Causal Inference of Carnitine on Blood Pressure and potential mediation by uric acid: A mendelian randomization analysis

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ARTICLE INFO

ABSTRACT

Background: Dietary change alters blood pressure (BP) but the specific causal dietary elements are unclear. Given previous observational data suggesting serum carnitine or uric acid affect BP, we investigated the role of serum carnitine and serum uric acid concentrations on BP, and considered mediation by lipids and insulin resistance using two-sample bi-directional Mendelian randomization (MR) analysis.

Methods: We performed MR to characterize bi-directional causal relationships of carnitine or uric acid on cardiometabolic traits. We performed two-sample MR using genome-wide association summary data from separate large-scale genomic analyses of carnitine, uric acid, BP, lipids, and glycemic traits. We used inverse variance weighted (IVW) meta-analysis and MR Egger regression to test for causal relations in the absence and presence of pleiotropy, respectively, and performed sensitivity analyses to identify confounders and intermediates.

Results: In our analysis, carnitine was directly, causally associated with systolic BP (IVW effect = 0.2, causal p-value = 0.03) but not diastolic BP (IVW causal p = 0.1). Our findings additionally support direct and indirect relationships of carnitine on TG and on uric acid. No causal associations of carnitine were found with glycemic traits. Uric acid was not associated with BP, nor TG.

Conclusion: Two-sample bi-directional MR demonstrated an unconfounded causal effect of carnitine, but not uric acid, on systolic but not diastolic BP, suggesting a role of carnitine in arterial stiffness. Carnitine, but not uric acid, also has direct and indirect effects on TG but are independent of the causal effect of carnitine on systolic BP.

1. Introduction

Dietary interventions such as in the Optimal Macronutrient Intake Trial for Heart Health trial (OMNIHeart) and Dietary Approaches to Stop Hypertension (DASH) have demonstrated clinically-meaningful reductions in blood pressure (BP) [1,2]. However, the specific causal components of diet plans that modify BP are not clear. For example, sodium is arguably the most widely agreed dietary component identified to affect BP from observational data, yet its relationship to cardiovascular health remains controversial [3,4]. Two other dietary components implicated in BP include carnitine and uric acid, which share genetic determinants.

In secondary analysis of the OMNIHeart trial carnitine was identified among circulating metabolites as associated with longitudinal changes in systolic BP, but not diastolic BP [5]. Carnitine is an amino acid derivative that is the key regulator of fatty acid oxidation and is ingested from food or endogenously produced [6]. Carnitine then affects at least

two metabolic domains that are implicated in vascular properties and BP: insulin resistance and dyslipidemia [7–16]. Carnitine dysregulation may cause insulin resistance, although reverse causation is also argued [7,12,17]. Insulin resistance is strongly associated with high triglyceride (TG), low high density lipoprotein cholesterol (HDL) atherogenic dyslipidemia [18,19]. Carnitine effects on BP and mechanisms thereof are unclear.

Uric acid is derived from endogenously produced purine metabolism as well as dietary sources [20]. While some observational data suggests uric acid is associated with blood pressure, others suggest the contrary or only a very modest effect [16,20,21]. Randomized controlled trials in obese youth showed uric acid modification reduced BP but in adults results are not consistent [15,16,21,22]. Thus, the effect of uric acid on BP is controversial.

Given complex interrelationships between cardiometabolic traits and dietary components, whether the putative effect of carnitine or uric acid on BP is causal, direct, or mediated through other mechanisms is

unknown, making investigatory techniques that identify unconfounded associations required [13,16,23]. While randomized interventional trials identify unconfounded relations, carnitine supplementation to unnatural levels would not directly answer the role of endogenous carnitine or uric acid. Instead, Mendelian randomization (MR) analysis leverages allelic assortment during meiosis to randomly assign persons to comparison groups prior to the development of traits of interest and thereby eliminate confounding and reverse causation [24]. To characterize unbiased causal associations we performed two-sample MR analyses of metabolomics, BP, TG, insulin response, and glycemic traits, using bi-directional testing. No previous genetic association studies exist on these relations. We identified genetic polymorphisms from large-scale genomic association studies to serve as instrumental variables for each exposure in this natural experiment, with careful attention to possible pleiotropic associations across multiple traits, including pleiotropic effects between carnitine and uric acid.

2. Methods

2.1. Genetic association summary data

This study was performed using publicly available data. We used genetic instrumental variables to test hypothesis-driven bi-directional causal relationships between serum carnitine and heritable metabolic traits (Fig. 1). To achieve optimal power for causal testing, we identified large population-based genome-wide association (GWAS) studies of each trait. We accessed genomic summary data through the MRBase platform, which curates publicly available genome-wide summary data through resources that include the NHGRI-EBI GWAS Catalog, consortium-based meta-analyses, and the UK Biobank. From MRBase, we identified the largest GWAS for each trait (as the exposure our outcome) among individuals of predominantly European ancestry. We additionally used PubMed to verify that no larger GWAS of predominantly European ancestry for these traits existed outside of the MRBase-available data at the time of analysis.

Serum carnitine and carnitine isoforms were assessed for genome-wide association among 7797 individuals of European ancestry in the TwinsUK and KORA cohorts [25]. We identified GWAS for serum uric acid concentration in over 140,000 individuals of European ancestry in the Global Urate Genetics Consortium [14]. Systolic (UKB-a:360) and diastolic BP (UKB-a:359), as well as self-report for high BP (UKB-a:437) and hypertension (UKB-a:61), were assessed for genome-wide association among up to 317,754 individuals of European descent in the UK Biobank [26]. The Global Lipids Genetics Consortium (GLGC) performed GWAS for lipid traits including normalized TG concentration, total

cholesterol, high density lipoprotein (HDL) cholesterol, and low density lipoprotein (LDL) cholesterol among up to 187,365 individuals [27]. The Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC), performed GWAS among 5318 individuals for corrected insulin response, 10,701 individuals for fasting proinsulin concentration, and 46,186 individuals for fasting glucose concentration [28,29]. Type 2 diabetes was assessed for association with genome-wide variants among 69,033 individuals of European ancestry in the DIAbetes Genetics Replication and Meta-analysis (DIAGRAM) consortium [30].

2.2. Selection of genetic instruments

We used the GWAS summary data for each trait to identify single nucleotide polymorphisms significant at the genome-wide level (P < 5e-8). We then pruned the genetic instruments to be independent using a linkage disequilibrium threshold of $r^2 < 0.001$ over a 10 Mb clumping distance. A final set of independent instrumental variables (IVs) for carnitine or a metabolic trait as the exposure of interest was then used to test its causal relationship with a phenotype.

2.3. Causal testing

MR was performed using the MRBase package [31]. We attempted to identify proxies for genetic instruments missing from the outcome summary data using a minimum linkage disequilibrium threshold of $r^2 > 0.8$. We additionally performed allele harmonization to align strands for palindromic SNPs and used a minor allele frequency threshold of <0.3. For each bi-directional relationship, we performed inverse variance weighted (IVW) meta-analysis and MR Egger regression to test a causal relationship in the absence and presence of pleiotropy, respectively. We used the MR Egger regression intercept to test for horizontal pleiotropy, where the genetic instruments for the exposure are related to the outcome either directly or through a mediator. When pleiotropy was present among the instrumental variables (intercept P-value <0.05), we relied on the MR Egger test for causation. In the absence of pleiotropy, we used the IVW approach to assess causation. All tests for causation used P < 0.05 to determine a causal relationship using the IVW approach or MR Egger test.

2.4. Weak instrument bias and sensitivity analyses

MR can be sensitive to the amount of variation in a trait that is explained by genetic instruments thereby biasing the causal effect estimate obtained through MR toward an observed confounded association [32]. Therefore, we additionally used MRBase to estimate the percent
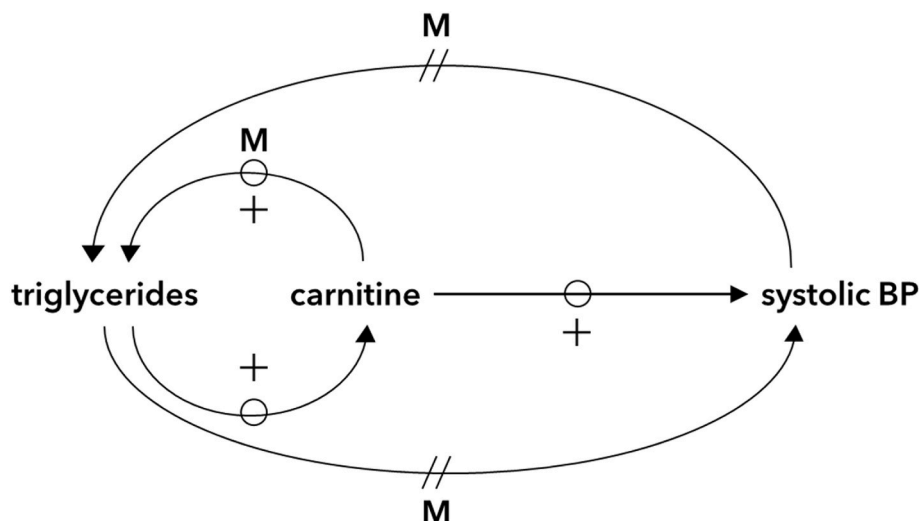
Fig. 1. Summary of causal relationships of carnitine with metabolic traits identified through Mendelian randomization. Direction of arrows represents significant causal directions identified through bi-directional Mendelian randomization. Pathways noted M demonstrate pleiotropic genetic effects and may be mediated by an unmeasured variable. Pathways with a circle demonstrate direct causal effects indicated by the arrow, whereas pathways with two hash marks are blocked when the causal intermediate is accounted in MR Egger regression.

variance of the exposure and outcome explained by each set of instrumental variables using the GWAS summary data for each trait. In order to avoid weak instrument bias, we did not consider results from causal tests when the genetic instruments explained less than 1% of the variance in an exposure or when the genetic instruments for an exposure explained more variation in an outcome than the exposure. We additionally performed leave-one-out sensitivity analyses to determine individual genetic instruments that may be driving observed causal relationships [24]. Similarly, for significant causal relationships identified using the IVW approach, we repeated the causal test iteratively excluding each genetic instrument. Causal tests determined to be influenced by individual variants had to show both a different magnitude of causal effect and loss of statistical significance when that variant was removed as a genetic instrument.

2.5. Intermediates in the causal pathway

Variants identified in sensitivity analyses may result from pleiotropic genetic effects that either capture intermediates in the causal pathway or represent the true exposure in the causal relationship. To assess these potential intermediate phenotypic traits, we first annotated variants flagged through "leave-one-out" sensitivity analyses for genome-wide associated traits in the GWAS Catalog. To determine the relationship of potential intermediates with carnitine or uric acid, we then assessed a bi-directional causal association of that intermediate with carnitine or uric acid. We further assessed potential causal relationships of the intermediate traits on outcomes that were used in the sensitivity analyses in order to identify direct causal effects of the potential intermediate. Taken together, this allowed us to determine whether potential intermediate traits were on the causal pathway between carnitine or uric acid and metabolic phenotypes.

3. Results

We identified 17 independent genetic variants to serve as instrumental variables in the assessment of serum carnitine levels (Table 1). The 17 carnitine genetic instruments explained 12.0% of the variance in serum carnitine levels. To comprehensively perform bi-directional MR between carnitine and metabolic traits, we additionally identified 3 to 130 independent genetic variants to serve as instrumental variables for each metabolic trait (Table 2). The genetic instruments for metabolic traits explained 1.3–6.2% of the variation in their respective metabolic traits (Table 2). To assess mediation by urate, there were 24 independent genetic instruments that explain 2.9% of the variation (Table 3).

Table 1
Independent genetic instruments for serum carnitine levels.

Carnitine			
SNP	Gene	Chr	Pos
rs1466788	Intergenic	1	110,618,730
rs735315	*LOC730100*	2	51,331,157
rs2279014	*SLC11A1*	2	219,261,176
rs9842133	*PEX5L*	3	179,664,102
rs4860022	*SPOCK3*	4	167,693,875
rs13182512	*JMY*	5	78,573,790
rs419291	*SLC22A4*	5	131,633,355
rs6862024	*TNIP1*	5	150,428,871
rs2396004	intergenic	6	43,355,851
rs12356193	*SLC16A9*	10	61,413,353
rs10821585	intergenic	10	61,516,587
rs11183620	*SLC38A4*	12	47,212,370
rs11620955	*CHURC1-FNTB/FNTB/MAX*	14	65,491,255
rs11620973	intergenic	14	96,018,052
rs2114713	*LINC01314*	15	80,528,373
rs3736438	*EFCAB13*	17	45,486,651
rs12709393	intergenic	17	55,292,115

Variants from Shin and colleagues [25].

We used genetic instruments to characterize the relationship of carnitine with measures of BP and metabolic traits using bi-directional MR (Table 2). When BP traits served as an exposure in MR testing, we identified weak instrument bias for each trait. The genetic instruments for diastolic BP explained 2.2% of the variation in both diastolic BP and carnitine, whereas the genetic instruments for systolic BP, high BP, and hypertension (1.8%, 1.9%, and 1.7%, respectively) all explained more variation in carnitine (2.0%, 2.4%, and 2.4%, respectively) than the BP trait. The genetic instruments for the other metabolic traits all explained at least twice the variation in the metabolic trait (1.3–6.2%) than carnitine (0.0–2.6%). We therefore did not further assess causal effects of BP traits on serum carnitine levels due to weak instrument bias and performed causal testing for metabolic traits on carnitine. The high heritability of carnitine (12.0%) enabled causal testing of carnitine on each of the BP and metabolic traits.

We identified carnitine as having evidence of a direct, causal effect on systolic BP (IVW causal p-value = 0.03), high BP (IVW causal p-value = 0.048), and hypertension (IVW causal p-value = 0.046). We further did not identify evidence of horizontal pleiotropy in the influence of any BP trait on carnitine (all intercept p-values <0.7).

Type 2 diabetes, insulin, proinsulin, and glucose levels did not demonstrate evidence of direct causal influences on serum carnitine levels (all IVW causal p-values >0.4), nor any evidence of horizontal pleiotropy (all intercept p-values >0.3; Table 2). Similarly, we observed no significant direct causal effects of serum carnitine levels on diabetes traits (all IVW causal p-values >0.05; all intercept p-values >0.4).

HDL cholesterol was observed to have a pleiotropic influence on serum carnitine levels (intercept p-value = 0.01; Table 2). The observed association of HDL cholesterol on carnitine levels (IVW effect = −0.011, p-value = 0.004) was entirely explained by an unobserved intermediate (MR Egger effect = 0.004, causal p-value = 0.6). We further identified pleiotropic effects captured by the carnitine IVs on HDL cholesterol levels (intercept p-value = 0.02). Serum TG levels had evidence of a direct, causal effect on serum carnitine levels (IVW effect = 0.011, causal p-value = 0.004) without evidence of horizontal pleiotropy (intercept p-value = 0.8). Additionally, we identified a direct causal effect of serum carnitine levels on TG (IVW effect = 0.27, p-value = 0.071) that is enhanced by an unmeasured intermediate (MR Egger effect = 0.70, causal p-value = 0.007, intercept p-value = 0.03). "Leave-one-out" sensitivity analyses identified the carnitine instrument *SLC16A9* rs12356193 as having a substantial influence on the causal test of carnitine on TG levels (Supplement).

We assessed uric acid levels for a mediating role in the relationship of BP, lipids, and carnitine. (Table 3). We were unable to assess a causal effect of urate on carnitine levels due to weak instrument bias (urate IVs explain 4.3% of the variation in carnitine). We identified evidence of an observed association of carnitine on urate levels (IVW effect = 1.6, p-value = 0.0004) that is a direct and mediated causal effect (MR Egger effect = 3.0, causal p-value = 0.002, intercept p-value = 0.047). We did not identify significant causal effects of urate on triglycerides (IVW causal p-value = 0.07) or systolic BP (MR Egger causal p-value = 0.1).

4. Discussion

In this two-sample bi-directional MR analysis using large-scale genome-wide summary data, we provide evidence that serum carnitine levels are causally associated with systolic BP. The lack of evidence for pleiotropic genetic effects and significant test for causation between systolic BP and carnitine suggest the effect of carnitine on systolic BP is both causal and direct. We additionally provide evidence of direct and indirect causal effects between carnitine and TG. Uric acid did not explain the estimated causal effect of carnitine on systolic BP nor have a direct effect on BP. Therefore, the role of carnitine on BP is not mediated through dyslipidemia nor uric acid on vasculature function (Figure).

Subanalysis of the OMNIHeart trial has shown that carnitine is one of multiple urinary metabolites associated with dietary-induced changes in

Table 2
Bi-directional causal testing for serum carnitine levels and metabolic traits.

Trait	Method	Trait → Carnitine								Carnitine → Trait				
		n IVs	PVE trait	PVE carnitine	Weak IVs	b	se	Causal p-value	Intercept p-value	n IVs	b	se	Causal p-value	Intercept p-value
Diastolic BP	MR Egger	130	0.022	0.022	Yes					17	0.22	0.19	0.266	0.711
	IVW	130								17	0.16	0.10	0.107	
Systolic BP	MR Egger	116	0.018	0.020	Yes					17	0.29	0.21	0.185	0.745
	IVW	116								17	0.23	0.11	0.032	
Hypertension	MR Egger	108	0.017	0.024	Yes					17	0.12	0.13	0.383	0.875
	IVW	108								17	0.14	0.07	0.046	
Type 2 diabetes	MR Egger	10	0.013	0.003	No	0.018	0.021	0.419	0.479	16	1.37	1.43	0.356	0.889
	IVW	10				0.003	0.004	0.470		16	1.19	0.71	0.091	
Insulin response	MR Egger	3	0.024	0.000	No	0.047	0.035	0.403	0.399	17	0.95	1.93	0.630	0.539
	IVW	3				0.000	0.006	0.988		17	−0.11	0.93	0.906	
Proinsulin	MR Egger	8	0.062	0.001	No	0.004	0.017	0.797	0.955	17	−0.31	0.44	0.486	0.783
	IVW	8				0.004	0.007	0.604		17	−0.41	0.23	0.072	
Glucose	MR Egger	14	0.028	0.003	No	0.007	0.027	0.809	0.539	17	−0.15	0.25	0.556	0.457
	IVW	14				−0.009	0.011	0.448		17	0.01	0.13	0.914	
HDL-C	MR Egger	87	0.044	0.020	No	0.004	0.007	0.593	0.010	16	−0.42	0.23	0.089	0.023
	IVW	87				−0.011	0.004	0.004		16	0.05	0.16	0.757	
Triglycerides	MR Egger	54	0.046	0.012	No	0.013	0.007	0.058	0.793	16	0.70	0.22	0.007	0.029
	IVW	54				0.011	0.004	0.004		16	0.27	0.15	0.071	

BP, blood pressure; HDL-C, high density lipoprotein cholesterol; IVs, instrumental variables; IVW, inverse variance weighted meta-analysis; LDL, low density lipoprotein; MR, Mendelian randomization; PVE, percent variance explained; se, standard error.

Table 3
Serum urate levels as a potential intermediate in the carnitine causal pathway.

Test	Method	n IVs	PVE exposure	PVE outcome	Weak IVs	b	se	Causal p-value	Intercept p-value
Urate– > Carnitine	MR Egger	24	0.029	0.043	Yes				
	IVW	24							
Carnitine– > Urate	MR Egger	16	0.120	0.001	No	2.99	0.77	0.0016	0.047
	IVW	16				1.56	0.44	0.0004	
Urate– > Triglycerides	MR Egger	24	0.029	0.009	No	−0.03	0.13	0.83	0.096
	IVW	24				0.15	0.08	0.07	
Urate– > Systolic BP	MR Egger	24	0.029	0.001	No	−0.03	0.02	0.11	0.001
	IVW	24				0.03	0.02	0.09	

BP, blood pressure; IVs, instrumental variables; IVW, inverse variance weighted meta-analysis; MR, Mendelian randomization; PVE, percent variance explained; se, standard error.

systolic BP but not diastolic BP [5]. In both aged adults and coronary artery disease patients acylcarnitines, carnitine derivatives, are associated with large artery stiffness [33,34]. Interventional studies show contrasting data on exogenous carnitine supplementation on vascular parameters but there is some evidence that open-label carnitine supplementation can lower systolic BP with no effect on diastolic BP [11,35,36]. Here, we add a naturally-randomized experiment to suggest an unbiased, direct association between carnitine and BP, and more precisely perhaps pulse pressure. Systolic BP is mathematically related to diastolic BP, and their difference is referred to as pulse pressure. Physiologically, pulse pressure reflects large artery function and aortic stiffness [37,38], and is a measure of arterial flow, arterial size, and arterial wall stiffness [39–41]. Taken together, these findings suggest that carnitine may have an effect on pulse pressure, and thus, aortic function.

In pursuing the root of possible mediators of carnitine to BP relations, our analysis showed pleiotropic association between serum carnitine and TG, but the association between carnitine and BP appeared to be direct and unconfounded, and thus independent of the influence of carnitine on TG. Previous publications showed baseline TG and baseline

glucose were associated with change in PP in middle aged adults over follow-up while lipids, obesity, and glucose were also associated with other measures of aortic stiffness [23]. Other cross sectional and longitudinal studies show both high TG and insulin resistance are known risk factor for arterial stiffness in adolescents and adults [23,42]. The present instrumental variable study design controls for the confounding effects of the complex interrelationships of cardiometabolic risk factors to suggest there is a causal relation between carnitine on TG in a pleiotropic fashion. TG elevation is generally attributable to either increased TG production due free fatty acids availability or decreased TG metabolism due to insulin resistance-mediated inactivation of catabolic enzymes [43]. In our analysis, carnitine associated genetic variants were not associated with glucose, insulin concentration, or insulin response. Carnitine associations with TG may thus be inferred to be mediated through another mechanism, perhaps free fatty acids. Supporting this contention is the known association between carnitine genetic variants and uric acid. This observation is supported by previous publications demonstrating the effect of carnitine on uric acid levels both by other genetic and interventional studies [14,44]. Uric acid and carnitine may be linked due to similar dietary sources and linked metabolic pathways

[45,46].

Uric acid is endogenously produced and converted from dietary sources such as red meat, wild game, organ meats and certain seafoods [20]. Observational and interventional studies are conflicting on the role of uric acid on BP [15,16,21,22]. Our analyses do not show an association of endogenous regulation of uric acid on BP or lipids. These results are consistent with recent Mendelian randomization analyses showing no or weak associations on CVD [47].

One limitation of our approach is the transformation of traits in large-scale genome-wide association studies. Such transformed traits do not allow for interpretable causal effect estimates. However, we are able to infer direction of causal relationship and magnitude of effect changes when examining adjustment for pleiotropy. This study leverages the natural assortment of genetic alleles prior to phenotype development, which allows for causal inference while minimizing confounding and bias and employs two independent cohort studies for each causal test. We used the type of meta-analysis appropriate for pleiotropy when present and performed sensitivity analyses to identify confounders or mediators that may be driving estimated causal effects. The present results cannot be generalized to exogenous carnitine or uric acid supplementation. The 17 genetic instrumental variables for carnitine explained 12% of the interindividual variation in carnitine but the MR association was more modest, perhaps suggesting the pathway is relevant for further investigation among many others relevant for blood pressure regulation.

In conclusion, this two-sample bi-directional MR analysis demonstrated an unconfounded causal effect of carnitine on systolic but not diastolic BP, which may suggest a role of carnitine in arterial stiffness. Carnitine also has direct and indirect effects on TG and uric acid, but these relationships are independent of the effect of carnitine on systolic BP. Uric acid had no association with BP or lipid traits, casting doubt on the utility of its modification on BP.

Subject codes

Risk factors, mechanisms, epidemiology.

Funding sources

Disclosures

We declare we have no financial disclosures nor conflicts of interest. No funding sources or other stakeholders had any impact on design, completion or decision to publish this manuscript.

Credit author statement

Melissa Richard: Conceptualization, Methodology, Formal analysis, Writing – original draft, Writing – review & editing; Philip Lupo: Methodology, Writing – review & editing, Supervision; Justin Zachariah: Conceptualization, Writing – original draft, Writing – review & editing, Supervision, Funding acquisition.

Acknowledgements

None.

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