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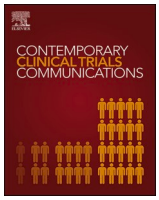

Effect of exogenous L-carnitine on aortic stiffness in dyslipidemic adolescents: Design of a quadruple-blind, randomized, controlled interventional trial

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ABSTRACT

Background: Atherosclerotic cardiovascular disease (ASCVD) risk factors including vascular remodeling leading to hypertension and dyslipidemia are prevalent among children and adolescents. Conflicting observational and Mendelian randomization data suggest endogenous carnitine may affect arterial stiffness and lipid traits. Because of this, we developed a study to evaluate the causal role for carnitine in arterial stiffness at a point when the lifecourse trajectory to hypertension can be modified.

Methods: This study is a mechanistic, double-blinded, randomized control trial (RCT) in 166 adolescents with dyslipidemia for the effect of 6 months of maximum dose 3 g daily oral L-carnitine supplementation (CS+) versus placebo (CS-) on aortic stiffness measured as carotid-femoral pulse wave velocity (CFPWV) and pulse pressure (PP); lipid concentrations (total cholesterol, HDL-C, triglycerides, and LDL-C) and serum fatty acid oxidation biomarkers by metabolomic analysis.

Conclusions: The simultaneous evaluation of endogenous carnitine genetic effects and exogenous L-carnitine supplementation may facilitate future therapies for youth with cardiometabolic derangement to arrest atherosclerotic changes.

1. Background

Atherosclerotic cardiovascular disease (ASCVD) risk factors have become highly prevalent among children, including 1 in 5 youth having abnormal cholesterol [1–3]. Childhood ASCVD risk factors track into adulthood [4], and predict CVD events and mortality, [5–8]. Therefore, targeting interventions for children may reduce ASCVD risk factors that accumulate over time [9].

One biophysical precursor to ASCVD is aortic stiffening, often measured as carotid-femoral pulse wave velocity (CFPWV) or pulse pressure (PP). Arterial stiffness predicts future CVD events even after adjustment for classic CVD risk factors [5,10,11].CFPWV is shown in longitudinal adult studies to predict future hypertension [12,13]. In youth, arterial stiffness is present in over 90% with elevated blood pressure(BP) [14,15].

Our previous study work suggests changes in CVD risk factors like obesity, serum glucose and serum triglycerides (TG) precede worsening arterial stiffness [16]. Obesity, dysglycemia and high TG are all manifestations of the high risk state called metabolic syndrome [17–19]. We showed insulin resistance is the key marker of CVD risk from metabolic syndrome [20–22]. Carnitine dysregulation may cause insulin resistance, although reverse causation has also been proposed [23–25]. Carnitine is a protein either consumed in the diet or synthesized [26–29]. The primary function of carnitine is to shuttle long chain fatty acids into the mitochondria [23,28–31]. With respect to carnitine and arterial stiffness, we demonstrated in a two-sample Mendelian randomization analysis in adults that genetic variants associated with circulating carnitine were associated in unconfounded fashion with categorical hypertension, continuous systolic BP but not diastolic BP, suggesting a PP effect as an index of arterial stiffness [32]. Pleiotropic

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associations with triglycerides were also noted. Given our previous data showing cholesterol is associated with aortic stiffness in adults and adolescents, we infer carnitine may have direct and indirect effects on aortic stiffness. This study is formulated to disambiguate previous observational findings and directly interrogate these hypotheses.

This study was designed as a mechanistic, quadruple blinded, placebo controlled RCT in adolescents with high serum TGs at risk of aortic stiffening. We will evaluate the effect of 6 months of maximum dose daily oral L-carnitine supplementation (CS+) versus matched placebo (CS-) on aortic stiffness measured as CFPWV and PP; conventionally measured lipid concentrations (total cholesterol, HDL-C, triglycerides, and LDL-C); and serum fatty acid oxidation biomarkers by metabolomic analysis. We hypothesize CS + will be associated with lower aortic stiffening, measured as a smaller increase in CFPWV and PP without effect modification by sex or race/ethnicity; altered fatty acid beta oxidation, measured as lower circulating long chain acylcarnitines; improved insulin resistance as homeostatic model assessment of insulin resistance (HOMA-IR); and decreasing TG levels. We will additionally characterize the role of endogenous carnitine genetic variants on aortic stiffness at baseline and on the effectiveness of CS+. We hypothesize by nature randomly assorted carnitine-associated genetic variants will be associated with higher aortic stiffness and that a carnitine genetic risk score will predict the change in arterial stiffness and/or modify the effect of CS + on change in arterial stiffness. Understanding the role of CS+ in arterial stiffness or other outcomes may facilitate targeting of future therapies on susceptible youth before irreversible atherosclerotic changes occur. Data reporting will adhere to the CONSORT guidelines for randomized trials, and the study is registered into ClinicalTrials.gov NCT04128969.

2. Material and methods

2.1. Participants

Participants are adolescents 11–21 years old (n = 166, 83 in each group) recruited from patients referred to Preventive Cardiology within the Texas Children's Hospital system. Preventive Cardiology treats youth according to NHLBI/American Academy of Pediatrics guidelines [33] after being identified by primary care physicians to have abnormal lipids or blood pressure. Inclusion criteria are: 1) 11–21 years of age, 2) TG levels 130–500 mg/dL, and 3) low density lipoprotein cholesterol (LDL-C) < 160 mg/dL. Lipid criteria are designed to exclude participants with lipid disorders [16,32,33]. Exclusion criteria are: 1) seizure disorder, 2) renal failure, 3) metabolic disorders, 4) type 1 or 2 diabetes mellitus, 5) congenital heart disease requiring surgical or catheterization intervention, 6) current pregnancy, and 7) incarceration/institutionalized/wards of the state. These exclusions represent ethical constraints and/or confounding conditions that can influence outcomes [34,35].

2.2. Intervention

Participants randomized to CS + arm will receive levocarnitine 1 g/10 mL oral liquid formulation, administered as 15 mL in the morning and 15 mL in the evening to achieve a steady state. Pharmacodynamic studies show 3 g/day of L-carnitine supplementation increase serum carnitine by 60% from baseline [28,29,36,37]. Participants randomized to CS- will receive a placebo similar in appearance, smell, and taste to CS+. The hospital Investigational Pharmacy Service(IPS) will be responsible for intervention randomization and standard dispensing procedures, including dispensing, inventory, and documentation.

2.3. Intervention safety and monitoring

The study will take a comprehensive view of possible adverse events during Run-In, Active, and Follow-up Phases. There are no previous reported sequelae from L-carnitine overdose in general or dyslipidemic populations [38,39]. Limited evidence suggests L-carnitine may provoke seizure in epileptics [40]. For this reason, we have excluded known seizure disorders. We will monitor hypersensitivity reactions, gastrointestinal complaints, breathing changes, rash, oral symptoms, changes in body odor, or other manifestations.

The Data Safety Monitoring Board will meet every 6 months up to the last participant visits, with one meeting 3 months later to adjudicate late events. DSMB will be comprised of 3 persons versed in pediatric medicine, cardiovascular disease, and metabolic disorders to adjudicate serious adverse events (life-threatening hypersensitivity, seizure, and/or physician diagnosed renal failure) likely related to study intervention and subsequent study discontinuation. The very wide safety margin of L-carnitine and exclusion criteria obviates emergency unblinding. The DSMB will perform an interim analysis when 50% of the total participants have completed a Final Visit. Utilizing conservative alpha-spending approach to determine a CFPWV difference between CS+/CS- at p value of 0.006 will be reported to IRB to determine if the study should be stopped by community standards or other considerations [41,42].

2.4. Procedures

The study team will screen potential participants per the inclusion and exclusion criteria from scheduled patients in Preventive Cardiology clinics [Fig. 1]. Potential participants will be approached to discuss the study along with potential risks and benefits. Interested candidates will have a Run-In Phase visit scheduled for 2 months later, corresponding to roughly 1 month before next scheduled clinic visit, allowing time for non-coercive due consideration. At the Run-In Visit, study background, inclusions/exclusions, and risks/benefits will be reviewed and consent will occur. Only consented young adult participants and minor participants with both parental consent and child assent will be enrolled in this study. Female participants will be urine tested for pregnancy exclusion criteria. During the one-month Run-In phase, consented participants will be instructed to take 15 mL of the placebo in the morning and 15 mL in the evening with or without food. Active Phase Initiate Visit will be scheduled one month later just before the next clinic visit.

2.5. Adherence/compliance procedures

During the Run-in phase, adherence will be randomly assessed at 12 ± 2 days and again at 22 ± 2 days. On the computer-randomly selected day, a HIPAA compliant messaging app or phone call will be used to verify the amount ingested and obtain photographic evidence of remaining CS- within 24 h. The expected remainder at each time point will be compared to the observed amount. Worsening non-adherence from >20% at the first check to >25% at second check will disqualify that individual from continuing the study prior to randomization.

2.6. Active Phase procedures

The Active Phase Initiate Visit will occur after a 10 h fast with reminders prior from study staff. Adherent participants succeeding in the Run-in phase will be randomly assigned to treatment arm and have study baseline assessments performed. The IPS will use computer-based permuted random block randomization to assign participants. The use of random block sizes will prevent treating providers from inferring the assignment. A three-month supply of the randomly assigned CS + or CS- will be dispensed.

2.7. Study measures

History taking will include age, sex, self-reported race, past medical diagnoses, inclusion/exclusion criteria, and medications. Anthropometric measures will include standardized measurement of height using

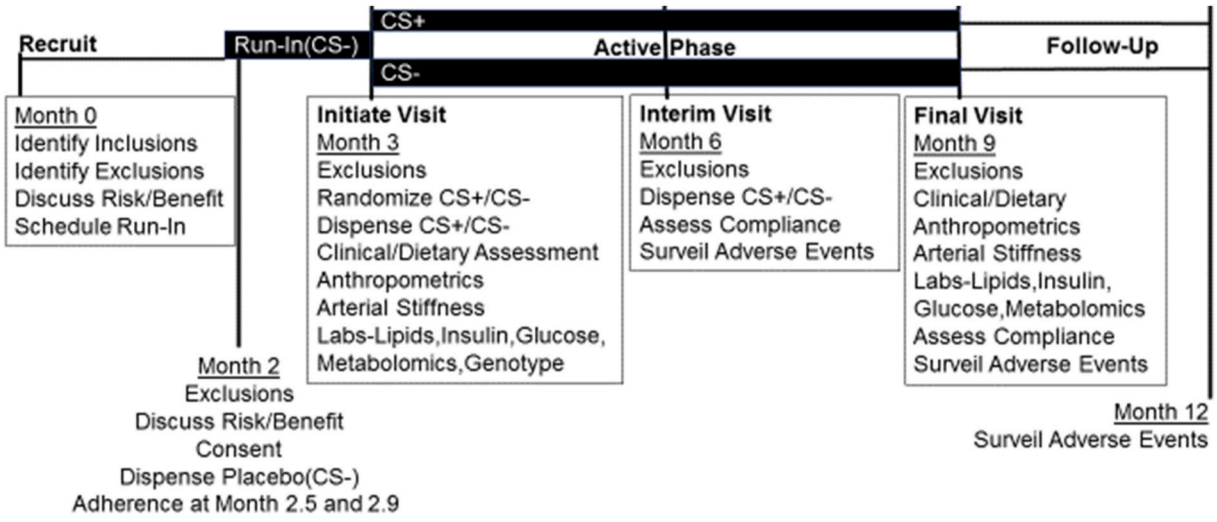

Fig. 1. Study Timeline Schematic with tasks listed under each timepoint.

stadiometer, weight using electronic scale, and waist circumference measured with non-stretching tape measure at the level of the iliac crest. Each measure will be performed in duplicate with average used.

Dietary assessment will occur through the National Cancer Institute's online Automated Self-Administered 24-Hour (ASA24) Dietary Assessment Tool. Study staff will be trained in non-interfering administration of ASA24 at the US Department of Agriculture Children's Nutrition Research Center (Houston, TX) who adapted the ASA24 to children. We specify the total carbohydrate intake as the most relevant measure for our dyslipidemic adolescents both due to the underlying inclusion criteria and the potential confounding effect on carnitine-associated change in insulin resistance. Additional measures of interest will be whole grain intake, refined carbohydrate intake, and vegetable total intake. We will also quantify dietary confounders that increase carnitine, including red meat intake quantified as composite of beef, veal, pork, lamb, game meat, cured meats, and organ meats [27,31].

Following a 5-min waiting period, seated brachial blood pressure will be measured twice to the nearest millimeters of mercury using automated sphygmomanometer. Applanation tonometry workstations will be used to measure hemodynamic variables of interest. A noninvasive micromanometer is placed on the skin overlying the right carotid and right femoral arteries to capture high fidelity, high frequency response, noninvasive pulse wave tracings. CFPWV is defined as the transit distance from carotid to femoral divided by the pulse transit time between those same sites. Transit distance will be calculated using a caliper held above the body as the difference in measured distance between suprasternal notch to femoral site minus the suprasternal notch to carotid site. Transit time is measured as the time from R wave on the ECG to the onset ("foot") of the pulse wave at femoral site minus the R wave to pulse foot at the carotid site. Commercial systems will be used with previous validation and cross checked between systems for comparability indicating a robust non-dependence on operators or system type [10,43]. Automated analysis of the carotid waveform allows for determination of central systolic and diastolic BP, the difference of which defines secondary outcome cPP [11,44].

2.8. Laboratory assessment

Venous blood draw will collect 15 mL into appropriate collection tubes for immediate processing, including centrifugation and aliquoting. Temporary storage will occur on dry ice for less than 4 h until full storage in −20 Celsius freezers. Ten percent of samples will be randomly selected and re-run for quality assurance. Samples will be immediately barcode labelled with freeze resistant labels. In all samples, freeze-thaw cycles will be avoided.

From this sampling, cholesterol, triglycerides, high density lipoprotein cholesterol (HDL-C), and glucosewill be analyzed using standard methods. Insulin will be measured using radio-immunoassay as total immunoreactive insulin. Insulin resistance will be defined as HOMA-IR calculated as the product of fasting serum glucose and serum insulin divided by 22.5 [45]. HOMA-IR will be classified as top quartile and also as continuous values [20].

The Baylor College of Medicine Metabolomics core (Houston,TX) will use standardized techniques to assess carnitine and acylcarnitine profiles as indices of fatty acid oxidation, along with secondary analyses of TCA cycle glycolysis, short and medium chain carnitines. Serum samples for metabolomics will be immediately placed in dry ice to keep the metabolite pattern stable [46]. designed to verify technical quality. Freeze-dried samples will be divided equally for either liquid chromatography mass spectrometry (LC/MS) or gas chromatography mass spectrometry (GC/MS) using standard techniques. Blood samples will be processed and stored for secondary genetic studies.

2.9. Interim assessments and Active Phase Interim Visit

Active Phase Interim Visit will be scheduled with timing similar to Initiate visit. After leaving the Initiate Visit, the study RA will engage in "compliance" assessments on the 28 ± 2 days and 56 ± 2 days after Initiate Visit similar to Run-In phase. Noncompliant participants will be encouraged to improve compliance but will NOT be removed from the study as the analysis will be intention-to-treat. Adverse events will also be assessed.

At the Active Phase Interim Visit adverse events and compliance will again be assessed. Another three-month supply of previous assignment will be dispensed. Transportation reimbursement will be given. The study team will schedule the Active Phase Final Visit to occur in 3 months timed similarly to Initiate Visit. Then compliance assessments and adverse events will again be assessed 28 ± 2 days and 56 ± 2 days after Interim Visit as per previous methods.

2.10. Active Phase Final Visit and follow-up visit

Active Phase Final Visit will also occur after a 10 h fast. Clinical history, anthropometrics, dietary assessment, fasting lab draw with analyses and hemodynamic assessments will be repeated (genotyping will not be part of this visit). Compliance and adverse event assessment will also be repeated. Participants completing all study tasks will receive a $100 participation reward and transportation reimbursement. A

Follow-up telecommunication only visit will occur 3 months after Final Visit to verify no lingering study related concerns or late adverse events.

2.11. Methods to enhance participant retention

Participant Retention in this study is enhanced by the timing of Research Visits to dovetail with the standard-of-care every three-month clinic visit schedule. RA reminders to attend research visits will prompt research activity and clinically required lipid lab draw at a convenient time for the family, thereby mutually enhancing research and clinical care [47]. Additional outreach from study staff will occur around holidays to troubleshoot atypical periods.

2.12. Methods to improve efficacy validity

All study staff, participants and families, and treating providers, will be blinded to intervention assignment. The placebo in CS- will be matched as closely as possible in appearance, smell and taste to CS + supplement thereby 'masking' treatment assignment. Since CS + effects on adolescents with dyslipidemia is unknown, treating providers will not be able to deduce the randomization by virtue of usual clinical care. To minimize bias, all data will be conscientiously recorded, ITT analysis will be used primarily, limits will be placed on variable database input fields to prevent spurious data entry, and analyzed for missing data. If missing data turns out to be consequential in sensitivity analyses then multiple methods will address the missing data including "imputation of last-observation," "last-rank-carried-forward," pattern-mixture models, or multiple imputation. We will also indirectly measure the effectiveness of the lifestyle modification with lab values, anthropometrics, and dietary assessment should randomization fail to evenly distribute confounders in this relatively small trial. We will consider sex as a biological variable by formally examining if effects of the intervention differ in females and will present sex-stratified outcomes as necessary.

2.13. Pandemic considerations

The severe acute respiratory syndrome coronavirus 2 (SARS COV2) pandemic is clinically serious and an obstacle to research. Modifications may include telephone or videoconferencing, urine pregnancy testing at local laboratories, and dispensation delivery or empty bottle return by mail or delivery service, thereby possibly rendering the Run-In Phase visit or Active Phase Interim Visit as virtual visits.

2.14. Analysis

Deidentified study data will be entered into secure, access restricted REDCap databases.

2.14.1.1. Aim 1: to compare CS change in arterial stiffness and monitor adverse events

H1a. CS+ is associated with lower arterial stiffening, measured as a smaller increase in CFPWV.

The primary analysis will use ANCOVA to compare CFPWV at Active Phase Final Visit (i.e., last measurement) between CS+ and CS-. Adjustment covariates include age, sex, height, heart rate and MAP, and CFPWV at Active Phase Initiate Visit (i.e., baseline measurement). The first 5 covariates are standard known confounders of CFPWV relations while the last is included to account for regression to the mean and covariance in the response variable [47,48]. To account for variability in clinical therapy effects, additional covariates will be added including alterations in dietary intake and change in weight status as a continuous or categorical normal weight versus above normal. As a secondary outcome we will use cPP in place of CFPWV.

H1b. The effect of CS + on arterial stiffness is not modified by sex or race/ethnicity.

We will investigate effect modification by sex using CS-by-sex interaction term in the H1a analysis structure, comparing self-identified Non-Hispanic White individuals versus all other race/ethnicities. These exploratory analyses may be underpowered, so attention will be paid to effect size to suggest a signal of effect modification should power preclude significance detection.

2.14.1.2. Aim 2: to compare the effect of CS on lipids, insulin resistance, and fatty acid metabolism

CS+ is associated with H2a) altered fatty acid beta oxidation, measured as lower long chain acylcarnitines; H2b) improved homeostatic assessment of HOMA-IR; H2c) decreasing TG levels.

H2a. The primary analysis will be ITT using ANCOVA with outcome being average osmolality-normalized concentration of long chain acylcarnitines at Final Visit. Estimates comparing CS + vs CS- will be adjusted for age, sex, and arithmetic mean of Initiate Visit long chain acylcarnitine levels to account for regression to the mean. In sensitivity analyses, body mass index percentile change and dietary changes will be added as covariates, with careful attention to avoid collinearity. In secondary analyses we will analyze alternate outcomes like free carnitine to acylcarnitine ratio change as an index of carnitine repletion and utilization; arithmetic mean fold change in short chain acylcarnitine (up to 7 carbons) or arithmetic mean fold change in medium chain acylcarnitines (8–12 carbons) to assess competitive changes on carnitine-independent energy metabolism; and similarly mean fold changes in TCA cycle intermediates as indirect changes in competitive energy substrate utilization and insulin resistance. In order to explore possible combinations of metabolomic alteration signaling unexpected pathways, principal components analysis will be used for group-wise classification. We will also use random forest analyses which create a set of classification trees based on continual sampling of the experimental units and compounds. Then each observation is classified based on the majority votes from all the classification trees.

H2b. The primary analysis will be ANCOVA with HOMA-IR at the Final Visit as outcome and using identical predictors and analogous covariates like Initiate Visit HOMA-IR as described H2a. In sensitivity analyses outcome will be substituted for glucose and for insulin to distinguish contrary effects in glycemia and insulin levels [49].

H2c. The primary analysis will be ANCOVA with TG at the Final Visit as outcome. Models will be adjusted analogously to H2a and H2b with adjustment for Initiate Visit TG.

2.14.1.3. Power

Sample Size was calculated using Quanto version 1.2.4 for the sample size needed to show a change in hemodynamic traits between CS + versus CS-, assuming equal sample sizes [42]. Previous studies suggest a 3 g supplement of L-carnitine will increase plasma carnitine by 60% [37,50]. We hypothesize a 60% increase in plasma carnitine could lead to a 5.0 mmHg change in PP, our more stringent hemodynamic outcome compared to PWV. To obtain 80% power we need 72 subjects enrolled in each treatment arm. Anticipating 15% attrition in our Active Phase participants after Run-In Phase adherence attrition, we anticipate starting with 83 in each group after randomization to end with 72.

3. Discussion

Novel approaches are needed to develop targeted interventions in youth at high risk of progressing to future ASCVD. Vascular remodeling is the consequence of changes in wall stiffness, thickness, luminal size and/or vasoactive response resulting from adaptive or maladaptive upstream stimuli, mediated by cellular and extracellular matrix changes [51]. Repeated ventricular ejection distends the artery and consequently increases stress on the vascular wall [52,53]. Wall stress induces structural changes including arterial stiffening, thickening, and dilation [54,

55]. In response to stress-strain, the major extracellular component of the aorta, elastin, is irreversibly disrupted [52,55]. With diminished elastin, the aorta can dilate, which further increases wall stress [56]. In response to wall stress, extracellular matrix remodeling leads to collagen deposition delimiting further expansion and reinforcing the cellular elements [51,55]. Directly supporting these biophysical mechanisms with respect to aging, CFPWV progressively increases over the lifecourse and the product of heart rate times PP was associated with higher PWV on 20 years follow-up, suggesting repeated cycles of stretch predispose to stiffer artery wall [13,44,53]. In obesity, blood volume is increased which increases flow [52]. Increased flow will distend blood vessels, placing the vessel high on the volume:pressure compliance curve [52]. Increased volume coupled with tissue changes is associated with higher aortic stiffness [15,47].

Insulin resistance drives arterial stiffening in obese adolescents [21]. Insulin resistance is a well-described consequence of and stimulant for excess body weight [17,19]. Insulin has trophic effects on vascular smooth muscle cells and induces fibrosis [22,51]. Our previous work suggested changes in obesity, serum glucose and serum TGs precede worsening CFPWV [16]. The Young Finns study showed lifecourse improvement in manifestations of insulin resistance syndrome mitigated arterial remodeling [57].

For adult arterial stiffness, acylcarnitines are associated with CFPWV [58]. CFPWV was cross sectionally associated with acylcarnitines in coronary artery patients but not controls [59]. The OMNIHeart dietary RCT found carnitine change was associated with systolic but not diastolic BP change, implying arterial stiffness change [60]. Open label L-carnitine supplementation also lowered PP, although contrary data exist [61,62]. L-carnitine supplementation RCTs in adult hemodialysis subpopulations showed reduced BP and CFPWV [63]. Therefore observational and trial data show relations between carnitine and arterial stiffness. Our published 2 sample Mendelian randomization analysis showed unconfounded causal effect of carnitine on systolic but not diastolic BP, suggesting a role on PP [32]. Carnitine also had direct and indirect effects on TG which were independent of the causal effect of carnitine on BP.

Carnitine is plausibly related to TG through insulin resistance. Serum carnitine does appear to be higher in men than women and acylcarnitine does appear to be lower in Non-Hispanic Blacks than Caucasians [64]. The primary function of carnitine is shuttling long chain fatty acid coenzyme A esters into the mitochondria [23,28,29,31]. Carnitine palmitoyl transferase 1(CPT1) transfers long chain acyl groups, but not short or medium chain, from CoenzymeA(CoA) to carnitine forming acylcarnitine which moves in to the mitochondria matrix [23,28,31]. There CPT2 transfers the long chain acyl back onto CoA and regenerates free carnitine [24]. The long chain acyl-CoA enters into fatty acid beta oxidation(FABo) [31]. FABo and glucose oxidation are under reciprocal control such that glucose oxidation inhibits CPT1 and thus FABo [23]. While CPT1 appears to be the rate limiting step of FABo, oral L-carnitine supplementation does increase FABo in general population [50].

Carnitine dysregulation may cause insulin resistance, although reverse causation is also argued [23–25]. A purported route from carnitine to insulin resistance starts with excessive fat intake producing excessive acyl-CoA and acyl-CoA sequestration in the cytosol [23,25,30]. Derivatives of excessive acyl-CoA like ceramides and gangliosides alter insulin receptor signaling [65,66]. To regenerate free CoA from sequestered acyl-CoA, some acyl groups are transferred to carnitine forming acylcarnitines which can be transported out into plasma as energy substrate for peripheral tissues or even eliminated from the body through bile and urine [66,67]. The long chain acylcarnitines can also incorporate into the cell membrane and directly interfere with insulin signaling [24,65]. Consistent with both pathways, plasma acylcarnitines are strong markers of insulin resistance [64,65]. The ability for physiologic carnitine to detoxify the cytosol of acyl-CoA derivatives can be overwhelmed [23,30]. Insulin resistance encourages free fatty acid production and inhibits lipoprotein lipase cleavage of TG leading to high serum TG [17]. Exogenous supraphysiologic L-carnitine supplementation in insulin resistant humans and analogous dosing in mice improved glucose homeostasis, perhaps by offering additional free carnitine to facilitate FABo or freeing CoA from acyl-CoA [23,25,30]. Success of L-carnitine supplementation on insulin resistance depends on patient subtype but dyslipidemic adolescents have never been tested [25]..

The complex interrelations between energy substrate and metabolites and the possibility of reverse or bidirectional causation highlights the need and utility of instrumental variable design *interventional* studies in relevant populations. Therefore, in adolescents with high TGs and thus at risk of accelerated arterial stiffening, this study aims to determine the causal effect of carnitine on arterial stiffness, lipids, insulin resistance, and metabolism through randomized, controlled, blinded intervention and through the effects of carnitine-driving genetic variants. By altering carnitine fatty acid metabolism will be altered, improving insulin resistance, leading to lower TGs, and improved arterial stiffness. Genetic variants that alter serum carnitine will be associated with arterial stiffness before any supplementation and modify the effect of supraphysiologic supplementation.

Subject codes

Risk factors, mechanisms, aortic stiffness.

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Declaration of competing interest

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.

Data availability

No data was used for the research described in the article.

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