

The effect of dietary nitrate on endothelial function and cardiovascular risk parameters in adults: a protocol for systematic review and meta-analysis

Daniel Forster¹, Gustavo Waclawovsky², Giuseppe Potrick Stefani³

¹ School of Health and Life Sciences, Pontifical Catholic University of Rio Grande do Sul (PUCRS), Porto Alegre, Rio Grande do Sul/Brazil.

² Instituto de Cardiologia do Rio Grande do Sul/Fundação Universitária de Cardiologia (ICFUC), Porto Alegre, Rio Grande do Sul/Brazil

³ Graduate Program in Nutrition Sciences, Federal University of Health Sciences of Porto Alegre (UFCSPA), Porto Alegre, Rio Grande do Sul, Brazil.

Open Access

NMJ

NUTRITION AND METABOLISM JOURNAL
Clinical and Experimental

Published: October 2, 2026



Copyright, 2026 by the authors. Published by Ascend Solutions and the work is licensed under the Creative Commons Attribution 4.0 International License. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>

[Nutrition and Metabolism Journal: Clinical and Experimental](#): 2026, Volume 2 (Issue 2)

DOI:
[10.66154/nmj.v2i2.13](https://doi.org/10.66154/nmj.v2i2.13)



Corresponding author:

Giuseppe Potrick Stefani (giuseppes@ufcspa.edu.br)

Federal University of Health Sciences of Porto Alegre (UFCSPA)

Graduate Program in Nutrition Sciences

Abstract

Objective: Cardiometabolic diseases comprise a group of conditions that include obesity, endothelial dysfunction (ED), dyslipidemia, diabetes mellitus type 2, systemic arterial hypertension and cardiovascular disorders and are the leading cause of death in the world. Given its high prevalence, it becomes important to investigate the potential of bioactive compounds such as the sodium nitrate (NO₃) present in vegetables and supplements in treating and preventing those conditions, once this substance has vasodilation properties, which may offer positive outcomes in cardiometabolic health.

Methods: Our review will include all randomized clinical trials (RCTs) published in English, Spanish and Portuguese without publication date restrictions. The databases searched will be MEDLINE (PubMed), Web of Science, Cochrane, Scopus, EMBASE, LILACS, SciELO and the grey literature. To be eligible the studies must be performed in adults over 18y, with ingestion of NO₃ in any form of administration or dosage, have a placebo group and assess endothelial function, arterial stiffness, blood pressure, lipid or glycemic profiles. The RoB2 tool will be used to evaluate the risk of bias and the GRADE system to evaluate the strength of evidence. Statistical analyses will be performed using RStudio for Windows and R package meta.

Perspectives: An updated meta-analysis evaluating the outcome of dietary nitrate in the endothelial function and control of cardiovascular parameters would fulfill a gap in the literature, facilitating access to the available information, helping healthcare professionals to make more assertive decisions when treating or preventing those conditions and evaluate the necessity for further RCTs.

Systematic review registration: PROSPERO registration no. CRD42024591474

Keywords: Sodium nitrate; Endothelial function; Cardiovascular health; Blood pressure; Fasting glycemia; HbA1C

Introduction

Epidemiological data on endothelial function at a global level are currently not fully explored by researchers, although it is known that endothelial dysfunction is a highly prevalent comorbidity in patients with several chronic noncommunicable diseases ¹. Conditions such as type 2 diabetes mellitus (T2DM), systemic arterial hypertension (SAH), coronary artery disease (CAD), obesity, metabolic syndrome (MS), and chronic obstructive pulmonary disease (COPD) are the most prevalent. Smokers, sedentary individuals, and the elderly ² and subjects with hypercholesterolemia ³ also show this pathology, being an important risk factor, as it is a precursor step in the development of atherosclerosis ⁴.

We searched the literature to find out more specific data on how common this condition is, and the results are alarming. The WISE study found a prevalence of 50% of ED in patients with an indication for angiography, climbing up to 75% of patients with acute coronary syndrome ⁵. Other observational studies indicate that 65%-90% of patients reporting angina and non-obstructive CAD have microvascular abnormalities ⁶ with 50%-70% of them having endothelial dysfunction ⁷.

As seen, there is a lack of specific data regarding the diagnosis of ED, although it is undoubtedly prevalent, once the comorbidities associated with the disease are widespread among the global population, with hypertension affecting 1,278 billion people worldwide ⁸, T2DM affects 6% of men and 5% of women ⁹, and, according to the WHO, in 2022, there were 2.5 billion overweight adults and 890 million of those were obese ¹⁰.

Given the alarming suggestive data on ED, it remains poorly known by the public and probably underdiagnosed and undertreated, making it important to investigate the effect of bioactive components as a possible intervention to treat and prevent this condition. This protocol for systematic review and meta-analysis aims to present the structure of a paper that will investigate the impact of inorganic nitrate (NO₃) on parameters such as endothelial function, lipidic and glycemic profile and body mass index (BMI). There's little biological plausibility that NO₃ impacts BMI directly, but we will add this variable to the study, once this parameter can influence endothelial health, so it is important to bring attention to changes in the weight of the participants of the trials to determine bias and adjust results for confounding factors.

Inorganic nitrate (NO₃) is a precursor of nitric oxide (NO) ¹¹, a substance with vasodilatory properties. The discovery of the role of NO as a signaling molecule in the cardiovascular system by the American pharmacologists Robert F. Furchgott, Louis J. Ignarro and Ferid Murad earned the 1998 Nobel Prize in Medicine. The work also highlighted the importance of the molecule beyond the endothelium, acting as a neurotransmitter in the central nervous system (CNS) and as an adjuvant in the metabolism of the immune system, activating macrophages to phagocytose bacteria and tumor cells ¹². NO also plays a fundamental role in mitochondrial respiration and skeletal muscle contraction ¹¹.

NO₃ can be found in vegetables like beetroot, cabbage, and spinach and in the form of a supplement, being widely used in sports as an ergogenic resource ¹³, promoting vasodilation, increasing tissue irrigation and, consequently, athletic performance. We hypothesized that the vasodilatory activity present in NO₃, could improve the endothelial function and attenuate or reverse the deleterious effects of the ED on the health of its carriers.

Methods

The study protocol was developed based on PRISMA-P (Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols) 2015 ¹⁴ and the Cochrane systematic review methodology ¹⁵. The protocol for the systematic review and meta-analysis was registered in the International Prospective Register of Systematic Reviews (PROSPERO) (CRD42024610581, November 16th, 2024). The databases used in the meta-analysis will be freely available in the Mendeley Data repository (<https://data.mendeley.com/>). Any amendments to this protocol will be documented in the main study.

Eligibility criteria

To establish the eligibility criteria, we will use the PICOS framework as the following:

- **Population:** human adults with 18y or older;
- **Intervention:** oral ingestion of inorganic nitrate (NO₃) without dosage or time administration limits;
- **Comparison:** powder, liquid, tablets, gels, bars or capsules containing placebo without vasodilation properties;

- **Outcomes:** the main outcome will be variation in the arterial diameter assessed by imaging exams such as Flow Mediated Dilation (FMD) (%) or Venous Occlusion Plethysmography (mL/100 mL) and the secondaries outcomes will be systolic and diastolic blood pressure (mmHg), arterial stiffness assessed by pulse wave velocity (PWV) or augmentation index (Aix), fasting glycemia (mg/dL), HbA1c (%), resting heart rate – RHR (bpm), body mass index - BMI (kg/m²) and lipid profile (total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides levels);
- **Study:** randomized clinical trials (RCTs) only.

The future article will present a comprehensive population of adults with or without established cardiometabolic risk factors (endothelial dysfunction, and/or SAH, and/or T2DM, and/or dyslipidemia, and/or overweight/obesity, and/or myocardial infarction/ stroke, and/or COPD). The inclusion criteria of our review will encompass studies with two or more arms; an "intervention" arm involving the consumption of sodium nitrate or beetroot juice or a nitrate-rich rich for at least 1 week and a "comparator" arm of placebo in any form or nitrate-depleted beverage.

It should be noted that the study populations, methods and interventions will be labelled according to the authors' description. When information is not available, authors will be contacted by email and asked to respond within 15 days (three attempts). For subgroup analyses in the meta-analysis, only RCTs reporting doses for the intervention group and the control group will be selected. The results of subgroup analyses comparing healthy individuals with those with cardiometabolic risk factors will also be discussed.

Inclusion and exclusion criteria

We will select studies involving adults aged 18 or older and consumption of sodium nitrate in any form (beetroot juice, enriched bars, capsules, powder, gel or supplement), with no limitation on the amount of NO₃, for at least 1 week with detailed assessments of cardiometabolic risk markers. This will include endothelium function measured using high-resolution Doppler ultrasonography or Venous Occlusion Plethysmography, arterial stiffness (AS) measured via PWV or Aix, systolic blood pressure (SBP), diastolic blood pressure (DBP), mean blood pressure, resting heart rate (RHR), BMI, waist circumference (WC). Lipid profile including total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides levels and glycemic profile (fasting blood glucose and HbA1c) will also be assessed. Studies combining other dietary interventions with a clearly defined intervention group of beetroot juice or supplementation NO₃ or dietary intake and a control group will be thoroughly reviewed for inclusion. Studies with participants undergoing medication or diet changes will be eligible if these changes were introduced at least four weeks prior to the start of the intervention or were consistent throughout the study to allow for accurate analysis. Regarding the 1-week intervention, there is no specific guideline or reference, as the outcomes are associated with different timeframes for promoting adaptations. Therefore, we decided to use 1 week as the minimum intervention period.

There will be excluded studies with participants undergoing treatments other than for cardiometabolic conditions; review or protocol studies; animal experimentation studies; and studies of conditions other than those related to cardiometabolic health, such as individuals diagnosed with cancer. Studies involving the same sample published in different journals will be thoroughly reviewed and excluded if duplicates.

Search strategy

We conducted a prior search in the MEDLINE database via PubMed to ascertain whether the research question of our review meets the FINER (feasible, novel, and relevant) criteria. A search strategy for RCTs was then developed and will be conducted by four independent reviewers (two pairs) in the databases recommended in the Cochrane Handbook for Systematic Reviews of Interventions¹⁵: MEDLINE, EMBASE (European literature), Web of Science, and Cochrane Central Register of Controlled Trials (to allow access to trials that are not indexed in MEDLINE and EMBASE). To broaden our search, we will conduct searches of publications from Latin America in LILACS (Latin American and Caribbean Health Sciences Literature/Virtual Health Library [VHL]) and SciELO (Scientific Electronic Library Online). We will also search the grey literature to minimize publication bias, including OpenGrey and the Brazilian Coordination for the Improvement of Higher Education Personnel (CAPES), Bank of Theses and Dissertations. For unpublished ongoing studies, we will conduct searches in the following clinical trial registries: ClinicalTrial.gov; and Brazilian Clinical Trials Registry (ReBEC). Preprint databases will also be included (preprints.org/, biorxiv.org and medrxiv.org). Table 1 details the search terms and search strategies used for each database. We will gather data through careful review of the articles retrieved and the authors will be contacted by email for additional information as needed. Articles in English, Portuguese and Spanish with no date of publication restrictions will be eligible for inclusion. Upon completion of the

review, we will undertake an additional search of all databases and registry platforms to ensure the inclusion of the most recent studies.

The main search terms will include “dietary nitrate,” “beta vulgaris,” and “beetroot” (Frame 1). To increase the accuracy and sensitivity of our searches, the search terms for the study design (RCT) will be entered in the databases MEDLINE¹⁶ and EMBASE¹⁷ (Frame 1). Four reviewers will independently select studies after an initial screening of titles and abstracts. When there is no sufficient information in the abstract, the reviewers will read the full text of the article. Any disagreements between the reviewers will be resolved through discussion and, if no consensus can be reached, a fifth reviewer will be consulted.

Frame 1: Search terms for searches in MEDLINE (PubMed), Web of Science, Cochrane, LILACS, Scopus, EMBASE, and SciELO.

MEDLINE (PubMed) ("nitration"[All Fields] OR "nitrates"[MeSH Terms] OR "nitrates"[All Fields] OR "nitrate"[All Fields] OR ("diet"[MeSH Terms] OR "diet"[All Fields] OR "dietary"[All Fields] OR "dietaries"[All Fields]) AND ("nitration"[All Fields] OR "nitrates"[MeSH Terms] OR "nitrates"[All Fields] OR "nitrate"[All Fields]) OR ("supplemental"[All Fields] OR "supplementating"[All Fields] OR "supplementation"[All Fields] OR "supplementation s"[All Fields] OR "supplementations"[All Fields] OR "supplementation"[All Fields]) OR ("beta vulgaris"[MeSH Terms] OR "beta"[All Fields] AND "vulgaris"[All Fields]) OR "beta vulgaris"[All Fields] OR "beet"[All Fields] OR ("beta vulgaris"[MeSH Terms] OR "beta"[All Fields] AND "vulgaris"[All Fields]) OR "beta vulgaris"[All Fields]) AND (("endothelialization"[All Fields] OR "endothelialize"[All Fields] OR "endothelialized"[All Fields] OR "endothelializing"[All Fields] OR "endothelial"[All Fields] OR "endothelium"[MeSH Terms] OR "endothelium"[All Fields] OR "endothelial"[All Fields]) AND ("functional"[All Fields] OR "functional s"[All Fields] OR "functionalities"[All Fields] OR "functionality"[All Fields] OR "functionalization"[All Fields] OR "functionalizations"[All Fields] OR "functionalize"[All Fields] OR "functionalized"[All Fields] OR "functionalizes"[All Fields] OR "functionalizing"[All Fields] OR "functionally"[All Fields] OR "functionals"[All Fields] OR "functioned"[All Fields] OR "functioning"[All Fields] OR "functionings"[All Fields] OR "functions"[All Fields] OR "physiology"[MeSH Subheading] OR "physiology"[All Fields] OR "function"[All Fields] OR "physiology"[MeSH Terms]) OR "FMD"[All Fields] OR ("flow-mediated"[All Fields] AND "dilatable"[All Fields] OR "dilatable"[All Fields] OR "dilating"[All Fields] OR "dilating"[All Fields] OR "dilatation"[MeSH Terms] OR "dilatation"[All Fields] OR "dilations"[All Fields] OR "dilate"[All Fields] OR "dilation"[All Fields] OR "dilations"[All Fields] OR "dilative"[All Fields] OR "dilator"[All Fields] OR "dilators"[All Fields]) OR ("plethysmography"[MeSH Terms] OR "plethysmography"[All Fields] OR "plethysmographies"[All Fields]) OR ("hyperemia"[MeSH Terms] OR "hyperemia"[All Fields] OR ("reactive"[All Fields] AND "hyperemia"[All Fields]) OR "reactive hyperemia"[All Fields]) OR ("vascular stiffness"[MeSH Terms] OR "vascular"[All Fields] AND "stiffness"[All Fields]) OR "vascular stiffness"[All Fields] OR ("arterial"[All Fields] AND "stiffness"[All Fields]) OR "arterial stiffness"[All Fields] OR "PWV"[All Fields] OR ("blood pressure"[MeSH Terms] OR "blood"[All Fields] AND "pressure"[All Fields]) OR "blood pressure"[All Fields] OR "blood pressure determination"[MeSH Terms] OR "blood"[All Fields] AND "pressure"[All Fields] AND "determination"[All Fields]) OR "blood pressure determination"[All Fields] OR "arterial pressure"[MeSH Terms] OR ("arterial"[All Fields] AND "pressure"[All Fields]) OR "arterial pressure"[All Fields] OR ("blood pressure"[MeSH Terms] OR "blood"[All Fields] AND "pressure"[All Fields]) OR "blood pressure"[All Fields] OR ("diastolic"[All Fields] AND "pressure"[All Fields]) OR "diastolic pressure"[All Fields] OR ("blood pressure"[MeSH Terms] OR "blood"[All Fields] AND "pressure"[All Fields]) OR "blood pressure"[All Fields] OR ("systolic"[All Fields] AND "pressure"[All Fields]) OR "systolic pressure"[All Fields] OR ("arterial pressure"[MeSH Terms] OR "arterial"[All Fields] AND "pressure"[All Fields]) OR "arterial pressure"[All Fields] OR ("arterial"[All Fields] AND "tension"[All Fields]) OR "arterial tension"[All Fields] OR ("body mass index"[MeSH Terms] OR "body"[All Fields] AND "mass"[All Fields] AND "index"[All Fields]) OR "body mass index"[All Fields] OR "BMI"[All Fields] OR ("body mass index"[MeSH Terms] OR "body"[All Fields] AND "mass"[All Fields] AND "index"[All Fields]) OR "body mass index"[All Fields] OR ("quetelet"[All Fields] AND "index"[All Fields]) OR "quetelet index"[All Fields] OR ("cholesterol"[MeSH Terms] OR "cholesterol"[All Fields] OR "cholesterol s"[All Fields] OR "cholesterole"[All Fields] OR "cholesterols"[All Fields] OR ("cholesterol, ldl"[MeSH Terms] OR "cholesterol"[All Fields] AND "ldl"[All Fields]) OR "ldl cholesterol"[All Fields] OR ("ldl"[All Fields] AND "cholesterol"[All Fields]) OR ("cholesterol, hdl"[MeSH Terms] OR "cholesterol"[All Fields] AND "hdl"[All Fields]) OR "hdl cholesterol"[All Fields] OR ("hdl"[All Fields] AND "cholesterol"[All Fields]) OR ("cholesterol, vldl"[MeSH Terms] OR "cholesterol"[All Fields] AND "vldl"[All Fields]) OR "vldl cholesterol"[All Fields] OR ("vldl"[All Fields] AND "cholesterol"[All Fields]) OR ("cholesterol, ldl"[MeSH Terms] OR "cholesterol"[All Fields] AND "ldl"[All Fields]) OR "ldl cholesterol"[All Fields] OR ("low"[All Fields] AND "density"[All Fields] AND "lipoprotein"[All Fields] AND "cholesterol"[All Fields]) OR "low density lipoprotein cholesterol"[All Fields] OR ("cholesterol, hdl"[MeSH Terms] OR "cholesterol"[All Fields] AND "hdl"[All Fields]) OR "hdl cholesterol"[All Fields] OR ("high"[All Fields] AND "density"[All Fields] AND "lipoprotein"[All Fields] AND "cholesterol"[All Fields]) OR "high density lipoprotein cholesterol"[All Fields] OR ("blood glucose"[MeSH Terms] OR "blood"[All Fields] AND "glucose"[All Fields]) OR "blood glucose"[All Fields] OR ("fasted"[All Fields] OR "fasting"[MeSH Terms] OR "fasting"[All Fields] OR "fastings"[All Fields] OR "fasts"[All Fields]) AND ("glycemia"[All Fields] OR "glycemias"[All Fields]) OR ("glycated hemoglobin"[MeSH Terms] OR "glycated"[All Fields] AND "hemoglobin"[All Fields]) OR "glycated hemoglobin"[All Fields] OR "glycohemoglobin"[All Fields] OR "glycohemoglobins"[All Fields] OR ("glycated hemoglobin"[MeSH Terms] OR "glycated"[All Fields] AND "hemoglobin"[All Fields]) OR "glycated hemoglobin"[All Fields] OR "hba1c"[All Fields] OR "A1c"[All Fields]) AND ("randomized controlled trial"[Publication Type] OR "controlled clinical trial"[Publication Type] OR "randomized controlled trials as topic"[MeSH Terms] OR "random allocation"[MeSH Terms] OR ("methods"[MeSH Terms] OR "single blind method"[MeSH Terms] OR "clinical trial"[Publication Type] OR "clinical trials as topic"[MeSH Terms] OR "clinical trial"[Text Word] OR "singl*[Text Word] OR "trebl*[Text Word] OR "tripi*[Text Word] OR "random*[Text Word] OR "cross over studies"[MeSH Terms] OR "control*[Text Word] OR "volunteer*[Text Word])
Web of Science 'Nitrate supplementation' OR 'dietary nitrate' OR 'Beta vulgaris' OR 'beet' OR 'beetroot' OR 'beet juice beverage' OR 'red beet juice' OR 'beetroot juice' AND 'endothelial function' OR 'blood pressure' OR 'arterial pressure' OR 'blood pressure monitoring' OR 'cholesterol' OR 'low density lipoprotein' OR 'high density lipoprotein' OR 'cholesterol blood level' OR 'very low-density lipoprotein' OR 'Glycated hemoglobin' OR 'HbA1c' OR 'A1c' OR 'fasting glycemia' OR 'Body Mass Index' OR 'BMI' AND 'randomized controlled trial' NOT 'medication' OR 'drug therapy'

Cochrane ((nitrate OR Beta vulgaris OR beet OR beetroot OR beet-it OR beet juice beverage OR red beet juice OR beetroot juice) AND (Glucose, Blood OR Blood Sugar OR Sugar, Blood OR Hemoglobin A1c, Glycated OR Hemoglobin A1c, Glycosylated OR Glycosylated Hemoglobin A1c OR Glycated Hemoglobin A1c OR Hemoglobin, Glycosylated A1b OR Glycosylated A1b Hemoglobin OR A1b Hemoglobin, Glycated OR A1b Hemoglobin, Glycosylated OR HbA1c OR Glycated A1b Hemoglobin OR Hemoglobin, Glycated A1b OR A1a-1 Hemoglobin, Glycosylated OR Hemoglobin, Glycosylated A1a-1 OR Hemoglobin, Glycosylated A1a 1 OR Glycosylated A1a-1 Hemoglobin OR Hb A1a-1 OR Hemoglobin, Glycated A1a 2 OR A1a-2 Hemoglobin, Glycated OR Hemoglobin, Glycated A1a-2 OR Hb A1a-2 OR Glycated A1a-2 Hemoglobin OR Glycosylated Hemoglobin OR Hemoglobin, OR fasting glycemia OR Glycosylated OR Glycated Hemoglobins OR Hemoglobins, Glycated OR Glycated Hemoglobin OR Hemoglobin, Glycated OR Hb A1c OR Hb A1 OR Glycohemoglobin A OR Hemoglobin A(1) OR Hemoglobin A, Glycated OR Glycosylated Hemoglobin A OR Hemoglobin A, Glycosylated OR Hb A1a+b OR HbA1 OR Blood Pressure OR Pressure, Blood OR Diastolic Pressure OR Pressure, Diastolic OR Pulse Pressure OR Pressure, Pulse OR Systolic Pressure OR Pressures, Systolic OR Pressure, Systolic OR Blood Pressure, Arterial OR Tensions, Arterial OR Arterial Blood Pressures OR Blood Pressures, Arterial OR Pressures, Arterial Blood OR Arterial Blood Pressure OR Pressure, Arterial Blood OR Arterial Pressures OR Arterial Tensions OR Pressure, Arterial OR Pressures, Arterial OR Arterial Tension OR Tension, Arterial OR Pressure, Mean Arterial OR Arterial Pressures, Mean OR Arterial Pressure, Mean OR Pressures, Mean Arterial OR Mean Arterial Pressure OR Mean Arterial Pressures OR Blood Pressure Monitoring, Home OR Home Blood Pressure Monitoring OR Ambulatory Blood Pressure Monitoring OR Monitoring, Ambulatory Blood Pressure OR Self Blood Pressure Monitoring OR Blood Pressure Monitoring, Self OR Body Mass Index OR Quetelet's Index OR Quetelet Index OR Index, Quetelet OR Quetelets Index OR Index, Body Mass OR Blood Cholesterol OR High Density Lipoprotein Cholesterol OR Cholesterol, alpha-Lipoprotein OR alpha Lipoprotein Cholesterol OR alpha-Lipoprotein Cholesterol OR HDL(2) Cholesterol OR HDL(3) Cholesterol OR HDL Cholesterol OR HDL2 Cholesterol OR Cholesterol, HDL2 OR Cholesterol, HDL3 OR HDL3 Cholesterol OR Cholesterol, LDL OR Low Density Lipoprotein Cholesterol OR beta Lipoprotein Cholesterol OR beta-Lipoprotein Cholesterol OR LDL Cholesterol OR Cholesterol, beta-Lipoprotein OR LDL Cholesteryl Linoleate OR Cholesteryl Linoleate OR Very Low Density Lipoprotein Cholesterol OR Cholesterol, Pre-beta-Lipoprotein OR Pre-beta-Lipoprotein Cholesterol OR Pre beta Lipoprotein Cholesterol OR Cholesterol, Pre beta lipoprotein OR Pre beta lipoprotein Cholesterol OR VLDL Cholesterol))
LILACS ((Dietary nitrate OR nitrate supplementation OR beetroot) AND (Blood Pressure OR systolic OR diastolic OR BMI OR Body Mass Index OR Blood Glucose OR Glycated Hemoglobin OR Cholesterol OR HDL OR LDL OR VLDL))
SCOPUS "Nitrate supplementation" OR "dietary nitrate" OR "Beta vulgaris" OR "beet" OR "beetroot" OR "beet juice beverage" OR "red beet juice" OR "beetroot juice" AND "endothelial function" OR "blood pressure" OR "arterial pressure" OR "blood pressure monitoring" OR "cholesterol" OR "low density lipoprotein" OR "high density lipoprotein" OR "cholesterol blood level" OR "very low-density lipoprotein" OR "Glycated hemoglobin" OR "HbA1c" OR "A1c" OR "fasting glycemia" OR "Body Mass Index" OR "BMI" AND "randomized controlled trial" NOT "medication" OR "drug therapy"
EMBASE 'beetroot'/exp OR 'beetroot juice'/exp OR 'beta vulgaris'/exp OR 'sodium nitrate'/exp AND 'blood pressure'/exp OR 'arterial pressure'/exp OR 'blood pressure monitoring'/exp AND 'endothelial function'/exp OR 'endothelial dysfunction'/exp OR 'arterial stiffness'/exp AND 'cholesterol'/exp OR 'low density lipoprotein'/exp OR 'high density lipoprotein'/exp OR 'cholesterol blood level'/exp OR 'very low-density lipoprotein'/exp AND 'hemoglobin A1c'/exp AND 'body mass'/exp AND 'randomized controlled trial'/exp
ScieLO ((Dietary nitrate OR nitrate supplementation OR beetroot) AND (Blood Pressure OR systolic OR diastolic OR BMI OR Body Mass Index OR Blood Glucose OR Glycated Hemoglobin OR Cholesterol OR HDL OR LDL OR VLDL))

Data extraction and management

After completing our searches in each database, all articles retrieved will be exported as “.ris”, “.txt” and “.enib” files and imported into Rayyan reference manager¹⁸. Duplicate studies will be removed using the deduplication function. Our blinded reviewers will manually check for remaining duplicates by reviewing titles, authors, year of publication and abstracts.

Our reviewers use Rayyan application “to include” articles that meet the eligibility criteria. Those that do not meet the inclusion criteria will be marked as “excluded” and categorized by reason for exclusion. (i.e., ineligible outcomes or population under the age of 18, non-RCT design, non-blinded, animal studies and intervention with organic pharmacologic nitrates). When it is not clear whether a study should be included, it will be marked as “undecided”. In the last step, our reviewers will compare the articles screened for any discrepancies. If there is not enough information in the abstract, we will retrieve and read the full-text article. When opinions about a paper diverge between the peers, it will be resolved by a fifth reviewer (WG).

The four blinded reviewers will independently conduct data extraction. When studies will be considered relevant and selected, the main data will be extracted and compiled in a prestructured Excel 2019 database divided into columns: reviewer; author; year; journal; country; inclusion and exclusion criteria; analysis strategy; number of individuals included

and excluded; number of individuals analyzed; sex; age; follow-up period; nutritional supplement taken; how it was supplemented; daily dose; and risk of bias. For the main analysis, baseline and post-intervention data will be extracted (mean and dispersion measures) for the following markers: EF, AS, SBP, DBP, MBP, RHR, body weight, BMI, WC, blood glucose, glycated hemoglobin, total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides. For the extraction of data from eligible studies with results presented in graphs, we will contact the authors by email to obtain these data or use GetData Graph Digitizer 2.26 to extract the data.

Risk of bias

The risk of bias of RCTs will be assessed using the Cochrane Risk of Bias (RoB) 2 tool included in the Cochrane Handbook ^{15,19,20}. The assessment is based on a set of five domains of bias rated as low, high, or unclear risk: bias arising from the randomization process; due to deviations from intended interventions; due to missing outcome data; in the measurement of the outcome; in the selection of the reported result. When participants are not blinded, studies will be classified as high risk of bias in the “blinding of participants and personnel” domain. No study will be excluded based on the risk of bias assessment. The risk will be analyzed for the primary outcomes of interest in our review.

Risk of overall bias in systematic reviews

We will assess certain of evidence using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) tool ^{19,20}. This tool assesses confidence in paired effect estimates and classifies treatment in a meta-analysis (high, moderate, low and very low) in the following domains ^{19,20}: study design, methodological limitations (risk of bias), inconsistency, indirectness of evidence, imprecision, publication bias, magnitude of effect, dose-response gradient, and residual confounders.

Analysis strategy

We will carry out statistical analyses to estimate the effects of dietary nitrate and/or NO₃ supplement consumption on endothelial function, arterial stiffness, resting heart rate, anthropometric measurements, BP, and lipid and glycemic profiles compared to a control group. For outcomes with the same unit, summary effect estimates will be expressed as mean difference (MD) and related 95% confidence interval (95% CI). For outcomes with different units, summary effect estimates will be expressed as standardized mean difference (SMD) and related 95% CI. The SMD expresses the size of the intervention effect in each study relative to the variability in outcome measurements observed in that study. Given that the SMD provides an overall interpretation of the results (absolute unit), we will convert SMD into units of the most common measurement instrument or proportions (%) so that the data are presented in a language more adequate to the outcome analyzed.

In studies involving humans, inconsistencies are expected due to different characteristics of the participants (clinical heterogeneity) or methods (methodological heterogeneity), with different effect sizes between studies. Therefore, to incorporate variability in the meta-analysis and to summarize the data, MDs or SMDs from individual studies will be pooled using a random-effects model. Therefore, we will use a random-effects model to incorporate variability in the meta-analysis. Since the 95% CI from random effects refer to uncertainty in the location of the mean effects across studies, we will consider the calculated values for a 95% prediction interval (95% PI) as they reflect the interval of uncertainty of the effects to be expected in future RCTs ²¹. To assess the consistency of NO₃ or dietary nitrate effects across studies, the degree of heterogeneity will be tested using the inconsistency test by Higgins (*I*²) for every pairwise comparison ^{15,22}.

To explore heterogeneity, we will conduct subgroup analyses and meta-regression analyses (≥ 10 studies) for effect modifiers with normal distribution in a quartile-quartile plot (*qq-plot*) and confirm it using the Shapiro-Wilk test ($p > 0.05$) ²³. Non-normal data will be normalized before performing meta-regression analysis. To remove discrepant data from the meta-analysis, forest plots will be constructed to display the effect estimates across studies and detect potential outliers based on not 95% CI overlapping that is due to heterogeneity ²². Potential effect modifiers (e.g., age, BMI, number of interventions and total NO₃ content) will be analyzed separately. If there is significant heterogeneity that cannot be explained, we will not perform a meta-analysis, but we will present individual intervention effect estimates from the studies. Considering that selective publication and/or suppression of specific results cause bias and consequently affect the validity of the results ²⁴, if applicable (≥ 10 studies; more than one study with significant statistical data; studies with different sample sizes), we will perform the Egger's test using a funnel plot to assess potential publication bias in the meta-analysis ^{25,26}. If publication bias is detected (Egger's test, $p < 0.1$), we will use the trim-and-fill method to identify and correct for funnel plot asymmetry by adding information of the bias-corrected data to the original data (before trimming and imputation of data by the trim-and-fill method) ^{27,28}.

Alternative analyses of the primary analysis of data including sensitivity analysis will be performed to assess the robustness of our decisions (e.g., imputation method used to impute missing values; inclusion of studies with high risk of bias, data from conference abstracts etc.)²⁹. Dispersion measures expressed as confidence intervals (CI) or standard errors (SE) will be converted to standard deviation ($SD = EP \times \sqrt{n}$) before the analysis. For eligible studies that do not report SD of differences, it will be estimated using an imputed correlation coefficient (CC) of 0.5³⁰ CC equation described in section 6.5.2.8 of Cochrane Handbook:

$$\Delta SD = SD_{baseline}^2 + SD_{final}^2 - (2 \times CC \times SD_{baseline} \times SD_{final})$$

Two-tailed tests will be used at a significance level of $p < 0.05$. Data modelling will be performed with RStudio (version 1.3.959) using the R package meta (version 3.6.1) for Windows.

Discussion and Conclusions

Beetroot juice and NO₃ supplementation are widely used among professional and amateur athletes, due to their unequivocal vasodilatory properties¹³. Organic pharmacologic nitrates are already a widely used intervention to treat a variety of cardiometabolic conditions, including endothelial dysfunction and hypertension³¹, but it's still unclear if NO₃ via dietary intake or supplementation can cause a positive impact in those conditions.

Furthermore, the sufficient dose of the compound to achieve satisfactory outcomes in human health is also not established, although the best ergogenic dose is already defined at a minimum of 400mg³², being the most studied dose-response for athletes. Since the NO₃ → NO pathway is widely studied and is known to influence the cardiovascular system¹¹ it becomes crucial to explore the potential of dietary nitrate as a supporting therapy to treat and prevent a series of conditions, as well as to determine the minimal and maximal effective dose for uses other than sports performance.

Author's Note

This article describes the original methodological protocol designed for this systematic review. The full study based on this protocol has already been completed, and the results can be found in the final published article³³.

References

1. Yilmaz MI, Romano M, Basarali MK, Elzagallaai A, Karaman M, Demir Z, et al. The Effect of Corrected Inflammation, Oxidative Stress and Endothelial Dysfunction on Fmd Levels in Patients with Selected Chronic Diseases: A Quasi-Experimental Study. *Sci Rep*. 2020;10(1). doi: <https://doi.org/10.1038/s41598-020-65528-6>
2. Neves JA, Neves JA, Oliveira R de CM. Biomarcadores de função endotelial em doenças cardiovasculares: Hipertensão. *J Vasc Bras*. 2016; 15(3):224-233. doi: <https://doi.org/10.1590/1677-5449.000316>
3. Bockus L, Kim F. Coronary endothelial dysfunction: from pathogenesis to clinical implications. *Open Heart*. 2022;9(2):e002200. doi: <https://doi.org/10.1136/openhrt-2022-002200>
4. Steyers CM, Miller FJ. Endothelial dysfunction in chronic inflammatory diseases. *Int J Mol Sci*. 2014;15(7):11324–11349. doi: <https://doi.org/10.3390/ijms150711324>
5. Sharaf BL, Pepine CJ, Kerensky RA, Reis SE, Reichek N, Rogers WJ, et al. Detailed angiographic analysis of women with suspected ischemic chest pain (pilot phase data from the NHLBI-sponsored Women's Ischemia Syndrome Evaluation [WISE] study angiographic core laboratory). *Am J Cardiol*. 2001;87(8):937-41. doi: [https://doi.org/10.1016/S0002-9149\(01\)01424-2](https://doi.org/10.1016/S0002-9149(01)01424-2)
6. Sara JD, Widmer RJ, Matsuzawa Y, Lennon RJ, Lerman LO, Lerman A. Prevalence of Coronary Microvascular Dysfunction Among Patients With Chest Pain and Nonobstructive Coronary Artery Disease. *JACC Cardiovasc Interv*. 2015;(11):1445-1453. doi: <https://doi.org/10.1016/j.jcin.2015.06.017>

7. Kumar S, Mehta PK, Eshtehardi P, Hung OY, Koh JS, Kumar A, et al. Functional coronary angiography in symptomatic patients with no obstructive coronary artery disease. *Catheter Cardiovasc Interv.* 2021;98(5):827-835. doi: <https://doi.org/10.1002/ccd.29237>
8. Zhou B, Carrillo-Larco RM, Danaei G, Riley LM, Paciorek CJ, Stevens GA, et al. Worldwide trends in hypertension prevalence and progress in treatment and control from 1990 to 2019: a pooled analysis of 1201 population-representative studies with 104 million participants. *The Lancet.* 2021;398:957-980. doi: [https://doi.org/10.1016/S0140-6736\(21\)01330-1](https://doi.org/10.1016/S0140-6736(21)01330-1)
9. Tinajero MG, Malik VS. An Update on the Epidemiology of Type 2 Diabetes: A Global Perspective. *Endocrinol Metab Clin North Am.* 2021;50(3):337-355. doi: <https://doi.org/10.1016/j.ecl.2021.05.013>
10. OMS. Obesity and overweight. 2024. <https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight#:~:text=Worldwide%20adult%20obesity%20has%20more,16%25%20were%20living%20with%20obesity>
11. Jones AM, Vanhatalo A, Seals DR, Rossman MJ, Piknova B, Jonvik KL. Dietary Nitrate and Nitric Oxide Metabolism: Mouth, Circulation, Skeletal Muscle, and Exercise Performance. *Med Sci Sports Exerc.* 2021;53(2):280-294. doi: <https://doi.org/10.1249/MSS.0000000000002470>
12. Smith O. Nobel Prize for NO research. *Nat Med.* 1998;4(11):1215. doi: <https://doi.org/10.1038/3182>
13. Macuh M, Knap B. Effects of nitrate supplementation on exercise performance in humans: A narrative review. *Nutrients.* 2021;13(9):3183. doi: <https://doi.org/10.3390/nu13093183>
14. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. *The BMJ.* 2021;372:n71. doi: <https://doi.org/10.1136/bmj.n71>
15. Higgins JPT, et al. *Cochrane Handbook for Systematic Reviews of Interventions* version 6.5 (updated August 2024). 2024. www.training.cochrane.org/handbook.
16. Robinson KA, Dickersin K. Development of a highly sensitive search strategy for the retrieval of reports of controlled trials using PubMed. *Int J Epidemiol.* 2002;31(1):150-3. doi: <https://doi.org/10.1093/ije/31.1.150>
17. Glanville J, Foxlee R, Wisniewski S, Noel-Storr A, Edwards M, Dooley G. Translating the Cochrane EMBASE RCT filter from the Ovid interface to Embase.com: a case study. *Health Info Libr J.* 2019;36(3):264-277. doi: <https://doi.org/10.1111/hir.12269>
18. Ouzzani M, Hammady H, Fedorowicz Z, Elmagarmid A. Rayyan-a web and mobile app for systematic reviews. *Syst Rev.* 2016;5(1):210. doi: <https://doi.org/10.1186/s13643-016-0384-4>
19. Hultcrantz M, Rind D, Akl EA, Treweek S, Mustafa RA, Iorio A, et al. The GRADE Working Group clarifies the construct of certainty of evidence. *J Clin Epidemiol.* 2017;87:4-13. doi: <https://doi.org/10.1016/j.jclinepi.2017.05.006>
20. Schünemann HJ, Brennan S, Akl EA, Hultcrantz M, Alonso-Coello P, Xia J, et al. The development methods of official GRADE articles and requirements for claiming the use of GRADE – A statement by the GRADE guidance group. *J Clin Epidemiol.* 2023;159:79-84. doi: <https://doi.org/10.1016/j.jclinepi.2023.05.010>
21. Int'Hout J, Ioannidis JPA, Rovers MM, Goeman JJ. Plea for routinely presenting prediction intervals in meta-analysis. *BMJ Open.* 2016;6(7):e010247. doi: <https://doi.org/10.1136/BMJOPEN-2015-010247>
22. Dias S, Sutton AJ, Welton NJ, Ades AE. Evidence synthesis for decision making 3: Heterogeneity - Subgroups, meta-regression, bias, and bias-adjustment. *Med Decis Making.* 2013;33(5):618-40. doi: <https://doi.org/10.1177/0272989X13485157>

23. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JPA, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. *BMJ*. 2009;339:b2700. doi: <https://doi.org/10.1136/bmj.b2700>
24. Deeks JJ, Macaskill P, Irwig L. The performance of tests of publication bias and other sample size effects in systematic reviews of diagnostic test accuracy was assessed. *J Clin Epidemiol*. 2005;58(9):882–93. doi: <https://doi.org/10.1016/J.JCLINEPI.2005.01.016>
25. Simmonds M. Quantifying the risk of error when interpreting funnel plots. *Syst Rev*. 2015;4(1):24. doi: <https://doi.org/10.1186/s13643-015-0004-8>
26. Page MJ, Higgins JPT, Sterne JAC. Assessing risk of bias due to missing results in a synthesis. In: *Cochrane Handbook for Systematic Reviews of Interventions*. 2019. doi: <https://doi.org/10.1002/9781119536604.ch13>
27. Mavridis D, Salanti G. How to assess publication bias: Funnel plot, trim-and-fill method and selection models. *Evid Based Ment Health*. 2014;17(1):30. doi: <https://doi.org/10.1136/eb-2013-101699>
28. Duval S, Tweedie R. Trim and fill: A simple funnel-plot-based method of testing and adjusting for publication bias in meta-analysis. *Biometrics*. 2000;56(2):455-63. doi: <https://doi.org/10.1111/j.0006-341X.2000.00455.x>
29. Boutron I. 2024. Chapter 7: Considering bias and conflicts of interest among the included studies [last updated August 2022]. In: Higgins JPT, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, Welch VA (editors). *Cochrane Handbook for Systematic Reviews of Interventions* version 6.5. Cochrane. www.training.cochrane.org/handbook
30. Balk EM, Earley A, Patel K, Trikalinos TA, Dahabreh IJ. Empirical Assessment of Within-Arm Correlation Imputation in Trials of Continuous Outcomes. *Methods Research Reports*. 2012;12. EHC141-EF.
31. Stanford Medicine Health Care. Endothelial Dysfunction. 2024. <https://stanfordhealthcare.org/medical-conditions/blood-heart-circulation/endothelial-dysfunction.html>
32. Mosher SL, Andy Sparks S, Williams EL, Bentley DJ, Naughton LRM. Ingestion of a nitric oxide enhancing supplement improves resistance exercise performance. *J Strength Cond Res*. 2016;30(12):3520-3524. doi: <https://doi.org/10.1519/JSC.0000000000001437>
33. Forster D, Wacławovsky G, Stefani GP. Effects of chronic nitrate supplementation on blood pressure in adults: a systematic review and meta-analysis of randomized clinical trials. *Nutr Res*. 2026;146:111–20. doi: <https://doi.org/10.1016/j.nutres.2025.08.002>