American Heart Journal Plus: Cardiology Research and Practice 23 (2022) 100219

Contents lists available at ScienceDirect

American Heart Journal Plus: Cardiology Research and Practice

journal homepage: www.sciencedirect.com/journal/american-heart-journal-plus-cardiology-research-and-practice

Review Article

Association of gut microbiota composition and their metabolites with subclinical atheromatosis: A systematic review

Konstantinos Grammatopoulos [a,1], Vaios-Dionysios Antoniou [a,1], Evangelos Mavrothalassitis [a], Dimitris Mouziouras [a], Antonios A. Argyris [a], Eleni Emmanouil [b], Charalampos Vlachopoulos [b], Athanase D. Protogerou [a,*]

[a] Cardiovascular Prevention and Research Unit, Clinic & Laboratory of Pathophysiology, Department of Medicine, Faculty of Health Sciences, National and Kapodistrian University of Athens, Greece
[b] 1st Department of Cardiology, National and Kapodistrian University of Athens, Greece

ARTICLE INFO

Keywords:
Gut microbiota
Intestinal microbiota
Gut microbiota metabolite
Endothelial dysfunction
Atheromatosis
Arterial stiffness

ABSTRACT

Study objective: The present systematic review investigates the hypothesis that specific components of the intestinal microbiome and/or their metabolites are associated with early stages of subclinical arterial damage (SAD).
Design: Based on the MOOSE criteria, we conducted a systematic review of the literature (Scopus, Medline) investigating the potential association between gut microbiota and the most widely applied arterial biomarkers of SAD.
Participants: All studies included individuals without established cardiovascular disease, either with or without SAD.
Intervention: No interventions were made.
Main outcome measures: Association between exposure (components/metabolites of microbiota) and outcome (presence of SAD).
Results: Fourteen articles met the predefined criteria. Due to the large heterogeneity, their meta-analysis was not possible. Our review revealed (a) two studies on endothelial dysfunction, out of which one found an inverse relation between plasma trimethylamine N-oxide levels and FMD and the other did not substantiate a statistically significant correlation with RHI. (b) Twelve studies on atheromatosis, assessed as intimal-medial thickness (IMT), coronary artery calcium (CAC) and arterial plaque, of which, seven studies showed statistically significant associations (negative or positive depending on the microorganism or microbiota metabolite) with IMT, one study revealed significant associations with coronary artery calcium, while one showed absence of correlation and four studies reported statistically significant correlations with arterial plaque. (c) Three studies on arterial stiffness (pulse wave velocity - PWV) with two of them concluding in statistically significant association while the third study did not. Some articles investigated multiple of the correlations described and therefore, belonged to more than one section.
Conclusion: Evidence of both positive and inverse associations of gut microbiota composition and their metabolites with different types of SVD has been found. However the small number and heterogeneity of available studies cannot allow to confirm or disprove the hypothesis.

1. Introduction

Multiple clinical studies in humans reveal striking associations between the composition of the intestinal microflora or its derivative metabolites, with the presence as well as the incidence of cardiovascular disease (CVD) [1]. However, the underlying pathogenetic mechanisms

* Corresponding author at: Cardiovascular Prevention & Research Unit, Clinic & Laboratory of Pathophysiology, Department of Medicine, School of Health Sciences, National and Kapodistrian University of Athens, 75, Mikras Asias Street (Building 16, 3rd floor, room 8), 115 27 Athens, Greece.
E-mail address: aprotog@med.uoa.gr (A.D. Protogerou).
[1] Equal contribution.

https://doi.org/10.1016/j.ahjo.2022.100219
Received 29 April 2022; Received in revised form 11 October 2022; Accepted 14 October 2022
Available online 17 October 2022

leading to CVD have not yet been elucidated [1]. Very few studies have provided evidence regarding the association of intestinal microbiome with the early stages of subclinical arterial damage (SAD), defined as the presence of: a) endothelial dysfunction; b) early atheromatosis, and c) early arterial stiffening in the general population or in populations free of established CVD.

A large number of non-invasive vascular biomarkers are currently accessible and available in clinical practice and research to detect SAD [2]. These biomarkers, e.g. flow-mediated dilatation (FMD) for endothelial dysfunction, intimal-medial thickening (IMT) or plaque for atheromatosis, pulse wave velocity (PWV) for early arterial stiffening (arteriosclerosis) detect distinct pathogenetic pathways leading to CVD, many of which provide incremental prognostic value over the currently available primary prevention prediction scores [2]. Therefore, they provide the opportunity: (i) in the case of clinical research to study factors and causes involved in the primary stages of the development of arterial disease, and (ii) in the case of clinical practice, to improve the stratification of CVD risk.

In the present study our hypothesis is that early SAD in individuals without established CVD is associated with either the composition of the gut microbiota or its metabolites. In order to examine this hypothesis, we performed a systematic review to identify all the available studies that investigated the association between the human gut microbiota - as assessed either by the composition of the intestinal microbes or by metabolites derived - and the presence or incidence of SAD, in individuals without established CVD.

2. Methodology

The methodology of the review was strictly based on the criteria MOOSE (Meta-analysis of Observational Studies in Epidemiology) [3]. Three independent authors (A.V. D., K.G., E.M.) systematically reviewed the Medline (via PubMed) and Scopus databases until 8 April 2021, to identify studies investigating the relationship between gut microbiota and its metabolites with endothelial function, subclinical atheromatosis and arterial stiffening. Differences in the choices of the three authors were resolved through discussion among team members and with the research team supervisor (A.D.P.) (Fig. 1).

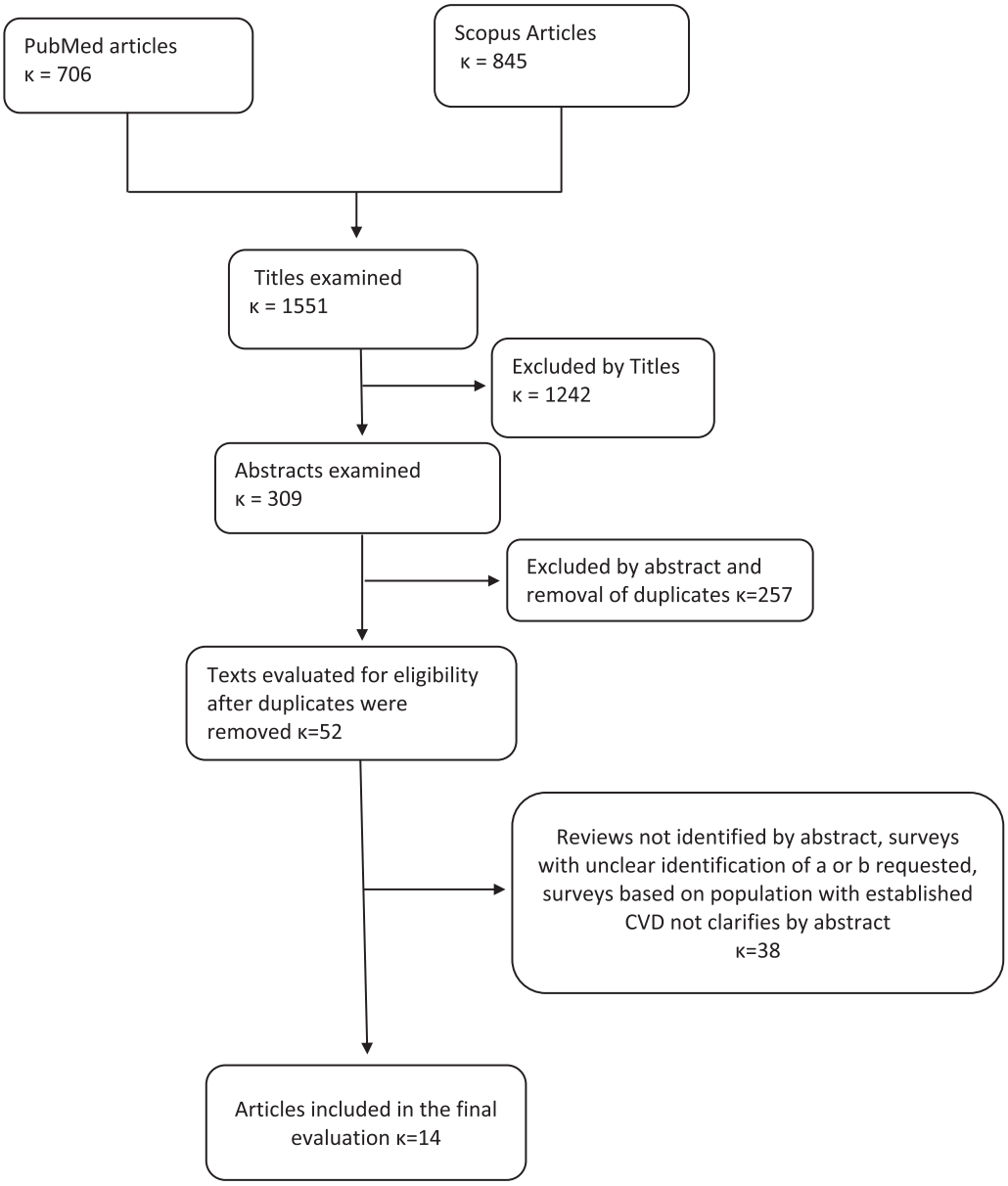

Fig. 1. Flow chart of the study selection process.

The following keywords were used to identify the exposure variables of interest:

(a1) for gut microbiota composition: "gut microbiota" OR "gut microbiome" OR "intestinal microbiota" OR "Intestinal microbiome" OR "gut microflora" OR "intestinal microflora" OR "gut microbiota composition" OR "gut microbiota diversity".
(a2) for gut microbiota metabolites: "gut microbiota derived metabolites" OR "gut microbiota metabolite" OR "gut microbiome metabolite" OR "gut microbiota endotoxins" OR "gut microbiome endotoxins" OR "microbial metabolites".

The following keywords were used to identify the outcome variables of interest:

(b1) for endothelial dysfunction: "flow mediated dilation" OR "FMD" OR "endothelial function" OR "endothelial dysfunction".
(b2) for atheromatosis: "atheromatosis" OR "atherosclerosis" OR "arterial plaque" OR "intimal-medial thickness" OR "IMT".
(b3) for arterial stiffness (arteriosclerosis): "arteriosclerosis" OR "arterial stiffness" OR "arterial stiffening" OR "arterial compliance" OR "arterial elasticity" OR "pulse wave velocity" OR "PWV".

The above keywords correspond to the most important non-invasive biomarkers or major terms used in clinical research and practice for the detection of endothelial dysfunction, atheromatosis and arterial stiffness. The final search terms were then built by matching as follows the above key words: (a1) & (b1); (a1) & (b2) & (a1) & (b3); (a2) & (b1); (a2) & (b2); (a2) & (b3).

The following inclusion criteria were used to select articles: observation or intervention studies in humans, English language. The exclusion criteria were: studies in populations with established CVD (defined as presence of coronary artery disease and/or heart failure, myocardial infraction, stroke, symptomatic peripheral arterial disease), studies without a clearly defined goal and without evaluation of the association between parameters the predefined terms a1, a2 & b1, b2, b3, as analyzed above.

3. Results

Following the display of 1551 articles produced by the bibliographic search from 2 bibliographic sources (Medline k = 706, Scopus k = 845), 1242 articles were initially excluded due to a title not related to the subject of our study. Of the remaining 309 articles, articles were excluded, as from their summary it was evident that they were either not related to our case or were systematic reviews and after the removal of duplicates, 52 articles remained. Those articles were assessed by their full text and reviews that had not been clarified by the summary were excluded, as well as surveys with unclear identification of the requested exposure terms or outcome terms, or surveys based on populations with any type of established CVD not mentioned in summary (total 38 articles excluded). As a result, 14 articles have been included in the present systematic review.

3.1. Description of the studies

3.1.1. Microbiota and endothelial dysfunction: assessed by flow mediated dilation (FMD) (Table 1)

The systematic review of literature identified one study that investigated the association of the intestinal microbiome, namely plasma Trimethylamine N-oxide (TMAO) levels, with endothelial dysfunction, assessed by FMD. TMAO is produced in the liver through conversion of trimethylamine (TMA). The latter is a substance produced through a gut microbiome-dependent conversion of ingested precursors such as and betaine choline, L-carnitine FMD refers to dilation (widening) of an artery when blood flow increases in that artery. The study was a cohort [4] including subjects aged 18 to 27 years (young controls group N = 22) or 50 to 79 years (middle-aged and older group N = 101). The study concluded in an inverse relation ($p < 0.001$) between TMAO levels and FMD in the healthy cohort, which persisted after adjusting for multiple traditional cardiovascular risk factors (systolic blood pressure, total and LDL cholesterol, etc.) (partial $p < 0.001$).

3.1.2. Microbiota and endothelial dysfunction: assessed by reactive hyperemia index (RHI) (Table 2)

Our systematic review revealed one interventional placebo-controlled study [5] examining the relation between microbiota and RHI, which was evaluated using peripheral arterial tonometry. The study's population was a group of 88 people, 40 % of which were males, with a mean age of 36 years. According to the results, the intervention with *B. subtilis* in adults caused moderate but non-statistically significant improvement in endothelial function ($p > 0.05$) compared to the placebo-controlled group. Moreover, a non-statistically significant effect on the endothelium in adults administered *B. lactis* was noted.

3.1.3. Microbiota and subclinical atheromatosis: assessed by IMT (Table 3)

The systematic review of the literature revealed 8 studies that investigated the correlation of intestinal microbiome with IMT - either femoral or carotid.

The first cross sectional observational study examined and compared elderly people aged from 97 to 100 years (20 % males). In this population Bifidobacteria/Coprococcus balance was positively and significantly associated with femoral IMT (corrected $p = 0.009$), while Coprococcus were inversely but not significantly associated with carotid IMT (corrected $p = 0.087$).

The second cross sectional observational study (n = 92) [7] examined patients with a median age of 52 years (29 % males, 37 % hypertensives, 23 % diabetics). In this population the highest concentrations of Serratia ($p = 0.004$) and Blautia ($p = 0.009$) were associated with increased IMT.

The third prospective observational study [8] (n = 817) examined adults aged 33 to 55 years, without hypertension, of which 52 % were males. In this population TMAO was not significantly associated with carotid IMT (beta coefficients [95 % confidence interval (CI)] from the linear regression model were - 0.009 [−0.03 to 0.01]).

The fourth cohort study [9] (n = 250) examined adults aged 25 to 50 years of age, without diabetes, with a median age of 48.6 years, of which 41 % were males. In this population it was observed that every 1 %

Table 1
Characteristics of studies that associate microbiome parameters with the presence of endothelial dysfunction, as studied with the marker flow mediated dilation (FMD).

Study author (year)	Location	Type of study	Population	Number	Mean age	Males (%)	Hypertension (%)	DM (%)	Main result
Vienna E Brunt et al. (2020) Trimethylamine-N-Oxide Promotes Age-Related Vascular Oxidative Stress and Endothelial Dysfunction in Mice and Healthy Humans	USA	Cohort study	Subjects aged 18 to 27 years or 50 to 79 years	123	–	–	–	–	Inverse relation between plasma TMAO levels and FMD in the healthy cohort that persisted after accounting for multiple traditional cardiovascular risk factors (systolic blood pressure, total and LDL cholesterol, etc.)

Table 2
Characteristics of studies that associate microbiome parameters with the presence of endothelial dysfunction, as studied with the marker reactive hyperemia index (RHI).

Study author (year)	Location	Type of study	Population	Number	Mean age	Males (%)	Hypertension (%)	DM (%)	Main result
R.E. Trotter et al. (2020) *Bacillus subtilis* DE111 intake may improve blood lipids and endothelial function in healthy adults	Fort Collins, USA	Interventional placebo-controlled study	Healthy adults aged 18–65 years	88	36	40	0	0	Moderate but statically insignificant improvements were observed in the endothelium of adults administered *B. subtilis*. No significant effects on the endothelium in adults administered *B. lactis*.

increase in the relative abundance of Aeromonadaceae (corrected p = 0.02) and Citrobacter (corrected p = 0.03) was associated with an increase of 18.2 μm and 97.3 μm in IMT respectively after correction for multiple confounders.

The fifth cohort study [10] examined 162 people living with HIV (PLWH) with a median age of 49 years, 91 % of which were males. From that population, 39 % had hypertension and 11 % diabetes mellitus. In the entire cohort, betaine and carnitine were significantly associated with baseline cIMT (8.2 % higher per 10-μmol/L increase of carnitine, p < 0.01; and 2.6 % higher per 10-μmol/L increase of betaine, p < 0.01) and progression of carotid IMT (cIMT) (1.3 % per year per 10-μmol/L increase of carnitine, p = 0.02 and 0.6 % per year for each 10-μmol/L increase of betaine, p = 0.04). As for the effectively treated and suppressed individuals, carnitine was associated with baseline cIMT, as well as progression of cIMT (8.2 % per 10-μmol/L increase of carnitine, p < 0.01; and 1.4 % per year per 10-μmol/L increase of carnitine, p = 0.05 respectively), whereas betaine showed no association with cIMT. Carnitine and betaine are predecessors of TMAO and are generated from the breakdown of dietary phosphatidylcholine by gut microbiota. However, TMAO was associated neither with baseline cIMT nor with cIMT progression.

The sixth cohort study [11] included 86 children aged 3 to 18 years old, with chronic kidney disease (CKD) of stages G1 (eGFR ≥ 90 mL/min/1.73 m^2), G2 (eGFR 60–89 mL/min/1.73 m^2) or G3 (eGFR 30–59 mL/min/1.73 m^2) from which 63 % were male. The median age of the children staged to G1 level disease was 9.5 years whereas the median age of those staged to G2 or G3 level was 13.7 years. The study resulted in a negative correlation between abundances of the Lactobacillus genus and cIMT (r = −0.284, p = 0.029).

The seventh (cohort) study [12] examined 28 asymptomatic monozygotic twins (14 pairs) of median age 65 years (29 % males), while 43 % of the population were hypertensives and 18 % were diabetics. The population was divided into two groups, one with normal IMT values (IMT < 0.9) and one with high IMT values (IMT > 0.9). The results reported an increased Firmicutes/Bacteroidetes ratio in subjects with increased carotid IMT (p = 0.031). Moreover, normal carotid IMT values were reported to be associated with a substantially higher fraction of Prevotellaceae (12.6 % against 2.1 % in the "higher IMT" group) without, however, using statistic procedures to support this assertion, such as p-values or r^2-values.

The eighth cohort study [13] included a population of 569 Chinese individuals aged 55–65 years having a median age of 59.8 years (42.7 % males, 56.9 % hypertensives, 11.6 % diabetics). Faecalicatena was negatively associated with carotid atherosclerosis as assessed by IMT > 0.9 mm (OR = 0.30, 0.12–0.65), while elevated Libanicoccus (OR = 2.43, 95 % CI: 1.46–4.20) and decreased Phocea (OR = 0.85, 95 % CI: 0.75–0.96) were related to increased IMT in the left carotid artery (LCA). Similar results were shown in the right carotid artery (RCA) analysis. Moreover, Helicobacter (OR = 1.15, 95 % CI: 1.03–1.29) was associated with increased risk of subclinical atherosclerosis in terms of increased IMT in the RCA. In addition, after the examination of 38 metabolites, it was found that metabolites are just marginally related to IMT with log2 fold changes ranging from −0.08 to 0.24.

3.1.4. Microbiota and subclinical atheromatosis: assessed by the presence of atheromatic plaque (Table 4)

The systematic review of the literature revealed 4 studies that assessed the presence of carotid plaque as outcome. In the first cohort study [10], cIMT was measured at baseline and at a median interval of 4 years in 162 adult People Living With HIV (PLWH). The mean age of the study's participants was 49 years (91 % males, 50 % treated with a suppressed viral load). It was found that only carnitine was strongly associated with baseline carotid plaque presence, even among effectively treated individuals.

The second cohort study (n = 737) [14], examined the association of 5 plasma choline metabolites, including TMAO, with the carotid plaque in 520 HIV-infected and 217 HIV-uninfected participants (112 incident plaque cases over 7 years). 46 % of the participants were males, whereas the median age for males was 42 years and for females 46 years. After multivariable adjustment, higher levels of plasma TMAO were associated with increased risk of carotid artery plaque among HIV-infected individuals (p = 0.01).

In the third (n = 222) cohort study [15], 155 HIV-infected and 67 non-HIV-infected subjects without known history of CVD were examined (mean age was 46.7 years, 53 % were males, 21 % had hypertension and 9 % diabetes mellitus). Serum TMA, but not serum TMAO, was associated with the presence of coronary plaque and more specifically calcified plaque in HIV-infected patients. Among the non-HIV-infected control subjects, no significant relationships with serum TMA or other metabolites were observed.

The fourth cohort study [16] recruited 345 individuals' representative of the general population. The median age was 67.3 years, 45.8 % were males, 45.5 % were under anti-hypertensive regiment and none of them was diabetic. The participants were categorized to the Subclinical Carotid Atherosclerosis (SCA) group and non-SCA group. SCA was defined as a mean IMT higher than 1.3 mm or as a presence of focal atherosclerotic lesions larger than 1.3 mm. According to the results, there was an increased relative abundance of members of Escherichia (p = 0.008) and Oscillospira (p = 0.013) genera in subjects with SCA.

3.1.5. Microbiota and subclinical atheromatosis: assessed by coronary artery calcium (CAC) (Table 5)

The first cohort study [8] (n = 817) examined the association of TMAO with CAC in middle aged adults (33 to 55 years, mean 40.2 years, 52 % male). After a 10 year follow up, no significant correlation between the baseline TMAO and the incidence (risk ratio = 1.03; 95 % CI: 0.71–1.52) or the deterioration (0.97; 0.68–1.38) of CAC was found. In the second (n = 222) cohort study [15], 155 HIV-infected and 67 non-HIV-infected subjects without known history of CVD were examined. The mean age of the study participants was 46.7 years, 53 % were males, 21 % had hypertension and 9 % had diabetes mellitus. Higher calcium score (p = 0.008) and calcified plaque mass (p = 0.007) were similarly related to increased serum TMA in HIV patients.

3.1.6. Microbiota and arterial stiffness: assessed by Pulse Wave Velocity (PWV) (Table 6)

The systematic review of the literature identified 3 studies that investigated the association of intestinal microbiome with arterial

Table 3
Characteristics of studies that associate microbiome parameters with the presence of subclinical atheromatosis, as studied with the marker intimal medial thickness (IMT).

Study author (year)	Location	Type of study	Population	Number	Mean age	Males (%)	Hypertension (%)	DM (%)	Main result
Daria A. Kashtanova et al. (2020) A cross-sectional study of the gut microbiota composition in moscow long-livers	Russia, Moscow	Cross sectional observational study	Elderly	42	97–100	20	-	-	Bifidobacteria were positively and significantly associated with femoral IMT, while Coprococcus were inversely but not significantly associated.
Daria Kashtanova et al. (2017) Gut microbiota and vascular biomarkers in patients without clinical cardiovascular diseases	Russia, Moscow	Cross sectional observational study	General population	92	52	29	37	23	Highest concentrations of Serratia and Blautia were associated with increased IMT
Katie A. Meyer et al. (2016) Microbiota-Dependent Metabolite Trimethylamine N-Oxide and Coronary Artery Calcium in the Coronary Artery Risk Development in Young Adults Study (CARDIA)	USA	Prospective observational study	Adults 33 to 55 years old	817	40,2	52	0	-	TMAO was not statistically significantly associated with carotid IMT
Fen Wu et al. (2019) The role of gut microbiome and its interaction with arsenic exposure in carotid intima-media thickness in a Bangladesh population	Bangladesh	Cohort study	Adults 25–50 years old without diabetes	250	48,6	41	-	0	Every 1 % increase in the relative abundance of Aeromonadacae and Citrobacter is associated with an increase of 18.2 μm and 97.3 μm in IMT, respectively
Arjun Sinha et al. (2019) Carnitine Is Associated With Atherosclerotic Risk and Myocardial Infarction in HIV -Infected Adults.	USA, San Francisco	Cohort study	People Living with HIV (PLWH)	162	49	91	39	11	Both betaine and carnitine were significantly associated with progression of cIMT in the entire cohort. Among the effectively treated and suppressed individuals, carnitine associated with baseline cIMT as well as progression of cIMT, betaine was not. TMAO did not associate.
Chien-Ning Hsu et al. (2018) Gut Microbiota-Dependent Trimethylamine N-Oxide Pathway Associated with Cardiovascular Risk in Children with Early-Stage Chronic Kidney Disease	Taiwan, Taoyuan	Cohort study	Children aged 3 to 18 years old with CKD stage G1-G3	86	G1 9,5 G2, G3 13,7	63	-	-	The abundances of the Lactobacillus genus were negatively correlated with cIMT.
Helga Szabo et al. (2021) Association between Gut Microbial Diversity and Carotid Intima-Media Thickness	Hungary	(Cohort study)	Asymptomatic MZ Hungarian twins	28	65	29	43	18	Increased Firmicutes/ Bacteroidetes ratio was reported in subjects with increased carotid IMT. Normal carotid IMT values were associated with a substantially higher fraction of Prevotellaceae
Sibo Zhu et al. (2021) The gut microbiome in subclinical atherosclerosis: a population-based multi-phenotype analysis	China	Cohort study	Han Chinese individuals aged 55–65 years	569	59.8	42.7	56.9	11.6	.Faecalicatena was negatively associated with carotid atherosclerosis, while elevated Libanicoccus and decreased Phocea were related to increased IMT in LAC. Presence of Helicobacter was associated with increased IMT in RCA

DM: diabetes mellitus, (-): not mentioned.

stiffness.

The first is a cross-sectional observational study [7] (n = 92) that examined individuals with a mean age of 52 years, of whom 29 % were male, 37 % had hypertension and 23 % had diabetes. The multivariate adjustment showed that the representation of Bacteroides was significantly higher in non-diabetic subjects with PWV $\geq$ 10 m/s (p = 0.0001).

The second cohort study [17] (n = 617) investigated middle-aged females from the Twins UK cohort with a median age of 61.4 years, 82 % of whom had high blood pressure. Examination of the association between PWV and bacterial genera or taxonomic groups identified 7 taxonomic groups that correlated inversely and significantly with PWV, after adjustment for multiple confounders (e.g., insulin resistance and

Table 4
Characteristics of studies that associate microbiome parameters with the presence of subclinical atheromatosis, as studied with the marker "plaque".

Study author (year)	Location	Type of study	Population	Number	Mean age	Males (%)	Hypertension (%)	DM (%)	Main result
Arjun Sinha et al. (2019) Carnitine Is Associated With Atherosclerotic Risk and Myocardial Infarction in HIV -Infected Adults.	USA, San Francisco	Cohort study	People living with HIV (PLWH)	162	49	91	39	11	Only carnitine was strongly associated with baseline carotid plaque even among effectively treated and suppressed adults
Zhilei Shan et al. (2018) Gut Microbial-Related Choline Metabolite Trimethylamine-N-Oxide Is Associated With Progression of Carotid Artery Atherosclerosis in HIV Infection	USA	Cohort study	People infected by HIV (520) and people uninfected (217)	737	42 and 46 for males and females respectively	46	-	-	Higher levels of plasma TMAO were associated with increased risk of carotid artery plaque in HIV-infected individuals
Suman Srinivasa et al. (2015) Plaque Burden in HIV-Infected Patients is Associated with Serum Intestinal Microbiota-generated Trimethylamine	USA, Boston	Cohort study	155 HIV-infected subjects and 67 non-HIV-infected subjects	222	46.7	53	21	9	Serum TMA, but not serum TMAO, is associated with the presence of coronary plaque, and more specifically calcified plaque in HIV-infected patients. Among the non-HIV-infected control subjects, no significant relationships with serum TMA or other metabolites were seen
Andrea Baragetti et al. (2021) Gut Microbiota Functional Dysbiosis Relates to Individual Diet in Subclinical Carotid Atherosclerosis	Italy, Milan	(Cohort study)	Subjects without previous clinically manifest ACVD representative of the general population	345	67.3	45.8	45.5 (use of anti-hypertensive drugs)	0	Increased relative abundance of members of Escherichia and Oscillospira genera in subjects with SCA.

DM: diabetes mellitus, (-): not mentioned.

Table 5
Characteristics of studies that associate microbiome parameters with the presence of subclinical atherosclerosis, as studied with the marker "coronary artery calcium" (CAC).

Study author (year)	Location	Type of study	Population	Number	Mean age	Males (%)	Hypertension (%)	DM (%)	Main result
Katie A. Meyer et al. (2016) Microbiota-Dependent Metabolite Trimethylamine N-Oxide and Coronary Artery Calcium in the Coronary Artery Risk Development in Young Adults Study (CARDIA)	USA	Prospective observational study	Adults 33–35 years old	817	40,2	52	0	-	TMAO was not significantly correlated with the incidence of 10-year CAC.
Suman Srinivasa et al. (2015) Plaque Burden in HIV-Infected Patients is Associated with Serum Intestinal Microbiota-generated Trimethylamine	USA, Boston	Cohort study	155 HIV-infected subjects and 67 non-HIV-infected subjects	222	46.7	53	21	9	Higher calcium score and calcified plaque mass were similarly related to increased serum TMA in HIV patients.

DM: diabetes mellitus, (-): not mentioned.

visceral fat). This study also showed that members of the families Ruminococcaceae, Rikenellaceae, Clostridiaceae, Actinobateria, Barnesiellaceae, and the genus Odoribacter were negatively correlated with PWV.

The population of the third cohort study [11] consisted of 86 children aged 3 to 18 years old, with chronic kidney disease (CKD) of stages G1 (eGFR $\geq$ 90 mL/min/1.73 m^2), G2 (eGFR 60–89 mL/min/1.73 m^2) or G3 (eGFR 30–59 mL/min/1.73 m^2) 63 % of whom were male. No correlation between the microbiota components and PWV ($p > 0.05$ for all the bacteria examined).

4. Discussion

In the present systematic review, we investigated the hypothesis that, in individuals without established CVD, early SAD is associated with either the gut microbiota composition or its metabolites, focusing on biomarkers of endothelial dysfunction, subclinical atheromatosis and arterial stiffening. It was found that: (a) the literature on this topic is limited; and (b) the studies not only present methodological heterogeneity but also their conclusions vary substantially; As a consequence, their meta-analysis was not feasible and the final conclusions remain uncertain.

Endothelial dysfunction is the first fundamental step in the

Table 6
Characteristics of studies that associate microbiome parameters with the presence of subclinical arterial sclerosis, as studied with the marker "Pulse Wave Velocity" (PWV).

Study author (year)	Location	Type of study	Population	Number	Mean age	Males (%)	Hypertension (%)	DM (%)	Main result
Daria Kashtanova et al. (2017) Gut microbiota and vascular biomarkers in patients without clinical cardiovascular diseases	Russia, Moscow	Cross sectional observational study	General population	92	52	29	37	23	It has been found that the representation of Bacteroides was significantly higher in non-diabetic subjects with PWV ≥ 10 m/s
Cristina Menni (2018) Gut microbial diversity is associated with lower arterial stiffness in women	United Kingdom	Cohort study	Middle-aged females	617	61.4	0	81.6	-	Ruminococcaceae, Rikenellaceae, Clostridiaceae, Actinobateria, Barnesiellaceae, Clostridiaceae and Odoribacter taxonomic units were negatively correlated with PWV.
Chien-Ning Hsu et al. (2018) Gut Microbiota-Dependent Trimethylamine N-Oxide Pathway Associated with Cardiovascular Risk in Children with Early-Stage Chronic Kidney Disease	Taiwan, Taoyuan	Cohort study	Children aged 3 to 18 years old with CKD stage G1-G3	86	G1 9,5 G2, G3 13,7	63	-	-	No correlation was found between the microbiota components and PWV

DM: diabetes mellitus, (-): not mentioned.

pathogenesis of atherosclerotic disease [2] and therefore the documentation of its association with the parameters of the gut microbiota or with its metabolites is essential for the etiological understanding of their relationship with CVD. In animal trials, it has been shown that TMAO impairs endothelial function by reducing the activation of the endothelial nitric oxide synthase and impairing nitric-oxide mediated dilation [4]. However, this systematic review of the literature provided only two human studies [4,5] examining the relation of the microbiota with endothelial dysfunction. Major differences in their study design (cohort study [4]; randomized intervention study [5]), the applied methodologies (FMD [4] and RHI [5]), the arterial beds and the population (healthy individuals [4]; healthy adults [5]) were identified, therefore, it come as no surprise the quite diverse outcome of the two studies. On the one hand, the inverse association of endothelial function with plasma TMAO, that persisted after adjusting for multiple traditional cardiovascular risk factors [4] and, on the other hand, the marginally beneficial evidence of microflora characteristics (*B. subtilis*) on endothelial function, were observed. Moreover, given the small sample size and all the above limitations, it is not possible to draw any firm conclusions or extrapolate these findings to other populations.

IMT is one of the most important non-invasive vascular biomarkers of arterial remodeling or atheromatosis. Increased IMT serves as a "proxy" for generalized atherosclerosis. In our review, eight studies [6–13] emerged (Table 3). Out of the 8 studies, 6 [6,7,9,11,12,13] aimed in correlating directly gut microbiota components with IMT. Those studies concluded in both positive (i.e., adverse) and negative (i.e., favorable) correlations between IMT and bacterial species. Positive associations were found with Bifidobacteria/Coprococcus balance, although in femoral IMT [6], Serratia, Blautia [7], Aeromonadacae, Citrobacter [9], the Firmicutes/Bacteroidetes ratio [12] and Libanicoccus [13]. Moreover, a higher fraction of Prevotellaceae was associated with normal carotid IMT values [12] and the presence of Helicobacter was associated with increased IMT [13]. Whereas, the negative associations concerned Lactobacillus [11], Faecalicatena and Phocea [13]. The most important limitation of all studies but one [7] was that they examined specific population rather than the general population, such as elderly [6] or other specific age groups [8,9,11,13] and patients with chronic diseases such as CKD [11] and PLWH [10], limiting their generalizability. Concerning the TMAO, the main metabolite studied, both studies [8,10] concluded in no association with carotid IMT. However, correlation between the level of two TMAO precursors were found (carnitine and betaine) with the progression of cIMT was found [10], which seemed to be stronger for carnitine. These results were validated from a rather large study (n = 817) performed by Meyer et al. [8], with - nevertheless - strict age criteria. Although these studies failed to show a statistically significant correlation between TMAO and cIMT, TMAO remains one of the possible links between gut microbiota and atherosclerosis as animal studies have found that it contributes to the development of atherosclerosis by promoting cholesterol accumulation within macrophages through scavenger receptors such as CD36 and SRA1[18].

The detection of early atheromatic damage as measured by high-resolution carotid ultrasound or CT angiography on coronary arteries, is not only a local phenomenon confirming the presence of atherosclerotic disease but also a biomarker with prognostic ability of future CVD events (stroke, coronary artery disease) [2]. Our research concluded in four studies [10,14–16] relating the gut microbiome with the presence of plaque. The three first studies included in their population PLWH [10,14,15] and emphasized on the relationship between the gut microbiota-related metabolites and the presence of plaque. Although HIV infection is possible factor for atherosclerosis per se, all three studies [10,14,15] have indicated a correlation between plaque and metabolites such as TMA, carnitine and TMAO. The present results, even though they derived from adequate sample sizes (162 [10], 520 [14], 155 [15] respectively), cannot be extrapolated to other populations, since HIV treatment may not only alter the pathophysiological mechanisms leading to plaque formation, but also because PLWHIV have microbiome alterations [19]. Regarding the fourth study [16] the findings revealed a relative increased in abundance of members of Escherichia and Oscillospira genera in a general population sample (=345); however, half of them were on anti-hypertensive treatment, with potentially effect on eth final result.

Finally, the literature review revealed two studies [8,15] (Table 4) that examined the association between CAC and the metabolites TMA and TMAO. Conflicting results were once more described by the two studies that both included PLWH. This inconsistency along with their respective limitations highlights the complicated relationship between CAC and gut microbiota metabolites.

Arterial stiffening is a distinct arterial disease from atheromatosis, both in terms of pathogenesis and pathophysiological consequences and complications in the cardiovascular system [2], as well as a major prognostic indicator of morbidity and mortality. The systematic review of the literature revealed three studies examining the relation of the gut microbiota with PWV. Although the design was similar across all three studies [17,7,11], the existing substantial differences in their samples sizes did not allow for consistency in the conclusions reached. To

elaborate, while a statistically significant inverse correlation was found between PWV and Ruminococcaceae, Clostridiaceae, Actinobateria, Odoribacter and two members of the Bacteroidetes phylum in women [17], on the contrary B subtilis (another member of this phylum) was shown to have the opposite association with PWV when both men and women were included [7]. At the same time these bacteria groups were not correlated with PWV in children [11], possibly due to the fact that arterial stiffness develops later in life. To conclude, the small sample size of two of the studies, in combination with both the lack of a standardized sample and the inconsistency of the results, limits the generalization of these findings.

The present systematic review of the literature has certain limitations. Some are inherent to the field of gut microbiota. The investigation performed was both direct (i.e. on the composition of the gut microbiota) and indirect (its metabolites); regarding the direct investigation, an additional limiting factor is the heterogeneity of bacteria species. Other limitations are related to the studies themselves. The number of articles was limited (n = 14), while there was great heterogeneity between them in terms of the study design, the characteristics of the populations and the methodology for assessing the microbiome and arterial damage. Consequently, a meta-analysis could not be performed. In addition, most studies focused on specific age groups, and, thus, the results cannot be generalized.

In conclusion, most studies provide evidence of an association between the composition and/or metabolites of the gut microbiota and different types of SAD (atherosclerosis and arteriosclerosis). These associations are positive or negative depending on the microorganisms studied. However, neither safe conclusions can be drawn, due to their large heterogeneity, nor causality can be inferred. Future studies should not only target on overt cardiovascular disease but also on SAD. These studies, apart from addressing existing methodological limitations, they have to be carried out on large, representative samples of the general population and additional patient groups.

Declaration of competing interest

All authors of the present scientific article “Association of gut microbiota composition and their metabolites with subclinical atheromatosis: A systematic review” declare that they have no conflicts of interest.

All authors disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work.

All authors agree on the submission and publication of the article titled “Association of gut microbiota composition and their metabolites with subclinical atheromatosis: a systematic review”.

References

[1] W.H. Tang, T. Kitai, S.L. Hazen, Gut microbiota in cardiovascular health and disease, Mar 31, Circ. Res. 120 (7) (2017) 1183–1196, https://doi.org/10.1161/CIRCRESAHA.117.309715. PMID: 28360349; PMCID: PMC5390330.

[2] C. Vlachopoulos, P. Xaplanteris, V. Aboyans, M. Brodmann, R. Cífková, F. Cosentino, M. De Carlo, A. Gallino, U. Landmesser, S. Laurent, J. Lekakis, D. P. Mikhailidis, K.K. Naka, A.D. Protogerou, D. Rizzoni, A. Schmidt-Trucksäss, L. Van Bortel, T. Weber, A. Yamashina, R. Zimlichman, P. Boutouyrie, J. Cockcroft, M. O'Rourke, J.B. Park, G. Schillaci, H. Sillesen, R.R. Townsend, The role of vascular biomarkers for primary and secondary prevention. A position paper from the European Society of Cardiology Working Group on peripheral circulation: endorsed by the Association for Research into Arterial Structure and Physiology (ARTERY) Society, Atherosclerosis 241 (2) (2015) 507–532. Aug.

[3] D.F. Stroup, J.A. Berlin, S.C. Morton, I. Olkin, G.D. Williamson, D. Ren¬nie, Meta-analysis of observational studies in epide¬miology: a proposal for reporting. Meta-analysis Of Observa¬tional Studies in Epidemiology (MOOSE) group, JAMA 283 (2000) 2008–2012.

[4] V. Brunt, R. Gioscia-Ryan, A. Casso, N. VanDongen, B. Ziemba, Z. Sapinsley, J. Richey, M. Zigler, A. Neilson, K. Davy, D. Seals, Trimethylamine-N-oxide promotes age-related vascular oxidative stress and endothelial dysfunction in mice and healthy humans, Hypertension 76 (1) (2020) 101–112.

[5] R.E. Trotter, A.R. Vazquez, D.S. Grubb, K.E. Freedman, L.E. Grabos, S. Jones, C. L. Gentile, C.L. Melby, S.A. Johnson, T.L. Weir, Bacillus subtilis DE111 intake may improve blood lipids and endothelial function in healthy adults, Nov 15, Benefic. Microbes 11 (7) (2020) 621–630, https://doi.org/10.3920/BM2020.0039. Epub 2020 Nov 9. PMID: 33161737.

[6] D.A. Kashtanova, N.S. Klimenko, I.D. Strazhesko, E.V. Starikova, O.E. Glushchenko, D.A. Gudkov, O.N. Tkacheva, A cross-sectional study of the gut microbiota composition in Moscow long-livers, Jul 30, Microorganisms 8 (8) (2020) 1162, https://doi.org/10.3390/microorganisms8081162. PMID: 32751673; PMCID: PMC7463576.

[7] Daria Kashtanova, Olga Tkacheva, Anna Popenko, Lilit Egshatyan, Alexander Tyakht, Dmitry Alexeev, Yulia Kotovskaya, Ekaterina Plokhova, Sergey Boytsov, Gut microbiota and vascular biomarkers in patients without clinical cardiovascular diseases, Oct 21, Artery Res. 18 (2017) 41–48, https://doi.org/10.1016/j.artres.2017.02.007. PMID: 27792658; PMCID: PMC5121500.

[8] K.A. Meyer, T.Z. Benton, B.J. Bennett, D.R. Jacobs Jr., D.M. Lloyd-Jones Jr., M. D. Gross Jr., J.J. Carr Jr., P. Gordon-Larsen Jr., S.H. Zeisel Jr., Microbiota-dependent metabolite trimethylamine N-oxide and coronary artery calcium in the coronary artery risk development in young adults study (CARDIA), Oct 21, J. Am. Heart Assoc. 5 (10) (2016), e003970, https://doi.org/10.1161/JAHA.116.003970. PMID: 27792658; PMCID: PMC5121500.

[9] F. Wu, L. Yang, M.T. Islam, F. Jasmine, M.G. Kibriya, J. Nahar, B. Barmon, F. Parvez, G. Sarwar, A. Ahmed, M. Eunus, T. Islam, V. Slavkovich, J. Hu, H. Li, J. H. Graziano, Z. Pei, H. Ahsan, Y. Chen, The role of gut microbiome and its interaction with arsenic exposure in carotid intima-media thickness in a Bangladesh population, Feb, Environ. Int. 123 (2019) 104–113, https://doi.org/10.1016/j.envint.2018.11.049. Epub 2018 Nov 29. PMID: 30503971; PMCID: PMC6371773.

[10] A. Sinha, Y. Ma, R. Scherzer, S. Rahalkar, B. Neilan, H. Crane, D. Drozd, J. Martin, S. Deeks, P. Hunt, P. Hsue, Carnitine is associated with atherosclerotic risk and myocardial infarction in HIV-infected adults, J. Am. Heart Assoc. 8 (9) (2019).

[11] C. Hsu, P. Lu, M. Lo, I. Lin, G. Chang-Chien, S. Lin, Y. Tain, Gut microbiota-dependent trimethylamine N-oxide pathway associated with cardiovascular risk in children with early-stage chronic kidney disease, Int. J. Mol. Sci. 19 (12) (2018) 3699.

[12] S. Helga, Association between gut microbial diversity and carotid intima-media thickness, Medicina (Kaunas) 57 (3) (2021) 195.

[13] S. Zhu, K. Xu, Y. Jiang, C. Zhu, C. Suo, M. Cui, Y. Wang, Z. Yuan, J. Xue, J. Wang, T. Zhang, G. Zhao, W. Ye, T. Huang, M. Lu, W. Tian, L. Jin, X. Chen, The gut microbiome in subclinical atherosclerosis: a population-based multiphenotype analysis, Rheumatology 61 (1) (2021) 258–269.

[14] J Infect Dis, in: Gut Microbial-Related Choline Metabolite Trimethylamine-N-Oxide Is Associated With Progression of Carotid Artery Atherosclerosis in HIV Infection 218(9), 2018, pp. 1474–1479.

[15] S. Srinivasa, K. Fitch, J. Lo, H. Kadar, R. Knight, K. Wong, S. Abbara, D. Gauguier, J. Capeau, F. Boccara, S. Grinspoon, Plaque burden in HIV-infected patients is associated with serum intestinal microbiota-generated trimethylamine, AIDS 29 (4) (2015) 443–452.

[16] A. Baragetti, M. Severgnini, E. Olmastroni, C. Dioguardi, E. Mattavelli, A. Angius, L. Rotta, J. Cibella, C. Consolandi, L. Grigore, F. Pellegatta, F. Giavarini, D. Caruso, D. Norata, A. Catapano, C. Peano, Individual diet relates to gut microbiota functional dysbiosis since subclinical stages of atherosclerosis, Atherosclerosis 331 (2021) e212–e213.

[17] C. Menni, C. Lin, M. Cecelja, M. Mangino, M.L. Matey-Hernandez, L. Keehn, R. P. Mohney, C.J. Steves, T.D. Spector, C.F. Kuo, P. Chowienczyk, A.M. Valdes, Gut microbial diversity is associated with lower arterial stiffness in women, Jul 1, Eur. Heart J. 39 (25) (2018) 2390–2397, https://doi.org/10.1093/eurheartj/ehy226. PMID: 29750272; PMCID: PMC6030944.

[18] Z. Wang, E. Klipfell, B.J. Bennett, R. Koeth, B.S. Levison, B. Dugar, A.E. Feldstein, E.B. Britt, X. Fu, Y.M. Chung, Y. Wu, P. Schauer, J.D. Smith, H. Allayee, W.H. Tang, J.A. DiDonato, A.J. Lusis, S.L. Hazen, Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease, Apr 7, Nature 472 (7341) (2011) 57–63, https://doi.org/10.1038/nature09922. PMID: 21475195; PMCID: PMC3086762.

[19] B. Williams, A. Landay, R.M. Presti, Microbiome alterations in HIV infection a review, Cell. Microbiol. 18 (2016) 645–651, https://doi.org/10.1111/cmi.12588.