Association of plasma polyunsaturated fatty acids with arterial blood pressure

A Mendelian randomization study

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Abstract

High polyunsaturated fatty acids (PUFAs) intake is recommended for primary and secondary prevention of cardiovascular disease (CVD). However, the association of PUFAs with blood pressure (BP) is still controversial. In the present study, two-sample Mendelian randomization (MR) analysis was performed to investigate the causal relationship of PUFAs with BP, including systolic blood pressure (SBP), diastolic blood pressure (DBP), and pulse pressure (PP).

Genetic instruments and summary statistics for two-sample MR analysis were obtained from 3 large-scale genome-wide association studies (GWASs). Eight single nucleotide polymorphisms (SNPs) significantly (P < 5 × 10−8) related to 6 PUFAs were used as instrumental variables. Conventional inverse-variance weighted method was adopted to evaluate the causality of PUFAs with BP; the Weighted Median, MR-egger, and Leave-one-out method were used for sensitivity analyses.

As a result, there was no evidence of a causal association between all PUFAs and SBP. In addition, arachidonic acid (AA, \( \beta = -0.04, P < .001 \)) and eicosapentaenoic acid (EPA, \( \beta = -0.47, P = .02 \)) were negatively associated with DBP, while linoleic acid (LA, \( \beta = 0.03, P = .005 \)) and \( \alpha \)-linolenic acid (ALA, \( \beta = 3.83, P < .001 \)) were positively associated with DBP. There was no evidence of a causal relationship between either docosapentaenoic acid (DPA) or docosahexaenoic acid (DHA) with DBP.

In conclusion, a genetic predisposition to plasma polyunsaturated fatty acid (PUFA) had a divergent effect on DBP, independent of SBP. It suggested that it is helpful for lower DBP level to supplemental intake of AA and EPA or promote the conversion of LA and ALA to AA and EPA respectively, which need to be further validated with randomized controlled studies.

Abbreviations: AA = arachidonic acid, ALA = \( \alpha \)-linolenic acid, BP = blood pressure, CI = confidence interval, CVD = cardiovascular disease, DBP = diastolic blood pressure, DHA = docosahexaenoic acid, DPA = docosapentaenoic acid, EPA = eicosapentaenoic acid, FADS = fatty acid desaturase, GWAS = genome-wide association study, ICBP = International Consortium for Blood Pressure, IV = instrumental variable, IVW = fixed-effects inverse-variance weighted, LA = linoleic acid, MR = Mendelian randomization, PP = pulse pressure, PUFA = polyunsaturated fatty acid, RCT = randomized controlled trial, SBP = systolic blood pressure, SD = standard deviation, SNP = single nucleotide polymorphism, UKBB = UK Biobank.

Keywords: blood pressure, Mendelian randomization, polyunsaturated fatty acid

1. Introduction

Hypertension is the most common public health issue and an important contributor to disability and mortality, which caused an estimated 10.4 million deaths worldwide in 2017.[3] Overall, the global burden of hypertension is persistently increasing and up to 1.56 billion in 2025.[1] However, epidemiological survey has shown that only 46.5% patients were aware of their condition, 36.9% received treatment, and 13.8% controlled blood pressure (BP) among the 1.39 billion hypertensive patients worldwide in 2010.[3] Since unhealthy lifestyle is an independent risk factor of hypertension, lifestyle-based medication is notably the best way to control BP and prevent cardiovascular events, including weight loss, increase of physical exercise, and change of dietary patterns.[4]

Polyunsaturated fatty acids (PUFAs) are straight-chain fatty acids, containing 2 or more double bonds and chains of 18 to 22 carbon atoms. They are mainly divided into 2 families, n-3 PUFAs and n-6 PUFAs. The former consists of \( \alpha \)-linolenic acid (ALA, \( C_{18:3} \)), eicosapentaenoic acid (EPA, \( C_{20:5} \)), docosapentaenoic acid (DPA, \( C_{22.5} \)), and docosahexaenoic acid (DHA, \( C_{22:6} \)), while the latter includes linoleic acid (LA, \( C_{18:2} \)) and arachidonic acid (AA, \( C_{20:4} \)). In human, longer-chain PUFAs are converted by LA and ALA through enzymatic cascades of desaturation,
elaboration, and oxidation, as shown in Figure 1. Since they cannot be self-synthesized in human, LA and ALA are referred as essential fatty acids and have to be provided in foods, such as flaxseed, sunflower, and soybean oil.[5] Besides, the synthesis of EPA and DHA in human is insufficient and need to be supplemented from marine creatures and algae.[5] Lack of PUFAs, especially for n-3 PUFAs, were associated with chronic diseases, such as inflammation, cancer, hypertension, and cardiovascular disease (CVD).[5]

However, there is still controversial about the relationship between PUFAs and BP. Some studies have indicated that increasing the intake of n-3 PUFAs reduced BP, including systolic blood pressure (SBP), diastolic blood pressure (DBP), and ambulatory BP.[7] Consumption of n-3 PUFAs has been reported to lower BP for both the young[8] and elderly[9] normotensive subjects, and to reduce the incidence of new-onset hypertension.[10,11] Systematic reviews have supported that dietary supplement of n-3 PUFAs may play a role in decreasing BP in hypertensive patients.[12–14] However, the recent reviews from Cochrane and the Agency of Healthcare Research and Quality (AHRQ) databases had suggested that supplementation with fish oils or highly purified PUFAs had mild or even no effect on BP, regardless of the dosage.[15–17] Due to complex confounding factors, such as selection bias, and loss of follow-up, traditional clinical studies could not illuminate whether PUFAs were causally linked to BP.[18] Furthermore, the n-3 and n-6 PUFAs are metabolically and functionally distinct.[5] Since the effects of PUFAs on BP are still confounded, it is worthy concerned about the causal association of each polyunsaturated fatty acid (PUFA) with BP.

Mendelian randomization (MR) refers to the random assortment of inherited alleles at conception and links to research subjects being randomly allocated to different genotypes, which has been proposed as a method to estimate causality.[18] Therefore, the MR analysis is considered as analogue to a "natural" randomized controlled trial (RCT), which is independent of confounding factors or reverse causation.[19] Based on the following basic principles of MR:

1. each selected variant is associated with the risk factor,
(2) variants are independent of confounders from other risk factors,
(3) each variant influences the outcome only through the risk factor,[19] we performed this study to investigate the nature of the association between PUFAs and BP.

2. Materials and methods

2.1. Genetic instruments selection
The exposure was genetically predicted plasma PUFAs level, and the single nucleotide polymorphisms (SNPs) distinctly associated with PUFAs were selected as instrumental variables (IVs). Genetic associations of 2 n-6 PUFAs, including LA and AA, were identified from a large-scale meta-analysis of genome-wide association study (GWAS) of 8631 European individuals from 5 prospective cohorts.[20] Genetic associations of 4 n-3 PUFAs, including ALA, EPA, DPA, and DHA, were identified from a meta-analysis of GWAS from the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium, including 8666 individuals of European ancestry.[21] The concentration of individual PUFAs was defined as the percentage of plasma total fatty acids and expressed for standard deviation (SD) as the unit, as described previously.[22] The selected SNPs were associated with one or more of the PUFAs at a genome-wide significance-threshold \( (P < 5 \times 10^{-8}) \) and involved in the PUFAs metabolic pathway. LD-Link (https://ldlink.nci.nih.gov/) was used to test linkage disequilibrium between 2 loci in the same chromosome based on European ancestries. Every targeted single nucleotide polymorphism (SNP) was searched in the PhenoScanner (http://www.phenoscanner.medschl.cam.ac.uk/phenoscanner)[24,25] for the known effects to restrict the potential pleiotropy. F-statistic were used to judge the strength of the IVs, and those with an F-statistic of 10 or less were regarded as weak instruments.[26]

2.2. Data source for BP
The summary statistics for BP (including SBP, DBP, and pulse pressure [PP]) were obtained from a meta-analysis of GWAS from the UK Biobank (UKBB) and International Consortium for Blood Pressure (ICBP) comprising a total of 757,601 subjects of European ancestry.[27] UKBB-GWAS (N = 458,577) was restricted to Europeans based on principal component analysis, and European ancestries.[27] UKBB-GWAS (N = 458,577) was restricted to Europeans based on principal component analysis, and UKBB-GWAS (N = 299,024) included European samples only, using a fixed-effects inverse-variance weighted (IVW) method.

2.3. Statistical analyses
The two-sample MR method was used to evaluate the causal relationship between genetically predicted PUFAs and BP traits. The causal effect estimates of SNPs on SBP, DBP, and PP were calculated using the Wald Estimator,[18] with standard errors estimated using the Delta method.[24] The effect estimates for each BP trait were analyzed using the IVW method to establish the overall causal effect estimates.[29] For IVs including more than 2 SNPs, sensitivity analyses were conducted using the Weighted Median, random-effects IVW method MR-Egger, and Leave-one-out method. The Weighted Median method allowed half of the information comes from invalid IVs.[30] The MR-Egger method not only detected pleiotropy with regression intercept but also provided stable estimates adjusted for directional pleiotropy.[31] Furthermore, the Leave-one-out analysis was performed to explore whether the association was influenced by a unique SNP. All statistical analyses were performed using R version 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria) and the \( P < .05 \) was considered as statistical significance.

2.4. Ethics approval
Studies included in the GWAS had been approved by respective institutional review board and participants had provided informed consent. The data used in this study were publicly available and ethical approval was not required.

3. Results

3.1. Genetic instruments for PUFAs
The details of GWAS used for this MR study were shown in Supplemental Table S1, http://links.lww.com/MD/F578. In total, 8 genome-wide significant \( (P < 5 \times 10^{-8}) \) and independent (linkage disequilibrium, \( r^2 < 0.1 \)) SNPs were selected, as shown in Supplemental Table S2, http://links.lww.com/MD/F579 and Table S3, http://links.lww.com/MD/F580. All SNPs have been selected as IVs for PUFAs in previous MR study, which well documented the validities of IVs by \( F \) test (\( F \)-statistic > 10).[32] The proportion of variance in plasma PUFAs level explained by each PUFA-specific instruments ranged from 0.65% (for DHA) to 33% (for AA). According to the third principle of the MR analysis mentioned above, rs10740118 was excluded when estimating the causal relationship between LA and BP. In addition, some SNPs were related to one or more phenotypes, such as blood cell count, blood lipids, and alcohol intake, which were obtained from the PhenoScanner and summarized in Supplemental Table S4, http://links.lww.com/MD/F581.

3.2. Effects of plasma n-6 PUFAs on BP
The associations between genetically predicted plasma PUFAs level and BP were shown in Figure 2. One SD increment of LA increased DBP by 0.03 mmHg (95% confidence interval (CI): [0.01, 0.06], \( P = .005 \)) and decreased PP by 0.07 mmHg (95% CI: [-0.39, 1.34], \( P < .001 \)). Similar results of LA affected PP were obtained by the Weighted Median and random-effects IVW method. There was no significant pleiotropy detected \( (\text{intercept} = 0.04, P = .51) \) by MR-Egger analysis as shown in Supplemental Table S5, http://links.lww.com/MD/F582. In addition, elevated AA was associated with decreased DBP \( (\beta = -0.04, 95\% \text{ CI}: [-0.06, -0.02], P < .001) \) and increased PP \( (\beta = 0.06, 95\% \text{ CI}: [0.06, 0.09], P < .001) \).

3.3. Effects of plasma n-3 PUFAs on BP
For primary outcomes obtained by the IVW method, genetically instrumented n-3 PUFAs were also mainly associated with DBP and PP, as shown in Figure 2. Increasing of ALA was positively associated with DBP \( (\beta = 3.83, 95\% \text{ CI}: [1.59, 6.07], P < .001) \) and inversely associated with PP \( (\beta = -6.78, 95\% \text{ CI}: [-9.44, -4.12], P < .001) \). However, for every additional SD of EPA, DBP decreased by 0.47 mmHg (95% CI: [-0.87, -0.07], \( P = .02 \)), and PP increased by 0.86 mmHg (95% CI: [0.39, 1.34], \( P < .001 \)). Plasma DPA was related to higher SBP (0.72 mmHg per SD, 95% CI: [0.01, 1.44], \( P = .05 \) and higher PP (1.04 mmHg per SD, 95% CI: [0.01, 2.08], \( P = .05 \))
Hg per SD, 95% CI: [0.56, 1.53], \( P < .001 \). Nevertheless, the estimation from the Weighted Median and random-effects IVW method were less consistent, and no pleiotropy was detected by MR-Egger analysis (intercept = 0.12, \( P = .47 \)), as shown in Supplemental Table S5, http://links.lww.com/MD/F582. In the Leave-one-out analysis, there was no correlation between plasma DPA and SBP after removing rs780094, as shown in Figure 3. The correlation between plasma DPA and PP was also inconsistent with the results from the Weighted Median and random-effects IVW method. Especially, plasma DPA was not associated with PP after omitting rs174547 by the Leave-one-out analysis. It suggested that the relationship between plasma DPA and BP needs to be drawn with caution. However, these MR analyses showed that plasma DHA was not associated with either SBP or DBP.

4. Discussion
As an important cellular component, PUFAs have been reported to be associated with many chronic diseases, such as hypertension, CVD, cancer, and neurodegenerative disease. However, it is still controversial whether each PUFA plays a critical role in BP control. Using the data from large-scale GWAS of European subjects, this MR study showed that higher plasma LA and ALA levels were causally associated with DBP elevation, while plasma AA and EPA levels were negatively associated with DBP. Six PUFAs enrolled in the present study had no causal relationship with SBP.

4.1. N-6 PUFAs and BP
N-6 PUFAs are abundant in the diet and easily obtained from vegetable oils, such as canola, soybean, and corn. Although n-6 PUFAs play an important role in regulating body homeostasis of inflammation, vasodilation, vasoconstriction, and lipid metabolism, their effect on BP is still unclear. The present results showed that increased plasma LA caused an increase of DBP, while 1 SD increment of plasma AA decreased DBP by 0.04 mm Hg. It suggested that both in vivo bioconversion of LA to AA and COX inhibitors for AA might be helpful for DBP control. Consistently, the INTERMAP study has demonstrated that 2-SD higher LA intake (9.0 g/day) reduced SBP/DBP by 1.42/0.91 mm Hg in 4680 participants from 17 populations of China, Japan, United Kingdom, and United States. Another cross-sectional observational study of 4033 healthy men has also reported that 2-SD increase of LA consumption reduced SBP by 1.9 mm Hg. Nevertheless, a systemic review of 19 RCTs from Cochrane database has shown that LA did not affect BP. Consequently, large-scale RCTs with strict control of confounding factors would be needed to clarify the effects of N-6 PUFAs on BP. LA was in vivo converted into AA through a series of reaction catalyzed by desaturase and elongase, resulting in an abundance of bioactive substances that played significant physiological roles. AA could be metabolized by cyclooxygenase (COX) and lipoxygenase (LOX) to produce pro-inflammatory eicosanoids, such as prostaglandin F2α (PGF2α), leukotriene B4 (LTB4), and thromboxane A2 (TXA2), which promoted vasospasm, vasoconstriction and platelet aggregation, thrombosis, and atherosclerosis. Contrarily, AA also could be metabolized by cytochrome P450 monoxygenase to produce epoxyeicosatrienoic acids (EET), beneficial for vasodilatation and thus BP down-regulation. Similarly, our study suggested that the vascular protective effect of lower LA and higher AA in plasma. Therefore, there might have a balance between pro- and anti-inflammatory metabolites in n-6 PUFAs that determine the persistence of inflammation and ultimately affect BP.
4.2. N-3 PUFAs and BP

Dissimilar to n-6 PUFAs, n-3 PUFAs mainly consist of fish oils, while their contents are low in natural fish oils, leading to the deficiency of n-3 PUFAs in the human body. Since their benefits for CVD were first described in the early 1970s, n-3 PUFAs have been widely studied. In the present study, each SD increase of ALA contributed to DBP increase by 3.83 mm Hg by gene-level analyses. Inconsistently, a meta-analysis revealed that flaxseed (mainly contained ALA) consumption could mildly reduce DBP. However, other 2 meta-analyses obtained from the AHRQ and Cochrane database pointed out that ALA did not affect BP. The discrepancy between the present MR study and previous studies might be explained by residual confounding. However, rs174547 was solely selected as instrumental variable (IV) of ALA, which limited sensitivity analyses by other MR methods, such as the MR-PRESSO, Weighted Median, and MR-Egger.

Inconsistent to the association of plasma ALA with DBP, the present MR study showed that plasma EPA level was associated with DBP reduction, as the similar conclusion from INTERMAP study. The result was also confirmed from a meta-analysis of 70 RCTs. Compared with placebo, EPA and DHA provision reduced SBP by 1.52 mm Hg and DBP by 0.99 mm Hg in the meta-analyses of all studies combined. Furthermore, EPA and DHA provision decreased SBP by 4.51 mm Hg and DBP by 3.05 mm Hg in untreated hypertensive subjects. The reductions of BP by EPA and DHA supplement (>2 g/day) were achieved in normotensive subjects as well. However, a lower dose (1–2 g/day) of EPA and DHA reduced SBP, independent of DBP. A updated review obtained from AHRQ database had supported that marine oil (0.3–6 g/day) did not affect BP, and similar finding was also revealed in another review of 15 studies (included >34,000 individuals) from Cochrane database. Consistently, our two-sample MR study provided with genetic evidence that plasma DHA or EPA level was not beneficial for BP. Mechanistically, the benefit of EPA on BP might be attributed to its cardiovascular protective properties. On the one hand, EPA could be metabolized by COX and LOX to produce eicosanoids, such as prostaglandins, thromboxane, leukotrienes, and resolvins, which had effects of anti-inflammation, vasodilatation, and anti-aggregation. N-3 PUFAs also were reported to reduce inflammatory biomarkers in patients with CVD, including interleukin-6 (IL-6), C-reactive protein (CRP), and tumor necrosis factor-α (TNF-α). Otherwise, EPA and DHA improved endothelial function and elasticity of large or small arteries. Taken together, EPA could improve vascular function and help control DBP in these ways.

4.3. Desaturases and BP

This study revealed that plasma AA and EPA decreased DBP, while their precursors, LA and ALA increased DBP, which supported that longer-chain PUFAs had vascular protective effects. Furthermore, most associations between PUFAs and BP were driven by SNPs within or near the fatty acid desaturase (FADS) and elongase of very-long chain fatty acids (ELOVL) gene, which encoded desaturase and elongase, respectively. In the human body, Δ5-desaturase and Δ6-desaturase are the key enzymes for PUFAs metabolism, while the latter is rate-limiting enzyme and requires zinc, magnesium, and pyridoxine as cofactors. Previous researches have suggested that decreased activity of desaturase was related to risk of CVD, hypertension, and metabolic syndrome. The activity of desaturase can be influenced by many factors, such as genetic variations, fatty acids, cholesterol, hormones, glucose, calorie intake, and so on.
Since n-6 and n-3 PUFAs compete for the same desaturase and elongase, bioconversion of LA and ALA to their corresponding longer-chain PUFAs is influenced by the ratio of n-6 and n-3 PUFAs. Upr grelation of desaturase activity by regulating these factors or exploring small molecules might be beneficial for BP control.

4.4. Strengths and limitations

The strengths and limitations of this MR study merits consideration. This MR analysis elucidated the relationship between PUFAs and BP without the interference of reverse causality and confounding factors. Only the SNPs in the functional genes strongly associated with PUFAs were selected, which could reduce the bias from weak IVs. Besides, data from large-scale meta-analysis of GWASs were used to perform MR and provided high statistical power. To avoid the influence of population stratification, only data from European populations were selected, which limited the ability to generalize our findings to other populations. More than 50% of the GWAS cohorts for PUFAs were contained in the GWAS for BP, while the overlapped samples were less than 0.1% of BP samples. Therefore, correlations of the genetic variants with unmeasured confounders in PUFAs were unlikely replicated in BP. However, only 3 or fewer independent SNPs were selected, limiting the conduct of sensitivity analyses, such as MR-PRESSO, Weighted Median, and MR-Egger method. In addition, this study failed to use the freely available data to assess whether the effects of PUFAs on BP vary by sex or age. Rs174547 in FADS1 and rs780094 in glucokinase regulatory protein (GCKR) gene are associated with heart rate and dyslipidemia, which might lead to pleiotropy. A major shortcoming of this study is that all PUFA-related SNPs were within or near the FADS gene and presented difficulties in evaluating the specific effects of each PUFA on BP.

5. Conclusion

In conclusion, this study provided genetic evidence that PUFAs had a divergent effect on DP independently of SBP. The findings suggested that supplementing AA and EPA or promoting LA and ALA convert to AA and EPA could reduce DBP. Future well-designed RCT studies need to be conducted to clarify the role of individual PUFA on BP.

Author contributions

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