistance.

Contributors

BBC and YH are joint first authors. XEP obtained funding. XEP, XL and SHX designed the study. XTP ,JHY,YFL and WJL collected the data. BBC and YH were involved in data cleaning and verification. BBC and XTP analyzed the data. BBC and YH drafted the manuscript. XEP ,YFL and JHY contributed to the interpretation of the results and critical revision of the manuscript for important intellectual content and approved the final version of the manuscript. All authors have read and approved the final manuscript.

Data sharing

No additional data are available.

Conflict of Interest

The authors declare that they have no conflict of interest.

Funding

This work was supported by the National Natural Science Foundation of China [grant number 81473047], the Training Program Foundation for Middle-aged and Young Talents from the Sanitation System of Fujian Province [grant number 2014-ZQN-ZD-23], and the Joint Innovation Project of Science and Technology of Fujian Province [grant number 2017Y9104].

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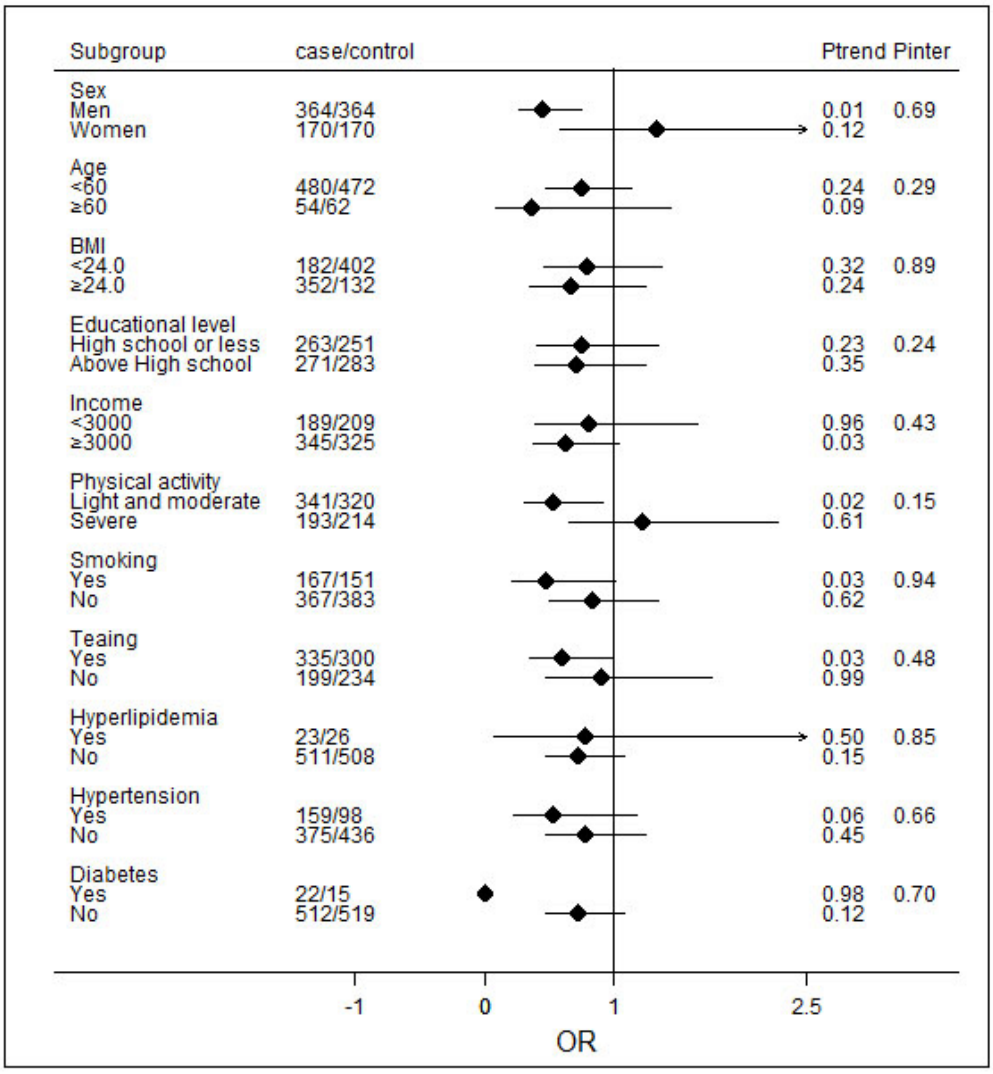
Figure legends

Figure 1. Odds ratios (OR) and 95% CIs for NAFLD across the strata of various factors.

The forest plot represents the ORs of the comparison of the lowest nut-intake category versus the highest nut-intake category, adjusting for age, income, smoking status, educational level, tea-drinking status, occupational status, marital status, BMI, physical activity, history of diabetes, hypertension and hyperlipidemia, total energy intake, and MUFA and PUFA intake. *P*-values are two-tailed; P_{inter} indicates *P* for the interaction between strata and nut intake; P_{trend} indicates *P* for the trend across levels of nut intake.



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148x159mm (96 x 96 DPI)

BMJ Open

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Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-028961.R1
Article Type:	Research
Date Submitted by the Author:	27-Apr-2019
Complete List of Authors:	Chen, Bing bing; Fujian Medical University, Han, Ying; The First Affiliated Hospital of Fujian Medical University Pan, Xinting; Department of Epidemiology and Health Statistics, Fujian Provincial Key Laboratory of Environment Factors and Cancer, School of Public Health, Fujian Medical University Yan, Jianhui; Department of Epidemiology and Health Statistics, Fujian Provincial Key Laboratory of Environment Factors and Cancer, School of Public Health, Fujian Medical University Liu, Wenjuan; Department of Epidemiology and Health Statistics, Fujian Provincial Key Laboratory of Environment Factors and Cancer, School of Public Health, Fujian Medical University li, yangfan; Department of Epidemiology and Health Statistics, Fujian Provincial Key Laboratory of Environment Factors and Cancer, School of Public Health, Fujian Medical University, Fujian, China, ; lin, xu; Key Laboratory of Ministry of Education for Gastrointestinal Cancer, Fujian Medical University, Fujian, China xu, shanghua; Department of Cardiology, Affiliated Nanping First Hospital, Fujian Medical University, Nanping, China Peng, Xian-E; Key Laboratory of Ministry of Education for Gastrointestinal Cancer, Fujian Medical University, Fujian,
Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Nutrition and metabolism, Public health
Keywords:	EPIDEMIOLOGY, NUTRITION & DIETETICS, Lipid disorders < DIABETES & ENDOCRINOLOGY

Association between nut intake and Non-alcoholic fatty liver disease risk: a retrospective case-control study in a sample of Chinese Han adults

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Running title： Nut intake and non-alcoholic fatty liver disease

Word count：2823

Abstract

Objectives: Nut consumption has been associated with a lower risk of type 2 diabetes, metabolic syndrome, and insulin resistance. However, its effect on the risk of non-alcoholic fatty liver disease (NAFLD) is unknown. Therefore, we investigated the relationship between nut consumption and NAFLD risk.

Setting and participants: We conducted a retrospective case-control study including 534 patients diagnosed with NAFLD and 534 controls matched by sex and age (± 5) from the Affiliated Nanping First Hospital of Fujian Medical University in China.

Main outcome measures: Information on dietary intake was collected using a semi-quantitative food frequency questionnaires and nut consumption was calculated. Nut consumption was categorized using quartiles based on the distribution of daily nut intake of the controls. Binary logistic regression models were used to estimate odds ratio (ORs) and the 95% confidence intervals (CIs) for the association between nut consumption and NAFLD risk.

Results: After adjusting for potential confounding variables, nut consumption was not associated with NAFLD risk in the overall sample. When the fully adjusted model was stratified by sex, a significant inverse association was found between high nut consumption and NAFLD only among the men in the highest quartile (OR = 0.45; 95% CI: 0.27–0.76; P_{trend} = 0.01). The inverse association of nut consumption with NAFLD risk in men remained significant after controlling for other known or suspected risk factors for NAFLD.

Conclusions: Diets with a higher intake of nuts may be associated with a decreased risk of NAFLD, particularly in men.

Strengths and limitations of this study

1.This study had a considerable sample size and several potential confounding variables such as energy intake and physical activity, were taken into account.

2.The study had sufficient power to investigate interactions between nut intake and other risk factors, for which a biologically plausible mechanism may exist.

3.This study was a case-control design, thus the causal association between nut intake and NAFLD could not be precisely identified.

4.This study was a case-control study , recall bias was inevitable and randomized controlled trial studies are therefore required for more accurate results.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is defined as macrovesicular steatosis in ≥5% of hepatocytes, in the absence of a secondary cause, such as alcohol or drug use. NAFLD has become a leading cause of chronic liver disease, with a 25% prevalence worldwide [1].Furthermore, a fatty liver is more prevalent in men compared with women[2]. The prevalence of NAFLD among the adults in China's general population is approximately 15% (6.3–27.0%), depending on the population studied [3]. Many studies suggest that diet and lifestyle may significantly influence the risk of NAFLD [4 5]. These studies indicate that type 2 diabetes (T2D) [6], metabolic syndrome (MetS), obesity [7], physical activity, and a high-fat diet [4] are associated with an increased risk for the development of NAFLD.

Nuts are nutrient-dense foods with complex matrices rich in unsaturated fatty and other bioactive compounds (e.g., high-quality vegetable protein, fiber, minerals, tocopherols, phytosterols, and phenolic compounds) [8]. The global intake of nuts has increased by 59% over the past decade [9]. China's per capita intake of nuts was 2.2 g/day in 1982, and increased to 3.8g/day in 2012 [10]. Although they are high in fat and

energy dense, a high intake of nuts has been associated with several health benefits, including reduced risk of cardiovascular disease [11], T2D [12], MetS[13], and insulin resistance[14] [15].Moreover, nuts have antioxidative effects by decreasing lipid peroxidation and protecting against oxidative DNA damage [16]. Beneficial health effects have been attributed to the macronutrient and micronutrient profiles of nuts [17].

NAFLD is regarded as the "hepatic manifestation of MetS." Few studies have assessed the effects of nut intake on NAFLD, despite previous findings of an inverse correlation between high nut consumption and the risk of T2D and cardiovascular disease. In this study, we analyzed the association between nut intake and NAFLD risk and the interactions between nut intake and other established NAFLD risk factors in a large case-control study with a sample of Han adults in China.

Methods

Study design

We conducted the retrospective case-control study in a health examination center at the Affiliated Nanping First Hospital of Fujian Medical University from April 2015 to August 2017. Data were obtained from subjects who underwent routine health examination in the examination center. Patients newly diagnosed with NAFLD using abdominal ultrasonography in accordance with the "Guidelines for the diagnosis and managementof nonalcoholic fatty liver disease: update 2010" were included in the study. Hepatic ultrasonography examination was performed by trained ultrasonographists who were blinded to the clinical and laboratory data. Hepatic steatosis was diagnosed by characteristic echo patterns according to conventional criteria, such as the evidence of diffuse hyper-echogenicity of the liver relative to the kidneys, ultrasound beam attenuation, and poor visualization of intrahepatic structures.

Sample size calculation

This study is a case-control design, thus we estimate the sample size based on the Case-control study formula. By consulting the literature[18],we estimate OR=0.74, ρ_0=0.8, the calculated sample size was N_{case} =$N_{control}$=489. Finally 1068 subjects (534 cases and 534 controls) were recruited in this study.

Outcome--eligibility of NAFLD cases and controls

All participants were of Chinese Han ethnicity.The cases were newly diagnosed with NAFLD. The exclusion criteria were as follows: (a) daily alcohol intake of >40 g (men) and >20 g (women), (b) a history of other liver diseases including drug-induced liver disease, viral hepatitis, autoimmune hepatitis, total parenteral nutrition, and hepatolenticular degeneration, (c) taking hypolipidemic or weight reduction drugs, (d) age <18 or >70 years, (e) non-resident of Nanping, or (f) not of Han ethnicity. Adults who reported extremely abnormal levels of energy intake (2 511.60 kJ [600 kcal] or 17 581.20 kJ [4 200kcal] per day for men; 2 093 kJ [500 kcal] or 14,651.00 kJ [3500 kcal] per day for women), and those who did not answer 25 or more food-related items or questions about nut intake on the questionnaire, were excluded from the study.

The controls were randomly selected from the same center during the study period. Their eligibility criteria were identical to those of the cases, except for the requirement of a diagnosis of liver steatosis; they were frequency-matched with cases by age (within 5-yr intervals), gender, ethnicity, and region of origin.

Potential confounders--Data measurements and data collection

Trained interviewers performed a comprehensive medical history on each participant that included eliciting information about their demographic and socio-economic characteristics (e.g., age, gender, education, income, marriage status

and history of diabetes, hypertension, hyperlipidemiaand), lifestyle habits (e.g., smoking, drinking (alcohol), tea drinking, and physical activity), anthropometric assessment (e.g., height, body weight and blood pressure). The data were obtained from participants using structured questionnaires during face-to-face interviews.

Exposure-Nut consumption

Information about participants' typical food consumption was collected using a semi-quantitative food frequency questionnaire that included 110 food items, which was developed and validated in a sample from southern China [19]. Participants were asked to estimate the average frequency of consumption of selected foods using the following response options: rarely, <once/month, 1–3 times/month, 1–2 times/week, 3–4 times/week, 5–6 times/week, once/day, twice/day, and >twice/day. Nut intake was defined as the consumption of "peanuts, walnuts, seeds, or other nuts." Data from a semi-quantitative food frequency questionnaire were used to calculate daily nut and energy intake. Nut consumption was converted to grams/day by multiplying the food-intake frequency by fixed portion sizes. Nutrient intake, such as mono-unsaturated fatty acids (MUFAs) and poly-unsaturated fatty acids (PUFAs) were calculated by multiplying the intake frequency of each food by the nutrient content of the specified portion, and summing the products of all the food items.

Statistical analyses

Participants' characteristics were analyzed using Pearson's Chi-square test for categorical variables, and Wilcoxon's rank-sum test and ANOVA for continuous variables. Nut consumption was categorized by quartiles based on the distribution of daily nut intake by the controls (Q1-Q4). Binary logistic regression models were used to estimate the odds ratios (ORs) and their 95% confidence intervals (CIs) for the associations between nut consumption and NAFLD risk. To account for the energy

content of the nut, our initial model adjusted for total energy intake by using the residuals method. The final model adjusted for potential confounders, including age, income, smoking status, educational level, and tea-drinking status, occupational status, marital status, body mass index (BMI), physical activity, and history of diabetes, hypertension, and hyperlipidemia. The final model also adjusted for MUFA and PUFA intake to control for their effects. The selection of covariates for the final model was based on clinical significance, results of previous studies. We tested for linear trends across categories of nut intake by assigning each participant the median value for each category and modeling this value as a continuous variable, consistent with prior studies[20-22].

We evaluated the influence of nut consumption across strata of other potential confounders, comparing participants in the highest nut-consumption category to the lowest nut-consumption category(reference). We also analyzed the interactions of nut consumption with age, sex, BMI, smoking status, educational level, tea-drinking status, and other factors. We used the medians of the continuous variables to categorize them and evaluate the interactions. The criteria for statistical significance of the likelihood-ratio test of interaction effects was $P < 0.05$. Statistical analyses were performed using SPSS version 22 (IBM, Armonk, NY, US). All P-values were 2-tailed, and $P < 0.05$ was considered statistically significant.

Patient and public involvement statement

The study was approved by the local ethics committees of Fujian Medical University (ethics number 2014096). All methods were performed in accordance with the relevant guidelines and regulations.In addition, each subject gave written informed consent before participation in the study.

Results

Baseline Characteristics

Table 1 presents health-related and demographic characteristics of the NAFLD cases and matched controls. Compared with the controls, the NAFLD cases tended to have a higher BMI, higher total intake of energy, higher MUFA and PUFA consumption, and a higher number of participants with a history of hyperlipidemia among both men and women. Patients with NAFLD had a lower educational level and the men engaged in less physical activity than the controls. No statistically significant associations were found for age, occupational status, income, marital status, smoking status, history of diabetes, or hypertension between the cases and the controls.

Table 1 General characteristics of cases and controls stratified by sex,*n*(%)

Variables	Men (*n*=728)			Women(*n*=340)		
	Cases	Controls(*n*=364)	*P*-value[a]	Cases (*n*=170)	Controls(*n*=170)	*P*-value[a]
Age			0.66			0.99
＜40	118(32.42)	116(31.87)		25(14.71)	22(12.94)	
40~60	217(59.61)	212(58.24)		123(72.35)	122(71.76)	
≥60	29(7.97)	36(9.89)		22(12.94)	26(15.30)	
Educational level			0.04			0.75
primary school and less than	10(2.75)	23(6.32)		30(17.65)	26(15.29)	
junior middle and high school	147(40.38)	128(35.16)		76(44.70)	74(43.53)	
junior college or above	207(56.87)	213(58.52)		64(37.65)	70(41.18)	
Occupational status			0.39			0.43
mental labour	108(29.67)	104(28.57)		50(29.41)	48(28.24)	
physical labor	86(23.63)	102(28.02)		25(14.71)	34(20.00)	
other	170(46.70)	158(43.41)		95(55.88)	88(51.76)	
Income(yuan/month)			0.35			0.85
＜2000	17(4.67)	17(4.67)		15(8.82)	18(10.59)	
2000~3000	87(23.90)	104(28.57)		70(41.18)	70(41.18)	
≥3000	260(71.43)	243(66.76)		85(50.00)	82(48.23)	
Marital status			0.24			0.52
single	45(12.36)	56(15.38)		4(2.35)	6(3.53)	
married or other	319(87.64)	308(84.62)		166(97.65)	164(96.47)	
Smoking status			0.30			0.16
never smoker	199(54.67)	213(58.52)		168(98.82)	170(100)	
smoker	165(45.33)	151(41.48)		2(1.18)	0(0.00)	
Tea-drinking status			0.30			0.01
yes	258(70.88)	245(63.31)		77(45.29)	55(32.35)	
no	106(29.12)	119(32.69)		93(54.71)	115(67.55)	
Physical activity			0.02			0.88
light	175(48.08)	137(37.64)		19(11.18)	19(11.18)	
moderate	100(27.47)	121(33.24)		47(27.64)	43(25.29)	
severe	89(24.45)	106(29.12)		104(61.18)	108(63.53)	
BMI(kg/m^2)			<0.001			<0.001
＜18.5	2(0.55)	11(3.02)		1(0.59)	9(5.29)	
18.5~24.0	107(29.40)	257(70.61)		72(42.35)	125(73.53)	
≥24.0	255(70.05)	96(26.37)		97(57.06)	36(21.18)	
History of diabetes			0.67			0.18
yes	12(3.30)	10(2.75)		10(5.88)	5(2.94)	
no	352(96.70)	354(97.25)		160(94.12)	165(94.06)	
History of hypertension			0.85			0.63
yes	15(4.12)	16(4.40)		8(4.71)	10(5.88)	
no	349(95.88)	348(95.60)		162(95.29)	160(94.12)	
History of hyperlipidemia			0.01			<0.001
yes	108(29.67)	77(21.15)		51(30.00)	21(12.35)	
no	256(70.33)	287(78.85)		119(70.00)	149(87.65)	
MUFA intake,g/d[b]	34.10(28.49,	31.39(26.55,36.79)	<0.001	29.99(26.37,36.37)	28.05(22.40,31.39)	<0.001
PUFA intake,g/d[b]	26.25(24.04,	23.90(22.01,26.02)	<0.001	24.52(22.11,27.12)	21.21(18.83,24.53)	<0.001
Energy intake,kJ/d[c]	10395.64±24	9890.97±2348.76	0.01	7509.73±1650.71	7323.78±1852.97	0.04

[a]*P*-values were calculated by using the Chi-square test for categorical variables and Wilcoxon rank sum test and ANOVA test for continues variables.

[b]Medians (IQRs).

[c]Mean(s.d).

The associations of dietary nut intake with NAFLD

The participants' nut consumption is summarized in Table 2. Among the women, those in the NAFLD group consumed a significantly higher amount of nuts (6.80 g/d vs. 2.50 g/d; P = 0.02) than those in the control group. No statistically significant differences were found between the cases and controls in the the sample or among the male participants. In order to adjust for potential confounding factors, quartile distributions of dietary nut consumption among the controls were used to categorize the nut intake of all the participants; the results are shown in Table 3. After adjusting for potential confounders, nut consumption was not associated with NAFLD risk among the participants in the sample. When the fully adjusted model was stratified by sex, a significant inverse association was found between nut consumption and NAFLD, but only among the men in the highest quartile (OR = 0.45; 95% CI: 0.27–0.76; P_{trend} = 0.01).

Table 2 Comparison of nut daily intake between the case and the control

Nut intake (g/d)	Case		Control		*P*-value[a]	*P*-value[b]
	Median	Quartile	Median	Quartile		
Total population	3.15	1.46-8.80	2.86	1.22-8.98	0.35	0.18
Men	2.68	1.15-8.43	2.86	1.22-8.98	0.36	0.94
Women	6.80	1.75-8.86	2.50	1.07-7.84	0.01	0.02

[a]*P*-values:calculated by using Wilcoxon rank sum test;before adjusting for energy.

[b]*P*-values :calculated by using Wilcoxon rank sum test; after adjusting for energy by using the residuals methods.

Table 3 Odds ratios (OR) and 95% confidence intervals (Cl) for NAFLD in relation to nut intake

Nut intake	Case *n*	Control *n*	Crude OR(95%CI)	Model 1[a] OR(95%CI)	Model 2[a] OR(95%CI)
Total population[b]	534	534			
Q1	125	133	1	1	1
Q2	112	134	1.30(0.93-1.81)	0.89(0.63-1.26)	0.98(0.65-1.47)
Q3	159	134	1.77(1.29-2.44)	1.26(0.90-1.77)	1.16(0.78-1.72)
Q4	138	133	1.17(0.81-1.68)	1.10(0.78-1.55)	0.85(0.57-1.27)
P for trend*			0.46	0.45	0.07
Men	364	364			
Q1	106	102	1	1	1
Q2	91	79	1.31(0.88-1.94)	0.90(0.60-1.35)	0.92(0.56-1.49)
Q3	74	123	1.81(1.24-2.65)	1.73(1.16-2.57)	1.36(0.85-2.18)
Q4	93	60	1.04(0.66-1.63)	0.67(0.44-1.02)	0.45(0.27-0.76)
P for trend*			0.81	0.04	0.01
Women	170	170			
Q1	23	27	1	1	1
Q2	33	43	1.28(0.69-2.37)	0.90(0.44-1.85)	0.86(0.37-2.02)
Q3	36	60	1.71(0.95-3.08)	0.70(0.35-1.41)	0.56(0.25-1.28)
Q4	78	40	1.41(0.76-2.64)	2.29(1.17-4.49)	1.34(0.59-3.04)
P for trend*			0.36	<0.001	0.12

[a]Model 1:Adjusted for total energy intake.

[a]Model 2:Adjusted further for age,income,smoking status,educational level, teaing status, occupationanl status,marital status,body mass index,physical activity,the history of diabetes,hypertension and hyperlipidemia,MUFA and PUFA intake.

[b]:further adiusted for sex.

*P for trend: Trend across intake levels with the categorical median.

For total population: Q1, <1.46 g/d; Q2, 1.46-3.15 g/d; Q3, 3.15-8.80 g/d; Q4, ≥8.80 g/d

For men: Q1, <1.15 g/d; Q2, 1.15-2.68 g/d; Q3,2.68-8.43 g/d; Q4, ≥8.43g/d

For women: Q1, <1.75 g/d; Q2, 1.75-6.80 g/d; Q3, 6.80-8.86 g/d; Q4, ≥8.86 g/d.

Stratified Analyses

In the stratified analysis, the inverse association between total-nut consumption and NAFLD risk was consistent across strata of age, sex, BMI, educational level, income, physical activity, smoking, tea drinking, and history of diabetes, hypertension, and hyperlipidemia. In addition to the association between nut consumption and NAFLD in men, we also found the highest nut-consumption category was associated

with a lower risk of developing NAFLD than the reference (OR = 0.53; 95% CI: 0.31–0.93) among the participants who engaged in light and moderate physical activity. No significant interactions of total nut consumption and the potentially confounding effects of NAFLD risk factors were identified (Figure 1).

Discussion

In this case-control study, we found a significant inverse relationship between high nut consumption and NAFLD risk among the males, but found either no or unclear associations among the females and the overall sample. Moreover, the associations seemed to be independent of other predictors, including diet and lifestyle factors. The effect of nut consumption remained among men after controlling for other known or suspected risk factors for developing NAFLD. Furthermore, no significant interactions between nut consumption and the potential modifying effects of NAFLD risk factors were identified. To the best of our knowledge, this was the first study to assess the association between nut consumption and the risk of NAFLD in a Chinese sample.

Our results are consistent with a previous study conducted in Korea, which found that a low intake of nuts and seeds (OR = 3.66; $P\text{-}_{trend}$ = 0.007) was associated with a significantly higher risk of developing NAFLD among male participants, but not among females [23]. Although another case–control study found an association between a high intake of nuts with a lower likelihood of NAFLD, after adjusting for the confounders age, sex, waist circumference, and the values of the homeostasis model assessment of insulin resistance (OR = 0.61; 95% CI: 0.38–0.98), the association disappeared after further adjustment for adiponectin and TNF-α (OR = 0.72; 95% CI: 0.41–1.25) [18]. However, the study did not examine nut intake separately; therefore, the results cannot be directly compared with those of our study. Although the results

of our study suggest nuts may play a protective role in the development of NAFLD in males, a significant association between nut intake and the risk of NAFLD was not found in females. NAFLD has been regarded as the "hepatic manifestation of MetS"; therefore, our findings are indirectly supported by several studies showing an inverse correlation between high nut intake and the risk of T2D and cardiovascular disease, which share common metabolic parameters with NAFLD [24-26]. Nevertheless, the relationship between nut intake and NAFLD warrants further exploration, and additional studies are needed to examine gender differences in the association between nut consumption and NAFLD, and its' possible mechanisms should be explored.

Several biological pathways have been proposed to explain the association between nut intake and NAFLD risk. Ellagic acid, a natural antioxidant polyphenol found in nuts, can suppress oxidative stress and inflammation [27] and improve hepatic insulin sensitivity and lipid metabolism [28]. Vitamin E and selenium in almonds and walnuts, as well as folic acid and resveratrol in pine nuts, have been reported to have beneficial effects on NAFLD. Numerous trials testing the therapeutic value of vitamin E in NAFLD prevention found that Vitamin E significantly improved liver function and histologic changes by significantly reducing aspartate aminotransferase, alanine aminotransferase, steatosis, and inflammation in patients with NAFLD/non-alcoholic steatohepatitis (NASH) [29]. Selenium is an antioxidant agent, and selenium deficiency is associated with insulin resistance in patients with chronic liver disease [30]. Folate plays an essential role in lipid metabolism and folic acid supplementation can attenuate steatosis and improve oxidative stress in rodent models of NAFLD. Folate can also blunt the increase of inflammatory cytokines secreted by immune cells [31]. Resveratrol has antioxidant, anti-apoptotic, and anti-inflammatory properties in

NAFLD patients [32]. Nuts, which are known to be rich in unsaturated fatty acids, are especially rich in n-3 PUFAs, a source of alpha-linolenic acid, which lowers LDL cholesterol [33], and has been found to have beneficial effects on NAFLD [34]. Each type of nut has many nutrients and phytochemicals that may be beneficial to health, and it is likely that unknown salubrious effects of nuts may be related to NAFLD prevention. Moreover, many studies have shown a beneficial association between high nut intake and decreased risk of obesity [35], T2D [36], and MetS [37], which are risk factors for NAFLD.

Our study has both strengths and limitations. The first strength is the large sample size, which may have reduced type II errors. Second, the collection of data using face-to-face interviews and a semi-quantitative food frequency questionnaire, which elicited extensive information on anthropometrics and lifestyle factors, allowed us to adjust for confounding factors. Finally, the study had sufficient power to investigate interactions between nut intake and other risk factors, for which a biologically plausible mechanism may exist. Nonetheless, several limitations should be considered when interpreting the study's findings. First, although we accounted for a wide range of socioeconomic characteristics and lifestyle factors, we cannot exclude the effects of unknown or poorly measured confounding variables or residual confounding attributable to other dietary/lifestyle factors, which might have influenced the observed associations. However, the associations persisted even after controlling for known and suspected predictors of NAFLD. Second, the intake of nuts was assessed by asking about using one question; therefore, we could not investigate the effects of different types of nuts, such as peanuts and walnuts, which contain different amounts of energy, fat content, and other nutrients[38]. Third, categorization of the intake of nuts in quartiles may lead to bias and inefficiency. Fourth, we did not consider

manufacturing methods (e.g., raw, roasted, or boiled) or extra ingredients (e.g., sugar, salt, seasoning). Different preparation methods before and after roasting, the amount of time roasted, and the temperature used can affect the nutrient composition and ingredients in the nuts[39 40]. What's more, the item'nuts' in the FFQ used in this study cannot represent all nuts, because it only included peanuts, walnuts, seeds. For this reason, direct comparisons with the results of other studies may be difficult. Fifth, because we used a case-control study design, recall bias is an inherent limitation. People who are health-conscious may over-report or under-report some food items. However, the protective effect of nuts on NAFLD was not generally known at the time of the survey, and thus, should be unrelated to recall bias. In addition, the stratified analyses was not corrected for multiple testing, which may inflate the risk for type I error. Finally, due to the small number of participants in the highest nut-intake category, we cannot rule out the possibility that some of our results are due to chance. However, the associations were consistent in the analyses stratified by some of the other factors, which reduces the likelihood of chance findings. In these stratified analyses, the statistical power needed to detect differences was limited by the sample size, and such analyses should be considered exploratory.

Our study had a retrospective design, which is lower on the evidence hierarchy than prospective studies. Nevertheless, case-control studies can provide evidence supporting the general relationship between diet and NAFLD, as there currently are no available prospective studies.

In conclusion, this case-control study indicated that high nut intake was associated with a significantly reduced risk of NAFLD among Han men in China. However, no relationship was found between total nut intake and NAFLD risk among the Han women. Our data contribute to the growing evidence showing that a relatively simple

prevention strategy of incorporating a modest amount of nuts in the diet may contribute to maintaining good health at both the individual and population levels.

Acknowledgements

We thank all study participants for their cooperation. We also thank our staff for recruiting participants and for their technical assistance.

Contributors

BBC and YH are joint first authors. XEP obtained funding. XEP, XL and SHX designed the study. XTP ,JHY,YFL and WJL collected the data. BBC and YH were involved in data cleaning and verification. BBC and XTP analyzed the data. BBC and YH drafted the manuscript. XEP ,YFL and JHY contributed to the interpretation of the results and critical revision of the manuscript for important intellectual content and approved the final version of the manuscript. All authors have read and approved the final manuscript.

Data sharing

No additional data are available.

Conflict of Interest

The authors declare that they have no conflict of interest.

Funding

This work was supported by the National Natural Science Foundation of China [grant number 81473047], the Training Program Foundation for Middle-aged and Young Talents from the Sanitation System of Fujian Province [grant number 2014-ZQN-ZD-23], and the Joint Innovation Project of Science and Technology of Fujian Province [grant number 2017Y9104].

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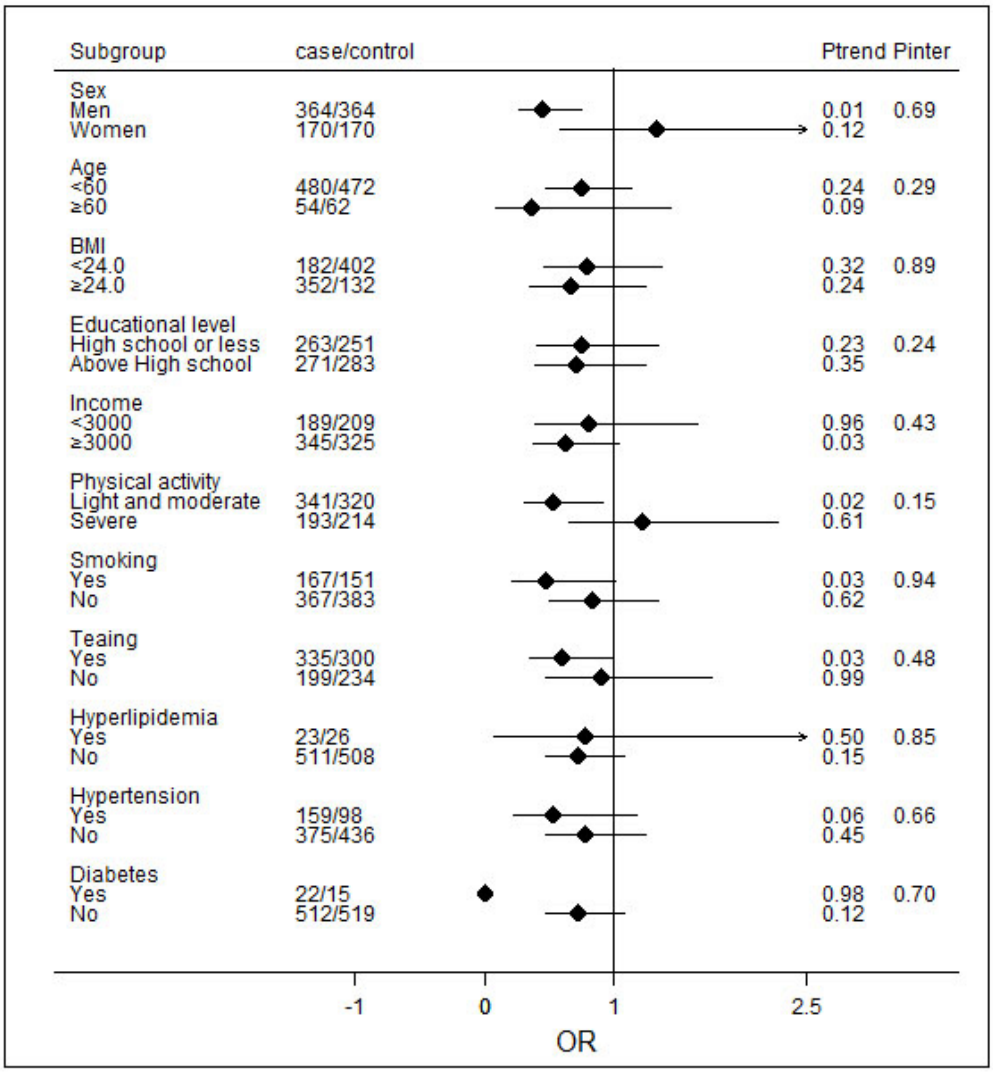
Figure legends

Figure 1. Odds ratios (OR) and 95% CIs for NAFLD across the strata of various factors.

The forest plot represents the ORs of the comparison of the lowest nut-intake category versus the highest nut-intake category, adjusting for age, income, smoking status, educational level, tea-drinking status, occupational status, marital status, BMI, physical activity, history of diabetes, hypertension and hyperlipidemia, total energy intake, and MUFA and PUFA intake. *P*-values are two-tailed; P_{inter} indicates *P* for the interaction between strata and nut intake; P_{trend} indicates *P* for the trend across levels of nut intake.



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148x159mm (96 x 96 DPI)

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *case-control studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	*(*a) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls	5
		(*b*) For matched studies, give matching criteria and the number of controls per case	5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5-6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5-6
Bias	9	Describe any efforts to address potential sources of bias	7

Study size	10	Explain how the study size was arrived at	5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5-6
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6-7
		(*b*) Describe any methods used to examine subgroups and interactions	7
		(*c*) Explain how missing data were addressed	
		(*d*) If applicable, explain how matching of cases and controls was addressed	5
		(*e*) Describe any sensitivity analyses	
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8-9
		(b) Indicate number of participants with missing data for each variable of interest	
Outcome data	15*	Report numbers in each exposure category, or summary measures of exposure	8-9
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9-10
		(*b*) Report category boundaries when continuous variables were categorized	10-11

		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	11
Discussion			
Key results	18	Summarise key results with reference to study objectives	12
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	14
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12-14
Generalisability	21	Discuss the generalisability (external validity) of the study results	15
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	17

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Association between nut intake and Non-alcoholic fatty liver disease risk: a retrospective case-control study in a sample of Chinese Han adults

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-028961.R2
Article Type:	Research
Date Submitted by the Author:	25-Jun-2019
Complete List of Authors:	Chen, Bing bing; Fujian Medical University, Han, Ying; The First Affiliated Hospital of Fujian Medical University Pan, Xinting; Department of Epidemiology and Health Statistics, Fujian Provincial Key Laboratory of Environment Factors and Cancer, School of Public Health, Fujian Medical University Yan, Jianhui; Department of Epidemiology and Health Statistics, Fujian Provincial Key Laboratory of Environment Factors and Cancer, School of Public Health, Fujian Medical University Liu, Wenjuan; Department of Epidemiology and Health Statistics, Fujian Provincial Key Laboratory of Environment Factors and Cancer, School of Public Health, Fujian Medical University li, yangfan; Department of Epidemiology and Health Statistics, Fujian Provincial Key Laboratory of Environment Factors and Cancer, School of Public Health, Fujian Medical University, Fujian, China, ; lin, xu; Key Laboratory of Ministry of Education for Gastrointestinal Cancer, Fujian Medical University, Fujian, China xu, shanghua; Department of Cardiology, Affiliated Nanping First Hospital, Fujian Medical University, Nanping, China Peng, Xian-E; Key Laboratory of Ministry of Education for Gastrointestinal Cancer, Fujian Medical University, Fujian,
Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Nutrition and metabolism, Public health
Keywords:	EPIDEMIOLOGY, NUTRITION & DIETETICS, Lipid disorders < DIABETES & ENDOCRINOLOGY

Association between nut intake and Non-alcoholic fatty liver disease risk: a retrospective case-control study in a sample of Chinese Han adults

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Running title： Nut intake and non-alcoholic fatty liver disease

Word count：3055

Abstract

Objectives: Nut consumption has been associated with a lower risk of type 2 diabetes, metabolic syndrome, and insulin resistance. However, its effect on the risk of non-alcoholic fatty liver disease (NAFLD) is unknown. Therefore, we investigated the relationship between nut consumption and NAFLD risk.

Setting and participants: We conducted a retrospective case-control study including 534 patients diagnosed with NAFLD and 534 controls matched by sex and age (± 5) from the Affiliated Nanping First Hospital of Fujian Medical University in China.

Main outcome measures: Information on dietary intake was collected using a semi-quantitative food frequency questionnaires and nut consumption was calculated. Nut consumption was categorized using quartiles based on the distribution of daily nut intake of the controls. Binary logistic regression models were used to estimate odds ratio (ORs) and the 95% confidence intervals (CIs) for the association between nut consumption and NAFLD risk.

Results: After adjusting for potential confounding variables, nut consumption was not associated with NAFLD risk in the overall sample. When the fully adjusted model was stratified by sex, a significant inverse association was found between high nut consumption and NAFLD only among the men in the highest quartile (OR = 0.43; 95% CI: 0.26–0.71; P_{trend} = 0.01). The inverse association of nut consumption with NAFLD risk in men remained significant after controlling for other known or suspected risk factors for NAFLD.

Conclusions: Diets with a higher intake of nuts may be associated with a decreased risk of NAFLD, particularly in men.

Strengths and limitations of this study

1.In this study, several potential confounding variables such as energy intake and physical activity, were taken into account.

2.The study had tried to investigate interactions between nut intake and other risk factors, for which a biologically plausible mechanism may exist.

3.This study was a case-control design, thus the causal association between nut intake and NAFLD could not be precisely identified.

4.This study was a case-control study , recall bias was inevitable and randomized controlled trial studies are therefore required for more accurate results.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is defined as macrovesicular steatosis in ≥5% of hepatocytes, in the absence of a secondary cause, such as alcohol or drug use. NAFLD has become a leading cause of chronic liver disease, with a 25% prevalence worldwide [1].Furthermore, a fatty liver is more prevalent in men compared with women[2]. The prevalence of NAFLD among the adults in China's general population is approximately 15% (6.3–27.0%), depending on the population studied [3]. Many studies suggest that diet and lifestyle may significantly influence the risk of NAFLD [4 5]. These studies indicate that type 2 diabetes (T2D) [6], metabolic syndrome (MetS), obesity [7], physical activity, and a high-fat diet [4] are associated with an increased risk for the development of NAFLD.

Nuts are nutrient-dense foods with complex matrices rich in unsaturated fatty and other bioactive compounds (e.g., high-quality vegetable protein, fiber, minerals, tocopherols, phytosterols, and phenolic compounds) [8]. The global intake of nuts has increased by 59% over the past decade [9]. China's per capita intake of nuts was 2.2 g/day in 1982, and increased to 3.8g/day in 2012 [10]. Although they are high in fat and

energy dense, a high intake of nuts has been associated with several health benefits, including reduced risk of cardiovascular disease [11], T2D [12], MetS[13], and insulin resistance[14] [15].Moreover, nuts have antioxidative effects by decreasing lipid peroxidation and protecting against oxidative DNA damage [16]. Beneficial health effects have been attributed to the macronutrient and micronutrient profiles of nuts [17].

NAFLD is regarded as the "hepatic manifestation of MetS." Few studies have assessed the effects of nut intake on NAFLD, despite previous findings of an inverse correlation between high nut consumption and the risk of T2D and cardiovascular disease. In this study, we analyzed the association between nut intake and NAFLD risk and the interactions between nut intake and other established NAFLD risk factors in a large case-control study with a sample of Han adults in China.

Methods

Patient and public involvement statement

The study was approved by the local ethics committees of Fujian Medical University (ethics number 2014096). All subjects underwent abdominal ultrasound and blood biochemical tests. Once cases and controls have been linked to the NAFLD, a letter of invitation and information about the study will be sent to each potential case and control to obtain consent. All methods were performed in accordance with the relevant guidelines and regulations. The subjects were not involved in the design and planning of the study .They were not involved in the recruitment to and conduct of the study.

Study design

We conducted the retrospective case-control study in a health examination cent er at the Affiliated Nanping First Hospital of Fujian Medical University from April 2015 to August 2017. Data were obtained from subjects who underwent

routine health examination in the examination center. Patients newly diagnosed with NAFLD using abdominal ultrasonography in accordance with the "Guidelines for the diagnosis and managementof nonalcoholic fatty liver disease: update 2010" were included in the study. Hepatic ultrasonography examination was performed by trained ultrasonographists who were blinded to the clinical and laboratory data. Hepatic steatosis was diagnosed by characteristic echo patterns according to conventional criteria, such as the evidence of diffuse hyper-echogenicity of the liver relative to the kidneys, ultrasound beam attenuation, and poor visualization of intrahepatic structures.

Sample size calculation

This study is a case-control design, thus we estimate the sample size based on the case-control study formula: $n = 2\overline{pq}(z_\alpha + z_\beta)^2/(p_1 - p_0)^2$, $p_1 = \frac{p_0 RR}{1 + p_0(RR-1)}$, $\overline{p} = 0.5 \times (p_1 + p_0)$, $\overline{q} = 1 - \overline{p}$. By consulting the literature[18],we estimate $OR_{nut\ intake}$ = 0.72, $\rho_0 = 0.50$, $\alpha = 0.05$, $\beta = 0.20$, the calculated sample size was $N_{case} = N_{control} = 609$. Finally 1068 subjects (534 cases and 534 controls) were recruited in this study.

Outcome--eligibility of NAFLD cases and controls

All participants were of Chinese Han ethnicity.The cases were newly diagnosed with NAFLD. The exclusion criteria were as follows: (a) daily alcohol intake of >40 g (men) and >20 g (women), (b) a history of other liver diseases including drug-induced liver disease, viral hepatitis, autoimmune hepatitis, total parenteral nutrition, and hepatolenticular degeneration, (c) taking hypolipidemic or weight reduction drugs, (d) age <18 or >70 years, (e) non-resident of Nanping, or (f) not of Han ethnicity. Adults who reported extremely abnormal levels of energy intake (2 511.60 kJ [600 kcal] or 17 581.20 kJ [4 200kcal] per day for men; 2 093 kJ [500 kcal] or 14,651.00 kJ [3500

kcal] per day for women), and those who did not answer 25 or more food-related items or questions about nut intake on the questionnaire, were excluded from the study.

The controls were randomly selected from the same center during the study period. Their eligibility criteria were identical to those of the cases, except for the requirement of a diagnosis of liver steatosis; they were frequency-matched with cases by age (within 5-yr intervals), gende, ethnicity, and region of origin.

Potential confounders--Data measurements and data collection

Trained interviewers performed a comprehensive medical history on each participant that included eliciting information about their demographic and socio-economic characteristics (e.g., age, gender, education, income, marriage status and history of diabetes, hypertension, hyperlipidemiaand), lifestyle habits (e.g., smoking, drinking (alcohol), tea drinking, and physical activity), anthropometric assessment (e.g., height, body weight and blood pressure). The data were obtained from participants using structured questionnaires during face-to-face interviews.

Exposure-Nut consumption

Information about participants' typical food consumption was collected using a semi-quantitative food frequency questionnaire that included 110 food items, which was developed and validated in a sample from southern China [19]. Participants were asked to estimate the average frequency of consumption of selected foods using the following response options: rarely, <once/month, 1–3 times/month, 1–2 times/week, 3–4 times/week, 5–6 times/week, once/day, twice/day, and >twice/day. Nut intake was defined as the consumption of "peanuts, walnuts, seeds, or other nuts." Data from a semi-quantitative food frequency questionnaire were used to calculate daily nut and energy intake. Nut consumption was converted to grams/day by multiplying the

food-intake frequency by fixed portion sizes. Nutrient intake, such as mono-unsaturated fatty acids (MUFAs) and poly-unsaturated fatty acids (PUFAs) were calculated by multiplying the intake frequency of each food by the nutrient content of the specified portion, and summing the products of all the food items.

Statistical analyses

Participants' characteristics were analyzed using Pearson's Chi-square test for categorical variables, and Wilcoxon's rank-sum test and ANOVA for continuous variables. Nut consumption was categorized by quartiles based on the distribution of daily nut intake by the controls (Q1-Q4). Binary logistic regression models were used to estimate the odds ratios (ORs) and their 95% confidence intervals (CIs) for the associations between nut consumption and NAFLD risk. To account for the energy content of the nut, our initial model adjusted for total energy intake by using the residuals method. The final model adjusted for potential confounders, including age, income, smoking status, educational level, and tea-drinking status, occupational status, marital status, body mass index (BMI), physical activity, and history of diabetes, hypertension, and hyperlipidemia. In order to reflect the matching protocol, when adjust for age, we entered a term for residual age into the regression analysis [20]. The final model also adjusted for MUFA and PUFA intake to control for their effects. The selection of covariates for the final model was based on clinical significance, results of previous studies. We tested for linear trends across categories of nut intake by assigning each participant the median value for each category and modeling this value as a continuous variable, consistent with prior studies[21-23].

We evaluated the influence of nut consumption across strata of other potential confounders, comparing participants in the highest nut-consumption category to the lowest nut-consumption category(reference). We also analyzed the interactions of nut

consumption with age, sex, BMI, smoking status, educational level, tea-drinking status, and other factors. We used the medians of the continuous variables to categorize them and evaluate the interactions. The criteria for statistical significance of the likelihood-ratio test of interaction effects was $P < 0.05$. Statistical analyses were performed using SPSS version 22 (IBM, Armonk, NY, US). All P-values were 2-tailed, and $P < 0.05$ was considered statistically significant.

Results

Baseline Characteristics

Table 1 presents health-related and demographic characteristics of the NAFLD cases and matched controls. Compared with the controls, the NAFLD cases tended to have a higher BMI, higher total intake of energy, higher MUFA and PUFA consumption, and a higher number of participants with a history of hyperlipidemia among both men and women. Patients with NAFLD had a lower educational level and the men engaged in less physical activity than the controls. No statistically significant associations were found for age, occupational status, income, marital status, smoking status, history of diabetes, or hypertension between the cases and the controls.

Table 1 General characteristics of cases and controls stratified by sex,*n*(%)

Variables	Men (*n*=728)			Women(*n*=340)		
	Cases	Controls(*n*=364)	*P*-value[a]	Cases (*n*=170)	Controls(*n*=170)	*P*-value[a]
Age			0.66			0.99
＜40	118(32.42)	116(31.87)		25(14.71)	22(12.94)	
40~60	217(59.61)	212(58.24)		123(72.35)	122(71.76)	
≥60	29(7.97)	36(9.89)		22(12.94)	26(15.30)	
Educational level			0.04			0.75
primary school and less than	10(2.75)	23(6.32)		30(17.65)	26(15.29)	
junior middle and high school	147(40.38)	128(35.16)		76(44.70)	74(43.53)	
junior college or above	207(56.87)	213(58.52)		64(37.65)	70(41.18)	
Occupational status			0.39			0.43
mental labour	108(29.67)	104(28.57)		50(29.41)	48(28.24)	
physical labor	86(23.63)	102(28.02)		25(14.71)	34(20.00)	
other	170(46.70)	158(43.41)		95(55.88)	88(51.76)	
Income(yuan/month)			0.35			0.85
＜2000	17(4.67)	17(4.67)		15(8.82)	18(10.59)	
2000~3000	87(23.90)	104(28.57)		70(41.18)	70(41.18)	
≥3000	260(71.43)	243(66.76)		85(50.00)	82(48.23)	
Marital status			0.24			0.52
single	45(12.36)	56(15.38)		4(2.35)	6(3.53)	
married or other	319(87.64)	308(84.62)		166(97.65)	164(96.47)	
Smoking status			0.30			0.16
never smoker	199(54.67)	213(58.52)		168(98.82)	170(100)	
smoker	165(45.33)	151(41.48)		2(1.18)	0(0.00)	
Tea-drinking status			0.30			0.01
yes	258(70.88)	245(63.31)		77(45.29)	55(32.35)	
no	106(29.12)	119(32.69)		93(54.71)	115(67.55)	
Physical activity			0.02			0.88
light	175(48.08)	137(37.64)		19(11.18)	19(11.18)	
moderate	100(27.47)	121(33.24)		47(27.64)	43(25.29)	
severe	89(24.45)	106(29.12)		104(61.18)	108(63.53)	
BMI(kg/m^2)			<0.001			<0.001
＜18.5	2(0.55)	11(3.02)		1(0.59)	9(5.29)	
18.5~24.0	107(29.40)	257(70.61)		72(42.35)	125(73.53)	
≥24.0	255(70.05)	96(26.37)		97(57.06)	36(21.18)	
History of diabetes			0.67			0.18
yes	12(3.30)	10(2.75)		10(5.88)	5(2.94)	
no	352(96.70)	354(97.25)		160(94.12)	165(94.06)	
History of hypertension			0.85			0.63
yes	15(4.12)	16(4.40)		8(4.71)	10(5.88)	
no	349(95.88)	348(95.60)		162(95.29)	160(94.12)	
History of hyperlipidemia			0.01			<0.001
yes	108(29.67)	77(21.15)		51(30.00)	21(12.35)	
no	256(70.33)	287(78.85)		119(70.00)	149(87.65)	
MUFA intake,g/d[b]	34.10(28.49,	31.39(26.55,36.79)	<0.001	29.99(26.37,36.37)	28.05(22.40,31.39)	<0.001
PUFA intake,g/d[b]	26.25(24.04,	23.90(22.01,26.02)	<0.001	24.52(22.11,27.12)	21.21(18.83,24.53)	<0.001
Energy intake,kJ/d[c]	10395.64±24	9890.97±2348.76	0.01	7509.73±1650.71	7323.78±1852.97	0.04

[a]*P*-values were calculated by using the Chi-square test for categorical variables and Wilcoxon rank sum test and ANOVA test for continues variables.

[b]Medians (IQRs).

[c]Mean(s.d).

The associations of dietary nut intake with NAFLD

The participants' nut consumption is summarized in Table 2. Among the women, those in the NAFLD group consumed a significantly higher amount of nuts (6.80 g/d vs. 2.50 g/d; P = 0.02) than those in the control group. No statistically significant differences were found between the cases and controls in the the sample or among the male participants. In order to adjust for potential confounding factors, quartile distributions of dietary nut consumption among the controls were used to categorize the nut intake of all the participants; the results are shown in Table 3. After adjusting for potential confounders, nut consumption was not associated with NAFLD risk among the participants in the sample. When the fully adjusted model was stratified by sex, a significant inverse association was found between nut consumption and NAFLD, but only among the men in the highest quartile (OR = 0.43; 95% CI: 0.26–0.71; P_{trend} = 0.01).

Table 2 Comparison of nut daily intake between the case and the control

Nut intake (g/d)	Case		Control		*P*-value[a]	*P*-value[b]
	Median	Quartile	Median	Quartile		
Total population	3.15	1.46-8.80	2.86	1.22-8.98	0.35	0.18
Men	2.68	1.15-8.43	2.86	1.22-8.98	0.36	0.94
Women	6.80	1.75-8.86	2.50	1.07-7.84	0.01	0.02

[a]*P*-values:calculated by using Wilcoxon rank sum test;before adjusting for energy.

[b]*P*-values :calculated by using Wilcoxon rank sum test; after adjusting for energy by using the residuals methods.

Table 3 Odds ratios (OR) and 95% confidence intervals (Cl) for NAFLD in relation to nut intake

Nut intake	Case *n*	Control *n*	Crude OR(95%CI)	Model 1[a] OR(95%CI)	Model 2[a] OR(95%CI)
Total population[b]	534	534			
Q1	125	133	1	1	1
Q2	112	134	1.30(0.93-1.81)	0.89(0.63-1.26)	0.85(0.56-1.29)
Q3	159	134	1.77(1.29-2.44)	1.26(0.90-1.77)	1.01(0.68-1.50)
Q4	138	133	1.17(0.81-1.68)	1.10(0.78-1.55)	0.67(0.44-1.02)
P for trend*			0.46	0.45	0.07
Men	364	364			
Q1	106	102	1	1	1
Q2	91	79	1.31(0.88-1.94)	0.90(0.60-1.35)	0.88(0.54-1.43)
Q3	74	123	1.81(1.24-2.65)	1.73(1.16-2.57)	1.34(0.84-2.16)
Q4	93	60	1.04(0.66-1.63)	0.67(0.44-1.02)	0.43(0.26-0.71)
P for trend*			0.81	0.04	0.01
Women	170	170			
Q1	23	27	1	1	1
Q2	33	43	1.28(0.69-2.37)	0.90(0.44-1.85)	0.84(0.36-1.97)
Q3	36	60	1.71(0.95-3.08)	0.70(0.35-1.41)	0.55(0.24-1.25)
Q4	78	40	1.41(0.76-2.64)	2.29(1.17-4.49)	1.30(0.57-2.95)
P for trend*			0.36	<0.001	0.12

[a]Model 1:Adjusted for total energy intake.

[a]Model 2:Adjusted further for age,income,smoking status,educational level, teaing status, occupationanl status,marital status,body mass index,physical activity,the history of diabetes,hypertension and hyperlipidemia,MUFA and PUFA intake.

[b]:further adiusted for sex.

P for trend: Trend across intake levels with the categorical median.

For total population: Q1, <1.46 g/d; Q2, 1.46-3.15 g/d; Q3, 3.15-8.80 g/d; Q4, ≥8.80 g/d

For men: Q1, <1.15 g/d; Q2, 1.15-2.68 g/d; Q3,2.68-8.43 g/d; Q4, ≥8.43g/d

For women: Q1, <1.75 g/d; Q2, 1.75-6.80 g/d; Q3, 6.80-8.86 g/d; Q4, ≥8.86 g/d.

Stratified Analyses

In the stratified analysis, the inverse association between total-nut consumption and NAFLD risk was consistent across strata of age, sex, BMI, educational level, income, physical activity, smoking, tea drinking, and history of diabetes, hypertension, and hyperlipidemia. In addition to the association between nut consumption and

NAFLD in men, we also found the highest nut-consumption category was associated with a lower risk of developing NAFLD than the reference (OR = 0.53; 95% CI: 0.31–0.93) among the participants who engaged in light and moderate physical activity. No significant interactions of total nut consumption and the potentially confounding effects of NAFLD risk factors were identified (Figure 1).

Discussion

In this case-control study, we found a significant inverse relationship between high nut consumption and NAFLD risk among the males, but found either no or unclear associations among the females and the overall sample. Moreover, the associations seemed to be independent of other predictors, including diet and lifestyle factors. The effect of nut consumption remained among men after controlling for other known or suspected risk factors for developing NAFLD. Furthermore, no significant interactions between nut consumption and the potential modifying effects of NAFLD risk factors were identified. To the best of our knowledge, this was the first study to assess the association between nut consumption and the risk of NAFLD in a Chinese sample.

Our results are consistent with a previous study conducted in Korea, which found that a low intake of nuts and seeds (OR = 3.66; $P\text{-}_{trend}$ = 0.007) was associated with a significantly higher risk of developing NAFLD among male participants, but not among females [24]. Although another case–control study found an association between a high intake of nuts with a lower likelihood of NAFLD, after adjusting for the confounders age, sex, waist circumference, and the values of the homeostasis model assessment of insulin resistance (OR = 0.61; 95% CI: 0.38–0.98), the association disappeared after further adjustment for adiponectin and TNF-α (OR = 0.72; 95% CI: 0.41–1.25) [18]. However, the study did not examine nut intake separately; therefore,

the results cannot be directly compared with those of our study. Although the results of our study suggest nuts may play a protective role in the development of NAFLD in males, a significant association between nut intake and the risk of NAFLD was not found in females. NAFLD has been regarded as the "hepatic manifestation of MetS"; therefore, our findings are indirectly supported by several studies showing an inverse correlation between high nut intake and the risk of T2D and cardiovascular disease, which share common metabolic parameters with NAFLD [25-27]. Nevertheless, the relationship between nut intake and NAFLD warrants further exploration, and additional studies are needed to examine gender differences in the association between nut consumption and NAFLD, and its' possible mechanisms should be explored.

Several biological pathways have been proposed to explain the association between nut intake and NAFLD risk. Ellagic acid, a natural antioxidant polyphenol found in nuts, can suppress oxidative stress and inflammation [28] and improve hepatic insulin sensitivity and lipid metabolism [29]. Vitamin E and selenium in almonds and walnuts, as well as folic acid and resveratrol in pine nuts, have been reported to have beneficial effects on NAFLD. Numerous trials testing the therapeutic value of vitamin E in NAFLD prevention found that Vitamin E significantly improved liver function and histologic changes by significantly reducing aspartate aminotransferase, alanine aminotransferase, steatosis, and inflammation in patients with NAFLD/non-alcoholic steatohepatitis (NASH) [30]. Selenium is an antioxidant agent, and selenium deficiency is associated with insulin resistance in patients with chronic liver disease [31]. Folate plays an essential role in lipid metabolism and folic acid supplementation can attenuate steatosis and improve oxidative stress in rodent models of NAFLD. Folate can also blunt the increase of inflammatory cytokines secreted by immune cells [32].

Resveratrol has antioxidant, anti-apoptotic, and anti-inflammatory properties in NAFLD patients [33]. Nuts, which are known to be rich in unsaturated fatty acids, are especially rich in n-3 PUFAs, a source of alpha-linolenic acid, which lowers LDL cholesterol [34], and has been found to have beneficial effects on NAFLD [35]. Each type of nut has many nutrients and phytochemicals that may be beneficial to health, and it is likely that unknown salubrious effects of nuts may be related to NAFLD prevention. Moreover, many studies have shown a beneficial association between high nut intake and decreased risk of obesity [36], T2D [37], and MetS [38], which are risk factors for NAFLD.

Our study has both strengths and limitations. The first strength is the large sample size, which may have reduced type II errors. Second, the collection of data using face-to-face interviews and a semi-quantitative food frequency questionnaire, which elicited extensive information on anthropometrics and lifestyle factors, allowed us to adjust for confounding factors. Finally, the study tried to investigate interactions between nut intake and other risk factors, for which a biologically plausible mechanism may exist. Nonetheless, several limitations should be considered when interpreting the study's findings. First, although we accounted for a wide range of socioeconomic characteristics and lifestyle factors, we cannot exclude the effects of unknown or poorly measured confounding variables or residual confounding attributable to other dietary/lifestyle factors, which might have influenced the observed associations. However, the associations persisted even after controlling for known and suspected predictors of NAFLD. Second, the intake of nuts was assessed by asking about using one question; therefore, we could not investigate the effects of different types of nuts, such as peanuts and walnuts, which contain different amounts of energy, fat content, and other nutrients[39]. Third, categorization of the intake of nuts

in quartiles may lead to bias and inefficiency. Fourth, we did not consider manufacturing methods (e.g., raw, roasted, or boiled) or extra ingredients (e.g., sugar, salt, seasoning). Different preparation methods before and after roasting, the amount of time roasted, and the temperature used can affect the nutrient composition and ingredients in the nuts[40 41]. What's more, the item'nuts' in the FFQ used in this study cannot represent all nuts, because it only included peanuts, walnuts, seeds. For this reason, direct comparisons with the results of other studies may be difficult. Fifth, because we used a case-control study design, recall bias is an inherent limitation. People who are health-conscious may over-report or under-report some food items. However, the protective effect of nuts on NAFLD was not generally known at the time of the survey, and thus, should be unrelated to recall bias. In addition, the stratified analyses was not corrected for multiple testing, which may inflate the risk for type I error. Sixth, NAFLD is a spectrum of disease ranging from essentially benign simple steatosis (fatty infiltration of the liver) to the more severe form non-alcoholic steatohepatitis (NASH- fat with inflammation and/or fibrosis), but we did not collect datas about the severity of NAFLD, so we cann't analysis the association between nuts intake with the the severity of NAFLD. Finally, due to the small number of participants in the highest nut-intake category, we cannot rule out the possibility that some of our results are due to chance. However, the associations were consistent in the analyses stratified by some of the other factors, which reduces the likelihood of chance findings. In these stratified analyses, the statistical power needed to detect differences was limited by the sample size, and such analyses should be considered exploratory.

Our study had a retrospective design, which is lower on the evidence hierarchy than prospective studies. Nevertheless, case-control studies can provide evidence

supporting the general relationship between diet and NAFLD, as there currently are no available prospective studies.

In conclusion, this case-control study indicated that high nut intake was associated with a significantly reduced risk of NAFLD among Han men in China. However, no relationship was found between total nut intake and NAFLD risk among the Han women. Our data contribute to the growing evidence showing that a relatively simple prevention strategy of incorporating a modest amount of nuts in the diet may contribute to maintaining good health at both the individual and population levels.

Acknowledgements

We thank all study participants for their cooperation. We also thank our staff for recruiting participants and for their technical assistance.

Contributors

BBC and YH are joint first authors. XEP obtained funding. XEP, XL and SHX designed the study. XTP ,JHY,YFL and WJL collected the data. BBC and YH were involved in data cleaning and verification. BBC and XTP analyzed the data. BBC and YH drafted the manuscript. XEP ,YFL and JHY contributed to the interpretation of the results and critical revision of the manuscript for important intellectual content and approved the final version of the manuscript. All authors have read and approved the final manuscript.

Data sharing

No additional data are available.

Conflict of Interest

The authors declare that they have no conflict of interest.

Funding

This work was supported by the National Natural Science Foundation of China [grant number 81473047], the Training Program Foundation for Middle-aged and Young Talents from the Sanitation System of Fujian Province [grant number 2014-ZQN-ZD-23], and the Joint Innovation Project of Science and Technology of Fujian Province [grant number 2017Y9104].

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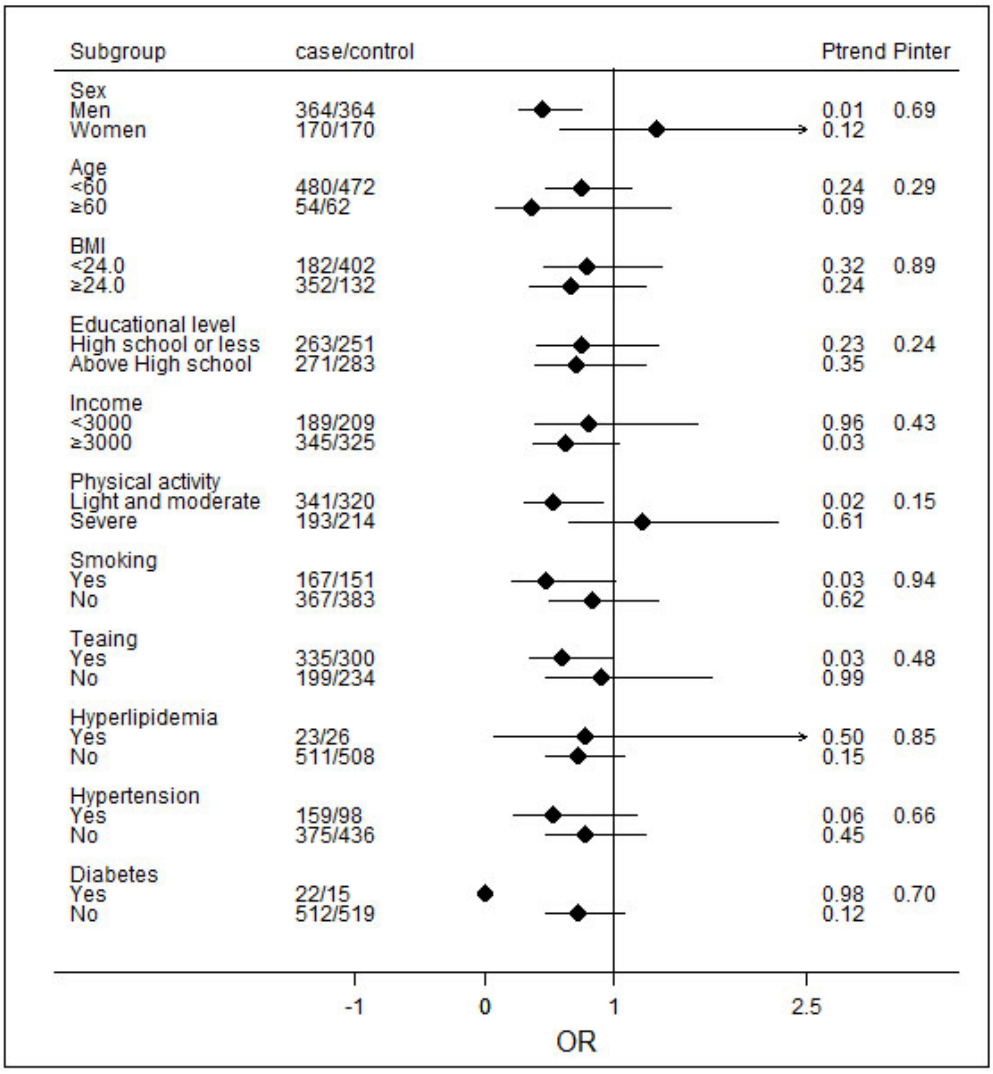
Figure legends

Figure 1. Odds ratios (OR) and 95% CIs for NAFLD across the strata of various factors.

The forest plot represents the ORs of the comparison of the lowest nut-intake category versus the highest nut-intake category, adjusting for age, income, smoking status, educational level, tea-drinking status, occupational status, marital status, BMI, physical activity, history of diabetes, hypertension and hyperlipidemia, total energy intake, and MUFA and PUFA intake. *P*-values are two-tailed; P_{inter} indicates *P* for the interaction between strata and nut intake; P_{trend} indicates *P* for the trend across levels of nut intake.



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148x159mm (96 x 96 DPI)

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *case-control studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	*(*a) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls	5
		(*b*) For matched studies, give matching criteria and the number of controls per case	5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5-6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5-6
Bias	9	Describe any efforts to address potential sources of bias	7

Study size	10	Explain how the study size was arrived at	5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5-6
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6-7
		(*b*) Describe any methods used to examine subgroups and interactions	7
		(*c*) Explain how missing data were addressed	
		(*d*) If applicable, explain how matching of cases and controls was addressed	5
		(*e*) Describe any sensitivity analyses	
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8-9
		(b) Indicate number of participants with missing data for each variable of interest	
Outcome data	15*	Report numbers in each exposure category, or summary measures of exposure	8-9
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9-10
		(*b*) Report category boundaries when continuous variables were categorized	10-11

		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	11
Discussion			
Key results	18	Summarise key results with reference to study objectives	12
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	14
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12-14
Generalisability	21	Discuss the generalisability (external validity) of the study results	15
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	17

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Association between nut intake and Non-alcoholic fatty liver disease risk: a retrospective case-control study in a sample of Chinese Han adults

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-028961.R3
Article Type:	Research
Date Submitted by the Author:	05-Aug-2019
Complete List of Authors:	Chen, Bing bing; Fujian Medical University, Han, Ying; The First Affiliated Hospital of Fujian Medical University Pan, Xinting; Department of Epidemiology and Health Statistics, Fujian Provincial Key Laboratory of Environment Factors and Cancer, School of Public Health, Fujian Medical University Yan, Jianhui; Department of Epidemiology and Health Statistics, Fujian Provincial Key Laboratory of Environment Factors and Cancer, School of Public Health, Fujian Medical University Liu, Wenjuan; Department of Epidemiology and Health Statistics, Fujian Provincial Key Laboratory of Environment Factors and Cancer, School of Public Health, Fujian Medical University li, yangfan; Department of Epidemiology and Health Statistics, Fujian Provincial Key Laboratory of Environment Factors and Cancer, School of Public Health, Fujian Medical University, Fujian, China, ; lin, xu; Key Laboratory of Ministry of Education for Gastrointestinal Cancer, Fujian Medical University, Fujian, China xu, shanghua; Department of Cardiology, Affiliated Nanping First Hospital, Fujian Medical University, Nanping, China Peng, Xian-E; Key Laboratory of Ministry of Education for Gastrointestinal Cancer, Fujian Medical University, Fujian,
Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Nutrition and metabolism, Public health
Keywords:	EPIDEMIOLOGY, NUTRITION & DIETETICS, Lipid disorders < DIABETES & ENDOCRINOLOGY

Association between nut intake and Non-alcoholic fatty liver disease risk: a retrospective case-control study in a sample of Chinese Han adults

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Running title： Nut intake and non-alcoholic fatty liver disease

Word count：3055

Abstract

Objectives: Nut consumption has been associated with a lower risk of type 2 diabetes, metabolic syndrome, and insulin resistance. However, its effect on the risk of non-alcoholic fatty liver disease (NAFLD) is unknown. Therefore, we investigated the relationship between nut consumption and NAFLD risk.

Setting and participants: We conducted a retrospective case-control study including 534 patients diagnosed with NAFLD and 534 controls matched by sex and age (± 5) from the Affiliated Nanping First Hospital of Fujian Medical University in China.

Main outcome measures: Information on dietary intake was collected using a semi-quantitative food frequency questionnaires and nut consumption was calculated. Nut consumption was categorized using quartiles based on the distribution of daily nut intake of the controls. Binary logistic regression models were used to estimate odds ratio (ORs) and the 95% confidence intervals (CIs) for the association between nut consumption and NAFLD risk.

Results: After adjusting for potential confounding variables, nut consumption was not associated with NAFLD risk in the overall sample. When the fully adjusted model was stratified by sex, a significant inverse association was found between high nut consumption and NAFLD only among the men in the highest quartile (OR = 0.43; 95% CI: 0.26–0.71; P_{trend} = 0.01). The inverse association of nut consumption with NAFLD risk in men remained significant after controlling for other known or suspected risk factors for NAFLD.

Conclusions: Diets with a higher intake of nuts may be associated with a decreased risk of NAFLD, particularly in men.

Strengths and limitations of this study

1.In this study, several potential confounding variables such as energy intake and physical activity, were taken into account.

2.The study had tried to investigate interactions between nut intake and other risk factors, for which a biologically plausible mechanism may exist.

3.This study was a case-control design, thus the causal association between nut intake and NAFLD could not be precisely identified.

4.This study was a case-control study , recall bias was inevitable and randomized controlled trial studies are therefore required for more accurate results.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is defined as macrovesicular steatosis in ≥5% of hepatocytes, in the absence of a secondary cause, such as alcohol or drug use. NAFLD has become a leading cause of chronic liver disease, with a 25% prevalence worldwide [1].Furthermore, a fatty liver is more prevalent in men compared with women[2]. The prevalence of NAFLD among the adults in China's general population is approximately 15% (6.3–27.0%), depending on the population studied [3]. Many studies suggest that diet and lifestyle may significantly influence the risk of NAFLD [4 5]. These studies indicate that type 2 diabetes (T2D) [6], metabolic syndrome (MetS), obesity [7], physical activity, and a high-fat diet [4] are associated with an increased risk for the development of NAFLD.

Nuts are nutrient-dense foods with complex matrices rich in unsaturated fatty and other bioactive compounds (e.g., high-quality vegetable protein, fiber, minerals, tocopherols, phytosterols, and phenolic compounds) [8]. The global intake of nuts has increased by 59% over the past decade [9]. China's per capita intake of nuts was 2.2 g/day in 1982, and increased to 3.8g/day in 2012 [10]. Although they are high in fat and

energy dense, a high intake of nuts has been associated with several health benefits, including reduced risk of cardiovascular disease [11], T2D [12], MetS[13], and insulin resistance[14] [15].Moreover, nuts have antioxidative effects by decreasing lipid peroxidation and protecting against oxidative DNA damage [16]. Beneficial health effects have been attributed to the macronutrient and micronutrient profiles of nuts [17].

NAFLD is regarded as the "hepatic manifestation of MetS." Few studies have assessed the effects of nut intake on NAFLD, despite previous findings of an inverse correlation between high nut consumption and the risk of T2D and cardiovascular disease. In this study, we analyzed the association between nut intake and NAFLD risk and the interactions between nut intake and other established NAFLD risk factors in a large case-control study with a sample of Han adults in China.

Methods

Patient and public involvement statement

Patients and public will not be involved in the development of the research question or in the design of the study. Subjects will receive oral and written information about this study, however, they will not be involved in the recruitment and conduct of the study. After signing an informed consent by the participants, they will be assessed for eligibility and data collection will begin. Eligible subjects will be interviewed face-to-face by investigators to collect data. In addition, all methods were performed in accordance with the relevant guidelines and regulations.

Study design

We conducted the retrospective case-control study in a health examination center at the Affiliated Nanping First Hospital of Fujian Medical University fro m April 2015 to August 2017. Data were obtained from subjects who underwe

nt routine health examination in the examination center. Patients newly diagnos ed with NAFLD using abdominal ultrasonography in accordance with the "Gui delines for the diagnosis and managementof nonalcoholic fatty liver disease: up date 2010" were included in the study. Hepatic ultrasonography examination was performed by trained ultrasonographists who were blinded to the clinical and laboratory data. Hepatic steatosis was diagnosed by characteristic echo patt erns according to conventional criteria, such as the evidence of diffuse hyper-e chogenicity of the liver relative to the kidneys, ultrasound beam attenuation, an d poor visualization of intrahepatic structures.

Sample size calculation

This study is a case-control design, thus we estimate the sample size based on the case-control study formula: $n = 2\overline{pq}(z_\alpha + z_\beta)^2/(p_1 - p_0)^2$, $p_1 = \frac{p_0 RR}{1 + p_0(RR-1)}$, $\overline{p} = 0.5 \times (p_1 + p_0)$, $\overline{q} = 1 - \overline{p}$. By consulting the literature[18],we estimate $OR_{nut\ intake}$ = 0.72, $\rho_0 = 0.50$, $\alpha = 0.05$, $\beta = 0.20$, the calculated sample size was $N_{case} = N_{control} = 609$. Finally 1068 subjects (534 cases and 534 controls) were recruited in this study.

Outcome--eligibility of NAFLD cases and controls

All participants were of Chinese Han ethnicity.The cases were newly diagnosed with NAFLD. The exclusion criteria were as follows: (a) daily alcohol intake of >40 g (men) and >20 g (women), (b) a history of other liver diseases including drug-induced liver disease, viral hepatitis, autoimmune hepatitis, total parenteral nutrition, and hepatolenticular degeneration, (c) taking hypolipidemic or weight reduction drugs, (d) age <18 or >70 years, (e) non-resident of Nanping, or (f) not of Han ethnicity. Adults who reported extremely abnormal levels of energy intake (2 511.60 kJ [600 kcal] or 17 581.20 kJ [4 200kcal] per day for men; 2 093 kJ [500 kcal] or 14,651.00 kJ [3500

kcal] per day for women), and those who did not answer 25 or more food-related items or questions about nut intake on the questionnaire, were excluded from the study.

The controls were randomly selected from the same center during the study period. Their eligibility criteria were identical to those of the cases, except for the requirement of a diagnosis of liver steatosis; they were frequency-matched with cases by age (within 5-yr intervals), gender, ethnicity, and region of origin.

Ethical considerations

All subjects who participated in this study provided written informed consent and the study was approved by the local ethics committees of Fujian Medical University (ethics number 2014096). In addition, all methods were performed in accordance with the relevant guidelines and regulations of the University.

Potential confounders--Data measurements and data collection

Trained interviewers performed a comprehensive medical history on each participant that included eliciting information about their demographic and socio-economic characteristics (e.g., age, gender, education, income, marriage status and history of diabetes, hypertension, hyperlipidemiaand), lifestyle habits (e.g., smoking, drinking (alcohol), tea drinking, and physical activity), anthropometric assessment (e.g., height, body weight and blood pressure). The data were obtained from participants using structured questionnaires during face-to-face interviews.

Exposure-Nut consumption

Information about participants' typical food consumption was collected using a semi-quantitative food frequency questionnaire that included 110 food items, which was developed and validated in a sample from southern China [19]. Participants were asked to estimate the average frequency of consumption of selected foods using the following response options: rarely, <once/month, 1–3 times/month, 1–2 times/week, 3–4 times/week, 5–6 times/week, once/day, twice/day, and >twice/day. Nut intake was defined as the consumption of "peanuts, walnuts, seeds, or other nuts." Data from a semi-quantitative food frequency questionnaire were used to calculate daily nut and energy intake. Nut consumption was converted to grams/day by multiplying the food-intake frequency by fixed portion sizes. Nutrient intake, such as mono-unsaturated fatty acids (MUFAs) and poly-unsaturated fatty acids (PUFAs) were calculated by multiplying the intake frequency of each food by the nutrient content of the specified portion, and summing the products of all the food items.

Statistical analyses

Participants' characteristics were analyzed using Pearson's Chi-square test for categorical variables, and Wilcoxon's rank-sum test and ANOVA for continuous variables. Nut consumption was categorized by quartiles based on the distribution of daily nut intake by the controls (Q1-Q4). Binary logistic regression models were used to estimate the odds ratios (ORs) and their 95% confidence intervals (CIs) for the associations between nut consumption and NAFLD risk. To account for the energy content of the nut, our initial model adjusted for total energy intake by using the residuals method. The final model adjusted for potential confounders, including age, income, smoking status, educational level, and tea-drinking status, occupational status, marital status, body mass index (BMI), physical activity, and history of diabetes, hypertension, and hyperlipidemia. In order to reflect the matching protocol, when

adjust for age, we entered a term for residual age into the regression analysis [20]. The final model also adjusted for MUFA and PUFA intake to control for their effects. The selection of covariates for the final model was based on clinical significance, results of previous studies. We tested for linear trends across categories of nut intake by assigning each participant the median value for each category and modeling this value as a continuous variable, consistent with prior studies[21-23].

We evaluated the influence of nut consumption across strata of other potential confounders, comparing participants in the highest nut-consumption category to the lowest nut-consumption category(reference). We also analyzed the interactions of nut consumption with age, sex, BMI, smoking status, educational level, tea-drinking status, and other factors. We used the medians of the continuous variables to categorize them and evaluate the interactions. The criteria for statistical significance of the likelihood-ratio test of interaction effects was $P < 0.05$. Statistical analyses were performed using SPSS version 22 (IBM, Armonk, NY, US). All P-values were 2-tailed, and $P < 0.05$ was considered statistically significant.

Results

Baseline Characteristics

Table 1 presents health-related and demographic characteristics of the NAFLD cases and matched controls. Compared with the controls, the NAFLD cases tended to have a higher BMI, higher total intake of energy, higher MUFA and PUFA consumption, and a higher number of participants with a history of hyperlipidemia among both men and women. Patients with NAFLD had a lower educational level and the men engaged in less physical activity than the controls. No statistically significant associations were found for age, occupational status, income, marital status, smoking status, history of diabetes, or hypertension between the cases and the controls.

Table 1 General characteristics of cases and controls stratified by sex,*n*(%)

Variables	Men (*n*=728)			Women(*n*=340)		
	Cases (*n*=364)	Controls(*n*=364)	*P*-value[a]	Cases (*n*=170)	Controls(*n*=170)	*P*-value[a]
Age			0.66			0.99
＜40	118(32.42)	116(31.87)		25(14.71)	22(12.94)	
40~60	217(59.61)	212(58.24)		123(72.35)	122(71.76)	
≥60	29(7.97)	36(9.89)		22(12.94)	26(15.30)	
Educational level			0.04			0.75
primary school and less than	10(2.75)	23(6.32)		30(17.65)	26(15.29)	
junior middle and high school	147(40.38)	128(35.16)		76(44.70)	74(43.53)	
junior college or above	207(56.87)	213(58.52)		64(37.65)	70(41.18)	
Occupational status			0.39			0.43
mental labour	108(29.67)	104(28.57)		50(29.41)	48(28.24)	
physical labor	86(23.63)	102(28.02)		25(14.71)	34(20.00)	
other	170(46.70)	158(43.41)		95(55.88)	88(51.76)	
Income(yuan/month)			0.35			0.85
＜2000	17(4.67)	17(4.67)		15(8.82)	18(10.59)	
2000~3000	87(23.90)	104(28.57)		70(41.18)	70(41.18)	
≥3000	260(71.43)	243(66.76)		85(50.00)	82(48.23)	
Marital status			0.24			0.52
single	45(12.36)	56(15.38)		4(2.35)	6(3.53)	
married or other	319(87.64)	308(84.62)		166(97.65)	164(96.47)	
Smoking status			0.30			0.16
never smoker	199(54.67)	213(58.52)		168(98.82)	170(100)	
smoker	165(45.33)	151(41.48)		2(1.18)	0(0.00)	
Tea-drinking status			0.30			0.01
yes	258(70.88)	245(63.31)		77(45.29)	55(32.35)	
no	106(29.12)	119(32.69)		93(54.71)	115(67.55)	
Physical activity			0.02			0.88
light	175(48.08)	137(37.64)		19(11.18)	19(11.18)	
moderate	100(27.47)	121(33.24)		47(27.64)	43(25.29)	
severe	89(24.45)	106(29.12)		104(61.18)	108(63.53)	
BMI(kg/m^2)			<0.001			<0.001
＜18.5	2(0.55)	11(3.02)		1(0.59)	9(5.29)	
18.5~24.0	107(29.40)	257(70.61)		72(42.35)	125(73.53)	
≥24.0	255(70.05)	96(26.37)		97(57.06)	36(21.18)	
History of diabetes			0.67			0.18
yes	12(3.30)	10(2.75)		10(5.88)	5(2.94)	
no	352(96.70)	354(97.25)		160(94.12)	165(94.06)	
History of hypertension			0.85			0.63
yes	15(4.12)	16(4.40)		8(4.71)	10(5.88)	
no	349(95.88)	348(95.60)		162(95.29)	160(94.12)	
History of hyperlipidemia			0.01			<0.001
yes	108(29.67)	77(21.15)		51(30.00)	21(12.35)	
no	256(70.33)	287(78.85)		119(70.00)	149(87.65)	
MUFA intake,g/d[b]	34.10(28.49,39	31.39(26.55,36.	<0.001	29.99(26.37,36.37)	28.05(22.40,31.39)	<0.001
PUFA intake,g/d[b]	26.25(24.04,29	23.90(22.01,26.	<0.001	24.52(22.11,27.12)	21.21(18.83,24.53)	<0.001
Energy intake,kJ/d[c]	10395.64±242	9890.97±2348.7	0.01	7509.73±1650.71	7323.78±1852.97	0.04

[a]*P*-values were calculated by using the Chi-square test for categorical variables and Wilcoxon rank sum test and ANOVA test for continues variables.

[b]Medians (IQRs).

[c]Mean(s.d).

The associations of dietary nut intake with NAFLD

The participants' nut consumption is summarized in Table 2. Among the women, those in the NAFLD group consumed a significantly higher amount of nuts (6.80 g/d vs. 2.50 g/d; P = 0.02) than those in the control group. No statistically significant differences were found between the cases and controls in the the sample or among the male participants. In order to adjust for potential confounding factors, quartile distributions of dietary nut consumption among the controls were used to categorize the nut intake of all the participants; the results are shown in Table 3. After adjusting for potential confounders, nut consumption was not associated with NAFLD risk among the participants in the sample. When the fully adjusted model was stratified by sex, a significant inverse association was found between nut consumption and NAFLD, but only among the men in the highest quartile (OR = 0.43; 95% CI: 0.26–0.71; P_{trend} = 0.01).

Table 2 Comparison of nut daily intake between the case and the control

Nut intake (g/d)	Case		Control		*P*-value[a]	*P*-value[b]
	Median	Quartile	Median	Quartile		
Total population	3.15	1.46-8.80	2.86	1.22-8.98	0.35	0.18
Men	2.68	1.15-8.43	2.86	1.22-8.98	0.36	0.94
Women	6.80	1.75-8.86	2.50	1.07-7.84	0.01	0.02

[a]*P*-values:calculated by using Wilcoxon rank sum test;before adjusting for energy.

[b]*P*-values :calculated by using Wilcoxon rank sum test; after adjusting for energy by using the residuals methods.

Table 3 Odds ratios (OR) and 95% confidence intervals (Cl) for NAFLD in relation to nut intake

Nut intake	Case *n*	Control *n*	Crude OR(95%CI)	Model 1[a] OR(95%CI)	Model 2[a] OR(95%CI)
Total population[b]	534	534			
Q1	125	133	1	1	1
Q2	112	134	1.30(0.93-1.81)	0.89(0.63-1.26)	0.85(0.56-1.29)
Q3	159	134	1.77(1.29-2.44)	1.26(0.90-1.77)	1.01(0.68-1.50)
Q4	138	133	1.17(0.81-1.68)	1.10(0.78-1.55)	0.67(0.44-1.02)
P for trend*			0.46	0.45	0.07
Men	364	364			
Q1	106	102	1	1	1
Q2	91	79	1.31(0.88-1.94)	0.90(0.60-1.35)	0.88(0.54-1.43)
Q3	74	123	1.81(1.24-2.65)	1.73(1.16-2.57)	1.34(0.84-2.16)
Q4	93	60	1.04(0.66-1.63)	0.67(0.44-1.02)	0.43(0.26-0.71)
P for trend*			0.81	0.04	0.01
Women	170	170			
Q1	23	27	1	1	1
Q2	33	43	1.28(0.69-2.37)	0.90(0.44-1.85)	0.84(0.36-1.97)
Q3	36	60	1.71(0.95-3.08)	0.70(0.35-1.41)	0.55(0.24-1.25)
Q4	78	40	1.41(0.76-2.64)	2.29(1.17-4.49)	1.30(0.57-2.95)
P for trend*			0.36	<0.001	0.12

[a]Model 1:Adjusted for total energy intake.

[a]Model 2:Adjusted further for age,income,smoking status,educational level, teaing status, occupationanl status,marital status,body mass index,physical activity,the history of diabetes,hypertension and hyperlipidemia,MUFA and PUFA intake.

[b]:further adiusted for sex.

P for trend: Trend across intake levels with the categorical median.

For total population: Q1, <1.46 g/d; Q2, 1.46-3.15 g/d; Q3, 3.15-8.80 g/d; Q4, ≥8.80 g/d

For men: Q1, <1.15 g/d; Q2, 1.15-2.68 g/d; Q3,2.68-8.43 g/d; Q4, ≥8.43g/d

For women: Q1, <1.75 g/d; Q2, 1.75-6.80 g/d; Q3, 6.80-8.86 g/d; Q4, ≥8.86 g/d.

Stratified Analyses

In the stratified analysis, the inverse association between total-nut consumption and NAFLD risk was consistent across strata of age, sex, BMI, educational level, income, physical activity, smoking, tea drinking, and history of diabetes, hypertension, and hyperlipidemia. In addition to the association between nut consumption and

NAFLD in men, we also found the highest nut-consumption category was associated with a lower risk of developing NAFLD than the reference (OR = 0.53; 95% CI: 0.31–0.93) among the participants who engaged in light and moderate physical activity. No significant interactions of total nut consumption and the potentially confounding effects of NAFLD risk factors were identified (Figure 1).

Discussion

In this case-control study, we found a significant inverse relationship between high nut consumption and NAFLD risk among the males, but found either no or unclear associations among the females and the overall sample. Moreover, the associations seemed to be independent of other predictors, including diet and lifestyle factors. The effect of nut consumption remained among men after controlling for other known or suspected risk factors for developing NAFLD. Furthermore, no significant interactions between nut consumption and the potential modifying effects of NAFLD risk factors were identified. To the best of our knowledge, this was the first study to assess the association between nut consumption and the risk of NAFLD in a Chinese sample.

Our results are consistent with a previous study conducted in Korea, which found that a low intake of nuts and seeds (OR = 3.66; $P\text{-}_{trend}$ = 0.007) was associated with a significantly higher risk of developing NAFLD among male participants, but not among females [24]. Although another case–control study found an association between a high intake of nuts with a lower likelihood of NAFLD, after adjusting for the confounders age, sex, waist circumference, and the values of the homeostasis model assessment of insulin resistance (OR = 0.61; 95% CI: 0.38–0.98), the association disappeared after further adjustment for adiponectin and TNF-α (OR = 0.72; 95% CI: 0.41–1.25) [18]. However, the study did not examine nut intake separately; therefore,

the results cannot be directly compared with those of our study. Although the results of our study suggest nuts may play a protective role in the development of NAFLD in males, a significant association between nut intake and the risk of NAFLD was not found in females. NAFLD has been regarded as the "hepatic manifestation of MetS"; therefore, our findings are indirectly supported by several studies showing an inverse correlation between high nut intake and the risk of T2D and cardiovascular disease, which share common metabolic parameters with NAFLD [25-27]. Nevertheless, the relationship between nut intake and NAFLD warrants further exploration, and additional studies are needed to examine gender differences in the association between nut consumption and NAFLD, and its' possible mechanisms should be explored.

Several biological pathways have been proposed to explain the association between nut intake and NAFLD risk. Ellagic acid, a natural antioxidant polyphenol found in nuts, can suppress oxidative stress and inflammation [28] and improve hepatic insulin sensitivity and lipid metabolism [29]. Vitamin E and selenium in almonds and walnuts, as well as folic acid and resveratrol in pine nuts, have been reported to have beneficial effects on NAFLD. Numerous trials testing the therapeutic value of vitamin E in NAFLD prevention found that Vitamin E significantly improved liver function and histologic changes by significantly reducing aspartate aminotransferase, alanine aminotransferase, steatosis, and inflammation in patients with NAFLD/non-alcoholic steatohepatitis (NASH) [30]. Selenium is an antioxidant agent, and selenium deficiency is associated with insulin resistance in patients with chronic liver disease [31]. Folate plays an essential role in lipid metabolism and folic acid supplementation can attenuate steatosis and improve oxidative stress in rodent models of NAFLD. Folate can also blunt the increase of inflammatory cytokines secreted by immune cells [32].

Resveratrol has antioxidant, anti-apoptotic, and anti-inflammatory properties in NAFLD patients [33]. Nuts, which are known to be rich in unsaturated fatty acids, are especially rich in n-3 PUFAs, a source of alpha-linolenic acid, which lowers LDL cholesterol [34], and has been found to have beneficial effects on NAFLD [35]. Each type of nut has many nutrients and phytochemicals that may be beneficial to health, and it is likely that unknown salubrious effects of nuts may be related to NAFLD prevention. Moreover, many studies have shown a beneficial association between high nut intake and decreased risk of obesity [36], T2D [37], and MetS [38], which are risk factors for NAFLD.

Our study has both strengths and limitations. The first strength is the large sample size, which may have reduced type II errors. Second, the collection of data using face-to-face interviews and a semi-quantitative food frequency questionnaire, which elicited extensive information on anthropometrics and lifestyle factors, allowed us to adjust for confounding factors. Finally, the study tried to investigate interactions between nut intake and other risk factors, for which a biologically plausible mechanism may exist. Nonetheless, several limitations should be considered when interpreting the study's findings. First, although we accounted for a wide range of socioeconomic characteristics and lifestyle factors, we cannot exclude the effects of unknown or poorly measured confounding variables or residual confounding attributable to other dietary/lifestyle factors, which might have influenced the observed associations. However, the associations persisted even after controlling for known and suspected predictors of NAFLD. Second, the intake of nuts was assessed by asking about using one question; therefore, we could not investigate the effects of

different types of nuts, such as peanuts and walnuts, which contain different amounts of energy, fat content, and other nutrients[39]. Third, categorization of the intake of nuts in quartiles may lead to bias and inefficiency. Fourth, we did not consider manufacturing methods (e.g., raw, roasted, or boiled) or extra ingredients (e.g., sugar, salt, seasoning). Different preparation methods before and after roasting, the amount of time roasted, and the temperature used can affect the nutrient composition and ingredients in the nuts[40 41]. What's more, the item'nuts' in the FFQ used in this study cannot represent all nuts, because it only included peanuts, walnuts, seeds. For this reason, direct comparisons with the results of other studies may be difficult. Fifth, because we used a case-control study design, recall bias is an inherent limitation. People who are health-conscious may over-report or under-report some food items. However, the protective effect of nuts on NAFLD was not generally known at the time of the survey, and thus, should be unrelated to recall bias. In addition, the stratified analyses was not corrected for multiple testing, which may inflate the risk for type I error. Sixth, NAFLD is a spectrum of disease ranging from essentially benign simple steatosis (fatty infiltration of the liver) to the more severe form non-alcoholic steatohepatitis (NASH- fat with inflammation and/or fibrosis). In the present study, the data about the severity of NAFLD was absent, therefore we did not analysis the association between nuts intake and the the severity of NAFLD. Seventh, lack of interactions association between nut intake and other risk factors may be due to lack of power, and a small interactive effect on NAFLD cannot be ruled out. Finally, due to the small number of participants in the highest nut-intake category, we

cannot rule out the possibility that some of our results are due to chance. However, the associations were consistent in the analyses stratified by some of the other factors, which reduces the likelihood of chance findings. In these stratified analyses, the statistical power needed to detect differences was limited by the sample size, and such analyses should be considered exploratory.

Our study had a retrospective design, which is lower on the evidence hierarchy than prospective studies. Nevertheless, case-control studies can provide evidence supporting the general relationship between diet and NAFLD, as there currently are no available prospective studies.

In conclusion, this case-control study indicated that high nut intake was associated with a significantly reduced risk of NAFLD among Han men in China. However, no relationship was found between total nut intake and NAFLD risk among the Han women. Our data contribute to the growing evidence showing that a relatively simple prevention strategy of incorporating a modest amount of nuts in the diet may contribute to maintaining good health at both the individual and population levels.

Acknowledgements

We thank all study participants for their cooperation. We also thank our staff for recruiting participants and for their technical assistance.

Contributors

BBC and YH are joint first authors. XEP obtained funding. XEP, XL and SHX designed the study. XTP ,JHY,YFL and WJL collected the data. BBC and YH were involved in data cleaning and verification. BBC and XTP analyzed the data. BBC and YH drafted the manuscript. XEP ,YFL and JHY contributed to the interpretation of the results and critical revision of the manuscript for important intellectual content and approved the final version of the manuscript. All authors have read and approved the final manuscript.

Data sharing

Extra data can be accessed via the Dryad data repository at http://datadryad.org/ with the doi:10.5061/dryad.8nn2j46

Conflict of Interest

The authors declare that they have no conflict of interest.

Funding

This work was supported by the National Natural Science Foundation of China [grant number 81473047], the National Natural Science Foundation of Fujian Province [grant number 2019J01316], and the Joint Innovation Project of Science and Technology of Fujian Province [grant number 2017Y9104].

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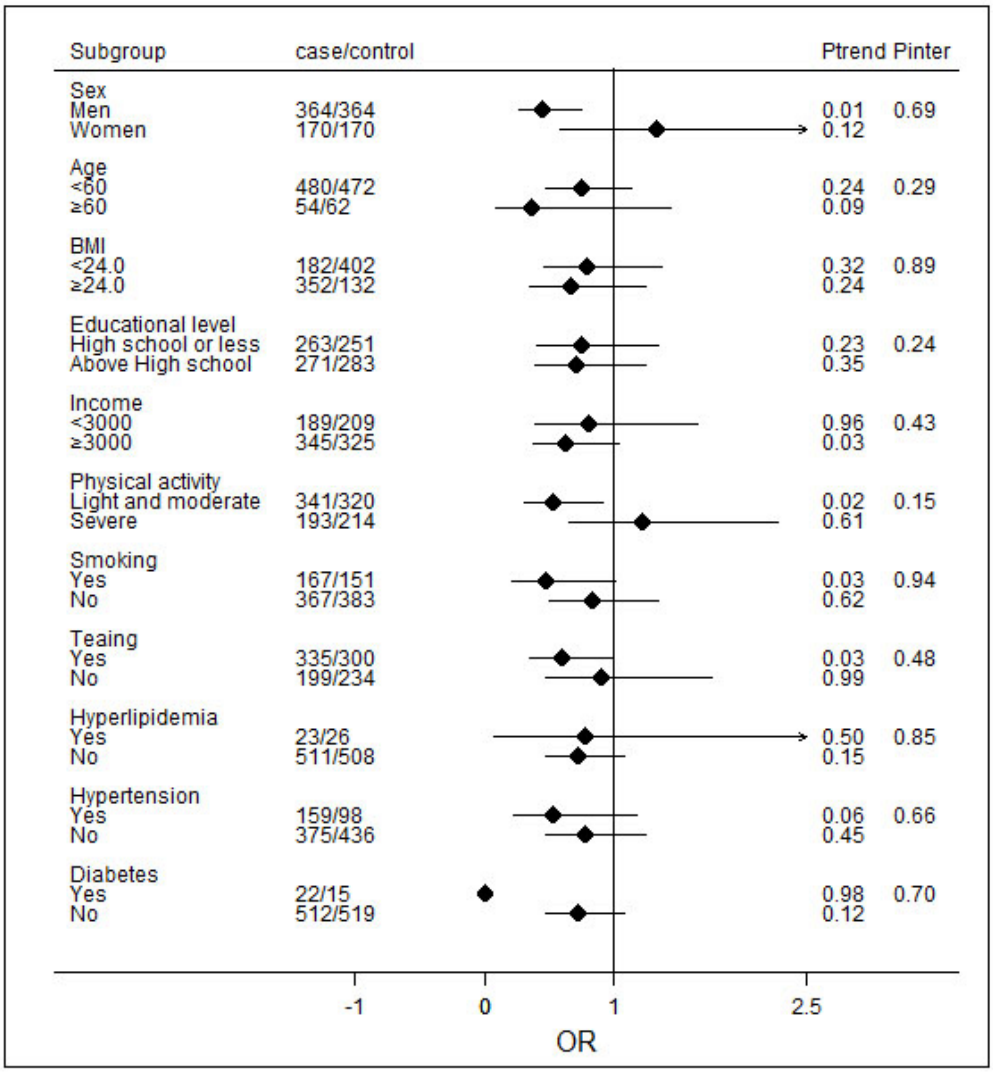
Figure legends

Figure 1. Odds ratios (OR) and 95% CIs for NAFLD across the strata of various factors.

The forest plot represents the ORs of the comparison of the lowest nut-intake category versus the highest nut-intake category, adjusting for age, income, smoking status, educational level, tea-drinking status, occupational status, marital status, BMI, physical activity, history of diabetes, hypertension and hyperlipidemia, total energy intake, and MUFA and PUFA intake. *P*-values are two-tailed; P_{inter} indicates *P* for the interaction between strata and nut intake; P_{trend} indicates *P* for the trend across levels of nut intake.



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148x159mm (96 x 96 DPI)

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *case-control studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	*(*a) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls	5
		(*b*) For matched studies, give matching criteria and the number of controls per case	5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5-6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5-6
Bias	9	Describe any efforts to address potential sources of bias	7

Study size	10	Explain how the study size was arrived at	5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5-6
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6-7
		(*b*) Describe any methods used to examine subgroups and interactions	7
		(*c*) Explain how missing data were addressed	
		(*d*) If applicable, explain how matching of cases and controls was addressed	5
		(*e*) Describe any sensitivity analyses	
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8-9
		(b) Indicate number of participants with missing data for each variable of interest	
Outcome data	15*	Report numbers in each exposure category, or summary measures of exposure	8-9
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9-10
		(*b*) Report category boundaries when continuous variables were categorized	10-11

		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	11
Discussion			
Key results	18	Summarise key results with reference to study objectives	12
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	14
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12-14
Generalisability	21	Discuss the generalisability (external validity) of the study results	15
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	17

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.