Fruit Intake and Alzheimer’s Disease: Results from Mendelian Randomization

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Research Article

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Abstract

Background

Alzheimer's disease (AD) is the leading cause of dementia in old age, recognized as a global health priority. The number of dementia patients is projected to reach 152 million worldwide by the mid-century. AD can damage thought, memory, and independence, increasing the risk of dependence, disability, and mortality. The aim of the present study is to explore precise causality from fruit intake on risk of AD.

Methods

To explore the causal effect of fresh fruit intake and dried fruit intake on the liability of AD, this study utilized a genome-wide association study (GWAS) developed by the MRC-IEU for the full UK Biobank and the GWAS from FinnGen round 8 to conduct a Mendelian randomization (MR) analysis. The instrumental variables (IVs) for each fruit intake were selected based on the InSIDE hypothesis and the exposure-outcome datasets were harmonized. The study used inverse variance weighted (IVW), MR-Egger, and weighted median (WM) approaches for MR estimates, and scatter plots, funnel plots, and leave-one-out plots were generated for statistical inspection of the stability of the results.

Results

MR analyses were conducted to investigate the causal effects of fresh fruit and dried fruit intake on Alzheimer's disease (AD) using targeted genome-wide association study datasets. Little evidence suggested a potential causal relationship between fresh fruit intake and AD (OR (95%CI) = 0.97 (0.50, 1.91), P-value = 0.939), while a significant and intensive causality was indicated between dried fruit intake and AD (OR (95%CI) = 4.09 (2.07, 8.10), P-value < 0.001). Stability evaluations showed no heterogeneity and pleiotropy affecting the interpretability and credibility of the primary analyses.

Conclusions

We strengthened the evidence supporting the positive causality from dried fruit intake to the liability of Alzheimer's disease, while the association between fresh fruit intake and the risk of Alzheimer's disease failed to be demonstrated. Further validation focusing on underlying molecular mechanisms and expansility of ethnicity could strengthen the reliability of the diet intervention throughout the prevention on AD.

1. Introduction

Alzheimer's disease is the leading cause of dementia in old age, accounting for 50–70% of cases, and is recognized by the WHO as a global health priority [1]. A recent report suggests that the number of dementia patients is projected to reach 152 million worldwide by the mid-century. AD can damage thought, memory, and independence, increasing the risk of dependence, disability, and mortality [2]. Numerous studies have demonstrated that several acquired factors increase the risk of developing AD,
including cerebrovascular diseases (the most commonly reported risk factor), diabetes, hypertension, obesity, and dyslipidemia [3]. In addition to those factors that can cause AD, there are also some protective factors between the effects of diet and the risk of developing AD that have an essential relationship, and morbidity can be reduced by controlling its associated factors [4]. A large number of articles indicate that some dietary components critically contribute to neurocognition protection, such as dietary fatty acids, including fish oil; antioxidants, such as vitamins E and C; fruits and vegetables; vitamins B6, B12, and folate [5]. Many nutrients, such as vitamin C and vitamin E, have antioxidant properties. A previous study noted that oxidative stress or inadequate antioxidant defense might affect the pathogenesis and progression of dementia [6]. Therefore, ingestion of dietary oxidants may mediate the development of cognitive impairment, and dietary sources of antioxidants are mainly fruit intake. Several studies indicated that elderly healthy subjects consuming a diet rich in fruits and vegetables had lower levels of biomarkers related to oxidative stress and scored higher on neuropsychological assessment compared to subjects with low intake of fruits and vegetables [7]. A prospective population-based cohort study in the Netherlands found that the incidence of Alzheimer’s disease was reduced in individuals aged 55 years or older with a high vitamin C intake [8]. The conclusions of previous studies are consistent in pointing to the possibility that certain components that are abundant in fruits, such as antioxidants, may influence the development of cognitive impairment [9]. The conclusions of previous studies are consistent in pointing to the possibility that certain components that are abundant in fruits, such as antioxidants, may influence the development of cognitive impairment.

Environmental confounding and reverse causation make assessing the causal relationship between exposures and outcomes difficult in observational studies. To solve this deficiency, we utilized Mendelian randomization (MR), a robust tool to genetically predict the causal associations in exposures and outcomes using genetic variants that were previously proven to be associated with exposures or outcomes. To date, previous studies have used MR to analyze the causal associations between AD and other diseases or metabolites [10, 11]. However, the causal association between fruit ingestion, dried fruit ingestion and AD is not clear. The aim of our study is to apply the MR approach to elucidate whether there are underlying causal relationships in fruit ingestion, dried fruit ingestion and AD. The conclusion may shed light on further studies investigating the association between AD and nutrients or metabolites and offer more references in clinical practice.

2. Materials and methods

2.1. Study design and participants enrolled

To explore the causal effect of fruit intake on the liability of Alzheimer’s disease (AD), the exposure was subdivided into fresh fruit intake and dried fruit intake to carry out subsequent analyses. Targeting the posterity of Western Hunter-Gatherers, the genome-wide association study (GWAS) developed by the MRC-IEU for the full UK Biobank (2018) and the GWAS from FinnGen round 8 were led into the Analysis architecture of the entire Mendelian randomization, while the latent overlap of participants was avoided at the same time.
2.2. Exposures and outcome measures

Summary-level statistical data for fresh fruit intake and dried fruit intake were downloaded from ieu open gwas (https://gwas.mrcieu.ac.uk). GWAS IDs for the exposures are ukb-b-3881 (sample size for fresh fruit intake = 446462) and ukb-b-16576 (sample size for dried fruit intake = 421764). The practical fresh fruit intake was defined by the question “About how many pieces of FRESH fruit would you eat per DAY? (Count one apple, one banana, 10 grapes, etc., as one piece; put ‘0’ if you do not eat any)”, while the question for dried fruit intake was “About how many pieces of DRIED fruit would you eat per DAY? (Count one prune, one dried apricot, 10 raisins as one piece; put ‘0’ if you do not eat any)”. Quality control was conducted regarding the participants with answers that eating over 50 pieces of fresh fruit or eating over 100 pieces of dried fruit was invalid.

The summary data for Alzheimer’s disease were stretched from FinnGen round 8 (released on 2022 Dec 1), id: finngen_R8_G6_ALZHEIMER. The sample size was 342499 (7759 cases versus 334740 controls). The diagnosis of Alzheimer’s disease in the GWAS included the senile and presenile forms reported in the phase of hospital discharge or serving as part of the cause of death, while unclassified degeneration of the brain and unspecified dementia were excluded during the phase of quality control.

2.3. Selection of instrumental variables of fruit intake.

The entire selection phase of instrumental single nucleotide polymorphisms (SNPs) was conducted under the InSIDE hypothesis: 1. The instrumental variables (IVs) must be intensively associated with the exposure. 2. Exposure is the only passage for the effect from IVs on the outcome, which means IVs ought to not be significantly associated with the outcome (P value > 0.05); 3. IVs are prohibited from being strongly related to the recognized confounders of the exposure-outcome correlation [12, 13] (Fig. 1). Only SNPs with exposure-associated P values < 5e-8 were stretched as the raw candidates, which were clumped to obtain independent loci with the threshold for linkage disequilibrium (LD) set $r^2 > 0.001$ and clump distance < 10000 kb. To fulfill the assumptions that IVs are not related to any confounders or the outcome itself, Phenoscanner was utilized to exclude the violative SNPs, with 11 SNPs significantly associated with Alzheimer’s disease or recognized risk factors (diabetes, midlife hypertension, midlife obesity, smoking, depression, cognitive inactivity or low educational attainment, and physical inactivity) excluded [14]. The F-statistics for all the IVs were calculated to avoid weak instrumental bias (F > 10). Exposure-outcome datasets were harmonized, palindromic SNPs were also considered, and reverse effects were precluded. MR-PRESSO was applied to exclude the outlier SNPs contributing to horizontal pleiotropy and estimate the stability of the result [15].

2.4. Statistical analysis for Mendelian randomization

All statistical analyses were conducted under R (4.2.1 ver.), with the package “TwoSampleMR” primarily utilized for performing the two-sample MR analysis.
Inverse variance weighted (IVW), MR–Egger, and weighted median (WM), as the three leading approaches for MR estimates, were introduced into the analysis. IVW enables the coefficients for IVs-exposure and IVs-outcome to be combined in a meta-analysis with effect scale rectified, which can be restated as a regression model where the IV-outcome coefficients are weighted by the IV-exposure coefficients, while the intercept is also constrained to be zero [16]. MR–Egger and weighted median were acknowledged to be more robust for horizontal pleiotropy in a loose set of scenarios while being less efficient in statistical power; thus, we regarded IVW as the primary approach to estimate the main result and included the methods to examine the consistency of the causal effect: only consistent effect directions of the methods above were found did we deem the effectiveness of the MR estimates [17, 18]. Scatter plots, funnel plots, and leave-one-out plots were generated for statistical inspection of the stability of the results (Fig. 3). Eventually, MR-PRESSO, Cochran’s Q test, and MR–Egger intercept test were conducted as the reexamination of the latent heterogeneity and pleiotropy test, and only P values all over 0.05 allowed the conclusions to be drawn.

3. Result

Targeted GWAS datasets, including GWAS on fresh fruit intake, dried fruit intake were retrieved from IEU analysis of UK Biobank phenotypes, and GWAS on AD was retrieved from FinnGen).

3.1. Fresh fruit intake on AD

Based on 24 SNPs screened out as IVs in Table 1, little evidence from MR estimates suggested a potential causal relationship between fresh fruit intake and AD (MR–Egger: OR (95% CI) = 0.26 (0.03, 2.58), P value = 0.260; Weighted median: OR (95% CI) = 0.74 (0.26, 2.06), P value = 0.554; IVW: OR (95% CI) = 0.97 (0.50, 1.91), P value = 0.939), and no statistical significance was detected.

3.2. Dried fruit intake on AD

Intriguingly, a relatively intensive causality from dried fruit intake on the liability of AD was indicated with statistical significance (MR–Egger: OR (95% CI) = 6.65 (0.12, 364.35), P value = 0.363; Weighted median: OR (95% CI) = 2.68 (1.05, 6.87), P value = 0.031; IVW: OR (95% CI) = 4.09 (2.07, 8.10), P value < 0.001), which revealed that with each standard difference (SD) of dried fruit intake per day, the risk of AD rises by approximately 309%. Twenty-four SNPs filtered as IVs in Table 2 were enrolled in the entire analysis pipeline.

3.2. Results stability evaluations

The results from MR-PRESSO, Cochran’s Q, and MR–Egger intercept tests were outperformed in Supplementary File, indicating that no heterogeneity and pleiotropy existed affecting the interpretability and credibility of the primary analyses (Fresh fruit intake on AD: Cochran’s Q-test derived P value: 0.601 for MR–Egger and 0.582 for IVW; MR–Egger intercept derived P value: 0.248; P value for MR-PRESSO: 0.95. Dried fruit intake on AD: Cochran’s Q-test derived P value: 0.845 for MR–Egger and 0.878 for IVW; MR–Egger intercept derived P value: 0.811; P value for MR-PRESSO: 0.89). In Fig. 3, directions for the
causal effect could be indicated in scatter plots. Based on leave-one-out plots, there did not exist any 95% confidence interval of IVs, including the invalid line in the effect of dried fruit intake on AD, illustrating that the removal of every single IV did not have an excessive effect on MR analysis, while the opposite appeared in causality from fresh fruit intake on AD. Visual judgment of the heterogeneity of each SNP was provided by funnel plots, corroborating the results of Cochran's Q-tests.

4. Discussion

In this study, we evaluated the causal association between fruit intake (including fresh and dried fruits) and Alzheimer's disease (AD) using MR. We found that the intake of fresh fruits had no causal effect on AD, while the intake of dried fruits had a promoting impact on AD. To our knowledge, this is the first study to focus on the relationship between fruit intake and Alzheimer's disease by using MR analysis.

Several observational studies have suggested an association between fruit intake and AD. In a prospective cohort study with 2613 participants (1288 men and 1325 women), habitual fruit intake was connected with the degree of age-related cognitive decline in middle-aged individuals [19]. In contrast to our conclusion, a case–control study using data from 3779 members of the cohort born between 1886 and 1925 in the Swedish Twin Registry found that more fruit consumption may reduce the risk of dementia and AD [20]. An animal study showed that lemon essential oil, a chemical extract from fruits, could inhibit cognitive dysfunction [21]. Likewise, during a randomized controlled trial, both the start and the duration of the neurocognitive test in total were shorter in intervention groups, which was more obvious in the cocoa-red berries group [22]. A meta-analysis that included 16 papers showed that the juices of fruits and some specific fruits could improve cognitive function and mood in individuals [23]. However, the relationship between fruit intake and AD has yet to be verified due to a lack of scientific research. In the present study, we discovered that dried fruit consumption was causally associated with AD, while fresh fruit intake was not associated with AD, which may account for their difference in nutrient and polyphenol contents [24, 25]. Many studies have demonstrated that polyphenols with antioxidant properties play an important role as antioxidants. Polyphenols exert their antioxidant effect by reducing the creation of free radicals, promoting endogenous antioxidant capacity, and upregulating antioxidant protective enzymes, including superoxide dismutase and catalase, aiming to rescue specific memory deficits and cognitive impairment [26,27]. Both fresh and dried fruit have antioxidant properties, whose ultimate antioxidant effect in vivo will be affected by bioavailability after ingestion of different fruits [28]. Moreover, most of the phenolics of apples are lost after different processes to varying degrees, which also influences the body’s absorption of phenolics [29]. A number of antioxidants alleviate cognitive decline, such as carotenoids and polyphenols, in fruits after drying [30]. As a result, bioavailability should be taken into consideration when studying the association between fruit and AD, probably causing differences between the influence of fresh and dried fruits. However, the risk of AD is affected by various nutrients and dietary patterns, making it difficult to explain the individual effects of nutrients and fruits that are correlated with each other [31].
Dried fruit is a kind of widely consumed snack that can influence our bodies’ health. Some studies have claimed that dried fruits can benefit our nervous system health [32, 33]. Fresh fruit consumption may be influenced by seasonal factors. However, dried fruit consumption could constantly influence people’s health [34]. A small sample randomized clinical trial conducted in 60 older adults showed that the consumption of cranberries could benefit visual episodic memory and decrease low-density lipoprotein [35]. Recently, some Mendelian randomization studies found that dried fruit decreases the incidence rate of some diseases, such as cancer and asthma, and keeps people healthy [36, 37]. Similarly, laboratory experiments found that some chemical extracts from dried fruit may prevent Alzheimer’s disease [38, 39]. By giving a whole food diet including fruits, researchers found that this dietary pattern worsened cognitive function in mice [40]. Another animal experiment indicated that long-term feeding of raspberries did not affect the microvascular architecture or cognition of mice with Alzheimer’s disease [41]. In addition, researchers found that some chemical components in currants may play a positive role in the nervous system, while long-term intake of currants may result in worse neuroinflammation [42]. This indicates that not all dried fruit can benefit the nervous system. Additionally, some chemical components may harm our nervous system. Researchers found that aflatoxin B1 in dried fruit could result in Wallerian degeneration of the sciatic nerve in rats [43]. The impact of dried fruit on neurological diseases is still unknown, and relevant studies are insufficient. Further clarification of the clinical significance of dried fruit intake, especially in the field of neuroscience, may have enlightening effects on preventing related diseases. Most of the studies only focus on the extracts from fruit and some specific fruit and ignore the effect of fruit itself. Our study needs more randomized controlled trials to explore the potential influence of dried fruit on human health and affirm our results.

Nevertheless, the results should be interpreted under several limitations. First, the GWAS used for analysis was stretched from MRC-IEU based on UK Biobank phenotypes, and the limited customer base may affect the accuracy of the conclusions drawn, while latent recall bias still existed in collecting self-reported information through questionnaires. Second, racial heterogeneity was ignored, as only Europeans were included in the study, differentiated susceptibility among various regions should be taken into consideration, and the significance of the results should be strengthened by future studies with population expansion. Third, the manual screening of SNPs violating the three assumptions may increase the false positives due to sample size limitations, although we tried to render the process of filtration sufficiently conservative [44]. Fourth, we were unable to examine whether the causal association between fruit intake and Alzheimer’s disease varied by covariates, including potential effect modifiers.

Our study also has noteworthy strengths. Two major European publicly available databases were utilized to obtain more accurate estimates and gain the maximum population representative power. Intensive causal effect from per SD increase in units of dried fruit intake on the risk of Alzheimer’s disease provided partial strong observative evidence on clinical instructions and avoided bias in interpretation of results due to weak effect scale. No pleiotropy or heterogeneity observed endowed the results with much more reliability.
5. Conclusion

In the study, we strengthened the evidence supporting the positive causality from dried fruit intake to the liability of Alzheimer’s disease, while the association between fresh fruit intake and the risk of Alzheimer’s disease failed to be demonstrated. The study has the potential on a causal level as well as the entire treatment course. Additionally, we foresee that further well-designed epidemiological research could validate the correlation between fruit intake and Alzheimer’s disease and explore more risk-relevant nutrients accompanied by overcoming bias caused by methodologies and other intrinsic properties.

Declarations

Data Share Statement

Data described in the manuscript, code book, and analytic code will be made available upon request pending application.

Ethical approval

No additional ethics approval was needed because all data in the study was previously collected, analyzed, and published.

Declaration of competing interest

The authors declare no conflicts of interest.

Consent for publication

Not applicable.

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Author contribution

LWZ contributed to data enrollment, statistical analysis, study design, manuscript writing, and proofreading, ZXF, XQ, MYT, and WLL contributed to the statistical analysis and composing of the manuscript, HXP contributed to the writing of the manuscript, and GXG contributed to the project design and administration. All authors have granted their approval for the manuscript.

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References


Figures

Figure 1

Three primary assumptions of MR analysis, required to avoid biased causal effect estimate. Assumption 1: IVs are strongly associated with the exposure variable; Assumption 2: IVs only affect the outcome via affecting the exposure; Assumption 3: IVs are stipulated not to be related to any confounders of the exposure-outcome relationship.
Figure 2

Forest plot for the effect of fresh fruit intake and dried fruit intake on risk of Alzheimer’s disease. OR, odds ratio; CI, confidence interval.
Figure 3

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFile.xlsx