Types of milk consumed and risk of essential hypertension: A 2-sample Mendelian randomization analysis

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ABSTRACT

Observational associations between milk consumption and essential hypertension have been reported. However, their causal inferences have not been proven, and the effects of different types of milk consumption on hypertension risk remain poorly characterized. The Mendelian randomization (MR) analysis was performed using public summary-level statistics from genome-wide association studies to determine whether the different types of milk consumption affect essential hypertension differently. Six different milk consumption types were defined as exposure conditions, whereas essential hypertension identified by the ninth and tenth revisions of the International Classification of Diseases was considered the outcome of interest. Genetic variants, which were genome-wide associated with the types of milk consumed, were used as an instrumental variable for MR analysis. In primary MR analysis, the inverse-variance weighted method was adopted followed by several sensitivity analyses. Our findings suggested that of the 6 common types of milk consumed, semi-skimmed and soya milk products were protective against essential hypertension, whereas skim milk had the opposite effect. Consistent results were also observed in sensitivity analyses that followed. The present study provided genetic evidence that a causal link between milk consumption and the risk of essential hypertension and a new reference for the diet antihypertensive treatment plan for patients with hypertension.

Key words: essential hypertension, Mendelian randomization, causal inferences, saturated fatty acids

INTRODUCTION

Milk, also known as lac in Latin, has been consumed by humans since the early seventh millennium BC (Evershed et al., 2008), has been an excellent source for obtaining essential nutrients, including protein, minerals, and vitamins, for most children and adults. Consequently, the relationship between milk consumption and human health has received much attention.

In recent decades, many studies have explored the relationship between milk consumption and hypertension risk reduction but have not been conclusive. Additionally, their causal relationship is unclear. For instance, Buendia et al. (2018) and Jung et al. (2022) reported that milk is associated with decreased hypertension risk in several ethnic populations. However, a French study showed no association between dairy products (including milk) and hypertension (Villaverde et al., 2020). These inconsistent results of research work led researchers to believe that determining the effect of fat content in milk and different processing methods on cardiovascular disease is necessary, rather than determining whether only milk consumption is related to the risk of hypertension (Drouin-Chartier et al., 2016; Zhang et al., 2021).

The high saturated fat content of milk has long been a concern since the lipid hypothesis was put forward (Herbert, 1977). In milk, approximately 70% of fat by weight is SFA, which may increase the level of low-density lipoprotein cholesterol (Artaud-Wild et al., 1993) and the risks of cardiovascular diseases. Therefore, through industrial skimming, various types of milk have been developed, and a variety of choices, such as whole milk, semi-skimmed milk, and skimmed milk products with different concentrations of SFA, are offered to consumers. At the same time, recommendations to choose semi-skimmed or skim milk products to reduce the risks of cardiovascular diseases have emerged (Mozaffarian et al., 2018). For example, Hypertension Canada (https://hypertension.ca/about-us/) recommended the Dietary
Approaches to Stop Hypertension diet to manage high blood pressure and stressed semi-skim milk and skim milk consumption (Karanja et al., 2004), and the Dietary Guidelines for Americans 2015–2020 also advised people to intake skimmed or semi-skim milk instead of whole milk (You, 2015). However, some studies provided evidence for these government approaches (Alonso et al., 2005), whereas others found that whole milk did not increase or even reduced the risk of hypertension (Ralston et al., 2012; Soedamah-Muthu et al., 2012; Wang et al., 2015). Therefore, the relationship between the types of milk consumed and improved hypertension becomes elusive, and a debate about which type of milk is right has emerged and continues to this day.

Recently, the Mendelian randomization (MR) analysis has been widely introduced based on the availability of large-scale human genetic projects (e.g., the data of GWAS) to investigate the causative relationship between exposure and outcome of interest (Bycroft et al., 2018). In the MR analysis, genetic variations with random distribution during meiosis are exploited as an instrumental variable (IV; Lawlor et al., 2008). Compared with that obtained by previous observational epidemiological and traditional retrospective studies, the phenotype proxied by such IV determined before one’s birth is less likely influenced by the bias from confounders and reverse causation. This analytical method has also been recently introduced to reveal the risk factors for hypertension (Ding et al., 2017; van Oort et al., 2020; Yang et al., 2021).

Here, we performed an MR analysis based on the summary-level statistic data obtained from the Neale Laboratory and the Medical Research Council-Integrative Epidemiology Unit consortia (Elsworth et al., 2020) to investigate the relationship between the types of milk consumed and the risk of essential hypertension.

**MATERIALS AND METHODS**

We designed this MR study following the Strengthening the Reporting of Observational Studies in Epidemiology Using Mendelian Randomization guidelines (Smith et al., 2019) and the manual for MR analysis (Burgess et al., 2019) without a preregistered or published analysis plan. All analyses were planned before study initiation. In this MR study, 3 IV assumptions were considered (Figure 1): (1) The genetic variants included in each instrument are strongly associated with the types of milk consumed by participants. (2) Each genetic variant is not associated with potential confounders. (3) Each genetic variant affects the risk of hypertension events only through the exposure condition (e.g., different types of milk consumption). The population stratification that the allele’s distribution varies in different ethnic populations is usually a common violation of the above second assumption. Focusing on homogeneous ancestry groups is recommended to overcome this issue (Haycock et al., 2016). Therefore, only individuals with European ancestry were included in this MR analysis. The details about the conception and assumption of MR are described in the online supplemental materials (https://doi.org/10.6084/m9.figshare.20025557.v2; Shi et al., 2022).

**Ascertainment of Exposure and Outcome**

In accordance with the data of the projects of the Neale Laboratory and Medical Research Council-Integrative Epidemiology Unit consortia, the types of milk consumed by 455,816 participants (ages from 40 to 79 years) were encoded as whole milk, semi-skimmed milk, skimmed milk, other types of milk, soya, and never/rarely have. These 6 types (Jiang et al., 2021) are introduced in the online supplemental materials (https:
Among these different types of milk products, the contents of SFA, which was previously looked at as a risk factor for hypertension, obviously vary (McCance and Widdowson, 2014). In the present study, these 6 types of milk consumption were defined as exposure conditions. The summary-level data on the relationship between genetic variants and people’s different types of milk consumed were obtained from the Medical Research Council-Integrative Epidemiology Unit database (Elsworth et al., 2020; Jiang et al., 2021). Essential hypertension was defined as our clinical outcome of interest. The summary-level data of essential hypertension GWAS, which included 54,358 patients diagnosed with essential hypertension in accordance with the ninth and tenth revisions of the International Classification of Diseases (https://www.who.int/classifications/icd/en/) and 408,652 general Europeans, were used.

Bias due to participants overlap in the 2 cohorts included in this MR study was estimated using a web tool available at https://sb452.shinyapps.io/overlap. As described in the online supplemental material (https://doi.org/10.6084/m9.figshare.20025557.v2; Shi et al., 2022), the sample overlap range was 0–100%. Moreover, no substantial inflation in bias and type I error rate was found. All GWAS studies cited in this MR analysis were approved by a relevant review board.

Two-Sample MR Analysis

The association between SNPs and the type of milk consumed was obtained from the summary statistics of a diet measurement GWAS (Jiang et al., 2021) with 455,816 participants in the UK Biobank-scale data. Within a clumping distance of 10,000 kb, the SNPs associated with special types of milk consumption ($P < 5e-6$) and unlikely linkage disequilibrium with other SNPs ($r^2 = 0.001$) were extracted using the extract_instruments command in the TwoSampleMR package in R. Consequently, 6 genetic variant sets were constructed with such SNPs and served as the independent genetic instrument for the type of milk consumed. The association of these IV to essential hypertension was extracted from Elsworth’s GWAS (Elsworth et al., 2020) and harmonized using the default settings of the harmonize_data command in the TwoSampleMR package in R to make the effect estimates consistent with the alleles. The variance ($R^2$) of each SNP included and their sum were calculated to estimate the variability of milk consumption that each genetic instrument represents. $F$-statistics, which could assess the strength of the selected instruments (Palmer et al., 2012), was also calculated as described in Supplemental Table S1 (https://doi.org/10.6084/m9.figshare.20025557.v2; Shi et al., 2022).

Statistical Analysis

In the primary analysis, an inverse-variance-weighted method, including fixed and random-effects models, was performed for its great power (Burgess et al., 2013). Cochrane’s Q statistics of the inverse-variance-weighted method (Burgess et al., 2013) and Rücher’s Q statistics of the MR-Egger method (Bowden et al., 2015) were used in the heterogeneity test. The result of the random-effects (default model) analysis of the inverse-variance-weighted method was accepted, given the significant heterogeneity between the estimates from different genetic variants.

The influence of outliers on the relationship between the types of milk consumed and one’s essential hypertension risk was estimated. The RadialMR analyses using modified second-order weights (Bowden et al., 2018) were used to identify the outliers. The threshold of $\alpha$ level was defined as 0.05 divided by the number of SNPs included in instruments. The RadialMR analysis was performed when the outliers were detected and removed. In addition to the heterogeneity test, a directional horizontal pleiotropy test was performed with mr_pleiotropy_test commands.

Considering the confounders of the SNP-outcome association, an SNP-phenotype scanning was performed by PhenoScanner v2 (Kamat et al., 2019). The SNPs associated with other cardiovascular diseases and hypertension-related traits (such as coronary artery disease, heart disease, diabetes, and obesity; Supplemental Methods, https://doi.org/10.6084/m9.figshare.20025557.v2; Shi et al., 2022) were defined as potential confounders because of their horizontal pleiotropies. Then, restrictive MR was performed with instruments that remove these pleiotropic SNPs. All statistical analyses were 2-sided and performed using the TwoSampleMR and RadialMR packages in the R software 4.2.0 (R Core Team, 2013). For statistical analyses, a $P$-value <0.05 was considered statistically significant.

Data Availability

Publicly available datasets were analyzed in this study. Data used for this study are available here: GWAS summary-level data on consumed milk type were downloaded from the NHGRI-EBI Catalog of human genome-wide association studies (https://www.ebi.ac.uk/gwas/home). Summary statistics of GWAS on essential hypertension were obtained from the GWAS summary data ecosystem of the MRC Integrative Epide-
miology Unit (IEU) at the University of Bristol (https://gwas.mrcieu.ac.uk/datasets/). Genome-level associated phenotype data for SNPs were extracted from the database of human genotype-phenotype associations (http://www.phenoscanner.medschl.cam.ac.uk/).

RESULTS

Genetic Instruments Based on the Statistically Driven Approach

A total of 11,842,647 autosome SNP-milk type associations were found in the GWAS of Jiang et al. (2021), of which 137 SNPs were significantly associated with different milk consumption types (whole milk, n = 30; semi-skim milk, n = 15; skim milk, n = 32; soya, n = 25; other milk types, n = 13; and never/rarely have, n = 22). Three SNPs (rs1426371, rs28384266, and 427118) were identified as significantly associated with the consumption of semi-skimmed and skim milk products with diametrically opposite directions of effects. Two or 3 of the 7 SNPs were located in a single gene (rs12149832 and 55872725 in FTO, HGNC: 24678; rs74849734 and rs79570398 in P12, HGNC: 35546; Supplemental Table S2; https://doi.org/10.6084/m9.figshare.20025557.v2; Shi et al., 2022). The 137 SNPs included was accounted for 12.6% of the variability in milk consumption types. Each subinstruments ranged from 0.3% to 4.1%. The mean F-statistic of each subinstructor, ranging from 86.24 to 1,435.23 with a minimum of 47.78, suggested a low probability of risk from weak instrument bias.

Causal Inference of Milk Types on Essential Hypertension

The primary MR analysis estimated that 3 types of milk consumption (whole milk, semi-skim milk, and skim milk) showed significant effects on the risk of essential hypertension (Table 1 and Figure 2). Compared with other milk consumption types, consuming whole milk and semi-skim milk had a protective effect (whole milk, OR = 0.860, 95% CI: 0.757–0.988, P = 0.034; semi-skim milk, OR = 0.892, 95% CI: 0.836–0.952, P = 2.9e–4) on essential hypertension. Soya consumption type also had a weak protective effect (OR = 0.844, 95% CI: 0.704–1.010) on the risk of hypertension. However, this effect did not reach a statistically significant level (P = 0.064).

<table>
<thead>
<tr>
<th>Genetic instrument</th>
<th>SNP (n)</th>
<th>OR1 (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole milk</td>
<td>30</td>
<td>0.865 (0.757–0.988)</td>
<td>0.034</td>
</tr>
<tr>
<td>Semi-skim milk</td>
<td>15</td>
<td>0.888 (0.834–0.945)</td>
<td>2.1e-4</td>
</tr>
<tr>
<td>Skim milk</td>
<td>32</td>
<td>1.126 (1.046–1.213)</td>
<td>1.5e-3</td>
</tr>
<tr>
<td>Soya</td>
<td>25</td>
<td>0.841 (0.704–1.010)</td>
<td>0.064</td>
</tr>
<tr>
<td>Other types of milk</td>
<td>13</td>
<td>1.045 (0.753–1.450)</td>
<td>0.793</td>
</tr>
<tr>
<td>Never or rarely have</td>
<td>22</td>
<td>1.129 (0.976–1.305)</td>
<td>0.100</td>
</tr>
</tbody>
</table>

1OR = odds ratio.
2 Analyzed by inverse-variance-weighted method with the fixed-effects model rather than the default model (random).

Table 1. Effects of different milk consumption types on essential hypertension risk

Outliers and Horizontal Pleiotropy Influences on Estimated Effects

RadialMR analyses were performed to estimate the influence of outliers among each genetic variant and obtain a robust result. Among 6 genetic instruments, one or up to 5 outliers were identified, except the instrument of semi-skim milk (Supplemental Table S4 and Figure S1; https://doi.org/10.6084/m9.figshare.20025557.v2; Shi et al., 2022). When the identified outlier SNPs were removed from each instrument, the protective effect of whole milk consumption estimated in primary MR analysis was lost (OR = 0.979, 95% CI: 0.888–1.080, P = 0.678), whereas the protective effect of soya consumption even became significant (OR = 0.813, 95% CI: 0.706–0.936, P = 3.8e-3). Similar effects of their primary MR tests were observed for the 4 other milk consumption types.

Ten pleiotropic SNPs were detected from the 137 milk-consumption-related SNPs scanned in the database of human genotype-phenotype associations by PhenoScanner v2 (Supplemental Method and Table S2; https://doi.org/10.6084/m9.figshare.20025557.v2). After removing these pleiotropic SNPs, the restrictive genetic instruments still showed a similar causal relationship with essential hypertension (Supplemental Table S5; https://doi.org/10.6084/m9.figshare.20025557.v2; Shi et al., 2022).

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DISCUSSION

For decades, milk consumption has been widely recommended. However, its health benefit and fat content of milk have not been established yet. Concerns about the risks of possible adverse health outcomes have been raised (Willett and Ludwig, 2020). Thus far, no convincing answer to the question “whole milk or skim milk”? is available. In the present study, a 2-sample-MR analysis was performed to estimate the causal relationship between one’s daily consumed milk types and the risk of essential hypertension. We observed the significant protective effect of consuming semi-skim milk and soya on essential hypertension risk and the negative effect of consuming skim milk.

In the 6 types of milk consumed in this study, the SFA content has long been a concern. Several current national guidance groups (such as the American Heart Association, European Society of Cardiology, American Diabetes Association, and European Association for Study of Diabetes) recommended minimizing the dietary intake of SFA by choosing semi-skimmed or skim milk to achieve the benefit of milk for cardiometabolic health. They believed that such milk consumption is beneficial for subjects with hypertension and can decrease the risk of hypertension for healthy people (Whelton et al., 2002; Chobanian et al., 2003; McCance and Widdowson, 2014). However, contrary to these national guidelines’ perspective, our findings indicated that the concentrations of SFA in whole milk, semi-skim milk, and skim milk products decreased sequentially, but the risk of essential hypertension in their corresponding consumption populations increased in sequence (whole milk: OR = 0.865, 95% CI: 0.757–0.998; semi-skim milk: OR = 0.888, 95% CI: 0.834–0.945; and skim milk: OR = 1.126, 95% CI: 1.046–1.213). This observation supported the new voice of some researchers, clinicians, and nutrition experts on this issue that the SFA content of milk should not be of such concern (Lordan et al., 2018). Furthermore, several meta-analyses of randomized trials and observational studies reported no significant evidence to conclude an association between SFA and increased risk of cardiovascular diseases (Siri-Tarino et al., 2010). Whole milk increases low-density lipoprotein cholesterol while increasing high-density lipoprotein (HDL-C). This means that whole milk may not affect or may even reduce the proportion of total cholesterol to HDL-C (Huth and Park, 2012). Thus, not only the milk fat contained in milk causes creaminess and delicious taste, but also it does not exert hypertensive effects on adult patients (Roy et al., 2019) and even has a protective effect on them (Chegini et al., 2017). Furthermore, in milk, other components besides SFA may have an impact on the varying effects of different types of milk on the risk of hypertension. For instance, compared with skim milk, the enriched milk protein in whole and semi-skim milk products is a good source of angiotensin-converting enzyme inhibitory peptides, which have an antihypertensive effect (FitzGerald et al., 2004). The calcium in different types of milk also has an antihypertensive feature (McCance and Widdowson, 2014).

Soya, also known as soy milk or soymilk, is a well-known cow’s milk replacement because of its high protein, vitamin, mineral, antioxidant contents, and health benefits (Idowu-Adebayo et al., 2022). In addition to its advantage of low SFA concentration, other active ingredients in soya milk may be responsible for its antihypertensive effect and have a better effect than semi-skim milk and skim milk products (Rivas et al.,

Figure 2. Scatter plots show the effects of different types of milk consumption on the risk of essential hypertension: (A) whole milk, (B) semi-skim milk, and (C) skim milk. HTN = hypertension.
lead to model over fitting and imprecise estimates because a large overlap between the 2 samples may exist. In the present study, the influence of overlap in samples between exposure and outcome data should not be overlooked. Substantial bias was observed from the sample overlap, particularly when the influence of outliers was considered. This finding was consistent with a recent point that plant-based milk can be a good alternative to milk (Vanga and Raghavan, 2018; Clegg et al., 2021). Plant-based milk is nutritionally adequate and has pleiotropic effects, which may be beneficial to patients with hypertension. Consistent with the principles of the Dietary Approaches to Stop Hypertension diet, our MR analysis proved that a diversified diet, such as increasing the proportion of plant-based protein, may be beneficial to patients with hypertension.

The plausibility of such a causal relationship between the milk consumption types and the essential hypertension risk was also confirmed by the following series of sensitivity analyses. First, in addition to the primary MR, heterogeneity, and pleiotropy analyses, we performed a RadialMR analysis (Bowden et al., 2018) considering the influence of outliers among each genetic instrument. Consistent with the above analysis, the protective effects of consuming semi-skimmed and soya milk products were observed, whereas an increased risk of hypertension was observed in subjects who consumed skim milk. Second, by controlling the bias from potential confounders and pleiotropic SNPs, we also observed similar effects on essential hypertension. These consistent results suggested a substantial causative link between the type of milk people consumed and the risk of essential hypertension.

Limitations still exist in this study, although the MR design was less susceptible to confounding compared with other observational studies. Caution should be exercised in interpreting this finding considering the following issues. First, our findings related only to the relationship between different types of milk consumed and the risk of essential hypertension and did not explain the effect of different intakes because the data on milk intake were not available. Moreover, the types and amounts of milk that some participants consumed might not be as distinct and regular. Second, although no substantial bias was observed from the sample overlap presented, the influence of overlap in samples between exposure and outcome data should not be overlooked because a large overlap between the 2 samples may lead to model over fitting and imprecise estimates toward observational relationships (Burgess et al., 2016). Third, this study still cannot absolutely rule out the influence of some genetically/epigenetically regulated factors (such as hypolactasia or lactose intolerance) and nongenetic elements (e.g., different forms of medical treatment). In addition, the official guidelines (e.g., Dietary Guidelines for Americans; Arnold et al., 2021), commercial development (Barbano, 2017), advertising, brands, and the cultural environment (Finnell and John, 2017) can easily sway a person’s choice of type of milk. Finally, given the effect of population stratification on the MR study and the available GWAS data, this study only estimated the relationship between milk consumption types and the risk of hypertension in the European population. Universal conclusions will be achieved by combining other MR studies in different ethnic populations in the future.

CONCLUSIONS

This MR study provides evidence of a causal relationship between the types of milk consumed and the risk of essential hypertension. The study also emphasizes that a varied diet plan may be effective in managing hypertension.

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