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Association between dietary tea consumption and non-alcoholic fatty liver disease: a study based on Mendelian randomisation and National Health and Nutrition Examination Survey (2005-2018) association between tea and non-alcoholic fatty liver disease

Shuyu Liu[1], Quanpeng Li[1], Peng Chen[2], Yuting Wang[1], Xianxiu Ge[1], Fei Wang[1], Mengyue Zhou[3], Jianing Xu[1], Yingting Zhu[1], Lin Miao[1]* and Xueting Deng[1]*

[1]*Medical Center for Digestive Diseases, Second Affiliated Hospital, Nanjing Medical University, Nanjing, People's Republic of China*

[2]*The Fourth Affiliated Hospital of Nanjing Medical University, Nanjing, People's Republic of China*

[3]*Department of Gastroenterology of Nanjing Pukou Hospital of Traditional Chinese Medicine, Nanjing, People's Republic of China*

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Abstract

Tea can improve the progression of some metabolic diseases through anti-inflammatory and antioxidant effects, but its impact on non-alcoholic fatty liver disease (NAFLD) is still controversial. The aim of this paper is to identify the relationship between tea and NAFLD by Mendelian randomisation (MR) and complete clinical validation using National Health and Nutrition Examination Survey (NHANES) database. MR used data from Genome Wide Association Study, with inverse-variance weighted (IVW) as principal analytical methods. The reliability of the results was verified by a series of sensitivity and heterogeneity tests. Subsequently, clinical validation was conducted using NHANES (2005–2018), involving 22 257 participants, grouped by the type of tea. Green tea drinkers were categorised into four groups (Q1–Q4) by quartiles of green tea intake, from lowest to highest (similar for black tea drinkers and other tea drinkers). Models were constructed by logistic regression to estimate the role of tea consumption (Q1–4) on NAFLD. Finally, using fibrosis-4 index (FIB-4) to evaluate the severity of hepatic fibrosis, the effect of tea consumption (Q1–4) on the degree of hepatic fibrosis was investigated by linear regression. IVW method (OR = 0·43, 95 % CI: 0·21, 0·85, $P = 0·01$) and weighted median method (OR = 0·35, 95 % CI: 0·14, 0·91, $P = 0·03$) revealed there was a causal relationship between tea and NAFLD. An array of sensitivity analyses validated the reliability of results. Analysis of NHANES indicated tea drinker present a slightly lower prevalence of NAFLD than non-tea drinker (green tea drinkers: 47·6 %, black tea drinkers: 46·3 %, other tea drinker: 43·2 %, non-tea drinkers: 48·1 %, $P < 0·05$). After adjusting for confounders, compared with the lowest black tea consumption (Q1), the population with the highest black tea consumption (Q4) was independently related to lower presence of NAFLD (Q4: OR = 0·69, 95 % CI: 0·50, 0·93, $P < 0·05$), such association remained stable in the overweight subgroup. As further analysed, Q4 also displayed a significant negative correlation with the level of hepatic fibrosis in patients with NAFLD ($\beta = -0·073$, 95 % CI: −0·126, −0·020, $P < 0·01$).Tea reduces the morbidity of NAFLD and ameliorates hepatic fibrosis degree in those already suffering from the disease.

Keyword: **Beverages: Tea: non-alcoholic fatty liver disease: National Health and Nutrition Examination Survey**

NAFLD is a metabolic disease typified by steatosis, vacuolisation and inflammatory infiltration of hepatocytes, etc[1]. In severe cases, NAFLD may be accompanied by liver fibrosis, hepatocyte necrosis or apoptosis and other pathological changes[2]. The morbidity rate of NAFLD is increasing over the years, especially in western developed countries, where the average morbidity rate reaches 20–30 %; thus, preventing and treating NAFLD is of great significance[3]. However, there is no specific drug for NAFLD, and life interventions (i.e. diet, exercise, etc.) with the ultimate target of weight loss are still the first-line treatment in clinical practice at present[2,4]. Tea, one of the most consumed beverages globally, has been proven to have strong antioxidant and apoptosis-inducing effect on inflammatory cells with its various bioactive components; it has also been proven to have remarkable effects on reducing body fat and improving NAFLD[5–9]. However, previous studies were

Abbreviations: FIB-4, fibrosis-4 index; IV, instrumental variables; IVW, inverse-variance weighted; MR, Mendelian randomisation; NAFLD, non-alcoholic fatty liver disease; NHANES, National Health and Nutrition Examination Survey; ALT, Alanine transaminase; AST, Aspartate aminotransferase.

* **Corresponding authors:** Xueting Deng, email 2094539575@qq.com; Lin Miao, email syljanelsy@sina.com. Institution email: 20203176@stu.hebmu.edu.cn

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mostly experimental and small clinical studies and were limited to one type of tea, lacking studies comparing the relationship between different types of tea and NAFLD[9,10].

Hitherto, the association between tea consumption and NAFLD, remains controversial. Hypothesis of this study is that tea has a protective impact on NAFLD. Mendelian randomisation (MR) is a novel approach of simulating randomised controlled trials using SNP as instrumental variables (IV), which effectively eliminates potential confounders or reverse causality that may introduce research bias[11]. The author first used MR to reveal a causal association between tea and NAFLD at genetic level and then used data from National Health and Nutrition Examination Survey (NHANES) for clinical validation, innovatively combining the two to enhance the credibility of this study. This study will provide novel insights and targets for the prevention and drug development of NAFLD from a novel genetic perspective.

Materials and methods

Study overview

Extraction of tea and non-alcoholic fatty liver disease SNP from summarised Genome Wide Association Study data. A convincing MR design should be compliant with following three basic hypotheses: Hypothesis 1, IV are strongly correlated to exposure; Hypothesis 2, genetic variation is irrelevant to confounding factors; Hypothesis 3, IV only influence outcomes through exposure. The outline of this study and three hypotheses are shown in Fig. 1(a).

Study Population in NHANES. NHANES is launched by USA National Center for Health Statistics on a two-year-cycle, consists of demographic information, diet and dietary supplement information, a variety of blood drawing indicators and test reports (e.g. colour ultrasound, etc.). All detailed information display on official websites and are available in http://www.cdc.gov/nchs/nhanes.htm. National Center for Health Statistics Research Ethics Review Committee approved the protocols of research. All individuals enrolled in the programme signed an informed consent form. All data collected for this study were coded, with personal identifiers removed, before being available to the public. Our study included a total of 7 cycles of surveys from 2005 to 2018, as shown in Fig. 1(b).The exclusion criteria included the following: (1) Under the age of 20; (2) pregnant; (3) abnormal hepatitis-related antigen or antibody tests, hepatocellular carcinoma/autoimmune hepatitis; (4) excessive alcohol consumption, with excessive alcohol consumption defined as alcohol intake of > 20 g/d for male or > 10 g/d for female; (5) abnormal energy intake, with total energy intake < 800 kcal/d, or > 4200 kcal/d; (6) participants who drank more than one type of tea; (7) participants who lack covariates such as age, sex and race.

Genome wide association studies (GWAS) data for non-alcoholic fatty liver disease

To make the genetic characteristics of the two samples similar, the database of European cohorts was selected for both exposure and outcome. Genetic information on NAFLD is accessible at the following website: https://gwas.mrcieu.ac.uk. Specifically, Professor Ghodsian conducted a meta-analysis involving cohorts from four databases (including 1 106 diseased and 8571 healthy individuals from eMERGE; 651 diseased and 176 248 healthy individuals from FinnGen; 2, 558 diseased and 395, 241 healthy individuals from UK Biobank; 4119 diseased and 190 120 healthy individuals from Estonian Biobank), resulting in a large-scale Genome Wide Association Study involving 8434 cases and 770 180 controls[12].

GWAS data for tea and selection of instrumental variables

Access to genetic data associated with tea was acquired through the following website: https://www.ebi.ac.uk/gwas/, totalling 434 171 cases with a SNP count of 5 733 790[13].To guarantee the validity of IV utilised in this MR research, author took the following steps to screen eligible instrumental SNP (Fig. 1(a)). First, given the limited amount of SNP that qualified for whole-genome significance of $P < 5 \times 10^{-8}$, authors obtained 3, 660 SNP associated with tea from the Genome Wide Association Study summary statistics for tea using $P < 1 \times 10^{-6}$ as the screening criterion[14–16]. Then, PLINK was utilised to conduct a linkage disequilibrium test ($r^2 < 0{\cdot}001$) to guarantee independency of the chosen IV. And the F-statistic of SNP ($F > 10$) was calculated to avoid bias resulting from the use of weak genetic instruments[17]. After above screening, 58 SNP were finally acquired as IV for this study (Fig. 2). Detailed information of above 58 IV was shown in Additional file 1: online Supplementary Table 1). We calculate F-statistic according to following equation:

$$F = R^2 \times (N - 2)/(1 - R^2),$$

R^2 represents the extent to IV can explain the exposure.

$$R^2 = [2 \times \text{Beta}^2 \times (1 - \text{EAF}) \times \text{EAF}]/[2 \times \text{Beta}^2 \times (1 - \text{EAF}) \times \text{EAF} + 2 \times \text{SE}^2 \times N \times (1 - \text{EAF}) \times \text{EAF}]$$

(EAF: effect allele frequency)[18].

Assessment of tea consumption in National Health and Nutrition Examination Survey

NHANES collected all food information consumed by participants 24 h before the survey. All participants underwent two 24-hour dietary review surveys. Since the 2nd survey was collected by telephone after an interval of 3–10 d with low accuracy, all dietary data in this study were derived from the first 24-hour dietary survey. Food types were refined based on codes came from the USA. Department of Agriculture food as well as nutrition database for dietary studies, with different codes representing different sorts of tea consumed. Green tea and black tea were directly extracted from all beverages. After eliminating black and green teas, the remaining teas are categorised into other types of teas (e.g. herbal teas, corn teas, etc.). According to the Measuring Guides for the Dietary Recall Interview, a typical cup of tea was 8 oz (226·7 g).

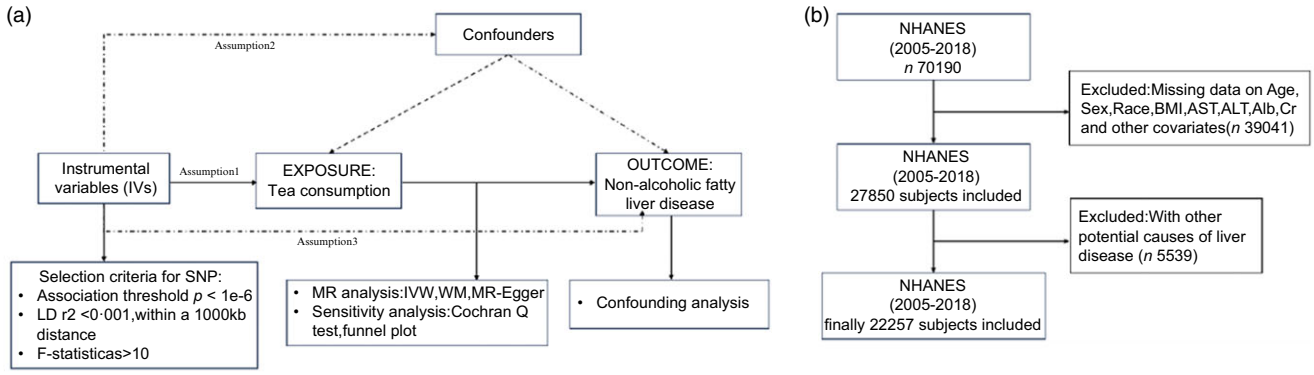

Fig. 1. (a) Overview of the current Mendelian randomisation (MR) study. (b) Flow chart of participants' selection.

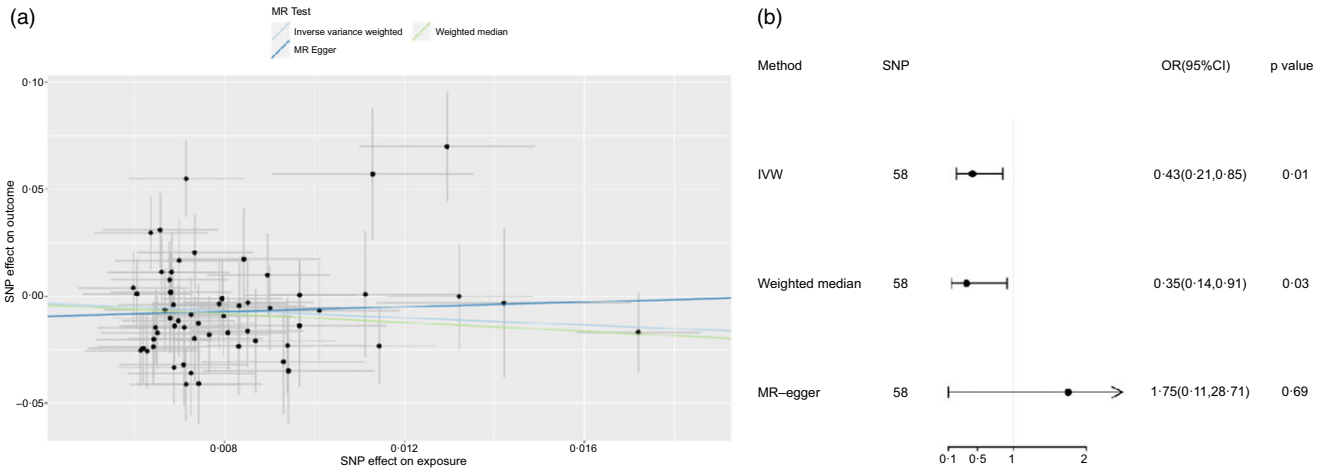

Fig. 2. (a) Scatter plot and (b) forest plot of Mendelian randomisation analyses for the associations of tea with risk of NAFLD. IVW, inverse-variance weighted method; MR, Mendelian randomisation; SNP, single nucleotide polymorphism.

Diagnostic criteria of non-alcoholic fatty liver disease. Fatty liver index, which consisted of TAG, glutamyltransferase, BMI and waist circumference, was utilised for an index to diagnose NAFLD in this study. If fatty liver index >= 60, the participant was considered to have NAFLD, and if <60, non-NAFLD[19].

Non-invasive evaluation indicators of liver fibrosis degree. Fibrosis-4 (FIB-4), which consisted of age, aspartate aminotransferase (AST), alanine transaminase (ALT) and platelet, was utilised for an index to evaluate the degree of liver fibrosis[20].

FIB-4 = (age × AST)/ (platelet × $ALT^{1/2}$), the higher the index value, the more severe the liver fibrosis.

Covariates

Participants' age, sex, race and prior disease history were collected by questionnaires. Diabetes, hypertension, heart failure, lung diseases, tumours are all diagnosed by doctors. Height, weight and waist circumference were collected by uniformly skilled health technicians using uniform measuring instruments at a mobile examination centre, dividing BMI into 4 groups: normal group (18·5 <= BMI < 25), overweight group (25 <= BMI < 30), obese group (BMI >= 30) and underweight group (BMI < 18·5). Laboratory parameters included TAG, glutamyltransferase, fasting glucose, HbA1c, creatinine (Cr) and albumin (Alb) that were obtained by blood drawing at the mobile examination centre by professionally medical technicians following standard operating procedures.

Statistical analysis

Mendelian randomisation. The two-sample MR package of version 4.3.0 of R software was used to conduct statistical analyses, and values were regarded as having statistical significance at $P < 0·05$. Inverse-variance weighted (IVW) was used as the principle method to assess if a causal association exists between tea and NAFLD, preceded with sensitivity analyses to valuate the bias of the MR hypothesis involving weighted median, MR-egger method and so on[21,22]. Outlier tests were performed by MR-PRESSO. Once outliers were found, we eliminated them and replicated the MR. The existence of heterogeneity or horizontal pleiotropy in the outcomes of this study depends on outcomes of Cochran Q-test and the significance of the intercept of MR-egger[23]. Leave-one-out analysis in order to assess if observed causality is strongly influenced by a single SNP. Finally, a search was performed at PhenoScanner (http://www.phenoscanner.medschl.cam.ac.uk) examining whether the IV utilised in present study were relevant

to additional NAFLD risk factors, primarily including hypertension[24], basal metabolism, BMI, smoke[25] and so on. After excluding, SNP associated with the above risk factors re-examine the causal effects with the aim of ascertaining if they are still significant.

National Health and Nutrition Examination Survey. R software version 4.3.0 was used to analyse the statistics, *and* $P < 0{\cdot}05$ was taken to be statistically difference. To avoid oversampling and reduce the rate of non-response, the weights in this analysis were adjusted with reference to the published NHANES article. Continuous and classified variables were characterised by weighted means and weighted percentages, separately[26,27].When comparing intergroup difference, weighed chi-square tests were utilised to classified variables and weighted linear regression models were utilised to the continuous variables. To investigate the connection between tea consumption and NAFLD, the author groups different types of tea separately according to the quartile of daily tea intake (Green tea: Q1 (0, 255) g, Q2 (255, 402·5) g, Q3 (402·5, 720) g, Q4 (> 720) g; Black tea:Q1 (0, 257·5) g, Q2 (257·5, 435) g, Q3 (435, 754·8) g, Q4 (> 754·8) g; Other tea: Q1 (0, 240) g, Q2 (240·0, 355·2) g, Q3 (355·2, 518·0) g, Q4 (> 518) g. With the lowest tea consumption group (Q1) as reference, crude model and models correcting for confounders were constructed by univariate and multivariate logistic regression, respectively. Subgroup analyses were performed based on age, sex, BMI, CHD, lung disease, diabetes, hypertension and tumour. Finally, the effect of tea consumption on hepatic fibrosis levels was also analysed by multivariate linear regression.

Result

Baseline characteristics

This study involved 22 557 subjects, of which 12 463 (47·7 %) were NAFLD patients. Average age of all involved participants was 47·9 ± 2·5 years and average daily tea consumption was 122·49 ± 367·71 g. There were 5134 tea drinkers in this USA population, with a total tea consumption rate of 20·09 %.Among them, the drinking rate of black tea was the highest (about 14·86 %), with an average daily tea consumption of 643·16 ± 648·30 g, followed by other teas (about 2·84 %), with an average daily tea consumption of 442·39 ± 393·42 g, and the drinking rate of green tea was the lowest (about 2·39 %), with an average daily tea consumption of 601·30 ± 569·47 g. In all three types of tea, the rate of tea consumption was higher in women than in men (Table 1). Among non-tea drinkers, there were 10 002 NAFLD patients (48·1 %), among green tea drinkers, 275 NAFLD patients (47·6 %), among black tea drinkers, 1868 NAFLD patients (46·3 %) and among other tea drinkers, 318 NAFLD patients (43·2 %) (Fig. 3).

Causal association between tea and non-alcoholic fatty liver disease

Utilising IVW as the principle method of MR analysis, the outcomes upheld a potentially negative causal association between tea and NAFLD (OR: 0·43, 95 % CI: 0·21, 0·85, $P = 0{\cdot}01$). It means that for every SD increase in tea consumption, the risk of developing NAFLD was reduced by 57 %.Weighted median method was used for sensitivity analysis, and the correlation and direction were consistent with those obtained by IVW (OR: 0·35, 95 % CI: 0·14, 0·91; $P = 0{\cdot}03$). Nevertheless, nothing significant was observed between tea consumption and NAFLD by utilising MR-egger (OR: 1·75, 95 % CI: 0·11, 28·70, $P = 0{\cdot}69$) (Fig. 2). The difference between IVW and MR-egger analysis lies in that MR-egger takes intercept term's presence in the regression into account, while $P = 0{\cdot}31$ of the MR-Egger intercept in this paper is of no significance. In addition, weighted median method has the advantage of retaining higher estimation precision in comparison with MR-egger analysis. And the results of weighted median method and IVW method in this study were consistent; therefore, the author concluded that there exists a negative causal relationship between tea and NAFLD. Heterogeneity test indicated no significant heterogeneity (Q-value (df) = 56, $P = 0{\cdot}08$). *P*-value of MR-egger intercept was 0·31, implicating non-existence of horizontal pleiotropy. Followed by leave-one-out analysis, after removing any SNP sequentially, the remaining SNP was on the left side of the invalid line, indicating that a single SNP had a limited contribution to the outcomes. Finally, through PubMed retrieval, a total of 6 SNP were found to have secondary phenotypes, and after excluding SNP with secondary phenotypes, MR analysis was re-conducted, the results were basically consistent (IVW method was adopted, OR: 0·46, 95 % CI: 0·21, 0·97, $P = 0{\cdot}04$), as shown in Additional file 1: online Supplementary Table 2).

Relationship between intake of different sorts of tea and non-alcoholic fatty liver disease

To further investigate the connection between tea consumption and NAFLD, the author grouped tea consumption by quartiles. The results showed that the highest black tea intake group (Q4) was significantly negatively associated with NAFLD in model1 which was not adjusted for any confounders (OR: 0·81, 95 % CI: 0·67, 0·96, $P < 0{\cdot}05$) and maintained negatively correlated with NAFLD after adjustment of age, sex and race in model2 (OR: 0·82, 95 % CI: 0·68, 0·99, $P < 0{\cdot}05$). This correlation remained steady after continuing to adjust for BMI, co-morbidity index, creatinine, albumin, FIB.4, AST to ALT (OR: 0·69, 95 % CI: 0·50, 0·93, $P < 0{\cdot}05$) (Table 2), but neither green tea nor other tea displayed significant association with NAFLD. Also, the prevalence of NAFLD was significantly lower in the highest black tea intake group (Q4) than in the other groups (Q1: 25·42 %, Q2: 25·32 %, Q3: 26·45 %, Q4: 22·81 %, $P = 0{\cdot}011$) (Fig. 4).

Subgroup analysis

Black tea is the most consumed tea beverage in developed countries; thus, authors further investigated the correlation between black tea intake (Q4) and NAFLD (Fig. 5). Following stratification of the study populations by age, sex, BMI and disease condition, independent multifactorial logistic regression analyses were performed for each subgroup. Except for the variables used for stratification, the variables adjusted in Model3 remain in this

Table 1. Baseline characteristics of study population (Numbers and percentages; Mean values and SD)

	Total		No tea		Green tea		Black tea		Other tea		*P*-value
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	
Count, *n*	22 257	100·00	20 423	79·91	611	2·39	3797	14·86	726	2·84	
Quantity of tea consumption, g/d											
Mean	122·49		–		601·30		643·16		442·39		
SD	367·71				569·47		648·30		393·42		
Age											
Mean	47·9		48·00		48·00		47·49		47·74		0·52
SD	2·5		0·28		1·05		0·49		1·20		
Sex											0·3
Male	12 233	47·40	9753	47·70	289	45·40	1830	45·90	361	48·00	
Female	13 344	52·60	10 670	52·30	322	54·60	1967	54·10	365	52·00	
Race											< 0·01
Non-Hispanic White	10 662	40·80	10 662	51·00	0	0·00	0	0·00	0	0·00	
Non-Hispanic Black	5363	18·60	2841	13·20	0·	0·00	2522·	61·70	0	0·00	
Mexican American	4294	18·80	4294	23·50	0·	0·00	0	0·00	0	0·00	
Other Hispanic	2626	10·50	2626	12·30	0·	0·00	0	0·00	0	0·00	
Other Race	2612	11·30	0·	0·00	611	100·00	1275	38·30	726	100·00	
BMI											0·1
Normal	6581	26·78	5237	26·45	174	28·64	970·	28·16	200·	27·26	
Overweight	8428	32·49	6650	32·16	203	31·78	1319·	33·32	256·	37·51	
Obese	10 175	39·19	8236	39·88	224	37·83	1458·	36·99	257	32·85	
Underweight	373	1·55	300	1·510	10	1·74	50	1·52	13	2·37	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
TAG, mg/dl	155·84	1·34	154·64	1·58	163·24	6·98	160·54	2·68	159·04	5·34	0·034
Albumin, g/dl	4·26	0·01	4·27	0·01	4·24	0·02	4·24	0·01	4·23	0·02	0·012
Creatinine, mol/l	0·89	0·01	0·88	0·01	0·97	0·04	0·90	0·01	0·92	0·01	< 0·01
Aspartate aminotransferase, U/l	24·70	0·12	24·58	0·13	25·54	0·71	24·97	0·21	25·65	0·88	0·052
Alanine aminotransferase, U/l	24·89	0·16	24·78	0·18	26·57	1·55	24·89	0·36	26·31	1·29	0·27
Aspartate aminotransferase/Alanine transaminase	1·11	0·01	1·11	0·01	1·09·	0·01	1·11	0·01	1·09	0·01	0·53
Fibrosis-4	1·08	0·01	1·12	0·01	0·95	0·03	0·98	0·01	0·94	0·04	< 0·01
γ-glutamyl transpeptidase, U/l	26·24	0·32	25·97	0·33	31·91	3·87	26·96	0·65	25·13	0·97	0·11
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	
Disease											
Heart failure	834	2·43	648	2·35	20	2·26	149	3·09	17	1·50	0·05
CHD	1094	3·60	866	3·63	31	3·60	173	3·63	24	2·59	0·7
Lung disease	1812	7·29	1403	7·03	36	6·86	310	8·28	63	9·80	0·02
Diabetes	4748	14·10	3878	14·5	94	10·7	671	12·8	105	11·4	0·01
Hypertension	9363	32·40	7564	32·6	200	30·5	1354	32·0	245	29·8	0·6
Non-alcoholic fatty liver disease	12 463	47·7	10 002	48·1	275	47·6	1868	46·3	318	43·2	0·05
Other tumour	2441	10·1	1958	10·4	54	9·18	363	9·16	66	8·27	0·2
Comorbidity index:											0·9
1	8078	29·9	1567	30·0	178	30·01	1167	29·2	222	29·0	
2	14 230	58·8	2895	58·7	361·	60·01	2116	59·0	418	60·6	
3 or more	3249	11·1	672	11·3	72	9·98	514	11·8	86	10·4	

analysis. The association remained stable among the overweight subgroup (OR: 0·67, 95 % CI: 0·47, 0·95, $P < 0·05$).

Relationship between tea consumption and the degree of hepatic fibrosis

NAFLD could evolve into liver fibrosis or even cirrhosis if it continued to progress. To further explore the connection between tea consumption and disease progression, linear regression analysis was used to analyse the effect of tea consumption on FIB-4, a non-invasive index of hepatic fibrosis (Table 3). After adjusting for confounders (Model3), with the non-tea drinker as the reference, tea drinkers was significantly negatively correlated with FIB-4 ($\beta = -0·039$, 95 % CI: −0·075, −0·003, $P < 0·05$). Further analysis showed that consumption of black tea > 754·8 g (Q4) had the most protective effect on fibrosis degree in NAFLD (Q4: $\beta = -0·073$, 95 % CI: −0·126, −0·020, $P < 0·01$).

Discussion

This study explored the association between tea consumption and NAFLD using genetic data from Genome Wide Association Study and clinical data from the large cross-sectional study NHANES. Our findings suggest that there exists a causal association between tea consumption and NAFLD based on genetic prediction. And when grouped according to the type of tea, it was found that consumption of black tea > 754·8 g per day reduced the risk of NAFLD by 24 % and had a protective effect against hepatic fibrosis. However, green tea and other types of

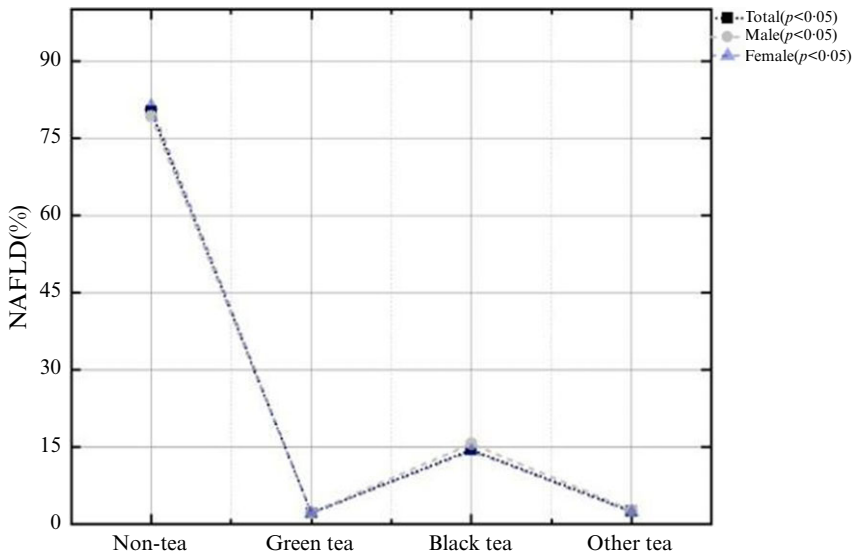

Fig. 3. Proportion of NAFLD in the different types of tea drinkers.

Table 2. Associations between tea consumption and non-alcoholic fatty liver disease (OR and 95 % CI)

	Participants	Model1		Model2		Model3	
Character	*n*	OR	95 % CI	OR	95 % CI	OR	95 % CI
Black tea consumption (g/d)							
Q1 (0–257·5)	949	Reference		Reference		Reference	
Q2 (257·5–435)	952	1·01	0·85, 1·21	1·06	0·88, 1·27	1·10	0·81, 1·49
P		0·889		0·537		0·527	
Q3 (435–754·8)	960	1·07	0·89, 1·28	1·07	0·91, 1·32	1·11	0·82, 1·50
P		0·465		0·310		0·507	
Q4 (> 754·8)	936	0·81	0·67, 0·96	0·82	0·68, 0·99	0·69	0·50, 0·93
P		< 0·05		< 0·05		< 0·05	
Green tea consumption (g/d)							
Q1 (0–255)	180	Reference		Reference		Reference	
Q2 (255–402·5)	127	1·32	0·82, 2·12	1·32	0·82, 2·14	1·68	0·81, 3·51
P		0·25		0·25		0·16	
Q3 (402·5–720)	165	0·83	0·54, 1·27	0·83	0·54, 1·29	0·98	0·50, 1·95
P		0·39		0·41		0·95	
Q4 (> 720)	139	1·08	0·70, 1·67	1·01	1·00, 1·02	0·84	0·43, 1·66
P		0·71		0·67		0·62	
Other tea consumption (g/d)							
Q1 (0–240)	178	Reference		Reference		Reference	
Q2 (240–355·2)	214	0·86	0·57, 1·28	0·88	0·59, 1·32	1·26	0·67, 2·37
P		0·45		0·54		0·47	
Q3 (355·2–518)	165	0·99	0·64, 1·53	1·02	0·65, 1·52	1·49	0·75, 2·98
P		0·95		0·53		0·25	
Q4 (> 518)	169	1·01	0·67, 1·54	1·00	0·66, 1·52	0·97	0·52, 1·82
P		0·97		0·98		0·93	

Model1: Crude model (without adjustment for any confounders). Model2 was corrected for age, sex and race. Model3 was corrected for variables included in Model2 and BMI, comorbidity index, albumin, creatinine fibrosis-4 index, aspartate aminotransferase, alanine transaminase.

tea were not significantly associated with a low prevalence of NAFLD.

Epidemiologic studies on the association between tea consumption and NAFLD are scarce, and we are aware of only 4 studies[9,28–30].In a large prospective cohort study in 2022[9], 372, 492 participants without liver disease at baseline were followed for 12 years, with 3527 cases of NAFLD, 1643 cases of cirrhosis and 669 cases of hepatocellular carcinoma occurring during follow-up. Grouped by different dietary groups to explore NAFLD risk ratios, it was found that higher tea consumption (HR: 0·85, 95 % CI: 0·77, 0·94) was significantly associated with a lower risk of NAFLD, and similar results were observed in cirrhosis and hepatocellular carcinoma. Despite the study type and statistical approach was different, this multivariable-adjusted HR was comparable to that in our analysis of black tea (OR: 0·69, 95 % CI: 0·50, 0·93), i.e. tea consumption was a protective factor for NAFLD.A cross-sectional study conducted by Soleimani et al of 170 NAFLD participants using Fibroscan to

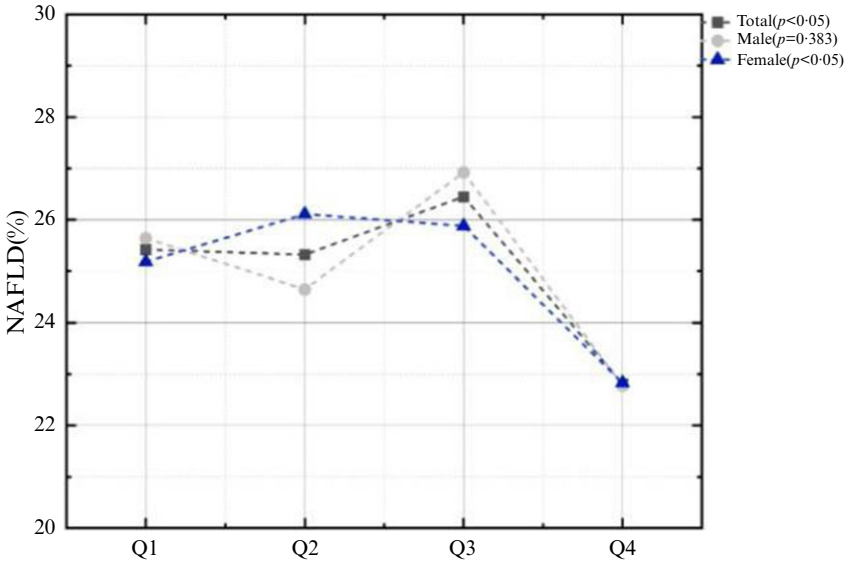

Fig. 4. Proportion of NAFLD in the quartile of black tea gram.

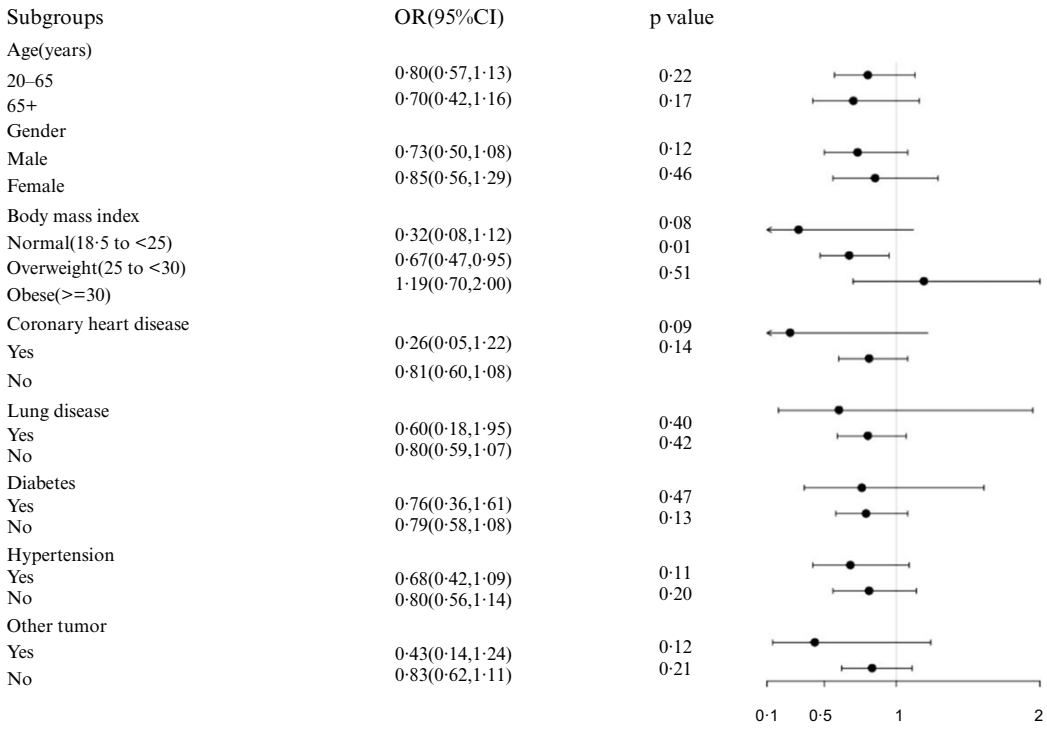

Fig. 5. Association between black tea (Q4) and NAFLD in different stratifications. The model adjusted for covariates such as age, gender, BMI, comorbidity index, albumin, creatinine, FIB-4, AST, ALT, but the model did not adjust for the stratification variables themselves.

assess hepatic fibrosis and using three 3-day dietary records during a 1-month period to assess diet showed that the risk of developing fibrosis was significantly lower in patients in the highest tertile of tea consumption (OR: 0·38; 95 % CI: 0·17, 0·71) than those in the lowest tertile, suggesting a protective role of tea on hepatic fibrosis[28]. Due to limited data, in our study we used FIB-4 rather than Fibroscan to assess hepatic fibrosis and obtained comparable results to their study, the highest quartile of black tea consumption significantly improved the hepatic fibrosis (β:–0·073, 95 % CI: –0·126, –0·020). Another cohort study enrolled 20, 051 participants without fatty liver at baseline. Using NAFLD onset as the outcome event, multivariate Cox analysis showed that tea consumption was a protective factor against NAFLD (OR: 0·86, 95 % CI: 0·78, 0·94)[30].The results of these studies support our conclusions, but it is important to notice that they do not detail the tea types. There is only one cross-sectional study exploring the association between different tea types and NAFLD. Yang et al found a positive association

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Table 3. Linear regression analysis of tea and black tea intake (classification) and degree of liver fibrosis (95 % CI)

	FIB-4			FIB-4		
	β	95 % CI	*P*	*β*	95 % CI	*P*
Age (years)						
20–65	Reference			Reference		
65+	0·756	0·735, 0·778	< 0·01	0·745	0·697, 0·792	< 0·01
Sex						
Male	0·237	0·219, 0·255	< 0·01	0·198	0·157, 0·240	< 0·01
Female	Reference			Reference		
Race						
Non-Hispanic White	0·140	0·094, 0·186	< 0·01	–	–	–
Non-Hispanic Black	0·064	0·027, 0·100	< 0·01	–0·013	–0·051, 0·024	0·492
Mexican American	0·035	–0·014, 0·083	0·162	–	–	–
Other Hispanic	0·152	0·101, 0·203	< 0·01	–	–	–
Other Race	Reference			Reference		
Body mass index, kg/m^2						
Underweight	–0·075	–0·145, –0·005	0·036	–0·026	–0·194, 0·142	0·759
Normal	–0·014	–0·035, 0·006	0·175	–0·020	–0·072, 0·031	0·441
Overweight	0·030	0·011, 0·048	< 0·01	0·041	–0·005, 0·086	0·078
Obese	Reference			Reference		
Drink tea				–	–	–
Yes	–0·039	–0·075, –0·003	0·033			
No	Reference					
Black tea (g)	–	–	–			
Q1				Reference		
Q2				–0·023	–0·076, 0·030	0·399
Q3				–0·045	–0·098, 0·007	0·093
Q4				–0·073	–0·126, –0·020	< 0·01
Comorbidity index:						
1	Reference			Reference		
2	–0·183	–0·202, –0·163	< 0·01	–0·160	–0·205, –0·115	< 0·01
3	0·124	0·097, 0·152	< 0·01	0·063	0·001, 0·125	0·045
Creatinine, mol/L	0·033	0·013, 0·052	< 0·01	0·127	0·078, 0·176	< 0·01
Albumin, g/dl	–0·156	–0·184, –0·129	< 0·01	–0·062	–0·125, 0·001	0·053
Aspartate aminotransferase/Alanine transaminase	0·629	0·603, 0·655	< 0·01	0·549	0·488, 0·610	< 0·01

Corrected for age, sex, race, BMI, tea consumption, comorbidity index, albumin, creatinine, aspartate aminotransferase and alanine transaminase.

between green tea (OR: 1·48, 95 % CI: 1·33, 1·65), oolong tea (OR: 1·50, 95 % CI: 1·33, 1·68),and black tea (OR: 1·28, 95 % CI: 1·13, 1·46) and the prevalence of NAFLD[29]. However, no significant association was found between tea consumption and the prevalence of NAFLD after correcting for factors that differed in baseline information and factors that were judged to be possible risk factors for NAFLD from a clinical perspective, suggesting that the confounding effect of common risk factors for NAFLD should not be underestimated. Our study took this fully into consideration when developing the regression model by including variables that did not differ in the baseline information, but were common risk factors for NAFLD. A prospective cohort study conducted in Yoshimi Town, Saitama Prefecture, recorded residents' disease history, health status, medication history and tea consumption (categorised as </= 3, 4–9, or >/= 10 cups per day).The result showed that tea consumption reduced hepatocyte injury. Maximum tea consumption (>/= 10 cups) was significantly and negatively correlated with AST and ALT[31]. However, the results of our study suggested that there was no significant difference in AST and ALT between tea drinkers and non-tea drinkers, which may be due to the fact that the distribution of the populations was not balanced in our study, with the number of tea drinkers being significantly smaller than the number of non-tea drinkers. In addition, Hoofnagle et al. found in their study that high doses of tea extracts may cause liver injury[32–34], so further longitudinal observational studies and randomised controlled trials are needed to evaluate the changes in liver function (such as AST and ALT) among tea drinkers. In conclusion, studies investigating the effect of tea consumption on NAFLD have inconsistent results, so in addition to observational studies using NHANES, we also used MR to provide a higher level of evidence[35]. Both the exposure and outcome SNP of this study were from European populations, potentially reducing population heterogeneity. Also, we used two models (IVW and weighted median model) to co-validate the existence of a causal relationship between tea consumption and NAFLD. All these make the results of this study more convincing.

Tea contains a variety of bioactive compounds, green tea being abundant in catechins and theanine, and black tea being abundant in theaflavins, theabrownin and caffeine, which could improve NAFLD via their anti-inflammatory, apoptosis-inducing and antioxidant abilities[5–7,36–38]. The mechanism of the protective effect of black tea on NAFLD can be explained as follows: theabrownin, a major component of black tea, was found to inhibit obesity and NAFLD in mice through serotonin-related signalling pathways and the gut-liver axis in animal studies[39]. Theaflavin, another major component of black tea,

directly binds to and inhibits the activation of plasma kinin-releasing enzyme, which further stimulates adenosine monophosphate-activated protein kinase and its downstream targets to reduce the lipid deposition of hepatocytes[40]. Theaflavin also regulates lipid metabolism through the Fads1/PPARδ/Fabp4 axis[41]. Caffeine in black tea may alleviate NAFLD by increasing low density lipoprotein receptor expression through direct binding to epidermal growth factor receptor (EGFR) and activating the EGFR-ERK1/2 signalling pathway[42].There are also studies that regard black tea as a whole. Shen et al. found that black tea alleviated high-fat diet-induced NAFLD by promoting the expression of PPARα in liver tissue and thereby promoting fatty acid β-oxidation and VLDL synthesis[43]. Moreover, black tea could significantly decrease the ratio of Firmicutes to Bacteroidetes, preventing NAFLD by modulating the intestinal microbiota[44].

There was no association between green tea or other teas and low prevalence of NAFLD in our study. Instead, Karolczak found that green tea reduced the prevalence of NAFLD in rats fed a high-fat diet[45]. Considering that among 22 257 participants included in our study, only 611 consumed green tea and 726 consumed other tea, which may result in poor statistical efficacy, subsequent studies in larger sample sizes are needed. Consistent with previous studies[9,28], high black tea consumption was negatively correlated with the degree of hepatic fibrosis, exerted a protective effect against NAFLD and had a therapeutic optimal effect in overweight NAFLD patients (OR: 0·67, 95 % CI: 0·47, 0·95).This may be because black tea polyphenols are more effective than green tea polyphenols in lowering body weight and anti-obesity[46], and weight control is an important measure in NAFLD disease management. Interestingly, in a study that included 1013 people with type 2 diabetes, it was found that drinking tea more than twice a day led to an increased risk of developing NAFLD[47]. In our study, high consumption of black tea also had no therapeutic effect on subgroups such as diabetes, probably because when NAFLD is co-morbid with diabetes, accompanied by worsening of insulin sensitivity and losing of glycaemic control[48], which can deteriorate the condition of NAFLD further, and so the therapeutic effect of tea may not be significant. The reasons for the differences in the effects of black tea on NAFLD with different underlying diseases still need to be further explored.

To the authors' knowledge, this study is the first to combine genetic inference from MR with clinical validation in a large NHANES cohort to elucidate the relationship between tea-drinking habit and the risk of developing NAFLD. In addition, this study is the first large cross-sectional study to focus on the association between black tea and NAFLD. However, some limitations do exist in this study. First, in MR analysis, only summary-level statistics from general population were available, so subgroup analyses based on age, sex, etc. could not be performed, and the results could not be mutually verified with the subgroup analyses of NHANES study. Second, due to limited data or insufficient number of subjects analysed, we could not further analyse the association between other types of tea (e.g. oolong tea, white tea, etc.) and NAFLD, or between tea consumption and different subtypes of NAFLD (e.g. simple fatty liver, non-alcoholic steatohepatitis, etc.). Third, the subjects of this study were adult Americans, excluding adolescents and children, which may affect the generalisation of the results.

In conclusion, our study revealed that high black tea consumption was a protective factor for NAFLD and negatively correlated with hepatic fibrosis. These findings provide new ideas for the clinical treatment of NAFLD and encourage people to reduce the prevalence of NAFLD by adopting the habit of drinking tea, and provide new insights on diet-based health interventions for NAFLD. Further prospective studies are necessary in large populations to determine the association between different types of tea and NAFLD.

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There are no conflicts of interest.

Supplementary material

For supplementary material/s referred to in this article, please visit https://doi.org/10.1017/S0007114524002277

References

1. Wesolowski SR, Kasmi KC, Jonscher KR, *et al.* (2017) Developmental origins of NAFLD: a womb with a clue. *Nat Rev Gastroenterol Hepatol* **14**, 81–96.
2. Younossi ZM, Koenig AB, Abdelatif D, *et al.* (2016) Global epidemiology of nonalcoholic fatty liver disease-meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology (Baltimore, Md)* **64**, 73–84.
3. Younossi Z, Anstee QM, Marietti M, *et al.* (2018) Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. *Nat Rev Gastroenterol Hepatol* **15**, 11–20.
4. Martel C, Esposti DD, Bouchet A, *et al.* (2012) Non-alcoholic steatohepatitis: new insights from OMICS studies. *Curr Pharmaceut Biotechnol* **13**, 726–735.
5. Yang CS & Wang ZY (1993) Tea and cancer. *J Natl Cancer Institute* **85**, 1038–1049.
6. Xia HM, Wang J, Xie XJ, *et al.* (2019) Green tea polyphenols attenuate hepatic steatosis, and reduce insulin resistance and inflammation in high-fat diet-induced rats. *Int J Mol Med* **44**, 1523–1530.
7. Wu Z, Huang S, Li T, *et al.* (2021) Gut microbiota from green tea polyphenol-dosed mice improves intestinal epithelial homeostasis and ameliorates experimental colitis. *Microbiome* **9**, 184.
8. Mokra D, Joskova M & Mokry J (2022) Therapeutic effects of green tea polyphenol (-)-Epigallocatechin-3-Gallate (EGCG) in relation to molecular pathways controlling inflammation, oxidative stress, and apoptosis. *Int J Mol Sci* **24**, 340.

9. Guo W, Ge X, Lu J, *et al.* (2022) Diet and risk of non-alcoholic fatty liver disease, cirrhosis, and liver cancer: a large prospective cohort study in UK Biobank. *Nutrients* **14**, 5335.
10. Tang G, Xu Y, Zhang C, *et al.* (2021) Green Tea and Epigallocatechin Gallate (EGCG) for the management of Nonalcoholic Fatty Liver Diseases (NAFLD): insights into the role of oxidative stress and antioxidant mechanism. *Antioxidants (Basel, Switzerland)* **10**, 1076.
11. Sekula P, Del Greco MF, Pattaro C, *et al.* (2016) Mendelian randomization as an approach to assess causality using observational data. *J Am Soc Nephrol: JASN* **27**, 3253–3265.
12. Ghodsian N, Abner E, Emdin CA, *et al.* (2021) Electronic health record-based genome-wide meta-analysis provides insights on the genetic architecture of non-alcoholic fatty liver disease. *Cell Rep Med* **2**, 100437.
13. Pirastu N, McDonnell C, Grzeszkowiak EJ, *et al.* (2022) Using genetic variation to disentangle the complex relationship between food intake and health outcomes. *PLoS Genetics* **18**, e1010162.
14. Li C, Niu M, Guo Z, *et al.* (2022) A mild causal relationship between tea consumption and obesity in general population: a two-sample Mendelian randomization study. *Front Genetics* **13**, 795049.
15. Liu X, Yu Y, Hou L, *et al.* (2023) Association between dietary habits and the risk of migraine: a Mendelian randomization study. *Front Nutr* **10**, 1123657.
16. Sun Y, Liang Z, Xia X, *et al.* (2023) Extra cup of tea intake associated with increased risk of Alzheimer's disease: genetic insights from Mendelian randomization. *Front Nutr* **10**, 1052281.
17. Brion MJ, Shakhbazov K & Visscher PM (2013) Calculating statistical power in Mendelian randomization studies. *Int J Epidemiol* **42**, 1497–1501.
18. Pierce BL & Burgess S (2013) Efficient design for Mendelian randomization studies: subsample and 2-sample instrumental variable estimators. *Am J Epidemiol* **178**, 1177–1184.
19. Bedogni G, Bellentani S, Miglioli L, *et al.* (2006) The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population. *BMC Gastroenterol* **6**, 33.
20. Pinzani M, Vizzutti F, Arena U, *et al.* (2008) Technology Insight: noninvasive assessment of liver fibrosis by biochemical scores and elastography. *Nat Clin Pract Gastroenterol Hepatol* **5**, 95–106.
21. Bowden J, Davey Smith G, Haycock PC, *et al.* (2016) Consistent estimation in Mendelian randomization with some invalid instruments using a weighted median estimator. *Genetic Epidemiol* **40**, 304–314.
22. Burgess S & Thompson SG (2017) Interpreting findings from Mendelian randomization using the MR-Egger method. *Eur J Epidemiol* **32**, 377–389.
23. Verbanck M, Chen CY, Neale B, *et al.* (2018) Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. *Nat Genetics* **50**, 693–698.
24. Xie J, Huang H, Liu Z, *et al.* (2023) The associations between modifiable risk factors and nonalcoholic fatty liver disease: a comprehensive Mendelian randomization study. *Hepatology (Baltimore, Md)* **77**, 949–964.
25. Yuan S, Chen J, Li X, *et al.* (2022) Lifestyle and metabolic factors for nonalcoholic fatty liver disease: Mendelian randomization study. *Eur J Epidemiol* **37**, 723–733.
26. Cai J, Zhang L, Chen C, *et al.* (2023) Association between serum Klotho concentration and heart failure in adults, a cross-sectional study from NHANES 2007–2016. *Int J Cardiol* **370**, 236–243.
27. Tao Z, Zhang R, Zuo W, *et al.* (2023) Association between dietary intake of anthocyanidins and heart failure among American adults: NHANES (2007–2010 and 2017–2018). Front Nutr 10, 1107637.
28. Soleimani D, Ranjbar G, Rezvani R, *et al.* (2019) Dietary patterns in relation to hepatic fibrosis among patients with nonalcoholic fatty liver disease. Diabetes, Metab Syndrome Obes: Targets Ther 12, 315–324.
29. Xia Y, Wang X, Zhang S, *et al.* (2019) Daily tea drinking is not associated with newly diagnosed non-alcoholic fatty liver disease in Chinese adults: the Tianjin chronic low-grade systemic inflammation and health cohort study. *Nutr J* **18**, 71.
30. Huo YB, Bai YN, Zhang DS, *et al.* (2021) Analysis on influencing factors for nonalcoholic fatty liver disease in Jinchang cohort. *Zhongbua liu xing bing xue za zhi = Zhonghua liuxingbingxue zazhi* **42**, 493–498.
31. Imai K & Nakachi K (1995) Cross sectional study of effects of drinking green tea on cardiovascular and liver diseases. *BMJ (Clin Res Ed)* **310**, 693–696.
32. Saleh IG, Ali Z, Abe N, *et al.* (2013) Effect of green tea and its polyphenols on mouse liver. *Fitoterapia* **90**, 151–159.
33. Hoofnagle JH, Bonkovsky HL, Phillips EJ, *et al.* (2021) HLA-B*35:01 and Green Tea-Induced Liver Injury. *Hepatology* **73**, 2484–2493.
34. Cho T, Wang X, Yeung K, *et al.* (2021) Liver injury caused by green tea extract in PD-1 (-/-) mice: an impaired immune tolerance model for idiosyncratic drug-induced liver injury. *Chem Res Toxicol* **34**, 849–856.
35. Smith GD & Ebrahim S (2003) 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? *Int J Epidemiol* **32**, 1–22.
36. Nehmi-Filho V, Santamarina AB, de Freitas JA, *et al.* (2022) Novel nutraceutical supplements with yeast β-glucan, prebiotics, minerals, and Silybum marianum (silymarin) ameliorate obesity-related metabolic and clinical parameters: a double-blind randomized trial. *Front Endocrinol (Lausanne)* **13**, 1089938.
37. Khan N & Mukhtar H (2018) Tea polyphenols in promotion of human health. *Nutrients* **11**, 39.
38. Ohishi T, Goto S, Monira P, *et al.* (2016) Anti-inflammatory action of green tea. *Anti-Inflamm Anti-Allergy Agents Med Chem* **15**, 74–90.
39. Li HY, Huang SY, Zhou DD, *et al.* (2023) Theabrownin inhibits obesity and non-alcoholic fatty liver disease in mice via serotonin-related signaling pathways and gut-liver axis. *J Adv Res* **52**, 59–72.
40. Zhang W, An R, Li Q, *et al.* (2020) Theaflavin TF3 relieves hepatocyte lipid deposition through activating an AMPK signaling pathway by targeting plasma kallikrein. *J Agric Food Chem* **68**, 2673–2683.
41. Valim V, Martins-Filho OA, Gouvea M, *et al.* (2022) Effectiveness, safety, and immunogenicity of half dose ChAdOx1 nCoV-19 COVID-19 vaccine: Viana project. *Front Immunol* **13**, 966416.
42. Huang YW, Wang LT, Zhang M, *et al.* (2023) Caffeine can alleviate non-alcoholic fatty liver disease by augmenting LDLR expression via targeting EGFR. *Food Funct* **14**, 3269–3278.
43. Shen Y, Xiao X, Wu K, *et al.* (2020) Effects and molecular mechanisms of Ninghong black tea extract in nonalcoholic fatty liver disease of rats. *J Food Sci* **85**, 800–807.
44. Li B, Mao Q, Xiong R, *et al.* (2022) Preventive effects of different black and dark teas on obesity and non-alcoholic fatty liver disease and modulate gut microbiota in high-fat diet fed mice. *Foods* **11**, 3457.

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45. Karolczak D, Seget M, Bajerska J, *et al.* (2019) Green tea extract prevents the development of nonalcoholic liver steatosis in rats fed a high-fat diet. *Polish J Pathol: Offic J Polish Soc Pathologists* **70**, 295–303.
46. Pan H, Gao Y & Tu Y (2016) Mechanisms of body weight reduction by black tea polyphenols. *Molecules* **21**, 1659.
47. Yang HH, Zhou H, Zhu WZ, *et al.* (2020) Green tea consumption may be associated with cardiovascular disease risk and nonalcoholic fatty liver disease in type 2 diabetics: a cross-sectional study in Southeast China. *J Med Food* **23**, 1120–1127.
48. Forst T, Botz I, Berse M, *et al.* (2024) Non-alcoholic fatty liver disease in obese subjects as related to increasing insulin resistance and deteriorating glucose control: three years of follow-up from a longitudinal survey. *J Diabetes Metab Disord* **23**, 999–1006.

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