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Mediators of the association between nut consumption and cardiovascular diseases: a two-step mendelian randomization study

Ruizhe Wang, Jinfang Sun & Xiaojin Yu✉

Previous observational studies have reported inconsistent associations between nut consumption and cardiovascular diseases (CVD). This study aims to identify the causal relationship between different types of nuts consumption and CVD, and to quantify the potential mediating effects of cardiometabolic factors. We utilized Genome-Wide Association Study (GWAS) data to assess the causal effects of nut consumption on CVD using two-sample Mendelian randomization (MR) and a two-step MR analysis. The inverse variance weighted (IVW) method indicated that processed (salted or roasted) peanuts were potentially and positively associated with ischaemic heart disease (IHD) (OR 1.4866; 95%CI 1.0491-2.1065). No causal relationships were found between nuts consumption and other CVD outcomes, including atrial fibrillation, angina, coronary atherosclerosis, coronary heart disease, IHD, myocardial infarction, subarachnoid hemorrhage, intracerebral haemorrhage and stroke. Both MR-Egger and median-based methods yielded similar results to IVW. Furthermore, in the two-step MR analysis, fasting insulin, low-density lipoprotein cholesterol and fasting blood glucose were identified as mediators in the potential causal relationship between processed peanuts and IHD, explaining 16.98%, 6.38% and 4.91% of the mediation, respectively. In total, these mediators accounted for 28.27% of the association between salted or roasted peanuts and IHD.

Keywords Nut, Peanut, Cardiovascular diseases, Mendelian randomization analysis, Mediation

Abbreviations

AF	Atrial fibrillation
BMI	Body mass index
CHD	Coronary heart disease
CVD	Cardiovascular diseases
DIAGRAM	DIAbetes genetics replication and meta-analysis
FG	Fasting blood glucose
FI	Fasting insulin
GIANT	Genetic Investigation of Anthropometric Traits
GLGC	Global Lipid Genetics Consortium
GWAS	Genome-Wide Association Study
HbA1c	Glycated hemoglobin levels
HDL	High-density lipoprotein cholesterol
HIP	Hip circumference
HIPadjBMI	BMI-adjusted HIP
ICH	Intracerebral haemorrhage
IHD	Ischaemic heart disease
IVs	Instrumental variables
IVW	Inverse variance weighted
IVW-FE	Fixed-effect inverse variance weighted
IVW-RE	Random-effect inverse variance weighted
LD	Linkage disequilibrium
LDL	Low-density lipoprotein cholesterol

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MAGIC	Meta-Analysis of Glucose and Insulin Correlated Traits Consortium
MI	Myocardial infarction
MR	Mendelian randomization
MR-PRESSO	Mendelian randomization pleiotropy residual sum and outlier
SAH	Subarachnoid hemorrhage
SNPs	Single nucleotide polymorphisms
TC	Total cholesterol
TG	Triglycerides
WC	Waist circumference
WCadjBMI	BMI-adjusted WC
WHR	Waist-to-hip ratio
WHRadjBMI	BMI-adjusted WHR

Cardiovascular diseases (CVD) remain the leading cause of global disease burden, with coronary heart disease (CHD), stroke and atrial fibrillation (AF) being the most prevalent conditions[1]. According to the latest statistics, the prevalence of CVD is nearly 127 million, affecting approximately 48.6% of adults, with the mortality rate of CVD continuing to rise in recent years, leading to 19 million global deaths in 2020 [2]. Therefore, it is essential to identify potential risk and protective factors to develop effective preventive measures, ultimately reducing the burden of CVD and improving global health[3].

The potential impact of dietary intake on CVD varies, and nuts consumption, as an important dietary habit, has been recommended in daily life guidelines due to its association with a reduced risk of CVD[4]. Previous studies have suggested that nut consumption may be beneficial in reducing the risk of total CVD, CHD[5], cardiovascular mortality[6], AF, non-fatal myocardial infarction (MI)[7] and hemorrhagic stroke[8]. Some observational studies and umbrella reviews have indicated that no significant association between nut consumption and fatal MI, ischaemic or hemorrhagic stroke[7–9] and AF[10], with the strength of evidence being limited for MI, AF, heart failure and stroke. Conclusions regarding different nut types, gender, and geographic region remain inconsistent[11,12]. Previous studies have also investigated the relationship between nut consumption and cardiovascular risk factors, including blood lipids, blood pressure, anthropometric factors and glycemic factors. A pooled analysis indicated that nuts have beneficial effects on total cholesterol (TC) and low-density lipoprotein cholesterol (LDL)[13]. Studies have shown that nuts do not lead to an increase in body weight, body mass index (BMI), or waist circumference (WC), but appear to improve glycemic control[12], which contrasts with findings from a random trial conducted on adults with Type 2 diabetes in America[14]. However, after adjusting for various confounding variables, no statistically significant association was found for other cardiovascular factors, except for obesity[15]. The China and SUN cohort study[16,17] found no association between nut and blood pressure, fasting blood glucose (FG), blood lipids and Framingham score. Two randomized trials[18,19] investigating the effects of various hazelnuts and peanuts flavors on blood pressure and lipids levels suggested no significant negative association. Approximately one-third of nuts and 68% of peanuts are processed, including salting and roasting[18]. The flavor of processed nuts is more palatable than that of raw nuts[20]. Therefore, the findings regarding the correlation between nut consumption and CVD in observational studies remain contradictory, with potential confounders, recalling bias, reverse causality, and limited data on nut processing contributing to these inconsistencies. It is of vital importance to assess the uncertain impact of various types of nut exposure on CVD and explore the mediating role of risk factors in this association.

Similar to the natural randomized trials, Mendelian randomization (MR) analysis is widely used as causal inference method for assessing the effects of specific exposures on disease outcomes[21]. Using genetic variants as instrumental variables (IVs) of dietary exposure, MR can overcome the risks of reverse causation and the distortion of confounding in conventional observational studies[22]. With key assumptions, MR analysis can estimate the causal relationship without the bias of unmeasured confounding providing more robust causal evidence. Recent MR studies have estimated the causal relationship between independent dietary habits and CVD, including the association between coffee consumption[23–25], tea consumption[26,27], raw and cooked vegetables intake[28], dried fruits intake[29] and the risk of stroke, AF, ischaemic cardio-cerebral vascular diseases and other CVD. Two-step MR can be used to estimate the mediating proportion of potential factors between exposure and diseases outcomes[30]. Currently, MR analysis has not been applied to investigate the relationship and mediating factors between nut consumption and CVD. Using two-sample MR and two-step MR analysis, this study aims to explore the causal relationship between nuts consumption and CVD (including AF, angina, coronary atherosclerosis, CHD, ischaemic heart disease (IHD), MI, subarachnoid hemorrhage (SAH), intracerebral haemorrhage (ICH) and stroke) and to quantify the potential mediating effects of cardiometabolic factors, including glycemic factors, lipids and anthropometric factors.

Materials and methods

Study design

In this study, a two-sample MR analysis (Fig. 1) was performed to investigate the causal relationship between four different types of nut consumption and various CVD. A two-step MR analysis was conducted to investigate the mediating pathways from nut consumption to CVD through 14 cardiometabolic factors. Single nucleotide polymorphisms (SNPs) were used as IVs to assess causality. Three key assumptions must be satisfied in MR analyses: (i) the IVs are strongly associated with the exposure, (ii) the IVs are independent of confounders, and (iii) the IVs influence the outcome only through the exposure.

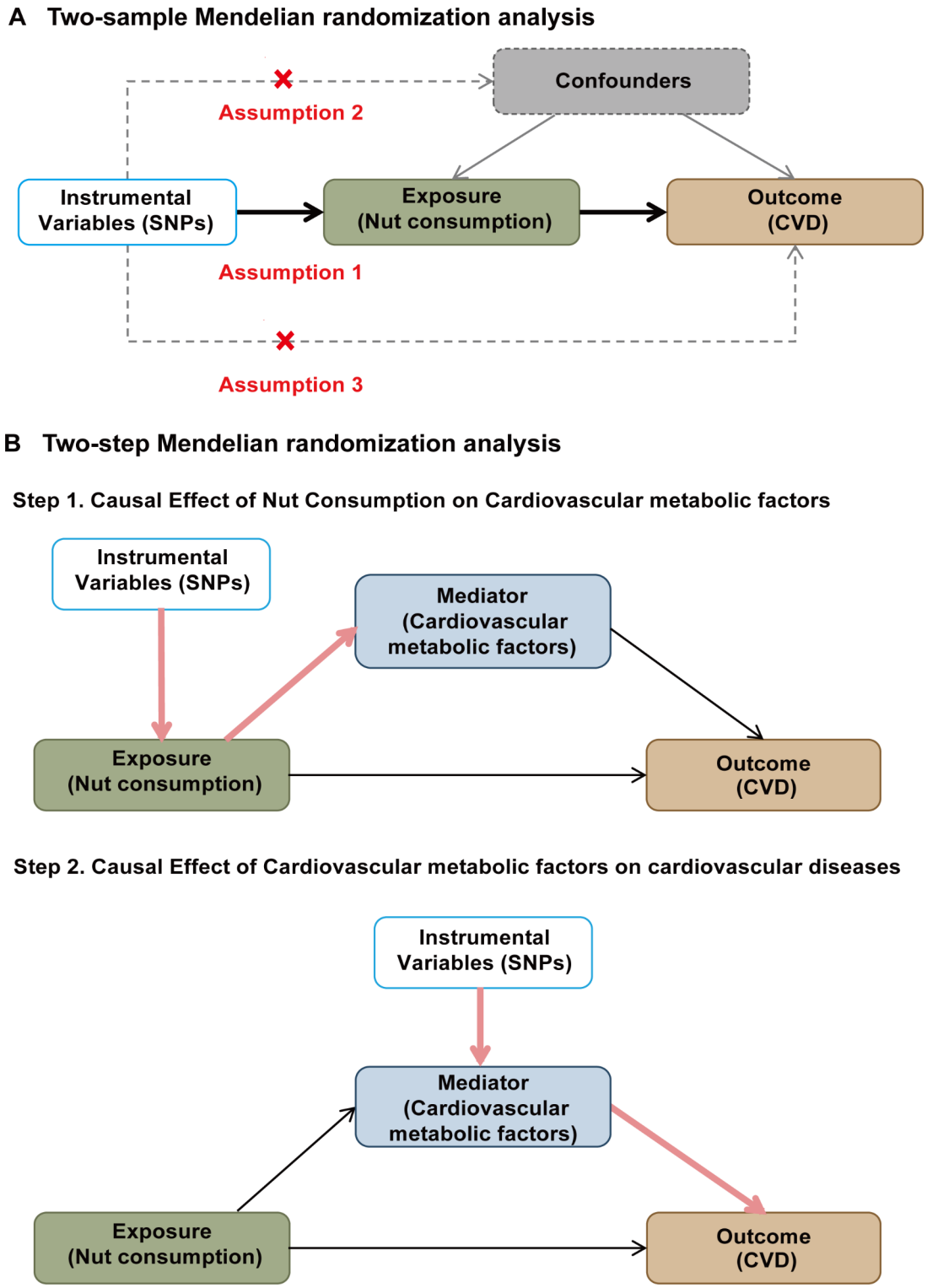


Fig. 1. MR study design for causal relationship between nuts consumption and CVD. (**A**) design of two-sample MR analysis. (**B**) design of two-step MR analysis. CVD, cardiovascular diseases; MR, Mendelian randomization; SNPs, single nucleotide polymorphisms.

Data sources

The exposure data for MR analysis were obtained from the UK Biobank, which is a large cohort study involving more than 500,000 participants. Participants were asked to report their nuts consumption (salted or roasted, unsalted) and peanuts consumption (salted or roasted, unsalted (monkey nuts)) based on 24-hour dietary recall questionnaires completed on the previous day. These questionnaires were administered during the assessment

center on a touch-screen device, along with four additional online rounds at three to four monthly intervals. The Genome-Wide Association Study (GWAS) summary data for the exposure can be accessed from the MRC IEU Open GWAS project database (https://gwas.mrcieu.ac.uk/). The summary statistics for CVD outcomes were extracted from the FinnGen consortium, which conducted a study in Finland involving a cohort of 500,000 participants from the Finnish biobank[31]. The R9 data were released in May 2023, with a total sample size of 377,277 (https://www.finngen.fi/en). Detailed information on the exposures and outcomes from the European population is provided in Supplemental Table 1.

Fourteen cardiovascular metabolic factors were identified as potential mediators, which were categorized into three groups: glycemic factors, lipids and anthropometric factors. Genetic variations in anthropometric factors were extracted from the Genetic Investigation of Anthropometric Traits (GIANT)[32,33] consortium, including BMI, hip circumference (HIP), BMI-adjusted HIP (HIPadjBMI), waist circumference, BMI-adjusted WC (WCadjBMI), waist-to-hip ratio (WHR) and BMI-adjusted WHR (WHRadjBMI). Summary statistics for lipemic factors were obtained from the Global Lipid Genetics Consortium (GLGC)[34], which included total cholesterol, triglycerides (TG), high-density lipoprotein cholesterol (HDL), and LDL. For blood glucose factors, data were obtained from the Meta-Analysis of Glucose and Insulin Correlated Traits Consortium (MAGIC)[35], which included glycated hemoglobin levels (HbA1c), fasting insulin (FI), and FG. Detailed information was provided in Supplemental Table 2.

Selection of genetic instrumental variants

SNPs associated with different types of nuts consumption were extracted from the GWAS datasets as significant IVs, with a threshold of $p < 5 \times 10^{-6}$[36]. In the two-step MR, SNPs that might serve as mediators were extracted at a threshold of $p < 5 \times 10^{-8}$. SNPs in linkage disequilibrium (LD) were excluded to ensure the independence of each IV within a window size of 10,000 kb and $R^2 < 0.001$. SNPs that were closely associated with the outcomes were selected, and missing SNPs were replaced by highly linkage proxies ($r^2 > 0.8$, MAF > 0.01). Potential confounders such as LDL, HDL, TC, FG, FI, type 2 diabetes, BMI, hypertension, insomnia and smoking were checked to see whether SNPs associated with the exposure were also linked to them ($p < 5 \times 10^{-8}$), by extracting data from GIANT, GLGC, DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) (https://diagram-consortium.org/about.html), MAGIC and UK Biobank. Similarly, mediators were examined to check if SNPs of mediators were associated with confounders, including BMI, hypertension, smoking, insomnia and type 2 diabetes ($p < 5 \times 10^{-8}$). Furthermore, all IVs related to potential confounding factors or directly associated with outcomes were assessed using PhenoScanner V2 [37].

To avoid weak instruments bias, F statistic ($F = beta^2/se^2$) was calculated for each single IV[38]. For each exposure (nuts and peanuts), a general F was calculated using the equation: $F = \left(\frac{n-k-1}{k}\right)\left(\frac{R^2}{1-R^2}\right)$, which is related to the proportion of variance in the exposure explained by the genetic variants (R^2), sample size (n) and the number of SNPs (k)[39]. R^2 for each exposure was calculated with the equation [40]: $R^2 = \frac{2\times EAF\times(1-EAF)\times beta^2}{2\times EAF\times(1-EAF)\times beta^2 + 2\times EAF\times(1-EAF)\times n\times se^2}$, where EAF is the effect allele frequency, $beta$ is the estimated genetic effect and se is the standard error of the genetic effect. If F-statistic was higher than 10, the correlation between the IV and exposure was considered sufficiently strong[39].

Statistical analysis

The fixed-effect inverse variance weighted (IVW-FE) method was used as the main analysis method for two-sample MR, supplemented by several other methods, including MR-Egger regression, median-based methods (simple median, weighted median and penalized weighted median) and random-effect inverse variance weighted (IVW-RE) method. The inverse variance weighted (IVW) method assumes that all the genetic variants are valid IVs and that there are no pleiotropic effects[41]. The IVW-RE is robust even in the presence of heterogeneity[42]. MR-Egger, using weighted linear regression with an intercept regression, was employed to obtain valid causal effect estimates in the presence of horizontal pleiotropy, assuming the independence of the associations between IVs and exposures[43,44]. Median-based methods assess the effect of the majority (or weighted majority) of IVs on the outcomes[43]. The weighted median provides a consistent estimate of the effect, even when half of the genetic variants are invalid[41].

Cochrane's Q statistic was computed to assess heterogeneity among different genetic variants[45]. The MR-Egger intercept was tested to detect horizontal pleiotropy[44]. If the intercept was not significantly different from zero with $p > 0.05$, no pleiotropic effects were assumed. Additionally, Mendelian randomization pleiotropy residual sum and outlier (MR-PRESSO) was used to obtain unbiased causal estimates by detecting and removing potential outliers[46]. Leave-one-out analyses were conducted to assess the robustness of the results with respect to single SNPs.

In the two-step MR analysis, SNPs associated with nuts consumption were used to estimate the causal effects of nuts consumption on various cardiometabolic factors, and the effects of these mediators on the corresponding diseases using the IVW method. The indirect effect of nuts consumption on CVD through specific mediators was estimated using the product of coefficients method[47]. To quantify the proportion of the effect mediated by each mediator, the indirect effect was divided by the total effect obtained from the two-sample MR, and the standard error was calculated using the delta method[48].

Statistical analyses were performed using R software (version 4.3.0) with the 'TwoSampleMR' (version 0.5.7) R package. Two-sided p-values < 0.05 were considered significant, while the Bonferroni-corrected threshold for statistical significance was set at $p < 0.0056$ (0.05/9). P-values between 0.05 and 0.0056 were considered suggestive of significance.

Results

After checking for the IVs closely associated with confounding factors, the SNP of rs3815692 and rs13284665 were removed, as both were closely related to the LDL and TC. 45, 16, 22 and 14 independent SNPs associated with unsalted peanuts, unsalted nuts, salted or roasted nuts and salted or roasted peanuts, respectively, were identified. Details of the exposure SNPs were presented in Supplemental Tables 3-6. All the *F* statistics of genetic variants were larger than 10.

The results of the causal relationship between four types of nuts consumption and CVD were analyzed by MR methods (IVW-FE) (Fig. 2). There was a suggestive significant causal relationship of salted or roasted peanuts consumption and IHD (OR: 1.4866; 95%CI: 1.0491-2.1065; $p=0.0258$), based on the method of IVW-FE, which was considered suggestive of significance. There was no significant evidence of causal relationship between nuts consumption and other CVD outcomes, including AF, angina, coronary atherosclerosis, CHD, MI, SAH, ICH and stroke. Since the statistical power of other methods, such as MR-Egger, weighted median, simple median and penalized weighted median, was lower than IVW, some of their results were found insignificant based on the *p*-value threshold. Without heterogeneity and pleiotropy was identified, these results were used to confirm consistent estimates in the same direction, demonstrating the robustness of IVW as complementary approaches[42,49], as presented in Supplemental Tables 7-10.

As shown in Supplemental Table 11, no evidence of heterogeneity was observed between the unsalted and salted or roasted nuts for CVD outcomes. Under the circumstances that some results for unsalted or salted or roasted peanuts of Cochrane's *Q* statistics showed heterogeneity (unsalted peanuts for angina, $Q=65.9822$; unsalted peanuts for CHD, $Q=61.0486$; unsalted peanuts for IHD, $Q=60.2355$; salted or roasted peanuts for MI, $Q=32.2798$), MR-PRESSO method with significant outliers removed was conducted and showed the same no-causal effect as IVW (removal of rs28868570, salted or roasted peanuts for MI, OR: 2.0880, 95%CI: 0.9734-4.4789, $p=0.0853$). Besides, for the situation that no significant outliers were identified and removed, the result of IVW-RE method provided consistent non-causal estimations (unsalted peanuts for angina, OR: 0.8805, 95%CI: 0.4869-1.5922, $p=0.6737$; unsalted peanuts for coronary atherosclerosis, OR: 1.2311, 95%CI: 0.7438-2.0379, $p=0.4187$; unsalted peanuts for IHD, OR:1.0679, 95%CI: 0.6860-1.6625, $p=0.7711$). The result of the MR-Egger intercepts suggested no significant evidence of horizontal pleiotropy with all *p*-values >0.05. The scatter plots and Funnel plots were showed in Supplemental Figs. 1-8. The result of the leave-one-out method were showed in Supplemental Figs. 9-12, and Supplemental Fig. 12 suggested that the association for salted or roasted peanuts on IHD was not influenced by a single SNP.

The two-step MR analysis was utilized to assess the mediation effects of 14 potential mediators in causal relationships that showed causal significance. The information of selected SNPs related to mediators was shown in Supplemental Tables 12-14. Figure 3 and Supplemental Table 15 showed the result of the causal effects of salted or roasted peanut consumption on 14 cardiometabolic factors estimated using the IVW method in the first step MR analysis. Furthermore, three mediators were identified in the association between salted or roasted peanuts and IHD. Salted or roasted peanut consumption was negatively correlated with FI (β_1: -0.1309; 95%CI: -0.2176 to -0.0441) and FG (β_1: -0.0928; 95%CI: -0.1589 to -0.0268), and positively associated with LDL (β_1: 0.0662; 95%CI: -0.0052 to -0.1272). No causal effect of salted peanut consumption on BMI, HIP, HIPadjBMI, WC, WCadjBMI, WHR, WHRadjBMI, TC, TG, HDL and HbA1c was found. Therefore, FI, FG and LDL may be mediators of the pathogenic pathways of peanuts and IHD and were included in the second step of the two-step MR analysis. In addition, FI (β_2: 0.5144; 95%CI: 0.1664 to 0.8625) and FG (β_2: 0.2099; 95%CI: 0.0776 to 0.3421) were positively related with IHD, and LDL (β_2: -0.3822; 95%CI: -0.4531 to -0.3113) was negatively associated with IHD (Fig. 4, Supplemental Table 16). Finally, the mediating role of three mediators in the association between salted or roasted peanut consumption and IHD was assessed, including the largest mediator, FI, explaining 16.98% of the mediation (95%CI: 0.90-33.06%), and LDL and fasting glucose, accounting for 6.38% (95%CI: 0.38-12.38%) and 4.91% (95% CI: 0.24-9.58%) of the mediation, respectively. Together, the mediators explained 28.27% of the association between salted or roasted peanuts and IHD, as presented in the Fig. 5, the detailed information was shown in Supplemental Table 17.

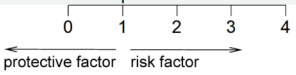
Discussion

In this study, MR analysis was used to estimate the causal relationship between different types of nuts consumption and CVD and potential mediation factors. The results indicated that processed (salted or roasted) peanuts consumption may be a potential risk factor for IHD, suggesting a potential causal relationship after Bonferroni correction. No significant causal relationship was observed between the consumption of nuts or peanuts and other CVD outcomes such as AF, angina, CHD, myocardial infarction, SAH and stroke. The mediation analysis explored three cardio-metabolic factors - FI, FG and LDL - as mediators in the causal relationship between processed peanuts and IHD.

Nut consumption is recommended in dietary guidelines for reducing CVD risk[4]. However, previous observational studies on the effects of nut consumption on CVD outcomes have yielded inconsistent results. A prospective study found inverse associations between nut consumption and the risk of total and non-fatal MI and AF, but no significant associations with fatal MI, ischaemic stroke or ICH[7]. The inverse associations with AF remained after adjusting for multiple risk factors. The Physicians' Health Study showed no association between nuts consumption and AF[10], total stroke or ischaemic stroke[8], but a suggestive J-shaped relationship between exposure and hemorrhagic stroke[8]. These findings only in male physicians may be related to the tendency of consuming more nuts in participants at risk of CVD due to medical knowledge. Cohort studies reported similar no significant relationship between total nuts and fatal or nonfatal stroke or ischaemic stroke[9], but a negative relationship between peanuts and walnuts and total CVD, CHD and stroke[5]. A review[50] of these studies found an inverse association between nut intake and CHD, but no such association with stroke risk.

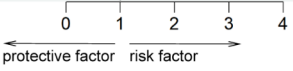
A

Exposure	Outcome	Method	SNPs	OR(95%CI)	P
Unsalted peanuts	AF	IVW-FE	44	1.3118(0.7796 to 2.2073)	0.3066
	Angina	IVW-FE	44	0.8805(0.5458 to 1.4204)	0.6019
	CHD	IVW-FE	44	1.0911(0.7112 to 1.6738)	0.6898
	Coronary atherosclerosis	IVW-FE	44	1.2311(0.8065 to 1.8793)	0.3353
	ICH	IVW-FE	44	1.7881(0.5172 to 6.1821)	0.3585
	IHD	IVW-FE	44	1.0679(0.7347 to 1.5522)	0.7306
	MI	IVW-FE	44	1.0805(0.6171 to 1.8919)	0.7865
	SAH	IVW-FE	44	1.1918(0.3172 to 4.4780)	0.7950
	Stroke	IVW-FE	44	1.3052(0.8359 to 2.0379)	0.2414



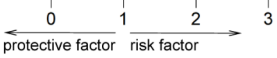
B

Exposure	Outcome	Method	SNPs	OR(95%CI)	P
Unsalted nuts	AF	IVW-FE	15	1.1743(0.7462 to 1.8481)	0.4873
	Angina	IVW-FE	15	0.8577(0.5907 to 1.2452)	0.4694
	CHD	IVW-FE	15	0.9514(0.3809 to 2.3764)	0.6029
	Coronary atherosclerosis	IVW-FE	15	1.1332(0.6904 to 1.8598)	0.8892
	ICH	IVW-FE	15	2.1983(0.4962 to 9.7397)	0.3034
	IHD	IVW-FE	15	1.0502(0.6448 to 1.7107)	0.6660
	MI	IVW-FE	15	1.4012(0.5369 to 3.6568)	0.8439
	SAH	IVW-FE	15	0.4712(0.1819 to 1.2202)	0.5662
	Stroke	IVW-FE	15	1.0113(0.6863 to 1.4902)	0.9548



C

Exposure	Outcome	Method	SNPs	OR(95%CI)	P
Processed nuts	AF	IVW-FE	21	0.7642(0.5199 to 1.1232)	0.1711
	Angina	IVW-FE	21	0.8610(0.6038 to 1.2279)	0.4086
	CHD	IVW-FE	21	1.0072(0.7337 to 1.3826)	0.9646
	Coronary atherosclerosis	IVW-FE	21	0.8944(0.6537 to 1.2238)	0.4855
	ICH	IVW-FE	21	0.4315(0.1724 to 1.0797)	0.0725
	IHD	IVW-FE	21	1.0158(0.7702 to 1.3397)	0.9115
	MI	IVW-FE	21	1.3941(0.9206 to 2.1112)	0.1166
	SAH	IVW-FE	21	0.6615(0.2484 to 1.7615)	0.4083
	Stroke	IVW-FE	21	0.9537(0.6861 to 1.3258)	0.7780



D

Exposure	Outcome	Method	SNPs	OR(95%CI)	P
Processed peanuts	AF	IVW-FE	13	1.1337(0.6985 to 1.8400)	0.6116
	Angina	IVW-FE	13	1.4895(0.9534 to 2.3272)	0.0801
	CHD	IVW-FE	13	1.3502(0.9060 to 2.0122)	0.1402
	Coronary atherosclerosis	IVW-FE	13	1.3829(0.9320 to 2.0519)	0.1074
	ICH	IVW-FE	13	1.1280(0.3544 to 3.5901)	0.8385
	IHD	IVW-FE	13	1.4866(1.0491 to 2.1065)	**0.0258**
	MI	IVW-FE	13	1.4428(0.8545 to 2.4361)	0.1702
	SAH	IVW-FE	13	1.1622(0.3385 to 3.9903)	0.8113
	Stroke	IVW-FE	13	1.2994(0.8577 to 1.9685)	0.2166

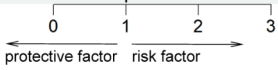


Fig. 2. MR assessment for causal relationship between different types of nuts consumption and CVD. All the SNPs associated with exposure were extracted as significant instrumental variants at a level of $p < 5 \times 10^{-6}$. (**A**) The exposure factor is unsalted peanuts consumption. (**B**) The exposure factor is unsalted nuts consumption. (**C**) The exposure factor is processed nuts consumption. (**D**) The exposure factor is processed peanuts consumption. AF, atrial fibrillation; CHD, coronary heart disease; ICH, intracerebral haemorrhage; IHD, ischaemic heart disease; MI, myocardial infarction; MR, Mendelian randomization; SAH, subarachnoid hemorrhage; IVW-FE, fixed-effect inverse variance weighted; SNPs, single nucleotide polymorphisms; OR, odds ratio.

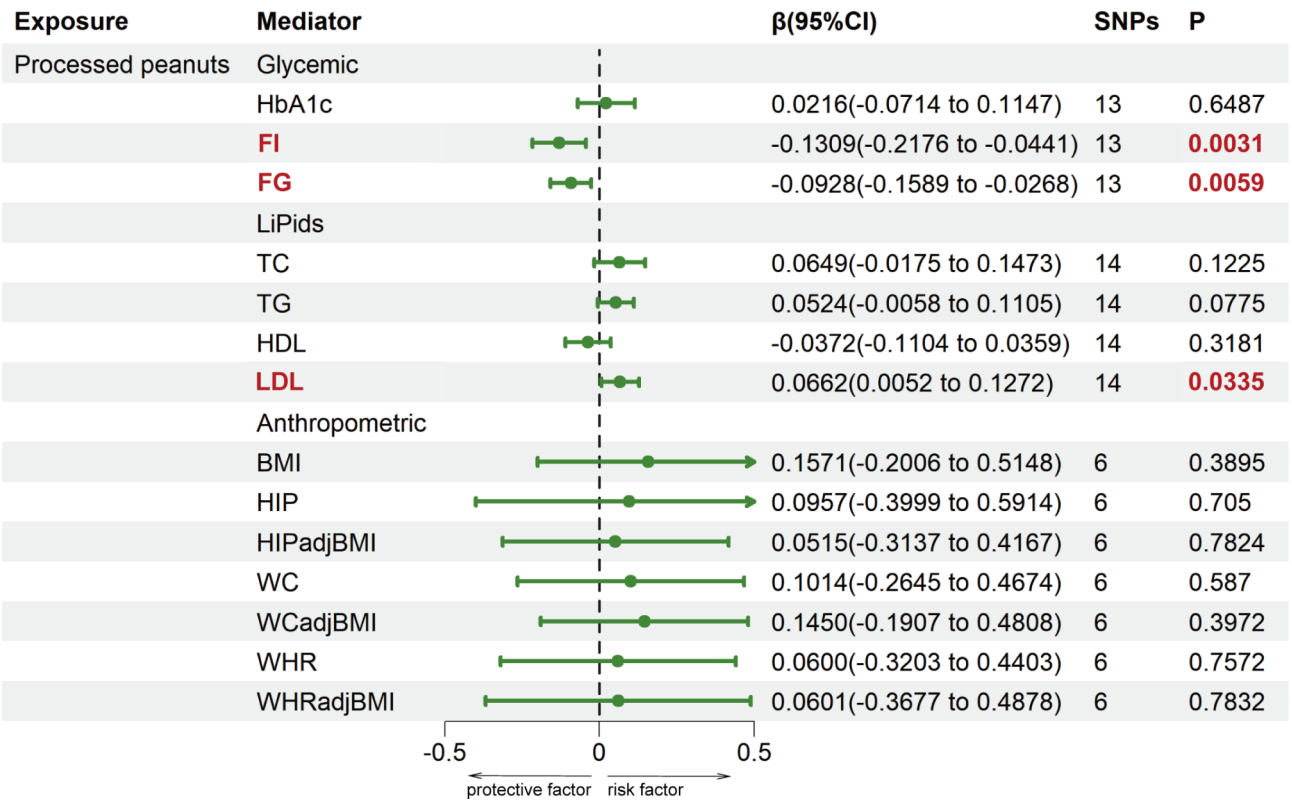


Fig. 3. First step of the causal relationship of processed peanuts with 14 Mediators. Mediators were classified into 3 categories: glycemic factors, lipids and anthropometric factors. BMI, body mass index; FG, fasting blood glucose; FI, fasting insulin; HbA1c, glycated hemoglobin levels; HDL, high-density lipoprotein cholesterol; HIP, hip circumference; HIPadjBMI, BMI-adjusted HIP; LDL, low-density lipoprotein cholesterol; SNPs, single nucleotide polymorphisms; TC, total cholesterol; TG, triglycerides; WC, waist circumference; WCadjBMI, BMI-adjusted WC; WHR, waist-to-hip ratio; WHRadjBMI, BMI-adjusted WHR.

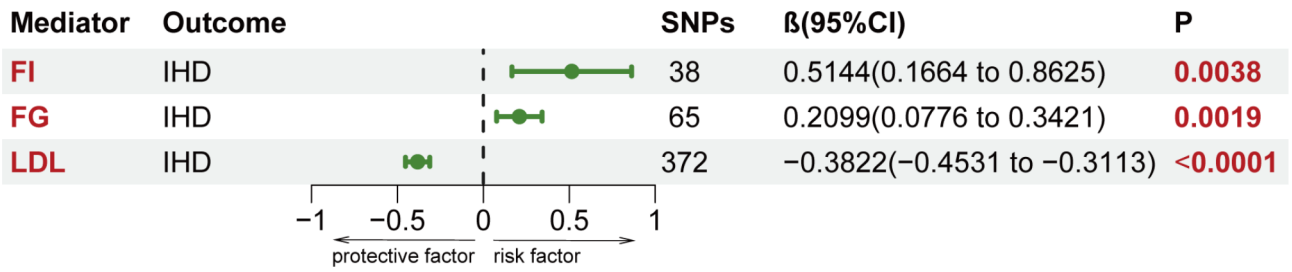


Fig. 4. Second step of the causal relationship of Mediators with IHD. All the SNPs associated with mediators were extracted as significant instrumental variants at a level of $p < 5 \times 10^{-8}$. FG, fasting blood glucose; FI, fasting insulin; IHD, ischaemic heart disease; LDL, low-density lipoprotein cholesterol; SNPs, single nucleotide polymorphisms.

The relationships between nuts and stroke remain inconsistent. A review[51] reported conflicting findings, with ten studies concluding no significant changes in vascular function, while others indicated improvements. This variation may be influenced by season and climates changes on the nutrient content of nuts. Umbrella reviews indicated that nut consumption reduces the risk of CHD and cardiovascular mortality, but evidence for MI, AF, heart failure and stroke was limited and varied based on nut types, gender, and geographic region, due to heterogeneity and potential confounders[11,12]. With limited information, these studies were not able to fully assess the influence of various nut processing methods, such as salted, roasted, or raw nuts. Our study suggested a potential relationship between long-term consumption of salted or roasted peanuts and the increased risk of IHD but no significant causal relationship was observed between consumption of nuts or peanuts and other CVD outcomes.

Nuts contain a variety of compounds, including macronutrients, micronutrients, unsaturated fatty acids such as monounsaturated fatty acids and polyunsaturated fatty acids, dietary fiber, water-soluble vitamins, non-

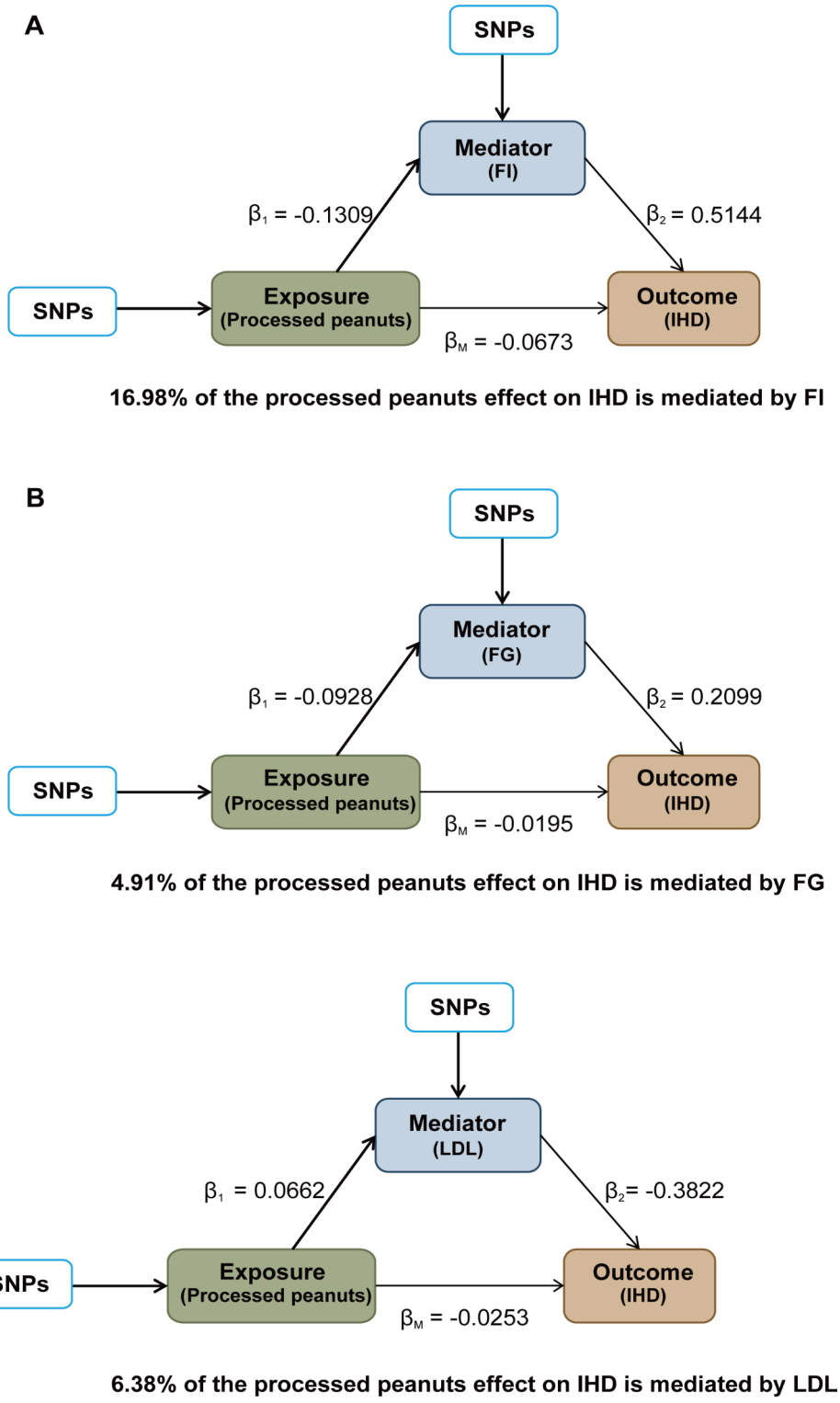


Fig. 5. The mediation effects of processed peanuts intake on IHD risks. (**A**) The mediating effect of FI. (**B**) The mediating effect of FG. (**C**) The mediating effect of LDL. FG, fasting blood glucose; FI, fasting insulin; IHD, ischaemic heart disease; LDL, low-density lipoprotein cholesterol; SNPs, single nucleotide polymorphisms.

sodium minerals and phenolic compounds, which have beneficial influences on antioxidant, anti-inflammatory and intermediate markers of cardiovascular risk such as blood cholesterol, glycaemia, blood pressure and vasomotion[52,53]. Due to the similarity of nutritional components, peanuts were also classified as nut foods[52]. Dose-response relationships suggested that the best nuts intake was 15-40 g/d, with limited benefits from intake beyond 28 g/d[12]. Recent studies have examined the effects of nut consumption on cardiovascular factors, yet the

results remain inconclusive. Meta-analysis and reviews found that nuts were associated with improvements in blood lipids[54], such as TC and LDL[13], TG and ApoB[55], but no significant effects were found for HDL[55]. Studies have shown that nuts do not increase body weight, BMI, WC, but they may improve glycemic factors[12], such as FI, while no effect on FG or HbA1c was observed[56]. This may be due to the replacement of unsaturated fatty acids and carbohydrates with unsaturated fatty acids. The fatty acids and other bioactive constituents in nuts may act by increasing insulin sensitivity or though non-insulin mediated mechanisms to promote increased glucose uptake, influencing FG. Our MR analysis confirmed that the potential protective association between salted or roasted peanuts and IHD was mediated by FI, FG and LDL. The type of population is quite important. A random trial involving individuals with Type 2 diabetes in America found that increasing peanuts intake could reduce the weight, BMI, and WC[14]. Meta-analysis indicated that walnut intake did not result in changes to body weight, BMI, fat mass or WC[57], which was not supportive for the improvement. In this MR analysis, genetic predictors linked to salted or roasted peanuts indicated no significant effect on anthropometric mediators, including BMI, WC, HIP and WHR. Besides, nuts may benefit to the patients with risk factors for CVD or some degree of impaired vascular function, while no benefit was observed in patients with healthy or poor vascular function. A study found women who consumed more nuts showed lower risk of left ventricular hypertrophy, which was also observed in women with or without hypertension and diabetes mellitus, but the cross-sectional design[58] could not provide the causative relationship. An inverse association between nut consumption and obesity remained after adjusting for various potential confounders but no significant associations were observed for other cardiovascular factors[15]. Similarly, the China cohort[16] and SUN prospective cohort[17] found no associations between nut consumption and Framingham score, systolic and diastolic blood pressure, HDL-cholesterol, LDL-cholesterol or FG.

The inconsistent results of previous studies may be related to the different processing of nuts. Roasting may enhance sensory characteristics, but it can also reduce antioxidant activity, nutritional content and total phenolic compounds due to blanching and peeling[53]. The skins of nuts, the main contributors to total phenolic content and antioxidant activity, are generally removed during the roasting process[59]. Studies found that the roasting affects lipids and other components such as linoleic acids and beneficial phytosterols. There is a significant loss of total phenolic compounds, especially tocopherols, and a decrease in thiamine in most types of nuts, while B vitamins showed high thermal resistance stability[59–61]. Nuts, especially walnuts and pistachios, were rich in alpha-linolenic acid (ALA), a shorter chain omega-3 fatty acid that can be converted to longer chain omega-3 fatty acids in body. Omega-3 fats were considered as protector for lowering blood pressure, modulating arterial lipoprotein lipase levels, producing anti-inflammatory and anti-arrhythmic effects. However, meta-analysis[62] concluded that increasing ALA intake make little or no impact on the risk of CHD, and may modestly reduce the risk of cardiovascular events and arrhythmia, but the effects of ALA on stroke is unclear.

Furthermore, in many European and American countries, especially in Spain[17], nuts and peanuts are often consumed as salted, grilled or fried ways[63],which may counteract the beneficial effects of other nutrients in raw nuts. Randomized trial of 72 participants compared the dry roasting and lightly salting hazelnuts with raw hazelnuts, both of which could improve the HDL, apolipoprotein A1 and systolic blood pressure, but only dry roasting and lightly salting ones showed significant effect of reduction on diastolic blood pressure[18]. Another study[19] of 151 participants reported no significance between four types of flavored peanuts and blood glucose and blood lipids. It was well established that excessive sodium intake is associated with an increased risk of CVD, especially hypertension[64,65], with processed foods contributing the majority of daily sodium intake. The sodium content of roasted nuts (145 mg/100 g) is approximately 10-20 times that of raw nuts (13 mg/100 g), with salted nuts containing 568 mg/100 g[18]. Salted almonds may contain up to 700 mg/100 g, which would represent 10% of the recommended daily salt intake in a 30 g serving[66]. Sodium ions may inhibit protease activity and increase lipid peroxidation[65]. Research suggested that the process of soaking nuts, particularly in the presence of salt, may leach out minerals and water-soluble vitamins, without reducing phytates[66]. Phytates, a strong chelator, reduces the bioavailability of important micronutrients such as zinc, iron, and calcium by inhibiting their absorption[67,68]. In addition, the heat processing of peanuts, including boiling, roasting and frying, caused the loss of nutrients, such as amino acids, total reducing sugar, sucrose and unsaturated fatty acids[69]. Harmful compounds may be produced such as 5-hydroxymethylfurfural and furan[69]. A research[18] suggested that it seemed no harmful impression when the sodium added in nuts $\leq$285 mg/d, which suggests the importance of proper sodium addition control. Due to limited availability for blood pressure data, this study was unable to incorporate relevant information into mediation analysis. Further exploration of potential mediators is necessary to explore whether the relationship between salt or roasted peanut and IHD through mediators can be altered by various processing methods. As an advantageous methodology, MR study could avoid bias from unobserved confounders of exposures and outcomes by using summary level data from GWAS. This approach provides more robust estimates of causality between salted or roasted or unsalted nuts and peanuts consumption and CVD outcomes compared to conventional observational epidemiology[70], which has not been assessed previously. Because two-sample MR analyses were performed at each step, measurement error and reverse causal error relationships could be overcome to identify the mediating effects. Secondly, with LD removal and F-statistics above 10, the study ensured the independence and strong association of SNPs with exposures, thus avoiding weak instrument bias. Sensitivity analyses using MR-Egger regression and median-based methods confirmed the robustness of the results. The results of these approaches, albeit less statistically powerful as compared to the primary IVW method, remained consistent with the directions of estimates, demonstrating the robustness of IVW[49].

The limitations were that summary statistics were extracted from European population, which may not apply to other ethnic groups. Additionally, the study was incapable of assessing the causal effects of specific nut types, including almonds, cashews and pistachios due to the insufficient data. The study could not test non-linear causal relationships between nut consumption and related outcomes. It is worth noting that there may be

differences between genetic hypotheses, clinical trials and actual nuts consumption. The suggestive significant of the association should be interpreted carefully. Furthermore, due to the low number of SNPs (less than three) after LD on the p-value of 5×10^{-8} and 5×10^{-7}, SNPs were extracted at a relaxed p-value threshold of 5×10^{-6}. Further analyses with specific types of nuts, more relevant SNPs, larger sample sizes, and more comprehensive and longitudinal dietary assessment are needed to confirm these findings and more accurately capture usual or habitual nut consumption patterns.

In conclusion, this MR analysis suggested that the evidence that processed (salted or roasted) peanuts consumption was a potential risk factor for IHD, with the potential causal relationship mediated by three cardiometabolic factors, FI, FG and LDL. Hence, it is necessary to evaluate the impact of nuts on health in further study.

Data availability

Data is provided within the manuscript or supplementary information files. The GWAS summary data related to exposure are available at https://gwas.mrcieu.ac.uk/. The GWAS summary data of FinnGen consortium are available at https://www.finngen.fi/en. The GWAS data of mediating factors are available at https://magicinvestigators.org/, http://csg.sph.umich.edu/willer/public/glgc-lipids2021/ and https://portals.broadinstitute.org/collaboration/giant/index.php/GIANT_consortium.

Received: 30 September 2024; Accepted: 31 December 2024
Published online: 04 January 2025

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Acknowledgements

We thank the following projects and institutions for making the summary data publicly available, including the IEU open GWAS project, the FinnGen consortium, GIANT, GLGC, MAGIC, DIAGRAM.

Author contributions

RW, XY conceived the ideas and designed the research; RW conducted the research and analyzed the data; and RW wrote the original draft. JS and XY reviewed and revised the manuscript critically. XY had primary responsibility for final content. All authors have read and approved the final manuscript.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declarations

Competing interests

The authors declare no competing interests.

Ethical approval

Ethical approval and participant agreement are not necessary because the public summary data has already been approved. All original studies had been authorized by corresponding ethical standards committee.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-024-85070-z.

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