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LIPIDOMICS ANALYSIS IN CEREBROVASCULAR DISEASE AND PERIPHERAL ARTERIAL DISEASE – A SYSTEMATIC REVIEW

by

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LIPIDOMICS ANALYSIS IN CEREBROVASCULAR DISEASE AND PERIPHERAL ARTERIAL DISEASE – A SYSTEMATIC REVIEW

Dissertation presented to *Escola Superior de Biotecnologia* of the *Universidade Católica Portuguesa* to fulfill the requirements of Master of Science degree in Biomedical Engineering

by

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To Mom and Dad

Thank you for your support and for believing in me

Abstract

Introduction: Cardiovascular diseases are the leading cause of death worldwide and one of contemporary medicine's most critical problems. Several classes of lipids are thought to contribute to the development of this disease.

Aim: This systematic review aims to summarize the current evidence on how lipid dysregulation affects these diseases, particularly, cerebrovascular disease and peripheral arterial disease.

Methods: This review was performed according to the PRISMA guidelines. A search was achieved at PubMed combining the word "Lipidomics" with keywords related to the diseases, yielding a total of 305 articles. Inclusion criteria were if there was an association between cerebrovascular disease or peripheral arterial disease and lipid markers and if the articles presented an analysis of the lipidomic profile of patients with the diseases under study. Exclusion criteria were if the articles studied another disease other than those under study and if the articles were a review or an editorial. A total of 15 articles were included in the qualitative analysis, which were divided into four groups with those studying atherosclerosis in general (n=6), those studying cerebrovascular disease (n=3), those studying peripheral arterial disease (n=2), and those who study both diseases (n=4). The Newcastle – Ottawa tool was used to assess the quality of each article.

Results: Among the 11 articles retained on human studies, 7 are cross-sectional studies and 4 case-control studies, and the other 4 are experimental animal studies. The diseases studied were analyzed together and separately, using different methodologies. For lipid extraction, two methods were used: the Liquid-Liquid Extraction, and the Solid-Phase Extraction, and ten distinct methods for the lipidomic analysis. There was greater lipid dysregulation in fatty acids, glycerolipids, glycerophospholipids, and sphingolipids.

Conclusion: Lipidomics is a fundamental tool in the identification of lipids that might play a relevant role in both cerebrovascular disease and peripheral arterial disease, and these biomarkers might be used to improve the individual cardiovascular risk stratification and to provide adequate clinical management. However, they need to be studied in larger settings, and further research is needed.

Keywords: Lipidomics, Atherosclerosis, Cerebrovascular Disease, Peripheral Arterial Disease

Resumo

Introdução: As doenças cardiovasculares são a principal causa de morte no mundo e um dos problemas mais críticos da medicina contemporânea. Acredita-se que várias classes de lípidos contribuam para o desenvolvimento dessa doença.

Objetivo: Esta revisão sistemática visa resumir as evidências atuais sobre como a desregulação lipídica afeta estas doenças, particularmente a doença cerebrovascular e a doença arterial periférica.

Métodos: Esta revisão foi realizada segundo as diretrizes PRISMA. Foi feita uma pesquisa no PubMed combinando a palavra "Lipidomics" com palavras-chave relacionadas às doenças, resultando num total de 305 artigos. Os critérios de inclusão foram se havia associação entre a doença cerebrovascular ou a doença arterial periférica e os marcadores lipídicos; e, se os artigos apresentavam uma análise do perfil lipidómico de doentes com as doenças em estudo. Os critérios de exclusão foram se os artigos estudavam outra doença que não as em estudo e, se os artigos eram uma revisão ou um editorial. Um total de 15 artigos foram incluídos na análise qualitativa, sendo os mesmos divididos em quatro grupos com os que estudam a aterosclerose em geral (n=6); os que estudam a doença cerebrovascular (n=3); os que estudam a doença arterial periférica (n=2); e os que estudam ambas as doenças (n=4). A ferramenta Newcastle – Ottawa foi usada para avaliar a qualidade de cada artigo.

Resultados: Dos 11 artigos retidos sobre estudos em humanos, 7 são estudos transversais e 4 de caso-controlo; e, os outros 4 são experimentais em animais. As doenças estudadas foram analisadas em conjunto e separadamente, utilizando diferentes metodologias. Foram utilizados dois métodos para a extração lipídica: a Extração Líquido-Líquido e a Extração em Fase Sólida; e dez métodos distintos para a análise lipidómica. Verificou-se maior desregulação lipídica nos ácidos gordos, nos glicerolípidos, nos glicerofosfolípidos e nos esfingolípidos.

Conclusão: A lipidómica é uma ferramenta fundamental na identificação de lípidos que podem desempenhar um papel relevante tanto na doença cerebrovascular como na doença arterial periférica, sendo que esses biomarcadores podem ser usados para melhorar a classificação do risco cardiovascular individual e fornecer um enquadramento clínico adequado. No entanto, dever-se-á alargar a amostra em estudo, sendo necessário mais investigação.

Palavras-chave: Lipidómica, Aterosclerose, Doença Cerebrovascular, Doença Arterial Periférica

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List of Abbreviations

CVDs	Cardiovascular Diseases
LDL	Low-Density Lipoprotein
CT	Computerized Tomography
MRI	Magnetic Resonance Imaging
PET	Positron Emission Tomography
SPECT	Single Photon Emission Computed Tomography
CTA	Computerized Tomography Angiography
MRA	Magnetic Resonance Angiography
PAD	Peripheral Arterial Disease
MG	Monoacylglycerols
DG	Diacylglycerols
TG	Triacylglycerols
MS	Mass Spectrometry
LC-MS	Liquid Chromatography with Mass Spectrometry
GC-MS	Gas Chromatography with Mass Spectrometry
GC	Gas Chromatography
LC	Liquid Chromatography
UHPLC	Ultrahigh Performance Liquid Chromatography
UPLC-Q/TOF-MS	Ultra-performance Liquid Chromatography coupled with Quadrupole Time-Of-Flight Mass Spectrometry
UPLC-MS	Ultra-performance Liquid Chromatography with Mass Spectrometry
MeSH	Subject Headings
$ApoE^{-/-}$	Apolipoprotein $E^{-/-}$

UHPLC-QTRAP	Hybrid Triple Quadrupole/ Linear Ion Trap Mass Spectrometry equipped with Ultra-High-Performance Liquid Chromatography
UPLC-QTRAP-MS/MS	Ultra-Performance Liquid Chromatography-Quadrupole Linear Ion Trap Mass Spectrometry
QqQ-MS	Triple-Quadrupole Mass Spectrometry
LC-MS/MS	Ultraperformance Liquid Chromatography-tandem Mass Spectrometry
NP/RP 2D LC-QToF/MS	Normal-phase/Reversed-phase Two-Dimensional Liquid Chromatography–Quadrupole Time-of-Flight Mass Spectrometry
LysoPL	Lysophospholipids
RvD1	Resolvin D1
DHA	Docosahexaenoic Acid
AA	Arachidonic Acid
PC	Phosphatidylcholines
LysoPC	Lysophosphatidylcholines
CAD	Coronary Artery Disease
Cer	Ceramides
CE	Cholesterol Esters
SM	Sphingomyelins
PS	Phosphatidylserines
PE	Phosphatidylethanolamines
LysoPE	Lysophosphatidylethanolamines
LDL-C	Low-Density Lipoprotein Cholesterol
HDL-C	High-Density Lipoprotein Cholesterol
PI	Phosphatidylinositols

PG	Phosphatidylglycerols
FFA	Free Fatty Acids
PUFA	Polyunsaturated Fatty Acids
GlcCer	Glucosylceramides
LysoPI	Lysophosphatidylinositols
LysoPG	Lysophosphatidylglycerols
GalCer	Galactosylceramides
LacCer	Lactosylceramides
PD1	Protectin D1
17-HDHA	17S-hydroxydocosahexaenoic Acid
14-HDHA	14S-hydroxydocosahexaenoic Acid
7-HDHA	7S-hydroxydocosahexaenoic Acid
HEPE	Hydroxyeicosapentaenoic Acid
LysoPS	Lysophosphatidylserines
oxCE	Oxidized Cholesteryl Esters
PE-Cer	Phosphatidylethanolamine-Ceramides
HETE	Hydroxyeicosatetraenoic Acid
DiHETrE	Dihydroxyeicosatrienoic Acid

1. Introduction

Cardiovascular diseases (CVDs) such as coronary heart disease, cerebrovascular disease, and peripheral arterial disease are a category of disorders of the heart and blood vessels [1]. CVDs are the world's greatest cause of death and are one of modern medicine's most serious issues. Genetic and/or lifestyle factors such as diabetes, hypertension, dyslipidemia, obesity, tobacco use, and sedentarism, are the key causes of this group of disorders.

Atherosclerosis is a common aspect of CVDs (Figure 1). The pathogenesis of atherosclerosis consists of three components: (1) biological environment, (2) hemodynamic factors, and (3) genetic or inherited factors. The main processes in atherosclerosis are lipid accumulation and intimal thickening in large and medium-sized arteries, caused by low-grade endothelial injury due to identified risk factors. The resulting endothelial dysfunction induces increased permeability to, and accumulation of, plasma lipoproteins (mainly low-density lipoprotein (LDL)) in the intimal wall, which are oxidized by free radicals generated in macrophages, endothelial cells, and smooth muscle cells [2].

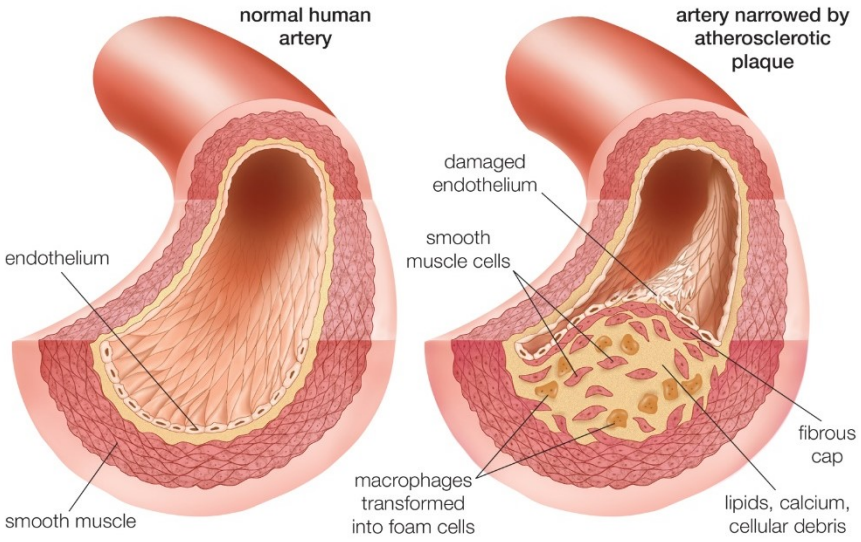

Figure 1 – Main pathophysiological features of atherosclerosis [3].

Recent research in CVDs has been trying to identify specific molecular markers that predict cardiovascular events and the answer to therapeutic strategies that will improve lifespans [4]. Lipidomic research, in particular, has increased rapidly in the last decade, as dysregulation of lipid metabolism is frequently seen in CVDs and is one of the major mechanisms that cause the development of these diseases. As such, lipidomic profiling might be a promising method for the investigation of novel biomarkers and mechanisms in CVDs, such as cerebrovascular disease and peripheral arterial disease [4].

1.1. Cerebrovascular Disease

Cerebrovascular diseases are one of the most common and debilitating diseases in adults. One in ten deaths worldwide is due to a stroke and more than half of the survivors remain dependent on others to perform daily activities [5]. Cerebrovascular disease is characterized as neurological deficits due to arterial insufficiency or occlusion; venous occlusive disease, or hemorrhage with typically focal deficits that can be multifocal in chronic diseases [6]. This type of disease is a condition of the cerebral blood vessels, cerebral blood flow, or the oxygen supply. The vessels that supply the blood to and from the brain are the cerebral vasculature. The brain usually receives its blood supply from four large extracranial arteries that originate from the great thoracic vessels, such as the aorta; these four large extracranial arteries are known as vertebral arteries and common carotid [7].

Atherosclerosis is a primary cause of cerebrovascular disease and may affect small and/or large size vessels. Atherosclerosis happens when high levels of cholesterol, along with inflammation in the brain arteries, induce the build-up of cholesterol as a dense, waxy plaque that may narrow or block blood flow in the arteries. This plaque can restrict the blood flow or completely block it, causing a stroke or transient ischemic attack [8]. Cerebrovascular disorders could occur in any section of the cerebral vasculature, from localized structures outside the brain as the great vessels of the heart or systemic problems. Even when the cerebral vasculature is completely healthy, the supply of oxygen to the brain could be compromised and a stroke could occur [7]. The most common causes of ischaemic, carotid territory stroke are thromboembolism from the internal carotid artery or the middle cerebral artery (25%) and small vessel intracranial disease (25%) [9]. Ischaemic stroke can also result from cardiac embolism (20%), other rarer causes (5%), and undiscovered causes despite investigation (25%) [9].

The major risk factors that can be addressed therapeutically are elevated blood pressure, cardiac disease, and diabetes *mellitus*. Potentially controllable risk factors include unhealthy diet or incorrect nutrition, cigarette smoking, alcohol abuse, obesity, physical inactivity, some blood disorders, lipid abnormalities, and drug abuse. Some studies have shown that a parental history of cerebrovascular disease and genetic predisposition are also important risk factors. The control of risk factors should aim to minimize the absolute risk of developing this type of disease [5].

A cerebrovascular problem may be diagnosed with neuroimaging, neurosonology, cardiac evaluation, and hematologic testing. Usually, these analyses are combined to have a better understanding of the disorder. Different techniques can be used, for example, computerized tomography (CT), magnetic resonance imaging (MRI), ultrasound and doppler ultrasound of the carotid and vertebral arteries, transcranial doppler ultrasound, positron emission tomography (PET), and single photon emission computed tomography (SPECT), and a catheter angiography [7].

CT and MRI are both techniques that belong in the neuroimaging field. CT is mainly used to evaluate the morphology of the brain. Computerized tomography angiography (CTA) uses rapid scanning with a specialized timing of the contrast agent so that the blood in the arteries becomes so "dense" that everything but the blood vessels can disappear [7]. MRI techniques are important for the visualization of the arteries that supply the brain. Magnetic resonance angiography (MRA) enables the visualization of intracranial and extracranial arteries of medium and large caliber. Ultrasound, doppler ultrasound of the carotid and vertebral arteries, and transcranial doppler ultrasound are techniques that belong to the neurosonology field and are noninvasive, reliable, and safe. Nuclear Medicine involves the techniques of PET and SPECT, and both require administration of a radionuclide, where the first emits positrons during radioactive decay and the second emits alpha particles. PET can be used to measure cerebral blood flow, brain metabolism, and the number of specific types of receptors within the brain. SPECT is a technique that is useful to measure cerebral blood flow, both quantitatively and qualitatively [7]. Finally, cerebral angiography requires the insertion of a catheter into the arterial system, the injection of iodinated contrast agents into the cerebral bloodstream, and the rapid acquisition of head plane radiographs [7]. Catheter angiography is less used for routine stroke treatment because the quality of CTA and MRA images has improved [7].

Medical therapy to address CVDs may include antiplatelet agents, anticoagulants, and/or thrombolytics, depending on the clinical status (acute vs chronic) and on the etiology. In selected cases, surgical interventions may be necessary such as in patients with significant carotid stenosis that benefit from carotid endarterectomy or stenting [7].

1.2. Peripheral Arterial Disease

Peripheral arterial disease (PAD) is a relatively common disease in many adults around the world. It is estimated that the incidence of the disease is highly dependent on the age of the population and, as the population ages, the prevalence of PAD is markedly increasing [10,11].

PAD is defined as the narrowing and obstruction of the antegrade flow of major systemic arteries other than cerebral and coronary arteries [12]. It is estimated to have a worldwide prevalence of up to 10%, rising to almost 30% in patients over 50 years of age [2]. Atherosclerosis, which is the accumulation of fats, cholesterol, and other substances in and on the artery walls (plaque), that may restrict blood flow, is the most common cause of PAD. Thus, the main risk factors for PAD include hypertension, smoking, diabetes *mellitus*, hyperlipidaemia, obesity, and a family history of vascular disease; where smoking and diabetes *mellitus* are the strongest predictors of morbidity and mortality, each conferring more than 2.5 times higher risk [12]. PAD is aggressive and more rapidly progressive in patients with diabetes than in non-diabetic patients, reflected in the significantly higher incidence of major amputations in this group, as well as accelerated atherosclerosis and higher rates of cardiac events, particularly in patients with poorly controlled blood glucose [2,12]. The prognosis of PAD patients is characterized by a greater risk of cardiovascular disease, affecting other arterial beds such as coronary heart disease and cerebrovascular disease, since atherosclerosis is a systemic process and therefore there is a strong correlation [11,12].

The cardinal symptom of PAD is intermittent claudication, which is a cramping leg pain caused by ambulation or exercise and relieved by rest [11]. Critical limb ischemia, characterized by persistent resting pain that worsens when the legs are lifted, is one of the most severe forms of the disease. In more serious cases, patients develop gangrene and ulceration and can undergo leg amputation or other surgical intervention [11]. Patients with PAD fit clinically into one of four categories depending on their symptoms: asymptomatic, intermittent claudication, chronic limb ischaemia, or acute limb ischaemia, according to the American College of Cardiology/ American Heart Association practice guidelines [12]. The majority of cases are asymptomatic, resulting in a clinically silent disease, with increased vascular morbidity and mortality. Strategies for diagnosis and treatment are straightforward and widely applicable, however, PAD remains undertreated [12].

In order to diagnose PAD, it is possible to do it with the ankle-brachial index, or with ultrasound, and CT or MRI combined with angiography. The Ankle-Brachial index is the ratio

of blood pressure at the brachial artery to blood pressure at the posterior tibial artery [12]. This method has high sensitivity, approximately 95% in symptomatic patients, and is non-invasive, which makes it an efficient PAD screening tool [2]. The main purpose of using ultrasound is to identify the arterial lesion that could be revascularized and is often used when there is uncertainty in the initial examination. The most commonly used ultrasound is the duplex ultrasound, a non-invasive imaging technique that measures the direction and velocity of blood flow through a vessel. Duplex ultrasound has also a high sensitivity, is readily accessible, and has high specificity [2]. Angiography is used because, depending on the pattern of the disease and intent of the proposed intervention, it could provide more comprehensive details. Angiography has been replaced by CTA and MRA. CTA is mainly used because of its rapid imagine acquisition times and non-invasiveness. Although there are some disadvantages, such as the use of intravenous contrast medium and high radiation exposure. MRA is the chosen second-line investigation for PAD since it does not require the use of contrast or exposure to radiation [2].

When a patient is diagnosed with PAD, there some certain goals to achieve, in order to increase their quality of life. These include improving symptoms and pain-free walking distance, avoiding amputation, and preventing more cardiovascular morbidity. To make this possible, the patient will require care in the form of a treatment, which is divided into 3 groups, lifestyle and risk factor modifications, drug therapy, and surgical intervention [2]. The first step is to change the patient's lifestyle and reduce risk factors. For that, the patient must quit smoking, exercise regularly, and have a healthy diet. It is also important to reduce cholesterol levels, hypertension, and control glycemic levels when the patient has diabetes. Usually, this change is combined with drug therapy, such as antiplatelet agents, statins, and vasoactive agents. In selected patients, surgery is advocated and may include open surgery for example endarterectomy or bypass, or endovascular techniques such as angioplasty with or without a stent, depending on the state of the disease [2].

1.3. Lipidomics

Lipidomics is an emerging field that aims to identify the main role of lipids in the cell; the study focuses on mapping the entire population of lipids in the biological system, defining their structure and biological function. Lipidomics may have a molecular signature to a certain pathway or a disease condition [13]. The role of lipids in cell membrane formation makes them

both ligand and protein substrate, indicating that developments in Lipidomics could have far-reaching consequences for metabolomics, proteomics, and genomics [13].

Generally, lipids are classified as a group of organic compounds that are insoluble in water but soluble in organic solvents, but more broadly described, in chemical terms, as small hydrophobic or amphiphilic small molecules that originate either in part or entirely from two distinct types of building blocks: ketoacyl and isoprene groups [14]. Many of these building blocks have similar structures or are homologous, and the homologies allow lipids to be divided into different major categories [15].

Lipids are crucial components of biological membranes and are a diverse class of metabolites, structurally and functionally. In the biological system, lipids play numerous and significant roles, including storing energy, producing signal transduction, composing membrane bilayer, and providing functional implementations of membrane proteins and their interactions. Lipids have been categorized into eight major categories: fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, sterol lipids, prenol lipids, saccharolipids, and polyketides [16] (Figure 2).

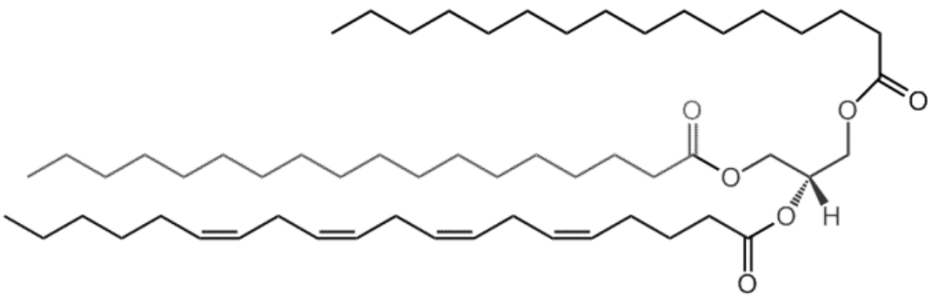

Figure 2 – Example of a Lipid [17].

According to LIPID MAPS, a database with the classification of lipids based on clearly defined biochemical and chemical principles, the fatty acyl structure is the main lipid building block of complex lipids, making it one of the most fundamental categories of biological lipids, characterized by a series of repetitive methylene groups that give this class a hydrophobic property, and the first subclass contains the straight-chain saturated fatty acids with a carboxylic acid in the end [17]. Glycerolipids are lipids with a 3-carbon glycerol backbone and one or two esterified acyl chains at positions sn-1 and sn-2, as well as a polar head at position sn-3. Glycerolipids consist predominantly of mono-, di-, and tri-substituted glycerols known as monoacylglycerols (MG), diacylglycerols (DG), and triacylglycerols (TG) [18]. The glycerophospholipids, most commonly referred to as phospholipids, are characterized by the presence of an esterified phosphate group. They are found everywhere in nature, are essential

components of the lipid bilayer of cells, and also being involved in signaling and metabolism. Sphingolipids are a complex family of compounds associated with the membranes surrounding cells, that share a similar structural feature, a sphingoid base backbone. Sterol lipids, such as cholesterol and its derivatives are essential components of membrane lipids. The sterol lipids and prenol lipids have a common biosynthetic pathway through polymerization of dimethylallyl pyrophosphate/isopentenyl pyrophosphate but have important differences in their structure and function. The category of saccharolipids was developed for lipids in which fatty acyl groups are directly connected to a sugar backbone, forming structures that are compatible with membrane bilayers. The final category is polyketides, which are a diverse group of metabolites from plants, microbial sources, and animals [17,19].

Lipidomics can be categorized into three analytical goals: focused lipidomics, targeted lipidomics, and untargeted lipidomics; wherein focused lipidomics the objective is to analyze a particular group of lipid metabolites, the objective in targeted lipidomics is to analyze a few important lipids, and the strategy in untargeted lipidomics is to analyze a very wide variety of lipids in biological samples [16].

To perform lipidomic analysis of biological samples, it is necessary to do sample preparation, mass spectrometry-based analysis, and data processing (Figure 3). The first step in the preparation of the sample is an extraction procedure because the lipids are embedded in a complex biological matrix and do not appear in their free form. The general procedures include the removal of any nonlipid component and the fractionation and separation of lipids from the extract [16].

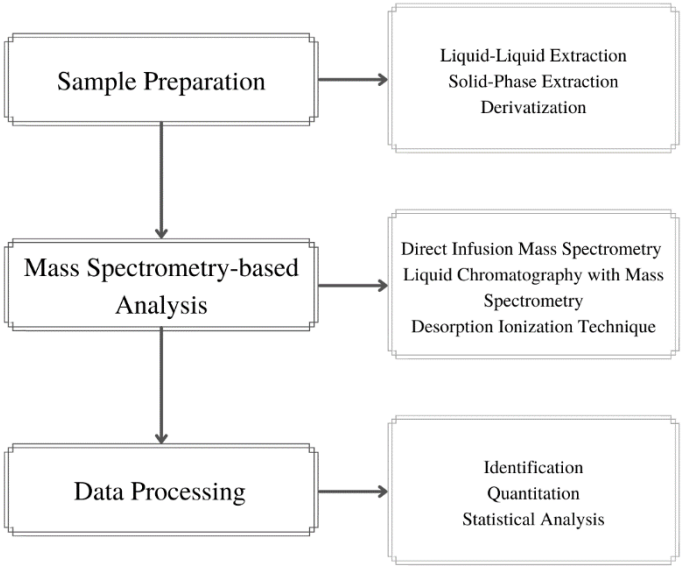

Figure 3 – Lipidomics Workflow.

There are various extraction methods used in Lipidomics and the selection of the technique depends on the analytical matrix. Usually, in Lipidomics, there are two main extraction methods, liquid-liquid extraction, and solid-phase extraction. During extraction, phase separation between immiscible solvents occurs, with lipids partitioning into the hydrophobic phase. Different solvents, whether single or mixed, were suggested as extracting solvents, for example, methanol and ethanol. In untargeted lipidomics, the most effective extraction method for more detailed lipids could be the liquid-liquid extraction and in targeted lipidomics, the method that can boost the specificity and efficiency of extraction is solid-phase extraction [16]. In liquid-liquid extraction, the chemical properties of the two immiscible solvents differ, so the distribution of the solute between the two solvents results in a separation of the components based on the distribution or partition between the solvents. Liquid-Liquid extraction selectively separates the substances from a mixture and removes impurities from the solution [20]. Solid-phase extraction is a fast extraction method that can reduce degradation and arrange automatic preanalytical facilities for the preparation of multiple samples at the same time. This method eliminated the need for solvent/water mixture separation, reducing solvent and time consumption [16].

In the past, thin-layer chromatography has also been used for lipid analysis, however today it is primarily used to prepare selected lipids or lipid (sub)classes for separation. For many decades, gas chromatography with mass spectrometry (GC-MS) has been used in lipid analysis. Gas chromatography (GC) is restricted for many nonvolatile lipids, as chemical components must be thermally stable with sufficient vapor pressure to volatilize during the injection, while derivatization may solve volatility and lability, the chemical derivation may be difficult for complex biological samples and may require multiple reactions steps [16].

After extraction, the sample is ready for analysis. The main approaches for lipidomic analysis include direct infusion mass spectrometry (MS) analysis, liquid-phase separations coupled to MS (usually liquid chromatography with mass spectrometry (LC-MS)), and desorption ionization techniques [21].

In MS, a process also known as Shotgun Lipidomics, a crude lipid extract is injected into the MS instrument, direct MS scans are usually used in high-resolution MS, but can also be done in conjunction with ion mobility on low-resolution instruments [21]. The fragmentation of lipid molecules creates lipid class-selective fragments typically found with lipid species of the same lipid class and in the negative ion mode, lipid molecules can be additionally classified

by fragment ions matching hydrocarbon chains and can be simplified as molecular lipid species [21]. The technique of the shotgun is simple and is fast, although its key disadvantage is ion suppression, which hinders the quantitative robustness and sensitivity of the determination [16]. Typically, the shotgun technique uses chemical standards to correct the matrix effects, but standards are not available for all lipids. In addition, isomeric and isobaric species are not differentiated by the shotgun process and often show identical fragmentation profiles of MS [16].

A significant advantage of LC−MS for lipidomic analysis is its relatively large scale of separation modes, which can be adapted to almost all known lipid isomers types. Reversed-phase liquid chromatography (LC) is the most popular method, as it can provide a complex separation based on the length of the fatty acyl and the number of double bonds and their positions [21]. The new approach in LC is the use of sub-2 mm particles and high-operating pressures, ultrahigh performance liquid chromatography (UHPLC), that give greater efficiency. The use of 2D LC is another common technique, in which two chromatographic modes with orthogonal separation selectivity can be combined to give higher peak capacities either in offline or online modes [21]. In current years, ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UPLC-Q/TOF-MS), which uses different types of mass spectrometers, has been commonly used for both targeted lipidomics and untargeted lipidomics, from a basic single quadrupole to hybrid instruments and high-resolution instruments [16]. Usually, the ultra-performance liquid chromatography with mass spectrometry (UPLC-MS) technique is used to isolate numerous lipids in the profiling studies of different biological samples [16].

The matrix-assisted laser desorption/ionization is part of the group of techniques for desorption ionization used to study biological tissues and cells including mass spectrometry imaging, providing information on the spatial distribution of individual molecules, especially lipids, small peptides, and metabolites [21].

The final step in Lipidomic analysis is data analysis, which is an important role in Lipidomics, especially in untargeted lipidomics with a very large amount of data. It is a major challenge to explain the data without adequate bioinformatics. In MS-based Lipidomics, the first step in data processing is lipid identification, many software packages, and lipid databases have been designed to reach this goal. The second step in data processing is to normalize the data using a series of internal standards, and after these measurements have been implemented, the lipids detected can then be quantified by comparison with the appropriate internal standards.

The final step is to conduct statistical analysis of complex data sets, univariate statistical analysis is typically used for comparison between experimental groups in Lipidomics; the principal component analysis method is becoming increasingly common in Lipidomics, an unsupervised analysis that enables any structure in a data set to be observed without any prior knowledge or manipulation [16]. Lipidomics attempts to compare differences between groups and it is important to use a supervised multivariate analysis to identify these significant differences [16].

Lipids play a crucial role in cellular structure, energy storage, and signaling, and several individual lipid molecules have been correlated with the evolution of various diseases [16]. Different studies have shown that lipids metabolic disorders or defects can lead to a diversity of human diseases. Lipidomics is a technique for the investigation of clinical applications, such as arteriosclerosis, brain injuries, and coronary heart disease.

1.4. Purpose of this work

In this dissertation, the goal is to study the available evidence on how the concentration of different lipids (dysregulation of lipid metabolism) affects cardiovascular disease, in more depth, cerebrovascular disease, and peripheral arterial disease. To accomplish the aims dissertation, we conducted a systematic review of articles where cardiovascular diseases were studied to understand how the concentration of certain lipids would affect the conditions studied.

Therefore, in the first phase, we performed a broad selection of literature found on the topic cardiovascular disease, and in a second phase, the list was reduced with the purpose of describing, specifically, the association between cerebrovascular disease, and peripheral arterial disease and lipid markers.

The structure of this dissertation is divided into 6 chapters: Introduction, where the diseases of the study were described and how/ what is Lipidomics; Methodology, the process through which we collected the articles for this review; Results, a general description, the methodology used in the articles and the identified lipids; Discussion, lipids categories and the lipids that correlate with the diseases and the role of the lipids in the human body as well as the limitations that were listed in this review; Conclusions, if Lipidomics is a viable method of studying CVDs; and Future Work, what needs to be improved in the future.

2. Methodology

This chapter is reserved for the presentation of the chosen methodology for this dissertation, the requirements for the selection of the articles, and the organization of the articles in groups to analyze how lipids can affect cardiovascular diseases. This systematic review was conducted under the recommendations of the PRISMA statement [22].

2.1. Literature Search

PubMed was searched from inception to 03/2020 using the medical subject headings (MeSH) term "Lipidomics" combined with keywords describing cerebrovascular disease or peripheral arterial disease. For cerebrovascular disease, the keywords were "Stroke", "Carotid Artery Diseases", and "Cerebrovascular Disorders". For peripheral arterial disease, the keywords used were "Atherosclerosis", "Peripheral Vascular Diseases", and "Vascular".

An article was included if it described, specifically, the association between cerebrovascular disease, or peripheral arterial disease and lipid markers. Studies were included if they presented an analysis of the lipidomic profile of patients suffering from cerebrovascular disease and peripheral arterial disease. Studies were excluded if they studied another disease other than cerebrovascular disease or peripheral arterial disease and if it was a review or an editorial.

This research yielded a total of 305 articles, where 36 to Lipidomics and Cerebrovascular Disease and 269 to Lipidomics and Peripheral Arterial Disease.

2.2. Article Selection

Two investigators independently screened the candidate articles by checking the title and abstract using Rayyan QCRI Software (Doha, Qatar). Articles that were still considered candidates by at least one investigator were scrutinized through full-text reading. The remaining discrepancies between the two investigators were solved by a third investigator. Final inclusion was obtained after that.

Rayyan QCRI is a free web application to assist investigators in determining whether or not to include the article in the dissertation work. The full list of references yielded in the PubMed search was transferred to Rayyan. Using Rayyan platform, the title and abstract from

each article were reviewed in order to determine if the articles were suitable or not. For each article, one of the following options was selected: include, maybe, or exclude. “Maybe” articles were later decided by revision of their full text. In the end of this phase, in the peripheral arterial disease folder, 99 articles were included and 170 excluded. In the cerebrovascular disease folder, 8 articles were included and 28 excluded (Appendix 1). The full text was obtained for all the included articles.

2.3. Data Collection

In the data collection stage, an Excel sheet was created to describe the characteristics of the included articles. These features included the following variables: Publication Date; Hypothesis; a study in Human/ Animal; Studied Population; study Target/ Untargeted; study with Follow-up/ No Follow-up; Study; Analysis done in; Study Disease; Technique used; Statistical Analysis; Results; and Conclusions (Appendix 2). A second wave of rejection was performed during this phase if the full text did not provide important information on the main outcomes of this review. The final list of included articles consisted of 15 publications divided into four groups (Appendix 3).

2.4. Bias Assessment

Following the selection of the articles, the next step was to assess the quality of each article. For this purpose, the quality tool selected was the Newcastle – Ottawa Scale.

The Newcastle – Ottawa Scale was created in collaboration with the Universities of Newcastle (Australia) and Ottawa (Canada) to analyze the quality of non-randomized studies to be used in systematic reviews [23]. The Newcastle – Ottawa Scale is available in two versions: one for cohort studies and one for case-control studies. Both versions include eight multiple-choice questions divided into three dimensions: selection, comparability, and outcome for cohort studies, or exposure for case-control studies. For each question, some response options are presented. In the Newcastle – Ottawa Scale a star system is used. This star system provides a semi-quantitative assessment of study quality, with the highest quality studies obtaining a maximum of one star for each response option, except the comparability question, which could receive up to two stars [24]. The Newcastle – Ottawa Scale has a scale of zero to nine stars.

The Newcastle – Ottawa Scale version used for this systematic review was the case-control studies version, and two questionnaires were created, one for human studies and one for animal studies (Appendix 4).

In the analysis on the human studies, in the questionnaire, the articles receive between four to nine stars, with the highest punctuation going to Paapstel *et al.* (2018) [25], and Stegemann *et al.* (2014) [26] and the lowest going to Mishra *et al.* (2020) [27] (Table 1).

Table 1 – Newcastle – Ottawa Scale for human studies.

Article	Selection	Comparability	Exposure	Total
Mishra *et al.*, 2020 [27]	★ ★		★ ★	4
Paapstel *et al.*, 2018 [25]	★ ★ ★ ★	★ ★	★ ★ ★	9
Bazan *et al.*, 2017 [28]	★ ★	★ ★	★ ★ ★	7
Yang *et al.*, 2017 [29]	★ ★ ★	★ ★	★ ★ ★	8
Caligiuri *et al.*, 2017 [30]	★ ★ ★		★ ★ ★	6
Vorkas *et al.*, 2016 [31]	★ ★		★ ★ ★	5
Vorkas *et al.*, 2015 [32]	★ ★	★ ★	★ ★ ★	7
Li *et al.*, 2014 [33]	★ ★ ★ ★		★ ★ ★	7
Stegemann *et al.*, 2014 [26]	★ ★ ★ ★	★ ★	★ ★ ★	9
Clària *et al.*, 2013 [34]	★ ★ ★		★ ★ ★	6
Stegemann *et al.*, 2011 [35]	★ ★	★ ★	★ ★	6

There was a low risk of bias in case definition adequate, representativeness of the cases, and in the ascertainment of exposure, it received 100%. In terms of control selection, 27% of the articles had a low risk of bias, while 73% had a high risk of bias, meaning that 27% of the articles employed community controls and 73% used hospital controls or did not have controls in the study. According to the definition of controls, 55% of the articles had a low risk of bias, while 45% had a high risk of bias, which means that 55% of the articles used controls without the disease in the study, whereas 45% of the articles used patients as controls for themselves, or studied different stages of the disease, or studied patients with pre-existing conditions, or did not have controls. In the comparability of cases and controls, 55% of the articles have a low risk of bias, while 45% have a high risk of bias, indicating that 55% of the articles employed the same design in the groups and the other 45% did not. In the last two options, both had 91% of the articles with a low risk of bias and 9% with a high risk of bias. The same method of ascertainment for cases and controls means that all the articles, except for Mishra *et al.* (2020) [27], utilized the same method of analysis for the case and the control. The non-response rate indicates that all of the population investigated was available for analysis, except Stegemann *et al.* (2011) [35] (Figure 4).

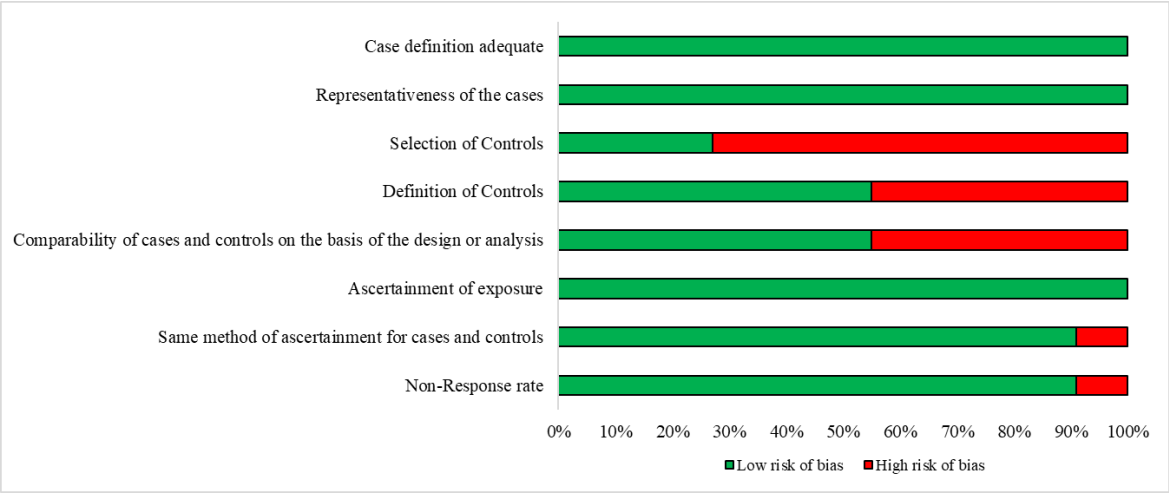

Figure 4 – Risk of bias in human studies.

In the analysis of animal studies, all of the articles obtained nine stars, suggesting that there was a low risk of bias for each response option in the questionnaire (Table 2).

Table 2 – Newcastle – Ottawa Scale for animal studies.

Article	Selection	Comparability	Exposure	Total
Yan *et al.*, 2019 [36]	★ ★ ★ ★	★ ★	★ ★ ★	9
Lee *et al.*, 2017 [37]	★ ★ ★ ★	★ ★	★ ★ ★	9
Chen *et al.*, 2017 [38]	★ ★ ★ ★	★ ★	★ ★ ★	9
Jové *et al.*, 2013 [39]	★ ★ ★ ★	★ ★	★ ★ ★	9

All the response options received 100%, so this analysis demonstrates that it is simple to control all factors in laboratory investigations, resulting in the best outcomes (Figure 5).

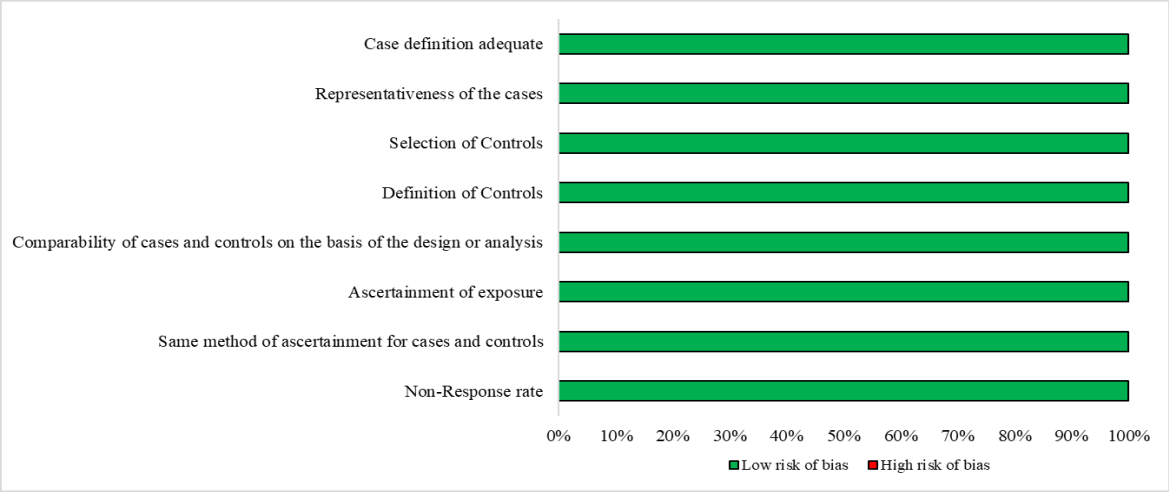

Figure 5 – Risk of bias in animal studies.

After analyzing the quality of each article, we can conclude that the articles chosen are suitable for this systematic review because they are complete and contain the majority of the material required to accomplish this assignment.

3. Results

3.1. General description

The included articles were published in the last 10 years, where 40% were performed in Europe, 20% in Asia, 33% in North America, and 7% together with North America and Europe.

Most studies were conducted in humans with four being conducted in animal models, two using the Male C57BL/6J and Apolipoprotein $E^{-/-}$ ($ApoE^{-/-}$) mice, one using the Male C57BL/6J mice, and one using the male Golden Syrian hamsters (*Mesocricetus auratus*).

The studies performed in humans were all observational studies where seven were cross-sectional studies and four case-control studies; the remaining four studies performed in animals were experimental.

3.2. Methodology

In lipidomic analysis, the collection of samples from the study population is followed by sample preparation, in which the lipids are extracted from the samples, sample analysis, and finally data processing and validation of the results. A brief description of the included studies is presented in Table 3 and the next paragraphs.

In Mishra *et al.* (2020) [27], the study aim was to conduct a system-level analysis of Lipidomics data to classify lipid species networks correlated with subclinical markers of both osteoporosis and atherosclerosis. The study begins with 3 596 children and adolescents and they were followed for about 40 years, but only 1 494 participants, aged 30 – 45, participated in the study, with four atherosclerotic and six osteoporotic markers.

In Paapstel *et al.* (2018) [25], the goal was to analyze serum phosphatidylcholines (PC) and lysophosphatidylcholines (LysoPC) for arterial stiffness, hemodynamic and endothelial dysfunction in patients with coronary artery disease (CAD), PAD, and in clinically healthy subjects. In this study, a total of 124 male subjects, where 32 patients with PAD, 52 with CAD, and 40 healthy controls.

In Bazan *et al.* (2017) [28], they wanted to establish whether circulating serum levels of resolvin D1 (RvD1), docosahexaenoic acid (DHA), and arachidonic acid (AA) represent the risk of atherosclerotic plaque rupture. For the study, there were 24 asymptomatic and 21 acutely

symptomatic patients, that were candidates for the carotid endarterectomy with ≥ 50% internal carotid stenosis. The samples were from peripheral blood.

In Yang *et al.* (2017) [40], the goal was to conduct a Lipidomics analysis to explore potential lipid biomarkers for lacunar infarction diagnosis. For this study, 56 human plasma samples, six lacunar infarction patients, and six controls were used in the discovery phase to identify biomarker candidates, and another 29 lacunar infarction patients and 15 control subjects to define plasma lipid biomarkers.

In Caligiuri *et al.* (2017) [30], it was investigated whether omega-6 fatty acid-based eicosanoids increase the chances of cardiovascular and cerebrovascular events in PAD patients. In total there were 98 patients (25 female and 73 male) with PAD, with the prevalence of transient ischemic attacks (n=16), cerebrovascular accidents (n=10), stable angina (n=16), and acute coronary syndrome (n=24). In these 98 patients, four had 3 of 4 potential events, 17 had 2 events, 20 had 1 event, and 57 had no cardiovascular or cerebrovascular event.

In Vorkas *et al.* (2016) [31], they researched whether, according to patient symptomatic status, a stenosing carotid plaque tissue will show a particular metabolic signature. The samples were obtained from five symptomatic patients undergoing carotid endarterectomy and five asymptomatic patients.

In Vorkas *et al.* (2015) [32], they researched the metabolic changes of the development from intimal thickening to stenosing plaque formation from human carotid and femoral endarterectomy. For this study, plaque tissues samples from a total of 78 patients were used, where 56 underwent carotid endarterectomy and 26 underwent femoral endarterectomy. The areas of intimal thickening were separated from the stenosing plaque segment, nine samples from the carotid, and seven from the femoral. The tissue of intimal thickening present in the proximal and distal extents of stenosing atheroma was used as control tissue.

In Li *et al*. (2014) [33], the goal was to separate and identify the lipid species in plasma to study atherosclerosis. In this study, the subjects were between 40 – 75 years old with carotid artery atherosclerosis, as well as subjects that were under stroke prevention therapy, so there were two groups, six controls, and six atherosclerosis patients.

In Stegemann *et al*. (2014) [26], the study focuses on a lipidomic analysis of the prospective population-based Bruneck Study and analyzes the connection of 135 different lipid species with the risk for CVDs over a 10-year observation period. The Bruneck Study is a

prospective, population-based survey of the epidemiology and pathogenesis of atherosclerosis and CVDs, conducted in the 1990 baseline assessment of the aged-and sex-stratified random sample of all Bruneck population. In 2000, there were still 702 subjects alive and participated in the follow-up. In the 2000 follow-up, after an overnight fast and 12 hours of smoking abstinence, citrate plasma samples were drawn. For 685 individuals, plasma samples were eligible for lipidomic analysis. A cardiovascular event occurred in 90 of the 685 individuals who were qualified for the lipidomic analysis.

In Clària *et al.* (2013) [34], they analyzed the profiles of lipid mediators in human adipose tissue from PAD patients who advanced to a clinical need for severe lower-extremity amputation and compared them with those collected from control subjects undergoing elective orthopedic procedures. A total of 26 subjects were used for the study, where 14 PAD patients undergoing lower extremity amputation and 12 control subjects with no history of PAD. In the PAD patients, a harvested adipose tissue was used to extract subcutaneous fat from the proximal portion of the amputation stump (n=13), perivascular fat surrounding one of the major arteries (n=14), and even subcutaneous fat from patients with open foot ulcers, peri-wound (n=9) and non-wound (n=7).

In Stegemann *et al.* (2011) [35], the goal was to reveal a lipid signature for plaque vulnerability, to compare samples from radial arteries, endarterectomy from symptomatic and asymptomatic patients, and stable and unstable areas within the same symptomatic lesion. For the study, 26 patients were included. The samples were taken from carotid or femoral endarterectomies and radial arteries. The stable and unstable samples were from eight carotid plaques. Plasma samples were also used, in this study, from 35 patients undergoing carotid endarterectomy.

In Yan *et al.* (2019) [36], the aim was to study lysophospholipids (LysoPL) profiles in $ApoE^{-/-}$ model group and wild type control group at various times. There were twelve $ApoE^{-/-}$ mice and twenty C57BL/6J 8-weeks-old wild type male mice. In the begging the mice were fed with a normal diet, after one week of acclimatization, the C57BL/6J mice were divided into two control groups, the normal diet, and the high-fat diet. The $ApoE^{-/-}$ mice were fed with a high-fat diet and served as the model group.

In Lee *et al.* (2017) [37], they used an integrated metabolomic and lipidomic analysis to explain cardiac metabolism in an atherogenic diet-induced atherosclerosis mouse model. The animals for this study were five-week-old male C57BL/6J mice. The mice were divided into a

normal diet (n=38) and an atherogenic diet (n=36) groups. After one week of adaptation, the atherogenic diet group did have a modification where was added 1.25% (w/w) cholesterol and 0.5% cholate (w/w).

In Chen *et al.* (2017) [38], the effects of a high-fat diet on plasma lipid profiles in ApoE$^{-/-}$ and wild-type mice were investigated and were proposed to identify lipid biomarkers that lead to atherosclerotic lesions comparing control and model animal groups, in combination with pattern recognition analysis methods. The mice used were male C57BL/6J and ApoE$^{-/-}$ mice with 10 – 11 weeks old. After one week of acclimatization, the mice were divided into three groups. The C57BL/6J mice were divided into two control groups, randomly and treated with a high-fat diet (n=15) and a normal diet (n=16). The ApoE$^{-/-}$ mice (n=15) were the model group fed with a high-fat diet and all groups were on diet for 16 weeks. Before the animals were sacrificed, plasma samples were obtained for biochemical and lipidomic analysis.

In Jové *et al.* (2013) [39], the aim was to analyze changes in the aortic and plasma lipidomes in a diet-induced model of early atheromatous. The atherogenesis was initiated in male Golden Syrian hamsters (n=15 – 16 per group). The diet of both control and atherogenic groups was maintained for three months. An additional group consumed the same diet as the atherogenic group but supplemented it with 0.2% grape seed extract to evaluate dietary modulation of atheromatous.

The main used extraction for each article was the Liquid-Liquid Extraction [25-27,31-33,35-40] and only 3 articles used the Solid-Phase Extraction [28,30,34].

Different methods for extracting lipids can be used in liquid-liquid extraction, and we will go over what that might be. An extraction begins with the application of internal standards to the sample, followed by the application of a solution, such as chloroform/methanol. After being sonicated or vortexed, the sample is centrifuged. Afterward, water is added to the supernatant, and the sample is centrifuged once more. The lower organic phase is then removed and dried under nitrogen evaporation before being stored. For lipidomic analysis, the dried extract is redissolved in solution, for example, chloroform/methanol [40].

We will also provide an example of how the solid-phase extraction procedure will work. For this example, the sample is adipose tissue, so for protein precipitation, two volumes of cold methanol are added to the frozen sample and stored. After centrifuging the sample, the supernatants are extracted. Following that, the organic solvent is removed with a nitrogen stream, and the sample is suspended in methanol and acidified with hydrochloric acid. The

acidified sample is then loaded into a C-18 solid-phase extraction column, where it is neutralized and eluted with hexane and methyl formate. Finally, the solvents are extracted using a nitrogen stream, and the residues are suspended in the mobile phase for lipidomic analysis [34].

For the Lipidomics analysis, four of the ten methods used were hybrid triple quadrupole/ linear ion trap mass spectrometry equipped with ultra-high performance liquid chromatography (UHPLC-QTRAP) [27], UPLC-Q/TOF-MS [38], ultra-performance liquid chromatography-quadrupole linear ion trap mass spectrometry (UPLC-QTRAP-MS/MS) [36] and flow injection analysis tandem mass spectrometry and liquid chromatography [25]. The method triple-quadrupole mass spectrometry (QqQ-MS) was used in 2 articles [26,35]. The method LC-MS was used in 2 articles [37,39]. The method ultraperformance liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used in 3 articles [28,30,34]. The method UPLC-MS was used in 2 articles [31,32]. The method normal-phase/reversed-phase two-dimensional liquid chromatography-quadrupole time-of-flight mass spectrometry (NP/RP 2D LC-QToF/MS) was used in two articles [33,40]. The methods GC and LC-MS/MS were both used in one article [30].

Table 3 – Main methodological features of the included studies.

Article	Human/ Animal	Studied Population	Targeted/ Untargeted	Follow-up	Study	Analysis done in	Studied Disease	Lipid Extraction	Used technique
Mishra *et al.*, 2020 [27]	Human	1 494 participants, aged 30 – 45	Untargeted	Yes	Lipidomic analysis	Serum samples	Osteoporosis and Atherosclerosis	Liquid-Liquid Extraction	UHPLC-QTRAP
Paapstel *et al.*, 2018 [25]	Human	A total of 124 male subjects	Targeted	No	Serum PC and LysoPC	Peripheral venous blood samples	Atherosclerosis	Liquid-Liquid Extraction	Flow Injection Analysis tandem MS and LC
Bazan *et al.*, 2017 [28]	Human	24 asymptomatic and 21 acutely symptomatic patients	Targeted	No	Serum RvD1, DHA, and AA levels	Peripheral blood samples	Risk of atherosclerotic plaque rupture	Solid-Phase Extraction	LC-MS/MS
Yang *et al.*, 2017 [40]	Human	A total of 56 participants	Untargeted	No	Lipid biomarkers	Plasma samples	Lacunar Infarction	Liquid-Liquid Extraction	NP/RP 2D LC-QToF/MS
Caligiuri *et al.*, 2017 [30]	Human	98 patients (25 female and 73 male) with PAD	Targeted	No	Oxylipins and Fatty Acids	Plasma samples	Cardiovascular/ Cerebrovascular events in patients with PAD	Solid-Phase Extraction	GC LC-MS/MS

Table 3 – Main methodological features of the included studies. (cont).

Article	Human/ Animal	Studied Population	Targeted/ Untargeted	Follow-up	Study	Analysis done in	Studied Disease	Lipid Extraction	Used technique
Vorkas *et al.*, 2016 [31]	Human	10 patients - symptomatic and asymptomatic	Untargeted	No	Metabolomic analysis	Carotid plaque tissue samples	Stroke risk	Liquid-Liquid Extraction	UPLC-MS
Vorkas *et al.*, 2015 [32]	Human	A total of 78 subjects	Untargeted	No	Metabolomic analysis	Plaque tissue samples	Atherosclerosis	Liquid-Liquid Extraction	UPLC-MS
Li *et al.*, 2014 [33]	Human	6 controls and 6 atherosclerosis patients, aged 40-75	Untargeted	No	Lipidomic analysis	Plasma samples	Atherosclerosis	Liquid-Liquid Extraction	NP/RP 2D LC-QToF/MS
Stegemann *et al.*, 2014 [26]	Human	685 participants	Untargeted	Yes	Lipidomic analysis	Plasma samples	Cardiovascular diseases	Liquid-Liquid Extraction	QqQ-MS
Clària *et al.*, 2013 [34]	Human	A total of 26 subjects	Untargeted	No	Lipid mediators	Adipose tissue samples	PAD	Solid-Phase Extraction	LC-MS/MS

Table 3 – Main methodological features of the included studies. (cont).

Article	Human/ Animal	Studied Population	Targeted/ Untargeted	Follow-up	Study	Analysis done in	Studied Disease	Lipid Extraction	Used technique
Stegemann *et al.*, 2011 [35]	Human	26 subjects and 35 patients	Untargeted	No	Lipidomic analysis	Endarterectomies, control radial arteries and plasma samples	Atherosclerotic Plaques	Liquid-Liquid Extraction	QqQ-MS
Yan *et al.*, 2019 [36]	Animal	Male C57BL/6J and ApoE$^{-/-}$ mice	Targeted	No	LysoPL	Plasma samples	Atherosclerosis	Liquid-Liquid Extraction	UPLC-QTRAP-MS/MS
Lee *et al.*, 2017 [37]	Animal	Male C57BL/6J mice	Untargeted	No	Lipidomic and Metabolomic analysis	Sera and Heart samples	Atherosclerosis	Liquid-Liquid Extraction	LC-MS
Chen *et al.*, 2017 [38]	Animal	Male C57BL/6J and ApoE$^{-/-}$ mice	Untargeted	No	Lipidomics analysis	Plasma samples	Atherosclerosis-induced dyslipidemia	Liquid-Liquid Extraction	UPLC-Q/TOF-MS
Jové *et al.*, 2013 [39]	Animal	Male Golden Syrian Hamsters	Untargeted	No	Lipidomic and Metabolomic analysis	Descending aorta and Plasma samples	Atherosclerosis	Liquid-Liquid Extraction	LC-MS

3.3. Lipids identified

After methodology analysis, the next step is to consider the results of each article and see how the lipids affected the diseases. The articles were divided into four groups, with the first group containing articles on atherosclerosis in general, the second containing articles on cerebrovascular disease, the third containing articles on PAD, and the fourth containing articles on both diseases, cerebrovascular and PAD.

a) Atherosclerosis, in general

In the first group, the articles analyzed were those researching atherosclerosis and for these groups, the articles were divided into subgroups, those that used human samples and those that used animal samples (Table 4).

Table 4 – Identified lipids in the first group.

Groups / **Lipids**	**Atherosclerosis, in general**					
	Mishra *et al.*, 2020 [27]	Stegemann *et al.*, 2014 [26]	Chen *et al.*, 2017 [38]	Jové *et al.*, 2013 [39]	Lee *et al.*, 2017 [37]	Yan *et al.*, 2019 [36]
Fatty Acyls	–	–	–	↗	↗	–
MG	–	–	CNA	↗	–	–
DG	↗	–	CNA	–	↗	–
TG	↗	↗	CNA	↗	↗	–
PC	↗	↗	↗	↗	↗	–
LysoPC	–	↗	↗	–	↗	↗
PE	–	↗	CNA	↗	↗	–
LysoPE	–	↘	–	–	↗	↗
PS	–	↘	CNA	–	–	–
LysoPS	–	–	–	–	–	–
PG	↗	–	CNA	↗	–	–
LysoPG	–	–	–	–	–	–
PI	–	–	CNA	–	–	–
LysoPI	–	–	–	–	–	↗
Sphingosine	–	–	CNA	↗	–	–
Sphinganine	–	–	↗	↗	–	–
Cer	↗	–	CNA	↗	↗	–
SM	–	↗	↗	–	↗	–
PE-Cer	–	–	–	–	–	–
GlcCer	–	–	–	↗	↗	–
LacCer	–	–	–	–	–	–
GalCer	–	–	–	–	–	–
Free Cholesterol	–	–	–	↗	–	–
CE	–	↗	–	–	–	–
oxCE	–	–	–	–	–	–

Legends: ↗ any correlation with the disease (positive or negative); ↘ no correlation; – no data; CNA – data not shown

In Mishra *et al.* (2020) [27], lipids modules were used, which are weighted networks of lipid species. These lipid profiles were summarized in each module by the module eigenlipid, which is known as the first major component of the modules' lipid profiles. The findings of this study were that of the twelve studied molecular lipid modules, one was identified with 105 lipid species that are substantially and jointly associated with both subclinical osteoporosis and atherosclerosis markers. Most lipid species in the module belong to the categories of glycerolipids (n=60), glycerophospholipids (n=13), sterol lipids (n=3), and sphingolipids (n=29), and this module was enriched with Ceramides (Cer) (n=20). In the glycerolipids category, it was identified 19 DG and 41 TG. In the glycerophospholipids category, it was identified 7 PC. In the multivariance analysis, there were 37 lipid species associated with markers in one module, and the most relevant biomarkers of both osteoporosis and atherosclerosis were TG, TG (18:0/18:0/18:1), TG (18:0/18:1/18:1), and TG (16:0/18:0/18:1) [27].

In Stegemann *et al*. (2014) [26], the shotgun Lipidomics identified 135 species of lipids, which belong to 8 different subclasses of lipids: TG, PC, LysoPC, sphingomyelins (SM), cholesterol esters (CE), phosphatidylserines (PS), phosphatidylethanolamines (PE), lysophosphatidylethanolamines (LysoPE). The risk of CVDs was significantly correlated with TG, CE, PE, PC, LysoPC, and SM when analyzing each lipid species. Only 28 lipid species remained significantly correlated with CVDs risk after data processing. Between the lipid species with the highest predictive value were TG and CE with a low carbon number and double-bond content, including TG (54:2) and CE (16:1), as well as PE (36:5) [26].

In both articles from the subgroup of human samples, the most relevant lipid species found belong to the categories of glycerolipids, glycerophospholipids, and sphingolipids.

Atherosclerosis was also studied in the next few articles, but in these cases, samples were taken from animals. There were used two types of animals, the C57BL/6J and $ApoE^{-/-}$ mice and Golden Syrian hamsters. The $ApoE^{-/-}$ mice are used in atherogenesis research since their lesion formation is similar to humans. Some studies have previously confirmed that Apolipoprotein E is a ligand for receptors accountable for plasma lipoprotein clearance, and its insufficiency can lead to cholesterol-rich remnants accumulation, which is an increased level of plasma TG, so, $ApoE^{-/-}$ mice may develop atherosclerosis [36].

In Chen *et al.* (2017) [38], from the lipidomic analysis, a total of 43 plasma samples from the high-fat diet, normal diet, and model group were collected, with three mice sacrificed in this

last group. Total cholesterol, low-density lipoprotein cholesterol (LDL-C), TG, and high-density lipoprotein cholesterol (HDL-C) levels were measured to investigate the impact of a high-fat diet on the total lipid profile in the plasma. Total cholesterol and LDL-C levels were higher in the model group than in the high-fat diet group. When compared to the normal group, the high-fat diet group had higher TG levels, while the model group had lower TG levels. The analysis of the plasma samples identified four categories of lipids, including glycerolipids, glycerophospholipids, sphingolipids, and sterol lipids. In the category of glycerolipids, 6 MG, 10 DG, and 127 TG were identified. In the category of glycerophospholipids, 41 PE, 64 PC, 6 phosphatidylinositols (PI), 6 PS, and 3 phosphatidylglycerols (PG) were identified. In the category of sphingolipids, it was identified 2 sphinganines, 12 SM, 5 Cer, and 2 sphingosines. In the category of sterol lipids, 2 cholesterols were identified [38].

When the normal diet group was compared to the high-fat diet group, seven lipid species with the most significant changes were identified, namely LysoPC (16:0), PC (16:0/22:6), PC (18:1/18:2), PC (16:0/18:1), PC (18:0/18:2), PC (22:2/14:0), and PC (18:0/18:1). The comparison between the results of the high-fat diet and the model group reveals that ten significantly altered lipid species associated with atherosclerosis were identified, namely C16 sphinganine, SM (d18:1/16:0), PC (18:2/20:4), PC (16:0/16:0), PC (16:0/18:1), PC (18:0/16:1), PC (18:0/18:1), SM (d18:1/24:1), SM (d16:0/28:5) and PC (17:1/22:6) [38]. The levels of PC (16:0/22:6) and PC (17:1/22:6), with a high degree of unsaturated fatty acyl chain, decreased in the high-fat diet group and the model group, respectively, which suggests that the reduction of unsaturated degree and the number of double bonds in the fatty acyl chains of phospholipids might change the function and the physicochemical properties of cell membranes [38].

In both comparisons, both PC (16:0/18:1) and PC (18:0/18:1) were selected, suggesting that their alterations are significantly influenced by the high-fat diet factor independent of the apolipoprotein E gene status. So, these 2 PC could be recognized, at an early stage of the atherosclerotic process, as possible indicators to predict the risk factor of a high-fat diet [38].

In Jové *et al.* (2013) [39], authors used the hamster because this type of animal can develop diet-induced atherogenesis, unlike the wild-type mice and rats, making the aorta one of the primary goals of the experimental atherogenesis process. The study began on the effects of the high-fat diet in aorta lipidome. Sample analyses were limited to the absence of morphologically detectable plaque but with chemical evidence of the absence of plaque (increased cholesterol concentration) [39]. In the aorta, levels of different lipid species belonging to the categories of

Free Fatty Acids (FFA), glycerolipids, glycerophospholipids, sphingolipids, and sterol lipids were altered by the high-fat diet [39]. Some of the lipids obtained in the plasma were similar to those obtained in the aorta. It was identified lipids belonging to the categories of FFA, glycerolipids, glycerophospholipids, and sphingolipids. Increased levels of Cer (d18:1/24:1) and polyunsaturated fatty acids (PUFA) DHA were present in both atherogenic plasma and aorta [39]. The glycerophospholipids and sphingolipids were both increased following the high-fat diet intake. Higher levels of high-density lipoproteins could explain the increase in levels of PE and PC [39].

In the metabolome analysis, 480 differential molecules were discovered between the control and atherogenic groups. Taurine and phenylalanine were found to be down-regulated by a high-fat diet, while taurocholic acid was found to be up-regulated. The levels of taurocholic acid in the aorta were found to be positively correlated with the levels of free cholesterol. As a result, taurocholic acid may be a potential biomarker of the atherogenesis process [39].

In Lee *et al.* (2017) [37], in the atherogenic diet group, the heart weight was significantly lower than in the normal diet group after 8, 16, and 25 weeks. In the atherogenic diet group, it was found a lower level of serum TG and HDL-C than in the normal diet group. On the other hand, higher levels of LDL-C and total cholesterol were found in the atherogenic diet group than in the normal diet group after 8, 16, and 25 weeks of feeding. The levels of myocardial and circulation metabolites have increased substantially in the atherogenic diet group than in the normal diet group. In the heart tissue of the atherogenic diet group, sulfur amino acid, energy, and short-chain fatty acids metabolism were severely disrupted. In the same group, the serum samples showed considerable metabolic changes in sulfur amino acid, energy, short-chain fatty acids, branched-chain amino acid, and aromatic amino acid metabolism. This demonstrates that an atherogenic diet affects both myocardial and circulating metabolism [37]. The lipids with major variations that were identified were the Cer, glucosylceramides (GlcCer), DG, FFA, LysoPC, LysoPE, PC, PE, SM, and TG [37].

The atherogenic diet group had lower FFA levels than the normal diet group. Nevertheless, at 25 weeks, the percentage of saturated fatty acids in heart tissue was substantially higher in the atherogenic diet group, but the levels of monounsaturated fatty acids and PUFA were significantly lower. There was a higher concentration of TG with a low degree of unsaturation and relatively short acyl chains in the atherogenic diet group, while there was a higher concentration of TG with a high degree of unsaturation and relatively long acyl

chains in the normal diet group. The Cer showed a significant decrease in the atherogenic diet group while GlcCer levels have increased in the atherogenic diet group. The atherogenic diet group had increased levels of SM with saturated fatty acids. These findings imply that in the atherogenic diet group, differences in heart lipid levels, such as FFA and TG, are determined by their degree of unsaturation [37]. The serum samples from the atherogenic diet group, showed significantly higher levels of LDL-C and total cholesterol and lower levels of HDL-C in the normal diet group, suggesting that the atherogenic diet group had an increased risk of developing atherosclerosis and disorders in fatty acid metabolism. The decreased levels of serum TG were possibly due to elevated levels of cholesterol and cholate in the diet [37].

In Yan *et al.* (2019) [36], after 18 weeks, the LysoPL profiles of the high-fat diet group and the model group were examined for arteriosclerosis-related differential metabolites. In $ApoE^{-/-}$ mice, there was meaningful lipid accumulation and cholesterol clefts in the aortic valves [36]. In the model group, the concentrations of TG, total cholesterol, and LDL-C were higher when compared to the ones in the high-fat diet group, but the concentration of HDL-C was lower. When comparing the high-fat diet group to the normal diet group, the levels of TG, total cholesterol, LDL-C, and HDL-C were higher in the high-fat diet group. After the analysis 37 qualified LysoPL were identified, of which 20 lipids were down-regulated and 17 lipids were up-regulated. LysoPE containing long and unsaturated fatty acyl chains (C22:6, C22:5, C20:4, C20:3) were down-regulated, while LysoPC (with C22:4, C22:5, C24:4) and lysophosphatidylinositols (LysoPI) (with C22:4, C22:5, C22:6) were up-regulated. After more research, 12 of the 37 differential LysoPL, including 2 LysoPE, 5 LysoPC, and 5 LysoPI, were classified as potential biomarkers. These 12 LysoPL correlated with total cholesterol, TG, and LDL-C levels, but only two LysoPE correlated with HDL-C levels [36].

In these four articles from the subgroup of animal samples, different lipids were identified, but some of them belong to the same category, the glycerophospholipids. Even though all of them study atherosclerosis, different methods were used and one of the articles used targeted lipidomics. So, their study focused on specific lipids, in that case, was the LysoPL, that is a category of biologically active metabolites and belongs to the glycerophospholipids.

b) Cerebrovascular Disease

In the second group, the articles focused on cerebrovascular disease and were divided into two subgroups, those analyzing the plasma from patients with carotid atherosclerosis and those that analyzing plasma from patients that have suffered a stroke (Table 5).

Table 5 – Identified lipids in the second group.

Groups / Lipids	Cerebrovascular Disease		
	Bazan *et al.*, 2017 [28]	Li *et al.*, 2014 [33]	Yang *et al.*, 2017 [40]
Fatty Acyls	↗	CNA	↗
MG	–	CNA	–
DG	–	CNA	↗
TG	–	CNA	↗
PC	–	CNA	–
LysoPC	–	CNA	↗
PE	–	CNA	↗
LysoPE	–	CNA	–
PS	–	CNA	–
LysoPS	–	–	–
PG	–	CNA	–
LysoPG	–	CNA	–
PI	–	CNA	–
LysoPI	–	–	–
Sphingosine	–	–	–
Sphinganine	–	–	–
Cer	–	CNA	–
SM	–	CNA	–
PE-Cer	–	–	–
GlcCer	–	↗	↗
LacCer	–	CNA	–
GalCer	–	↗	–
Free Cholesterol	–	–	–
CE	–	–	–
oxCE	–	–	–

Legends: ↗ any correlation with the disease (positive or negative); ↘ no correlation; – no data; CNA – data not shown

In Bazan *et al.* (2017) [28], the results obtained show that the concentration of the sera RvD1 was lower in acutely symptomatic patients than in asymptomatic patients. The concentration of DHA, a precursor of RvD1, was lower in sera of acutely symptomatic patients,

than in asymptomatic patients, and the serum concentration of AA, the major ω6 PUFA in human blood, was higher in asymptomatic patients. The RvD1 concentration was higher in stable plaques and lower in acute rupture, with the strongest correlation for acutely symptomatic patients. The concentrations of the ratios AA:DHA, AA:RvD1, and DHA:RVD1 were also analyzed, and the ratios of AA:DHA and AA:RvD1 were associated with acutely symptomatic patients, both of which were higher in the sera of acutely symptomatic patients, and no substantial difference in the ratio DHA:RvD1.

In Li *et al*. (2014) [33], it was developed a novel method to analyze four categories of 17 subclasses of lipids: FFA, Cer, MG, DG, TG, PI, PG, lysophosphatidylglycerols (LysoPG), PE, LysoPE, PS, PC, LysoPC, SM, galactosylceramides (GalCer), GlcCer and lactosylceramides (LacCer). The results obtained show that the two groups could be separated, the GalCer levels in the plasma of atherosclerosis patients were much higher than those of the control subjects, but GlcCer was the opposite [33]. The authors concluded that this method is suitable for lipid profiling since it identifies the 17 subclasses of lipids, and the variations between the groups were possible to compare. Considering that the isomers of GalCer and GlcCer were successfully isolated, it was possible to notice that only the levels of GalCer were higher in the atherosclerosis group and that the levels of GlcCer were higher in the control group. This shows that the method chosen could be useful for Lipidomics and clinical research due to a comprehensive separation of lipids [33].

In these articles about cerebrovascular disease from the subgroup of patients with atherosclerosis in the carotid, different results were obtained because one did targeted lipidomics and one did untargeted lipidomics, but the category that was involved in every study was the fatty acids.

Cerebrovascular disease is also studied in this next article, although the plasma used was from lacunar infarction patients.

In Yang *et al.* (2017) [40], it was analyzed 17 subclasses of lipids, FFA, PC, LysoPC, PE, LysoPE, PG, LysoPG, PI, PS, SM, Cer, GalCer, GlcCer, LacCer, MG, DG, and TG. They obtained in the preliminary screening results, a total of 90 lipids that were selected as biomarker candidates. The 44 plasma samples, with 29 lacunar infarction patients and 15 control subjects, were used to validate these 90 lipids selected as biomarker candidates. The proposed lipids as potential biomarkers were FFA (16:1), GlcCer (38:2), and PE (35:2) that were down-regulated in the lacunar infarction group, and the other ten lipid species were DG (38:6), LysoPC (20:5),

LysoPC (20:4), LysoPC (22:6), LysoPC (24:0), TG (52:5), TG (54:5), TG (54:4), TG (54:3), and TG (56:5), that were up-regulated in the lacunar infarction group. Several of the lipids in the 13 promising biomarkers are TG [40]. An optimization algorithm was developed to differentiate lacunar infarction patients from healthy controls with 93.3% sensitivity and 96.6% specificity, from four lipids in the "plasma biomarker model", GlcCer (38:2), PE (35:2), FFA (16:1), and TG (56:5) [40].

In the lacunar infarction group, four LysoPC were up-regulated. LysoPC is a phospholipase A2 hydrolysis product of PC and there is significant evidence that phospholipase A2 is triggered in transient ischemia and contributes to neuronal damage. As a result of the up-regulated, phospholipase A2 hydrolyzes the phospholipids of the membrane and increases the levels of LysoPL [40]. At the end of this study, the authors concluded that with this method it was possible, following a comprehensive screening and validation, to propose 13 lipid species as potential biomarkers, the majority of which were TG and LysoPC. Also, a model with GlcCer (38:2), PE (35:2), FFA (16:1), and TG (56:5) was finally determined as a novel plasma biomarker [40].

c) Peripheral Arterial Disease

The third group is about PAD and it is divided into an article that used plasma samples and an article that used adipose tissue samples (Table 6).

Table 6 – Identified lipids in the third group.

Groups / **Lipids**	**PAD**	
	Paapstel *et al.*, 2018 [25]	Clària *et al.*, 2013 [34]
Fatty Acyls	–	↗
MG	–	–
DG	–	–
TG	–	–
PC	↗	–
LysoPC	↗	–
PE	–	–
LysoPE	–	–
PS	–	–
LysoPS	–	–
PG	–	–
LysoPG	–	–

PI	–	–
LysoPI	–	–
Sphingosine	–	–
Sphinganine	–	–
Cer	–	–
SM	–	–
PE-Cer	–	–
GlcCer	–	–
LacCer	–	–
GalCer	–	–
Free Cholesterol	–	–
CE	–	–
oxCE	–	–

Legends: ↗ any correlation with the disease (positive or negative); ↘ no correlation; – no data

In Paapstel *et al.* (2018) [25], even though PAD and CAD result from atherosclerosis it appears that their serum PC and LysoPC levels can vary in relation to other biochemical and functional parameters. Compared to CAD patients only, CAD patients with comorbid PAD have a higher overall burden of atherosclerosis and inflammation, enhanced arterial stiffness, poorer endothelial function, and reduced physical activity [25].

The results showed a link between LysoPC (16:0), LysoPC (17:0), LysoPC (18:0), LysoPC (20:4), and PC (32:2) for the three study groups. For the carotid-femoral pulse wave velocity, there was no significant relationship with the lipids. For the resting heart rate, an independent predictor of morbidity and mortality, the significant lipid was LysoPC (17:0) for the PAD group. For the endothelial dysfunction, LysoPC (20:4) was the only significant determinant of both asymmetric dimethylarginine/arginine and asymmetric dimethylarginine for the PAD group. For the CAD group, LysoPC (18:0) was a significant determinant of both asymmetric dimethylarginine/arginine and asymmetric dimethylarginine [25].

The results also show that LysoPC (16:0) has a positive correlation with total cholesterol, a negative correlation with interleukin-6, and high-sensitivity C-reactive protein in the PAD group, and a positive correlation with total cholesterol and LDL-C in the CAD group. The LysoPC (17:0) was negatively correlated in the PAD group for interleukin-6 and high sensitivity C-reactive protein; in the CAD and healthy group with interleukin-6 and insulin, respectively. In the PAD group, the LysoPC (18:0) have a positive correlation to total cholesterol, LDL-C, and HDL-C and a negative correlation with interleukin-6, high-sensitivity

C-reactive protein, and insulin. In the CAD group, the LysoPC (18:0) have a positive correlation with total cholesterol and LDL-C and a negative correlation with interleukin-6. In the healthy group, the LysoPC (18:0) have a negative correlation with insulin. The LysoPC (20:4), in the PAD group, has a negative correlation with interleukin-6, and in the healthy group a positive correlation with HDL-C and a negative with insulin. Finally, the PC (32:2) has a positive correlation with total cholesterol and LDL-C and a negative with interleukin-6 in the PAD group [25].

In Clària et al. (2013) [34], RvD1, resolvin D2, and protectin D1 (PD1) were discovered in the subcutaneous adipose tissue. It was also discovered 17S-hydroxydocosahexaenoic acid (17-HDHA), 14S-hydroxydocosahexaenoic acid (14-HDHA), the monohydroxy markers for RvD1, PD1, and maresin 1 biosynthesis pathways, 7S-hydroxydocosahexaenoic acid (7-HDHA), and was also discovered the monohydroxy markers for resolvin E1 [18-hydroxyeicosapentaenoic acid (HEPE)] and 15-epi-LXA_5 [15-HEPE].

When the subcutaneous adipose tissue profile of control subjects was compared to the subcutaneous fat profile of PAD patients, the concentrations of 17-HDHA, which has potent protective actions in vascular inflammation, and PD1 were found to be significantly lower in PAD patients, as were the concentrations of 15-HEPE [34]. The results when comparing perivascular fat with subcutaneous adipose tissue indicate that the concentrations of PD1, 17-HDHA, 18-HEPE, and Lipoxin A4 were higher, and the concentrations of 7-HDHA, 14-HDHA, and 15-HEPE were lower [34]. Lastly, in PAD patients' peri-wound subcutaneous fat, the production of specialized pro-resolving mediators was considerably higher. Peri-wound fat also contained increased concentrations of leukotriene B4, prostaglandin E2, prostaglandin D2, and 5-lipoxygenase, 12-lipoxygenase, and 15-lipoxygenase products from AA [34].

In this study, a crucial finding was made, which is the results confirm the existence of an improved biosynthetic ability of adipose tissue-derived pro-resolving mediators in perivascular fat. Perivascular adipose tissue surrounding systemic vessels has specific significance to vascular biology due to its tissue mass, anatomical proximity, and emerging role in vascular pathologies. In the group of PAD patients, it was found that perivascular adipose tissue showed higher pro-resolving mediators levels compared to the subcutaneous, indicating activation of resolution circuits in this area. This in favor of a different distribution of bioactive lipid mediators based on the location of human fat depots and reveal a particular pattern of pro-resolving mediators closely linked to PAD [34].

d) Cerebrovascular Disease and Peripheral Arterial Disease

Finally, the fourth group analyzes articles that examine both cerebrovascular disease and PAD (Table 7).

Table 7 – Identified lipids in the fourth group.

Groups / Lipids	Cerebrovascular Disease + PAD			
	Stegemann *et al.*, 2011 [35]	Vorkas *et al.*, 2015 [32]	Vorkas *et al.*, 2016 [31]	Caligiuri *et al.*, 2017 [30]
Fatty Acyls	–	↗	↗	↗
MG	–	–	–	–
DG	–	–	↗	–
TG	↘	–	↗	–
PC	↗	–	↗	–
LysoPC	↗	–	↗	–
PE	↗	–	↗	–
LysoPE	↘	–	–	–
PS	↗	–	–	–
LysoPS	↗	–	–	–
PG	–	–	–	–
LysoPG	–	–	–	–
PI	–	–	–	–
LysoPI	–	–	–	–
Sphingosine	–	–	–	–
Sphinganine	–	–	–	–
Cer	–	↗	↗	–
SM	↗	–	↗	–
PE-Cer	–	↗	–	–
GlcCer	–	–	–	–
LacCer	–	–	–	–
GalCer	–	–	–	–
Free Cholesterol	–	↗	–	–
CE	↗	–	–	–
oxCE	–	↗	↗	–

Legends: ↗ any correlation with the disease (positive or negative); ↘ no correlation; – no data

In Stegemann *et al.* (2011) [35], when the control samples (radial arteries) were compared to the carotid and femoral endarterectomies samples, 150 lipids were identified. The results obtained for the tissue extract were a few amounts of TG in the radial arteries samples, and for the endarterectomy samples were SM, CE, PC, and TG species. It was also detected: LysoPC,

PC, SM, CE, LysoPE, PE, lysophosphatidylserines (LysoPS), TG, and PS [35]. The main differences between control and diseased arteries were accounted for by lipid subclasses: CE, SM, LysoPC, and PC. The relative amount of CE with linoleic acid and other PUFA such as AA [CE (20:4)] and eicosapentaenoic acid [CE (20:5)] when compared to control arteries, showed remarkable differences [35].

The following section of the analysis was based on plaques from symptomatic and asymptomatic patients, as well as stable versus unstable regions within the same lesion, and the results show that stable and unstable regions were separated [35].

The results obtained from plasma samples were 10 CE, 9 SM, 8 LysoPC, and 31 PC. After the analysis, as suspected, plasma samples and plaque samples were well-differentiated using the principal component analysis [35]. The highest relative enrichment in plaques in comparison with plasma was the polyunsaturated CE with long-chain fatty acids and some SM species and formed part of the lipid signature for sensitive and stable plaque areas in systems-wide network analysis [35].

In Vorkas *et al.* (2015) [32], the results have shown that samples of intimal thickening and plaque lesions from carotid endarterectomy and femoral endarterectomy were separated. The same happens to the samples from carotid and femoral tissue.

The free cholesterol was present in both carotid endarterectomy and femoral endarterectomy at higher concentrations compared to intimal thickening; several oxidized cholesteryl esters (oxCE) were also found at higher concentrations. A significant amount of purine and pyrimidine was also detected in lower concentrations in both plaque groups. Purine and pyrimidine control the vascular tone in a process similar to hypoxia and some metabolites, such as adenosine and inosine, are known for their anti-inflammatory effects, also their inhibition can lead to apoptosis [32]. Cer was also found in higher concentrations in both carotid endarterectomy and femoral endarterectomy, but SM was found in lower concentrations, and the glycosphingolipids were dysregulated [32]. The levels of two phosphatidylethanolamine-ceramides (PE-Cer) were also identified in this study and were observed to be strongly associated with plaque groups. The two PE-Cer identified were PE-Cer (d18:1/16:0) and PE-Cer (d18:1/24:1). The findings also demonstrate alterations in acylcarnitine metabolism in plaque tissue, which are implicated in the translocation of fatty acyl chains by the mitochondrial membrane [32].

In Vorkas *et al.* (2016) [31], when compared to asymptomatic patients with symptomatic patients, these are at a significantly higher risk of suffering a stroke in the immediate period following a symptom initiation [31]. The result shows that there are significant differences between the symptomatic and asymptomatic groups. Different lipids were obtained, such as PC, LysoPC, PE, Cer, SM, oxCE, TG, DG, and fatty acids. The main differences were observed in five metabolites, PC (16:0/20:4), PC (16:0/18:1), PE (18:1/18:0), AA, and a not yet identified feature, which were significantly higher in the symptomatic group [31].

The main differences found between groups were the higher concentrations of PC (16:0/20:4) and AA, carrying PC, in symptomatic atherosclerosis. After being hydrolyzed by the enzyme phospholipase A2, the PC (16:0/20:4) will release AA. The function of AA as a precursor molecule of a broad range of inflammation-related eicosanoids [31].

In this next article, cerebrovascular disease indeed is studied but in patients who already have PAD, since PAD patients are more prone to cardiovascular events.

In Caligiuri *et al.* (2017) [30], the results were that 24 plasma fatty acids were detected and 12 of these 24 were PUFA, also 39 plasma oxylipins were quantified. In the plasma oxylipins, four had major variations between the presence or absence of an event, such as plasma 16-hydroxyeicosatetraenoic acid (HETE), which was four times higher in patients that experienced a cerebrovascular accident. Based on the measured physiological concentration range, the results suggest that 16-HETE resulted in the largest increase in the chance of angina and cerebrovascular accidents [30]. The plasma oxylipins with a significant increase in the acute coronary syndrome prevalence were 8,9-dihydroxyeicosatrienoic acid (DiHETrE). When plasma oxylipins were compared between those who had multiple events and those with no events, 16-HETE, thromboxane B2, and 11,12-DiHETrE increased the chances of having multiple events. β-blockers were found to be substantially linked with the incidence of angina, acute coronary syndrome, and transient ischemic attack, but not with the prevalence of a cerebrovascular accident. Age, gender, smoking status, hyperlipidemia, hypertension, diabetes mellitus, and other drugs were not shown to be substantially related to any event. 16-HETE and 8,9-DiHETrE nevertheless had a significant impact on the probability of occurrences in the presence of these factors [30]. The ratio of thromboxane B2 and 6k-prostaglandinF1α had previously been found to have predictive value for cardiovascular disease, so it was tested as a predictor of events in this study. The results show that the ratio increased the likelihood of suffering a transient ischemic attack as well as multiple events [30].

The finding indicates that there was no substantial association relationship between fatty acid profiles and cardiovascular events, but, their products, the plasma oxylipins, had a significant effect on the presence of events. Oxylipins could have a role in cardiovascular and cerebrovascular events due to their actions on inflammation and vascular tone [30].

The results also show that patients with previous clinical events did not have substantially different omega-6 fatty acid concentrations relative to patients with no events, but patients with events had significantly higher levels of many pro-inflammatory and vasoconstrictive oxylipins derived from omega-6 fatty acids [30].

In this study, one important conclusion was obtained that there is an important association between certain plasma oxylipins, such as 16-HETE and 8,9-DiHETrE, for cardiovascular and cerebrovascular events if the risk factors are well-controlled. Therefore, oxylipins could have the potential to be used as new risk markers or therapeutic targets [30].

At the end of this group, different analyses were performed, so most results are heterogeneous, but the categories where the major differences were identified are in glycerophospholipids, in the fatty acids, and in the sterol lipids.

4. Discussion

Across the articles, it was clear that several lipids were dysregulated, thus it is now necessary to understand the biological function of each lipid and how the dysregulation affects the human body.

Lipids, as discussed in the first chapter, are important components of membrane structures; they are integrated into protein complexes and perform a variety of biological processes such as signal transduction, energy transfer, apoptosis, cell growth, and others. There are eight categories of lipids: 1) fatty acyls; 2) glycerolipids; 3) glycerophospholipids; 4) sphingolipids; 5) sterol lipids; 6) prenol lipids; 7) saccharolipids and 8) polyketides [18].

Even though five of the eight categories appear throughout the articles, it is possible to see that the lipids that have a major correlation with the studied CVDs belong to four of the eight categories of lipids: fatty acyls, glycerolipids, glycerophospholipids, and sphingolipids (Figure 6).

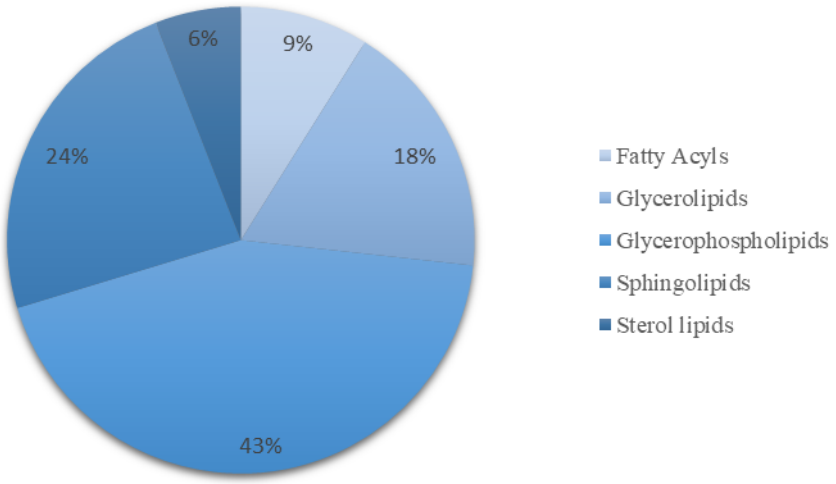

Figure 6 – Distribution of the dysregulated lipids found in the studies among the lipid categories.

In the articles on atherosclerosis, the main lipid class that appears in the articles that used human plasma are TG and PC; the ones that used animal plasma, the lipids that most correlate with the disease are fatty acyls, TG, PC, LysoPC, PE, LysoPE, sphinganine, Cer, SM and GlcCer. In the articles on cerebrovascular disease, the lipids that appear the most are the fatty acyls and GlcCer. In the articles on PAD, different studies were conducted, therefore different lipids were obtained, but important results were achieved, mainly in the fatty acyls, PC and LysoPC. In the articles on both diseases, the lipids that show the most correlation with the disease are fatty acyls, PC, Cer, SM, and oxCE.

4.1. Fatty Acyls

The category of fatty acyls is divided into fatty acids and conjugates, octadecanoids, eicosanoids, docosanoids, fatty esters, fatty amides and fatty acyl glycosides [18]. Throughout the articles, the fatty acyls that appear are fatty acids and conjugates, eicosanoids, docosanoids, and fatty esters.

Throughout biological responses, fatty acids are synthesized and metabolized as energy substrates. Long-chain fatty acids and medium-chain fatty acids are obtained primarily from dietary TG, and the short-chain fatty acids are the main source of FFA in the metabolic system. FFA are energy sources for the majority of the body tissue and also perform important functions, like gene expression and receptor signaling [41]. FFA affects different physiological and biochemical processes, some studies indicate that FFA levels have been associated with the risk, recurrence, and functional outcome of stroke [40].

Omega-3 PUFA, like eicosapentaenoic acid and DHA, belonging to the class of fatty acids and conjugates, are found in some fish oils and vegetable oils and are thought to reduce the risk of a variety of human metabolic diseases and inflammatory disorders such as diabetes, atherosclerosis, inflammatory bowel disease, and rheumatoid arthritis. By acting as an alternative substrate for cyclooxygenase or lipoxygenase, omega-3 PUFA hinders the transformation of AA to pro-inflammatory eicosanoids, leading to the production of less potent products [18]. The mechanisms for omega-3 PUFA's anti-atherothrombotic effects involve improved lipid metabolism, including a decrease in TG and an increase in HDL-C, as well as glucose metabolism, anti-platelet activity, anti-inflammatory effects, improved endothelial function, and the normalization of atherosclerotic plaque [18].

Essential omega-6 and omega-3 long-chain PUFA are used to create lipid-derived mediators. The activation of cytosolic phospholipase A2 and/or other phospholipases induced by a variety of stimuli releases omega-6 AA from phospholipid pools that is a precursor to several lipid mediators. The FFA is then used as a substrate for cyclooxygenase, lipoxygenase, and cytochrome P450 enzymes, leading to the generation of eicosanoid mediators [18].

Free AA, that belongs to the class of fatty acids and conjugates, is oxidized by cyclooxygenase-1 or cyclooxygenase-2, resulting in the formation of prostaglandin H2. prostaglandin H2 is then metabolized into a variety of biological prostaglandins (prostaglandin E2, prostaglandin D2, prostaglandin F2, and prostaglandin I2) as well as thromboxane A2. Furthermore, lipoxygenases can metabolize free AA, resulting in hydroperoxyeicosatetraenoic

acids [18]. Such metabolites are then reduced to their correspondent HETEs or further processed into leukotrienes or multiple lipoxins. Lastly, cytochrome P450 enzymes can oxidize the free AA at double bonds or in the ω-terminus, resulting in epoxyeicosatrienoic acids or HETEs. These bioactive lipid mediators control a wide range of pathological and physiological mechanisms, including pro-inflammatory effects, tumor proliferation, and diabetes [18].

4.1.1. Lipid mediators

Lipid mediators, like pro-resolving mediators, are frequently pro-inflammatory autacoids. In various inflammatory situations, pro-resolving mediators can resolve acute inflammatory responses with the least damage to the surrounding tissue [34]. An important factor in atherosclerosis plaque rupture is the unresolved chronic inflammation, resolvins are endogenous inflammation-resolving lipid mediators induced by inflammation, likely to serve as self-defense, for sure where resolvins are not properly expressed, the pro-inflammatory state is dominant [28]. Prostaglandin E2 and related prostanoids, such as prostaglandin I2, act as vasodilators during the first stage of the inflammatory response, recruiting immune cells like neutrophils, macrophages, and mast cells from the bloodstream to the area of inflammation or tissue injury [18].

Lipoxins, resolvins, and protectins are examples of specialized pro-resolving mediators that are essential for the shift from inflammation to resolution. The upper respiratory tract produces lipoxins and severe airway allergy is related to reduced lipoxin production [18].

Resolvins are generated from eicosapentaenoic acid and DHA and come in two chemically distinct structural forms: the E-series and the D-series of resolvins. Resolvin E1 and resolvin E2 are E-series mediators and are biosynthesized by polymorphonuclear leukocytes from 18-HEPE through the arachidonate 5-lipoxygenase pathway. In mouse peritonitis, eicosapentaenoic acid-derived resolvins exhibit potent anti-inflammatory activity by inhibiting neutrophil recruitment and regulating cytokine production. DHA is broken down into two types of resolvins: the 17S and 17R D-series resolvins. The 17S-D series includes four resolvins indicated as RvD1 to resolvin D4. RvD1 is a powerful anti-inflammatory mediator that hinders interleukin 1β expression and hinders neutrophil transmigration in response to neuronal injury. Resolvin D2 has been discovered to block the NLRP3 inflammasome in the peritonitis model, resulting in a decreased release of interleukin 1β into exudates through the inflammation resolution process [18].

Protectins and maresins are two additional families of specialized pro-resolving mediators that are derived from n-3 PUFA. PD1 protects mice against liver injury by inhibiting inflammatory cytokines and immune cell infiltration in the liver. Furthermore, PD1 has potent neuroprotective effects in neuronal systems by promoting cell survival following neural injury or stroke, and it is also known as neuroprotectin D1. Maresin 1 is involved in the treatment of inflammatory diseases like peritonitis and colitis. Maresin 1 has anti-inflammatory properties by suppressing the development of pro-inflammatory cytokines induced by LysoPS, such as interleukin 6, tumor necrosis factor α, and interleukin 1β [18].

4.2. Glycerolipids

Glycerolipids are classified into MG, DG, TG, glycosylmonoradylglycerols, and glycosyldiradylglycerols [18]. The ones mentioned in this dissertation are MG, DG, and TG.

As a component of the lipid bilayer, DG helps to keep the cell membrane structure stable and acts as a second messenger by regulating the activation of protein kinase C, that is involved in T cell activation and proliferation. The buildup of DG can cause lipotoxicity, which could lead to cell malfunction and apoptosis, as well as diabetes, cancer, and coronary heart disease [18]. In the biosynthesis and degradation of TG, DG are important intermediates; some studies have shown that low production of ATP and uncontrolled outflow of ions through the membrane stimulate the production of different phospholipase groups, generating excess amounts of DG [40]. Contrary to TG, that is mainly an energy storage molecule, some DG and MG species have additional intracellular signaling functions [20].

In the human body, TG is an important source of energy and an essential component of lipoprotein. In a water-free environment, most of TG is accumulated in adipocytes and acts as an energy storage reservoir with tightly packed energy-rich fatty acids [20]. Inflammation and insulin resistance are linked to TG metabolism. The inflammatory state in adipose tissues has a significant impact on lipid metabolism, causing a decrease in TG synthesis and an increase in lipolysis, resulting in higher levels of circulating FFA [18].

Some studies have shown a substantially higher level of CE in the arterial wall of atherosclerotic lesions and a higher amount of TG is also considered to be a significant biomarker in the development of cardiovascular disease. The lipoproteins that transport TG in the bloodstream accumulate in the artery wall intima and are taken up by macrophages to form

foam cells that lead to the formation of plaque along the walls of the artery. There is solid evidence of the connection between TG and CVDs, but it remains controversial if TG levels are merely a marker of proatherogenic lipoprotein dynamics and composition [26,27].

4.3. Glycerophospholipids

Glycerophospholipids are divided into various classes, including glycerophosphates, PG, PC, PI, PS, PE, and cardiolipins. In this dissertation, PC, PE, PS, PG, and PI, as well as their subclasses, are the ones that appear. The pathophysiology of metabolic diseases is linked to the composition of membrane lipids. There is evidence that changes in membrane-constituting lipids, like polyunsaturated and saturated PC and LysoPC, in mouse livers contribute to the development of high-fat diet-induced hepatic insulin resistance. Phospholipids can affect cell signal transduction in two ways: by interacting with specific proteins and by changing the composition of the lipid acyl chain, which controls downstream signaling [18].

The PC have choline as the head group. The biological functions are fuel and energy storage/ source, membrane integrity/ stability, cell signaling and are also part of glycerophospholipids metabolism [38]. The PC molecule may have different combinations of fatty acids varying lengths and saturation attached to the glycerol backbone in the positions sn-1 and sn-2, and variations in the composition of individual PC molecular species may be caused at least in part by an underlying (patho)physiological condition [25]. Some reports indicate that the more unsaturated degree of fatty acyl chains present in the structure of the phospholipids, the weaker interaction of fatty acid, and a greater membrane fluidity that can minimize the risk of atherosclerotic plaque [38].

The PE have amino alcohol ethanolamine as a head group. In some tissues, like the liver and the brain, it is the primary component of the phospholipid pool. PE are an essential precursor for PC, where PE are methylated three times to produce PC [20].

The PS includes the proteinogenic amino acid serine as the head group [20]. PS is one of the most prevalent lipids on the inner leaflet of the plasma membrane. When PS is exposed to the external leaflet and phospholipid asymmetry is lost, apoptotic cells, such as lymphocytes, neutrophils, and tumor cells might occur [18]. In mammals, only an existing glycerophospholipid such as PC or PE can synthesize PS. The PS containing the docosahexaenoyl fatty acid influences the normal function, the development, and the prevention of neural cell apoptosis [20].

The PG can be found in plant and animal membranes. PG contains two glycerol molecules connected by ester bonds with a phosphate group and two fatty acids. In animal tissues, it is found in small amounts but it accounts for up to 11% of the overall phospholipid pool in the lung surfactant. A significant function of PG is related to the lung where PG is a key functional component of pulmonary surfactant [20].

The PI are found in all eukaryotic cells with the highest concentrations found in the nervous system and it is involved in cellular signaling. PI are the main source of AA in animal tissue, which is necessary for the synthesis of eicosanoids. The fatty acid composition of PI is primarily stearic acid at sn-1 and AA at sn-2. Furthermore, PI are the primary source of DG, which acts as signaling molecules in plant and animal cells [20].

LysoPL are present in all cell types and act as second messenger molecules, influencing intracellular signaling pathways involved in a variety of biological processes including inflammation, nervous system regulation, atherosclerosis, and cancer [18]. The LysoPL that contain a single fatty acid chain result from phospholipase A2 hydrolysis [18].

The LysoPC is a lipid species of the category glycerophospholipids and it is involved in cell signaling [25]. PC releases LysoPC by phospholipase A2 [18]. It is a pro-inflammatory lipid formed by multiple pathological activities and is a component of oxidized LDL. Studies have shown that oxidized LDL promotes atherosclerosis by stimulating their adherence to the endothelial cells and by initiating the formation of foam cells through a chemotactic and proliferative process on monocytes. It has also shown that it proliferates and stimulates the migration of smooth muscle cells into the tunica media, which stimulates the development of collagen, thereby contributing to the fibrous lining in the atherosclerosis plaque [27]. LysoPC is known to have both pro-atherogenic and anti-atherogenic properties. PC and LysoPC are the reservoirs and transporters for the components of glycerophospholipids: glycerol, choline, fatty acids, and phosphate [25].

LysoPE can be produced by the action of the phospholipase A2 on PE on cell membranes. Many studies have shown that LysoPE is involved in a variety of cellular activities, including differentiation and migration of MDA-MB-231 breast cancer cells, SK-OV3 ovarian cancer cells, and PC-12 neuronal cells. Since LysoPE is a zwitterionic lipid, protein transporters can aid in its transport across the plasma membrane [42].

LysoPS can be present in a variety of tissues including the peripheral lymphoid tissues, thymus, colon, and central nervous system. There is significant evidence that LysoPS plays a

role in the immune response. These include suppression of T cell proliferation, increased histamine release, promotion of cell clearance by macrophages, and stimulation of mast cell degranulation [42].

LysoPG was recently classified as a bioactive lipid. The role of LysoPG in the pathogenesis of cardiovascular diseases has been attributed to elevated LysoPG concentrations in acute coronary syndrome. LysoPG can cause chemotactic migration and tube formation in human umbilical vein endothelial cells, implying a role in endothelial cell activity modulation, also they enable natural killer cells trafficking [42].

Obese people have elevated levels of LysoPI. Therefore, its physiological function in obesity was related to the upregulation of lipogenic genes, which stimulate lipid storage and promote adipocyte differentiation. LysoPI has been shown to stimulate signaling pathways involved in cellular migration, proliferation, and tumorigenesis. Furthermore, this bioactive lipid acts as a mitogenic factor [42].

4.4. Sphingolipids

Sphingolipids are classified into several classes, including sphingoid bases, Cer, phosphosphingolipids, neutral glycosphingolipids, and acidic glycosphingolipids [18]. Throughout this dissertation, the classes of sphingolipids that appear are sphingoid bases (sphingosine and sphinganine), Cer, phosphosphingolipids (SM and PE-Cer), and neutral glycosphingolipids (GalCer, GlcCer, and LacCer).

Sphingolipids are a lipid category that has an aliphatic amino alcohol backbone [18]. Sphingolipids are involved in various intracellular and extracellular signaling processes, as both secondary messengers and signaling molecules [39]. The sphingoid bases, also known as long-chain bases, form the backbone of all sphingolipids, sphingosine and sphinganine are some examples. Sphingoid bases are the key constituents for more complex sphingolipids like Cer, glycosphingolipids, and phosphosphingolipids. Usually, free sphingoid bases can only be observed at trace amounts. The sphingoid bases, in addition to being substrates for ceramide synthesis, serve as mediators in several cellular processes. They also hinder cell growth and influence cell proliferation and apoptosis [20].

Cer is an important metabolite and precursor in the synthesis of sphingolipids. The addition of distinct head groups to Cer results in the formation of SM and distinct

glycosphingolipids. Cer is generated via three key pathways: the *de novo* pathway, SM hydrolysis, and the salvage pathway. In the *de novo* pathway of production of Cer, palmitoyl CoA and serine, that are produced from the breakdown products of saturated lipids and proteins, undergo a four-step, conserved response in the endoplasmic reticulum. Cer can be produced through the hydrolysis of SM by the sphingomyelinase catalysis. In plasma membranes, sphingomyelinase hydrolyzes the phosphocholine group of SM to create Cer and PC. The salvage pathways recycle sphingosine by breaking down complex sphingolipids and glycosphingolipids through ceramide synthase to create Cer. It must be emphasized that the sphingosine–ceramide salvage pathway contributes 50% to 90% of sphingolipid biosynthesis [18].

Cer are abundant in most mammalian tissues, especially in the skin, but their levels in lipid membranes are relatively low. Cer have an effect on membrane curvature based on the length of their fatty acid chains [20]. The presence of Cer are usually linked to the activity of the enzyme sphingomyelinase in the atheromatous plaque, especially when there is pro-atherogenic oxidized LDL [39]. They are known for their involvement in atherosclerosis, their role in apoptosis, and their connection to pro-inflammatory factors [32].

SM has a similar structure to PC and a significant role in the membrane. In animal membranes, it is possible to find SM at high concentrations, but not at levels higher than PC. SM has a strong affinity to sterols, in the biological membrane [20].

The role of PE-Cer in the development of plaques in both carotid endarterectomy and femoral endarterectomy was clear. PE-Cer are present in mammalian cells in trace concentrations and have not been linked with any human disease [32].

Glycosphingolipids are involved as signaling molecules in vascular cell proliferation under oxidative conditions, as well as platelet activation and adhesion to vessel wall [32].

In mammals, the most abundant glycosphingolipids are GalCer, GlcCer, and LacCer. GalCer is in a higher concentration in tissues of the central nervous system. GlcCer can be transformed to LacCer and acts as a precursor for a variety of glycosphingolipid species. GalCer and GlcCer play a role in protein trafficking and sorting, in the regulation of cell growth, and modulation of cell morphogenesis and cell adhesion [43].

GlcCer is found in animals, fungi, and plants, it is necessary for the survival of multicellular organisms. They play a distinct and sometimes unclear role in mammalian cells.

Variations in GlcCer levels, in tissues and cells, have been observed in response to diabetes, cancer, cardiovascular disease, and skin disorders. The GlcCer upregulation is going to provide cellular defense and prime specific cells for proliferation [44].

LacCer is a glycosphingolipid that is found everywhere and plays an important role in the biosynthesis of specific glycosphingolipids. A recent breakthrough in LacCer research indicates that this molecule controls important phenotypic changes, in mammalian cells like cell adhesion and proliferation [45].

GalCer has been correlated to immune responses and some studies have shown that GalCer induces rapid and robust cytokine development of natural killer T cells, secondary activation of a variety of innate and adaptive immune cells, and modulation of T cell responses. The ability of the natural killer T cells to respond to lipid antigens and modulate innate and acquired immunity indicates that they could have a role in atherogenesis. Therefore, GalCer could be considered a reliable marker and a new pathway for better understanding atherosclerosis [33].

4.5. Sterol lipids

Lastly, in the category of sterol lipids, there are the sterols, which include cholesterol.

Cholesterol derivatives and free cholesterol are well-known risk factors for atherogenesis [32]. The accumulation of free cholesterol, a known marker of early atherogenesis, within macrophages and endothelial cells, may cause structural changes in the cell membranes, as well as cytotoxicity, in the case of macrophages, higher levels of free cholesterol are correlated with foam cells [39]. Cholesterol is a cyclic hydrocarbon and when it is esterified with a fatty acid, forms CE. In eukaryotic cells, cholesterol is fundamentally crucial for maintaining membrane fluidity, and it is solubilized as a consequence of the interactions with membrane phospholipids or with phospholipids and bile acid micelles in the gall bladder [46]. Excess CE are stored in the cytosol as lipid droplets and are common in steroidogenic tissues. Lipoprotein complexes are needed in blood for the solubilization of cholesterol and CE. The lipoproteins include LDL, very-low-density lipoprotein, ultra-low-density lipoproteins, and HDL that transport insoluble lipids around the body [46]. The accumulation of CE is thought to be a significant contributor to plaque formation, while free cholesterol is accumulated in advanced atherosclerotic plaques [32].

Lipid oxidation has been described as a reactive oxygen species-induced process that affects unsaturated fatty acids, CE, and free cholesterol. Lipid oxidation, which has a well-known role in the development of atherogenesis, will potentially lead to an oxidation chain reaction and cell membrane damage. Furthermore, as macrophages are less effective for oxCE degradation, oxCE are associated with the contribution of foam cell formation [32].

4.6. Limitations

Following the analysis of each article, it is clear that many lipid species have a significant relationship with cerebrovascular disease and PAD. It should be noted that new knowledge has emerged that has not been captured by traditional biochemical measurements of TG, total cholesterol, and HDL-C and LDL-C.

There is a lack of effective biomarkers for early prediction and diagnosis of acute symptomatic plaques, which hinders the ability of doctors to rapidly diagnose and treat patients with atherosclerotic plaque rupture, so it is important to reduce the time between plaque rupture and treatment for reducing morbidity [28].

Even though there is strong evidence that Lipidomics could aid in the diagnosis of CVDs among these articles, there were some limitations that made analyzing the impact of each lipid in the disease difficult. The drawbacks were that some articles did not state whether any of the obtained lipids had an impact or not on the disease and did not include all of the methodology details in the article itself.

Also, for a better comprehension of the results, if all the authors included the concentration of the obtained lipids in the articles, it will aid in understanding how the dysregulation affects the diseases in the review.

5. Conclusion

Cerebrovascular disease and PAD are very common diseases and it is important to provide an early diagnosis so that the patients can receive the best care available. Lipidomics is where the lipids in the biological system are mapped and certain diseases can dysregulate the lipid metabolism. This systematic review examined both diseases separately and together to see how lipid dysregulation affects them. The methodologies used in the articles differed, even though the target was the same: how do lipids correlate with the disease. Regardless of the disease and/or the type of study, many studies identified the same lipids. Some of the detected lipids did correlate with disease, while others did not.

The articles were divided into four groups, in the atherosclerosis group, the lipids that were identified the most and did have a correlation with the diseases were TG and PC; in the cerebrovascular disease group was the fatty acyls; in the PAD group, different analyses were performed, resulting in different results; and in the group on cerebrovascular disease and PAD, were the fatty acyls, PC, Cer, SM and oxCE. To sum up, the main categories that correlate with cerebrovascular Disease and/or PAD are fatty acyls, glycerolipids, glycerophospholipids, and sphingolipids.

In conclusion, Lipidomics has the potential to be used in the diagnosis of cerebrovascular disease and PAD. Therefore, further analysis and study are needed since we do not yet know the impact of all lipids in the human body, it is also necessary to improve the best method to analyze the samples and to determine if Lipidomics can be used in other diseases.

6. Future Work

Following this review of literature, one of the work extensions would be the proper examination of biological samples from patients with both the diseases under study and other types of CVDs. Aiming to broaden the group of CVDs that are characterized by Lipidomics with the intention of better understanding their pathophysiology and clinical significance.

When examining biological samples, one must keep in mind how important it is to have a large number of subjects, both control and disease subjects. It is also critical to have a wide range of subjects in terms of age, gender, and ethnicity.

Appendices

Appendix 1 – Flowchart

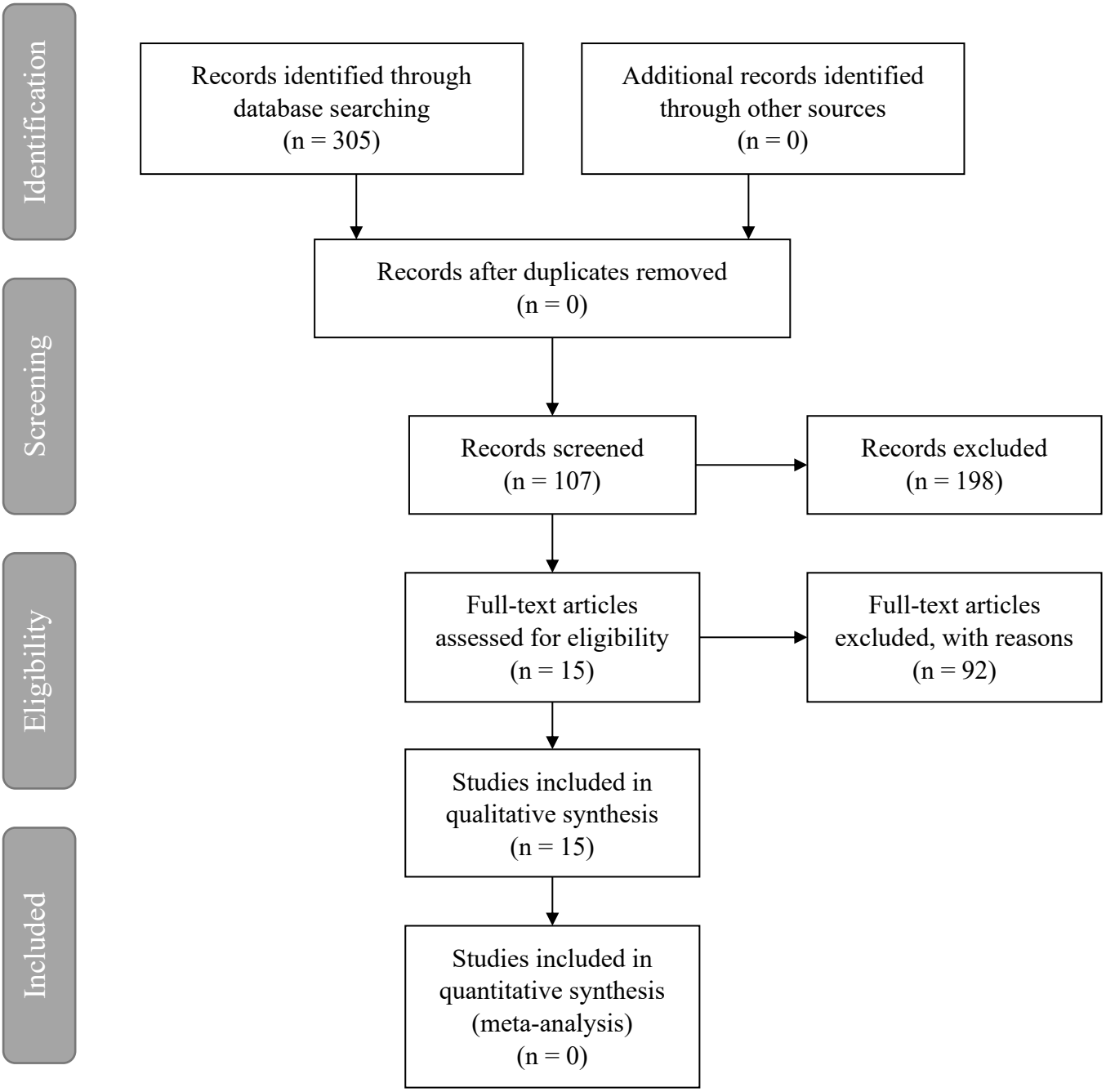

Figure A1 – PRISMA 2009 Flow Diagram [22].

Appendix 2 – Excel data

Table A1 – Data from the Cerebrovascular Disease folder's articles.

Reference	Alshehry *et al.* (2016) [47]	Bazan *et al.* (2017) [28]
Hypothesis	Independent of current risk factors, specific lipid species will be correlated with potential cardiovascular events in Type 2 Diabetes Mellitus	Determine if circulating serum RvD1, DHA, and AA levels indicate an increased risk of atherosclerotic plaque rupture
Human// Animal	Human	Human
Studied Population	Cohort with 3779 participants; all participants had type 2 diabetes mellitus with ≥1 additional cardiovascular risk factors; 35% had a history of macrovascular disease.	Cohort with 24 asymptomatic and 21 acutely symptomatic patients
Targeted// Untargeted	Targeted	Targeted
Follow-up// No Follow-up	5 years follow-up	No
Study	Plasma lipid species	Serum RvD1, DHA, and AA levels
Analysis done in	Plasma	Peripheral blood
Study Disease	Identify lipid species that are linked to future cardiovascular events and death	Risk of atherosclerotic plaque rupture
Technique used	LC electrospray ionization–tandem MS	LC-MS/MS
Statistical Analysis	Weighted Cox regression; Principal component analysis	SPSS software
Results	3 of the 22 lipid subclasses - related to the risk of cardiovascular events; 2 subclasses - increased risk of cardiovascular death; 32 different lipid species - related to both potential cardiovascular events and death.	RvD1 concentrations were lower in acutely symptomatic than in asymptomatic carotid patients; DHA concentration were lower in acutely symptomatic, compared to asymptomatic carotid patients; AA concentration was higher in asymptomatic vs. acutely symptomatic
Conclusions	Phospholipids (including lyso- and ether- species), sphingolipids, glycerolipids and CEs were linked with future cardiovascular events and death	Elevated AA:RvD1 and AA:DHA is linked with stroke and acutely symptomatic carotid disease

Table A1 – Data from the Cerebrovascular Disease folder's articles (cont).

Reference	Caligiuri *et al.* (2017) [30]	Pechlaner *et al.* (2017) [48]
Hypothesis	Novel eicosanoids can increase the chances of cardiovascular and cerebrovascular events in PAD patients	MS is used to quantify apolipoproteins and compare their interactions with incident CVD, as well as to achieve a system-level of apolipoprotein correlations with the plasma lipidome and proteome
Human// Animal	Human	Human
Studied Population	98 patients with PAD; prevalence of transient ischemic attacks, cerebrovascular accidents, stable angina and acute coronary syndrome was n= 16, 10, 16, and 24	702 subjects; 6.4% has a history of CVD, and 9% were prescribed statins
Targeted// Untargeted	Targeted	Targeted
Follow-up// No Follow-up	No	10-year follow-up
Study	Plasma oxylipins and fatty acids	Apolipoproteins
Analysis done in	Plasma	Plasma samples
Study Disease	Cardiovascular/cerebrovascular events in PAD patients	If 13 apolipoproteins, 135 lipid species, and 211 other plasma proteins are linked to incident CVD (91 events)
Technique used	GC and HPLC-MS/MS	MRM-MS
Statistical Analysis	Logistic regression	R 3.2.0
Results	24 plasma fatty acids were observed, 12 of which were PUFAs and 39 plasma oxylipins were quantified	apoC-II, apoC-III, and apoE were found to have the strongest correlations with incident CVD, followed by apoL-I, apoB-100, and apoH. ApoL-I was found to have a strong correlation with stroke; ApoC-II, ApoC-III, and ApoE were found to have strong direct associations with CEs, PC, PE, and, in particular, TG
Conclusions	None of 24 fatty acids observed were related to events; 39 plasma oxylipins were identified and 8 were significantly linked with events	Very-low-density lipoprotein-associated apolipoproteins have good associations with incident CVD supporting the idea of reducing CVD risk by targeting triacylglycerol-rich lipoproteins

Table A1 – Data from the Cerebrovascular Disease folder's articles (cont).

Reference	Trepanier, Eiden, Morin-Rivron, Bazinet & Masoodi (2017) [49]	Vorkas *et al.* (2016) [31]
Hypothesis	Create a system that combines MS/MS, high-energy head-focused microwave fixation, and statistical modeling to quantify lipids and lipid mediators to eradicate ischemia-induced fatty acid release and classify the rat neurolipidome	Depending on the patient's symptomatic status, stenosing carotid plaque tissue will have a distinct metabolic signature.
Human// Animal	Animal	Human
Studied Population	The animals were divided into 4 groups; 1) microwave fixation (n=10); 2) CO2 asphyxiation (n=10); 3) CO2 asphyxiation and then microwave fixation (n=9); and 4) lipopolysaccharide 3hr before microwave fixation (n=9)	Carotid plaque tissue samples were collected from patients with carotid-related cerebrovascular symptoms (n = 5) and from asymptomatic patients (n = 5). From each tissue, two adjacent biological replicates were collected.
Targeted// Untargeted	Untargeted	Untargeted
Follow-up// No Follow-up	No	No
Study	Neurolipidome	Metabolic phenotyping
Analysis done in	Brain tissue	Three transverse segments of stenosing carotid plaque tissue
Study Disease	Ischemia-induced	Stroke risk in stenosing carotid plaque
Technique used	(LC)-MS/MS	UPLC-MS
Statistical Analysis	Partial Least Squares Discriminant Analysis	Principal components analysis
Results	PGE2 concentration was higher in the CO2 group compared to the MW; PGE2 concentration was also higher in the CO2+MW group compared to the MW. Most mediators, including AEA, TXB2, 17S-HDoHE and 12-HETE, presented similar increases in the CO2 and CO2+MW groups, but lipopolysaccharide had no impact	The two research groups revealed distinct plaque phenotypes and metabolites that distinguished the two groups. Metabolites of the eicosanoid pathway and three acylcarnitine species, intermediates of b-oxidation, were found in higher concentrations in symptomatic patients
Conclusions	The effect of CO2-induced-ischemia is more powerful than that of lipopolysaccharide injection and it is mitigated by microwave-fixation. The use of microwave fixation reduces variability in measurements, allowing for higher sensitivity in detecting subtle variations between experimental groups	Elevated AA:RvD1 and AA:DHA is linked with stroke and acutely symptomatic carotid disease

Table A1 – Data from the Cerebrovascular Disease folder's articles (cont).

Reference	Yang *et al.* (2017) [40]	Yang *et al.* (2018) [50]
Hypothesis	Possible lipid biomarkers for the diagnosis of lacunar infarction, as well as providing insight into disrupted pathways of the underlying pathophysiology	Isosteviol sodium treatment was used to examine the lipid profiles of stroke rats
Human// Animal	Human	Animal
Studied Population	6 Lacunar Infarction patients and 6 control subjects - identify biomarker candidates; 29 Lacunar Infarction patients and 15 control subjects - plasma lipid biomarkers	Sprague-Dawley male rats were randomly assigned to one of 4 groups: 1) sham group (n=10); 2) model group (n=10); 3) STV-Na group (n=10); 4) EDA group (n=10). TTC staining was examined using additional rats (n = 24, six rats in each group)
Targeted// Untargeted	Untargeted	Untargeted
Follow-up// No Follow-up	No	No
Study	Lipid biomarkers	Lipid profiles
Analysis done in	Plasma samples	Brain samples
Study Disease	Lacunar infarction	Stroke rats with STV-Na treatment
Technique used	NP/RP 2D LC-QToF/MS	UHSFC-IT-TOF/MS
Statistical Analysis	IBM SPSS Statistics software, Version 20	PLS-DA
Results	Total of 90 lipids were chosen as biomarker candidates; 3 of these lipids' species were down-regulated, while the other ten, were up-regulated in the patient group; these lipids were suggested as potential lipid biomarker	Following STV-Na treatment, 15 lipids were found to be trending towards normal levels. STV-Na dramatically reversed the levels of 6 lipids to normal. STV-Na protective effects may be linked to the regulation of AA metabolism, sphingolipid metabolism and glycerophospholipid metabolism
Conclusions	Following a thorough screening and validation process, 13 lipid species were identified as potential lipid biomarkers	This approach proved to be a quick and efficient way to investigate the protective effects of STV-Na against stroke and it could be applied to other lipidomics studies

Table A2 – Data from the PAD folder's articles.

Reference	Alibhai *et al.* (2018) [51]	Allen-Redpath *et al.* (2019) [52]
Hypothesis	Role of CLOCK, a central regulator of cardiac gene and protein rhythms and a core component of the circadian system, was studied in female cardiac aging	The control of coagulation by eoxPL can play a role in the development of an abdominal aortic aneurysm. So, researchers used genetic murine models, oxylipidomics mapping of whole blood, and analysis of human abdominal aortic aneurysm tissue to characterize the generation and function of the procoagulant surface given by eoxPL in angiotensin II-driven disease
Human// Animal		Both
Studied Population		Six patient samples collected from subjects undergoing open AAA repair; On C56BL/6, Alox12-/- and Alox15-/- mice were crossed with an ApoE-/- strain
Targeted// Untargeted		Targeted
Follow-up// No Follow-up		No
Study		eoxPL regulation of coagulation
Analysis done in		Aortic tissue; aortic wall, inner thrombus (closest to lumen), and outer thrombus (closest to aneurysm wall)
Study Disease		Abdominal aortic aneurysm
Technique used		LC/MS/MS
Statistical Analysis		SIMCA-P version 12.0 (Umetrics)
Results		AAA development is substantially slowed in ApoE−/− mice deficient Alox12 or Alox15; Coagulation is disrupted in wild-type or ApoE-/- mice with Alox12 or Alox15 deficiency; Alox12-/- or Alox15-/- deficiency improves PE externalization on the surface of platelets or eosinophils.; HETE-PEs are higher in ApoE-/- during clotting but a lower ApoE-/- when backcrossed to Alox12-/- or Alox15-/-
Conclusions		This approach proved to be a quick and efficient way to investigate the protective effects of STV-Na against stroke and it could be applied to other lipidomics studies

Table A2 – Data from the PAD folder's articles (cont).

Reference	Alshehry *et al.* (2016) [47]	Anroedh *et al.* (2018) [53]
Hypothesis	Independent of current risk factors, specific lipid species will be correlated with potential cardiovascular events in Type 2 Diabetes Mellitus	Ten previously reported high-risk molecular lipid species and three Cer ratios are linked to the incidence of significant adverse cardiac events
Human// Animal	Human	Human
Studied Population	Cohort with 3779 participants; all participants had type 2 diabetes mellitus with ≥1 additional cardiovascular risk factors; 35% had a history of macrovascular disease.	581 patients with stable angina pectoris or acute coronary syndrome who required diagnostic coronary angiography and/or percutaneous coronary intervention - Cohort with 574 patients
Targeted// Untargeted	Targeted	Targeted
Follow-up// No Follow-up	5 years follow-up	4.7 years follow-up
Study	Plasma lipid species	Ten previously described high-risk molecular lipid species and three ceramide ratios
Analysis done in	Plasma	Plasma
Study Disease	Identify lipid species that are linked to future cardiovascular events and death	CAD
Technique used	LC electrospray ionization–tandem MS	MS
Statistical Analysis	Weighted Cox regression; Principal component analysis	Mean± standard deviation; median and their logarithm; SPSS software
Results	3 of the 22 lipid subclasses - related to the risk of cardiovascular events; 2 subclasses - increased risk of cardiovascular death; 32 different lipid species - related to both potential cardiovascular events and death.	When compared to SAP patients, ACS patients had substantially higher serum LDL cholesterol levels, higher serum non-HDL cholesterol levels, and lower serum TG levels; Except for LacCer plasma concentration, all of the above-mentioned lipid species plasma concentrations were considerably higher in ACS patients relative to SAP patients, in patients with an incident (MACE); The Cer(d18:1/16:0) concentration in ACS patients was considerably higher in the MACE cohort relative to the non-MACE cohort
Conclusions	Phospholipids (including lyso- and ether- species), sphingolipids, glycerolipids and CEs were linked with future cardiovascular events and death	Circulating ceramide lipids were found to be associated with poor cardiac outcomes during long-term follow-up, regardless of clinical risk factors

Table A2 – Data from the PAD folder's articles (cont).

Reference	Bazan *et al.* (2017) [28]	Caligiuri *et al.* (2017) [30]
Hypothesis	Determine if circulating serum RvD1, DHA, and AA levels indicate an increased risk of atherosclerotic plaque rupture	Novel eicosanoids can increase the chances of cardiovascular and cerebrovascular events in PAD patients
Human// Animal	Human	Human
Studied Population	Cohort with 24 asymptomatic and 21 acutely symptomatic patients	98 patients with PAD; prevalence of transient ischemic attacks, cerebrovascular accidents, stable angina and acute coronary syndrome was n= 16, 10, 16, and 24
Targeted// Untargeted	Targeted	Targeted
Follow-up// No Follow-up	No	No
Study	Serum RvD1, DHA, and AA levels	Plasma oxylipins and fatty acids
Analysis done in	Peripheral blood	Plasma
Study Disease	Risk of atherosclerotic plaque rupture	Cardiovascular/cerebrovascular events in PAD patients
Technique used	LC-MS/MS	GC and HPLC-MS/MS
Statistical Analysis	SPSS software	Logistic regression
Results	RvD1 concentrations were lower in acutely symptomatic than in asymptomatic carotid patients; DHA concentartion were lower in acutely symptomatic, compared to asymptomatic carotid patients; AA concentration was higher in asymptomatic vs. acutely symptomatic	24 plasma fatty acids were observed, 12 of which were PUFAs and 39 plasma oxylipins were quantified
Conclusions	Elevated AA:RvD1 and AA:DHA is linked with stroke and acutely symptomatic carotid disease	None of 24 fatty acids observed were related to events; 39 plasma oxylipins were identified and 8 were significantly linked with events

Table A2 – Data from the PAD folder's articles (cont).

Reference	Chan *et al.* (2018) [54]	Chatterjee *et al.* (2017) [55]
Hypothesis	Since altered lipid metabolism can be a key mechanism connecting vascular disease and depression, phospholipids can help determine depressive symptoms in CAD patients	Intraplatelet lipid metabolism in patients with symptomatic CAD, and the effect of platelet–CXCL12 and CXCR-4/-7 on lipid-induced pro-oxidative and prothrombotic functions of platelets
Human// Animal	Human	Human
Studied Population	Major depressive symptoms (n=37) and those without (n=49)	175 patients with symptomatic CAD and 15 healthy volunteers
Targeted// Untargeted	Targeted	Targeted
Follow-up// No Follow-up	No	No
Study	Phospholipid species	Platelet oxidixed LDL
Analysis done in	Blood	Blood
Study Disease	Depression in patients with CAD	CAD
Technique used		UHPLC–ESI–QTOF–MS/MS
Statistical Analysis	IBM SPSS Statistics; Linear regressions	GraphPad Prism software; SPSS version
Results	Biosignature scores were substantially higher among those with major depressive symptoms relative to CAD controls; AA with a PS head group, lignoceric acid with a SM head group, Stearic Acid with a PS head group, and Palmitic Acid with a PI head group were correlated with higher CES-D scores	In vitro, oxLDL–LDL increased CXCL12 surface exposure and differentially modulated CXCR4 and CXCR7 surface expression, decreasing CXCR4 while increasing CXCR7 surface exposure; LDL–oxLDL induced intracellular ROS and intraplatelet mitochondrial superoxide development; Cer (d18:1/16:0), Cer (d18:1/18:0), Cer (d18:1/24:0), and Cer (d18:1/24:1) plasma levels were significantly up-regulated in CAD platelets, along with high levels of SM
Conclusions	The 10-phospholipid model could tell the difference between CAD patients who had major depressive symptoms and those who did not	Platelet–oxLDL levels are increased in CAD patients; LDL-induced ROS generation causes intraplatelet oxidative conversion into lipid peroxides and oxidized metabolites; CXCL12 increases LDL–oxLDL absorption while also improving LDL–oxLDL-induced pro-oxidative and prothrombotic platelet functions via CXCR-4/-7

Table A2 – Data from the PAD folder's articles (cont).

Reference	Chen *et al.* (2017) [38]	Cheng *et al.* (2015) [56]
Hypothesis	To reveal Atherosclerosis-induced dyslipidemia, it was examined the lipid compositions in the plasma of apolipoprotein E deficient mice fed with a high-fat diet	Examine the link of high-risk molecular lipids with coronary plaque characteristics measured by IVUS-VH imaging, coronary lipid core burden evaluated by NIRS imaging, and 1-year cardiovascular outcome in CAD patients
Human// Animal	Animal	Human
Studied Population	C57BL/6J mice were assigned to one of two control groups and fed a normal diet (NDC, n = 16) or a high-fat diet (HFDC, n = 15). ApoE-/- mice (n = 15) were also fed a high-fat diet	581 patients had diagnostic coronary angiography or percutaneous coronary intervention for acute coronary syndrome or stable angina pectoris
Targeted// Untargeted	Targeted	Targeted
Follow-up// No Follow-up	No	No
Study	Apolipoprotein E	Molecular lipids
Analysis done in	Plasma samples	Blood samples
Study Disease	Atherosclerosis-induced dyslipidemia	CAD
Technique used	UPLC-Q/TOF-MS	UHPLC
Statistical Analysis	MetaboAnalyst 3.0	SPSS software
Results	1 LysoPC and 6 PC were reported to differentiate C57BL/6J mice fed a normal and high-fat diet; PC (16:0/18:1) and PC (18:0/18:1) were also obtained when the ApoE-/- and C57BL/6J mice, both fed a high-fat diet, were compared; Then 2 PC, 1 sphinganine, 4 PC and 3 SM were reported, of which PC (16:0/16:0), PC (18:0/16:1), SM (d16:0/28:5), SM (d18:1/24:1), and SM (d18:1/16:0) showed positive associations with plasma levels of TC and LDL-C	Numerous CEs, Cer, LacCer and Cer ratios were linked to prone plaque characteristics on IVUS-VH and NIRS imaging, also with 1-year major adverse cardiac events; Ceramide d18:1/16:0 was frequently correlated with a higher LCBI on NIRS, a higher necrotic core fraction on IVUS-VH, and a higher MACE rate
Conclusions	Sphinganine, SM, and PC are the most altered lipid species in the ApoE -/- model, suggesting a disruption in sphingolipid and glycerophospholipid metabolism during the development of atherosclerotic dyslipidemia	Numerous CEs, Cer, and LacCer plasma concentrations were linked to the fraction of necrotic heart tissue on IVUS-VH imaging and the lipid core pressure on NIRS imaging of coronary atherosclerosis

Table A2 – Data from the PAD folder's articles (cont).

Reference	Claria, Nguyen, Madenci, Ozaki & Serhan (2013) [34]	De Leon, Boue, Szostak, Peitsch & Hoeng (2015) [57]
Hypothesis	The lipid mediator profiles in human adipose tissue of PAD patients who had progressed to the clinical need for significant lower-extremity amputation were compared to those of control subjects undergoing elective orthopedic procedures	Approaches based on lipidomics are a strong technique for characterizing the molecular lipid species and pathways involved in CVD onset and progression
Human// Animal	Human	
Studied Population	14 PAD patients undergoing major lower extremity amputations; Control subcutaneous adipose tissue samples were collected from 12 participants undergoing elective hip or knee replacement who had no history of PAD	
Targeted// Untargeted	Untargeted	
Follow-up// No Follow-up	No	
Study	Lipid mediators	
Analysis done in	Adipose tissue	
Study Disease	PAD	
Technique used	LC-MS/MS	
Statistical Analysis	continuous variables between groups - Mann-Whitney U-test; paired continuous variables - the Wilcoxon signed-rank test	
Results	SC fat from PAD patients had significantly lower levels of PD1 and 17-HDHA; When compared to SC, perivascular adipose tissue had higher pro-resolving mediators levels, indicating that this fat depot has a higher resolution capability	
Conclusions	it was identified a particular pro-resolving mediators pattern strongly linked with PAD by studying the distribution of bioactive lipid mediators based on the localization of human fat depots	

Table A2 – Data from the PAD folder's articles (cont).

Reference	de Mello *et al.* (2009) [58]	Djekic, Pinto, Repsilber, Hyotylainen & Henein (2019) [59]
Hypothesis	The relationship between circulating levels of IL-6, C-reactive protein and TNF-α and total plasma content of Cer and DG in CHD patients with Insulin Resistance	Determine the lipids that are most suitable to be linked in the biochemical process of CAC and its severity
Human// Animal	Human	Human
Studied Population	33 patients with CHD	No coronary calcification (NCC = 26), Mild coronary calcification (MCC = 27) and Severe coronary calcification (SCC = 17)
Targeted// Untargeted	Targeted	Untargeted
Follow-up// No Follow-up	No	No
Study	Serum levels of the inflammatory markers and plasma lipid metabolites	Lipidomic approach
Analysis done in	Plasma	Blood (serum)
Study Disease	Coronary heart disease	Coronary artery calcification
Technique used	UPLC coupled to electrospray ionisation MS	LC-MS
Statistical Analysis	SPSS software	MATLAB®; Principal Components Analysis
Results	Both plasma Cer and serum IL-6 levels were found to be linked to HOMA-IR; There was no association observed within TNF-α or hsCRP levels and plasma DG; Cer and the SM pathway have been linked to the regulation of IL6 gene expression	PC(16:0/20:4) at higher levels and PC(18:2/18:2), PC(36:3), and PE(20:0/18:2) at lower levels were classified as having a correlation with SCC than NCC; Individual TG levels did not vary significantly between the 3 groups, but gathering the lipid profiles revealed a pattern for higher saturated and monounsaturated TG levels in SCC than in NCC; SCC had lower TG(49:2), TG(51:1), TG(54:5), and TG(56:8) levels as opposed to MCC
Conclusions	The inflammatory marker IL-6 is linked to Cer, IR, and inflammation; Cer can play a role in the initiation of inflammation in IR states, which frequently coexist with CHD	103 lipids were included in 4 major lipid classes: 38 glycerolipids, 49 glycerophospholipids, 15 sphingolipids, and 1 sterol lipid; The mechanism of coronary calcification is linked to autophagy dysfunction

Table A2 – Data from the PAD folder's articles (cont).

Reference	Djekic, Pinto, Vorkas & Henein (2016) [60]	Estronca *et al.* (2012) [61]
Hypothesis	Lipidomics findings obtained by two separate centers in serum samples from patients with calcific coronary artery disease (CCAD) and their corresponding controls were evaluated for reproducibility and replication.	Evaluating the effect of LDL charge on Nat-LDL due to the inclusion of a cholesteryl hemiester, cholesteryl hemisuccinate (Chs); When macrophages were exposed to Chs-loaded Nat-LDL (Chs-LDL), the role of Chs in the development of a lipidosis phenotype and cell death was investigated
Human// Animal	Human	
Studied Population	No calcification (CS=26), mild calcification (CS=27) and severe calcification (CS=17); Each patient had two serum samples taken and tested by two different laboratories in different countries and at different times	
Targeted// Untargeted	Untargeted	
Follow-up// No Follow-up	No	
Study	Lipidomic approach	
Analysis done in	Blood (serum)	
Study Disease	Calcific coronary artery disease	
Technique used	LC-MS	
Statistical Analysis	Principal component analysis; ANOVA	
Results	Six similar metabolites that distinguished patients with severe coronary artery calcification from those with no calcification; The relative intensities of the two studies showed strong correlation coefficients; In the diseased group serum, PC moieties with 18-carbon FAC were found in lower concentrations and 20:4 FAC in higher concentrations, also, 3 common sphingomyelins were discovered	
Conclusions	Six distinct metabolites distinguished patients with severe calcification from those with no calcification; Those with mild observable calcification were not different from the ones with no calcification	

Table A2 – Data from the PAD folder's articles (cont).

Reference	Feng *et al.* (2018) [62]	Garcia *et al.* (2018) [63]
Hypothesis	Find new candidate lipid biomarkers to improve the diagnosis of myocardial I/R injury, to determine the seriousness of myocardial I/R, and discover novel lipid-related mechanisms	HDL2 and HDL3 antiplatelet effects in ACS patients and non-CAD control subjects were compared, as well as the signaling pathways modulated by both HDL subfractions were characterized
Human// Animal	Human	Human
Studied Population	40 patients (42-75y) diagnosed with ST-segment elevation myocardial infarction (STEMI)	ACS patients (n = 30) and non-CAD (n=30)
Targeted// Untargeted	Untargeted	Targeted
Follow-up// No Follow-up	No	No
Study	Lipid biomarkers	Measured platelet aggregation and ex vivo thrombus formation
Analysis done in	Blood (serum)	Blood (serum)
Study Disease	Myocardial ischemia reperfusion (I/R) injury	Acute coronary syndrome
Technique used	LC-ESI-MS/MS	MS equipped with electrospray ionization operating in negative mode
Statistical Analysis	SPSS 17.0 software	Pearson's chi-square test; Mann–Whitney U-test or Student's t-test; Wilcoxon or ANOVA tests
Results	Bioinformatics analysis revealed 16 possible lipid biomarkers over three time periods, as well as complex changes in these lipid contents before and after reperfusion	Hydroxylated fatty acids may be essential biologically active lipids that contribute to HDL2 particles' enhanced antithrombotic effect
Conclusions	Lipidomics analysis revealed differences before and after reperfusion, implying that some of these lipids could be used as biomarkers to improve the diagnosis of myocardial I/R	The findings highlight the complexities of HDL particle composition and point to a physiological modification of HDL properties in ACS patients, which may result in a decrease in platelet-dependent thrombotic risk

Table A2 – Data from the PAD folder's articles (cont).

Reference	Gollasch *et al.* (2019) [64]	Gong *et al.* (2020) [65]
Hypothesis	RBC epoxy fatty acids may be affected by acute, maximal physical activity; Bruce test is used to guarantee that healthy individuals had an adequate and robust metabolic and hemodynamic response	Investigate the possible biomarker of metabolic syndrome in depth by analyzing changes in plasma metabolic profile in subjects with metabolic syndrome symptoms
Human// Animal	Human	Human
Studied Population	6 healthy volunteers (5 male and 1 female; BMI 27.9 ± 6.6 kg/m2) signed informed consent forms outlining the protocols to be followed and the potential risks involved	MetS patients and healthy controls; Untarget study on 80 subjects (40 MetSs and 40 HCs); Target study on 160 subjects (80 MetSs and 80 HCs)
Targeted// Untargeted	Targeted	Untargeted
Follow-up// No Follow-up	No	3 years follow-up
Study	Epoxy fatty acids	Metabolic profile
Analysis done in	Venous blood	Plasma
Study Disease	Cardiovascular function	Metabolic syndrome
Technique used	LC–MS/MS	UHPLC-Q-TOF/MS
Statistical Analysis	ANOVA	PCA and PLS-DA
Results	Variations in the total amounts of epoxyoctadecenoic acids, epoxyeicosatrienoic acids and epoxydocosapentaenoic acids; The data show an accumulation 5,6-EET, 11,12-EET, 14,15-EET, 16,17-EDP, 19,20-EDP 9,10-EpOME, and 12,13-EpOME concentrations in RBCs at exhaustion, which are active vasodilators	The interior standard method was used to verify the 55 candidate metabolites; When compared to the HC group, PC(18:1/P-16:0), PC(o-22:3/22:3), and PC(P-18:1/16:1) were significantly lower in MetS patients; Sphingolipid metabolism and glycerophospholipid metabolism were the primary metabolic pathways
Conclusions	Intense exercise may affect the levels of a variety of CYP epoxy-metabolites in RBCs; RBCs may serve as a reservoir for EETs and probably other epoxide fatty acids, which when released may have a vasoregulatory effect	Based on the rigorous screening and validation protocol, a panel of three plasma metabolites was discovered: PC (18:1/P-16:0), PC (o-22:3/22:3), and PC (P-18:1/16:1); MetS was discovered to have a major impact on choline metabolism pathways; MetS biomarker model established in the study serves as a foundation for potential diagnostic or therapeutic growth

Table A2 – Data from the PAD folder's articles (cont).

Reference	Guo *et al.* (2019) [66]	Halade *et al.* (2018) [67]
Hypothesis	It was discovered and quantified major oxCEs in human plasma from various forms of CVD, as well as observed changes in oxCE levels in human plasma with CVD	For structure recognition and quantification of lipid species imaged on mouse infarcted and non-infarcted left ventricle, molecular mass spectrometry imaging was related to LC-MS/MS
Human// Animal	Both	Animal
Studied Population	49 human subjects were classified into 4 groups: healthy control group (con=10), coronary heart disease group (CHD=14), coronary heart disease and cerebrovascular disease group (CHD+CBD=15), and myocardial infarction group (MI=10); Male C57BL/6 mice were assigned to one of four: control group, ch-13(c, t)-HpODE group with varying concentrations (1 mg/kg, 1.5 mg/kg, 2.2 mg/kg)	8–12-week-old male C57Bl/6J mice; The left atrium and ventricle were examined; After removing the thin pericardium layer, the left anterior descending artery was permanently ligated
Targeted// Untargeted	Targeted	Untargeted
Follow-up// No Follow-up	No	No
Study	oxCE	Lipid molecules localized in the infarcted myocardium
Analysis done in	Human plasma samples; Liver, peritoneal macrophages and plasma	Cardiac structure
Study Disease	CVD	Infarcted myocardium
Technique used	LC-MS	LC-MS and LC-MS/MS
Statistical Analysis	ANOVA	PCA; (ROC) curve analysis
Results	A complex mixture of oxCE is easily detectable in human plasma from various forms of CVD using a targeted lipidomics approach; Besides that, it was discovered that oxCE levels in human plasma were positively correlated with various forms of CVD and increased significantly in MI patients	On day 1 post-MI, different lipid biomarkers were identified at high levels within failing LV tissue when infarcted heart tissue was compared to control heart tissue
Conclusions	A new mechanism whereby endogenous oxCE in oxLDL leads to CVD pathogenesis has been established	it was possible to calculate the concentrations and types of lipid molecules localized in the infarcted myocardium on day 1 post-MI using a MI model and molecular MSI coupled with LC-MS/MS

Table A2 – Data from the PAD folder's articles (cont).

Reference	Halade, Kain, Tourki & Jadapalli (2019) [68]	Havulinna *et al.* (2016) [69]
Hypothesis	identify the unknown and underappreciated metabolite profiles in acute and chronic leukocyte reprogramming as a component of molecular mechanisms leading to HF	if ceramides are linked to significant adverse cardiovascular events (MACEs) in otherwise healthy people
Human// Animal	Animal	Human
Studied Population	MI was caused in wild type and 12/15LOX−/− male mice through surgical biding of the left anterior descending coronary artery to mimic human HF	men and women between the ages of 25 and 74 were recruited; incident MACE (n=813); secondary tests were carried out for MACE death (n=116) in the absence of a prior nonfatal MACE and for chronic MACE (n=226) in survivors of a previous incident MACE
Targeted// Untargeted	Targeted	Targeted
Follow-up// No Follow-up	No	Yes
Study	Lipoxygenase's function in the temporal kinetics of lipidomic and metabolic reprogramming in HF	Quantified 4 circulating Cer, Cer(d18:1/16:0), Cer(d18:1/18:0), Cer(d18:1/24:0), and Cer(d18:1/24:1)
Analysis done in	Heart	Blood (serum)
Study Disease	Heart failure	Cardiovascular events (MACEs)
Technique used	LC-MS/MS	LC-MS/MS
Statistical Analysis	GraphPad Prism 5; (ANOVA), followed by Newman-Keuls post hoc test; Kaplan-Meier and the logrank test	R version 3.2.2
Results	In 12/15LOX null mice, cardioprotective plasma metabolome with lower levels of 2-aminoadipic acid and cypoxin-enriched lipidome reconfigured leukocyte profiling in acute cardiac injury, improving LV efficiency and recovery in chronic HF	Cer(d18:1/18:0)/Cer(d18:1/24:0) and Cer(d18:1/24:1)/Cer(d18:1/24:0) were elevated in MACE subjects opposed to asymptomatic subjects; Ceramide (d18:1/18:0) is connected to incident MACE; Ceramide (d18:1/16:0) and (d18:1/24:1) have been linked to recurrent MACE
Conclusions	The deletion of a specific lipoxygenase alters lipidomic and metabolic signatures, resulting in altered leukocyte profiling that delays HF progression and improves survival	In healthy people, specific circulating ceramides are linked to an increased risk of incident MACE

Table A2 – Data from the PAD folder's articles (cont).

Reference	Hilvo *et al.* (2018) [70]	Hinterwirth, Stubiger, Lindner & Lammerhofer (2013) [71]
Hypothesis	characterize the changes in the lipidome of plasma and lipoprotein particles following administration of a PCSK9 inhibitory antibody to patients with proven CAD	The benefit of antibody specificity for OxLDL extraction and enrichment was coupled with the sensitive identification and quantification of OxPLs using LC-ESIMS/MS
Human// Animal	Human	
Studied Population	A randomized, double-blind study involving 40 non-diabetic patients with proven CAD	
Targeted// Untargeted	Untargeted	
Follow-up// No Follow-up	No	
Study	Lipidome of plasma and lipoprotein particles	
Analysis done in	Plasma	
Study Disease	CAD	
Technique used	Hybrid triple quadrupole/linear ion trap mass spectrometer	
Statistical Analysis	SAS version 9.4 and R version 3.3.0	
Results	PCSK9 inhibition drastically reduced plasma levels of sphingolipids (dihydroceramide, GlcCer, SM, Cer), CE, and free cholesterol; The administration of PCSK9 antibody changes the lipid composition of plasma and lipoprotein particles significantly	
Conclusions	PCSK9 inhibition through antibodies affects not only LDL cholesterol metabolism as well as the global lipidome at the plasma and circulating lipoprotein particle levels	

Table A2 – Data from the PAD folder's articles (cont).

Reference	Holcapek *et al.* (2015) [72]	Hu *et al.* (2011) [73]
Hypothesis	Lipidomic analysis of CVD, healthy normal, and healthy obese subjects using three MS-based methods: HILIC-UHPLC/ESI-MS for polar lipid analysis, NP-UHPLC/APCI-MS for nonpolar lipid analysis, and MALDI-MS for rapid lipidomic screening	Centered on lipidomics technology, LC-MS was used to investigate the distinction of plasma lipid profiles in patients with treated/untreated critical hypertension and subjects with normotension
Human// Animal	Human	Human
Studied Population	58 men aged 40 to 55 years old: group 1: healthy subjects with BMI of 25.1 kg/m2; group 2: healthy subjects with BMI of 30 to 35 kg/m2; group 3: subjects with non-ischemic dilated cardiomyopathy; group 4: subjects with chronic atrial fibrillation; group 5: subjects with ischemic heart diseases, post-myocardial infarction	Male subjects aged 35 to 55; The study population included 30 male patients with uncomplicated primary hypertension who were either treated or not and 28 men with normotension who served as controls
Targeted// Untargeted	Untargeted	Untargeted
Follow-up// No Follow-up	No	No
Study	Lipidomic study	Lipidomic study
Analysis done in	Blood	Plasma
Study Disease	CVD	Hypertension
Technique used	HILIC-UHPLC/ESI-MS; NP-UHPLC/APCI-MS; MALDI-MS	LC–MS
Statistical Analysis	Unsupervised PCA and supervised OPLS methods	SPSS statistical
Results	The PC 32:0 concentration is down-regulated in CVD patients, with group 3 showing the greatest decrease but also the greatest difference; The TG 52:3 concentration is up-regulated in CAD patients, where the greatest shift is reported for group 3, yet again with the greatest variation for this group	The antihypertensive drugs used in the study not only reduced hypertensive patients' blood pressure to target levels but also caused a slight modification in the metabolism of neutral plasma lipids such as CE and TG in the patients
Conclusions	In CVD groups, the two most up-regulated lipids are 1,3-DG 32:1 and 1,3-DG 34:1, while the two most down-regulated species are SM 34:2 and 1,3-DG 32:0	Systematic and detailed characterization of lipid metabolism about hypertension and drug therapy

Table A2 – Data from the PAD folder's articles (cont).

Reference	Huynh *et al.* (2019) [74]	Jove *et al.* (2013) [39]
Hypothesis	By analyzing associations with anthropometric characteristics related to risk of cardiometabolic diseases, such as age, gender, and BMI, using a subcohort of the Australian Diabetes, Obesity, and Lifestyle research, it will be able to gain a better understanding of biological processes	The key pathological mechanism leading to CVD is atherosclerosis, and diet being the most important element associated; Comparative lipidomics and metabolomics in plasma in early atherogenesis may contribute to the identification of plasma biomarkers that can be used to monitor not only disease progression but also disease prediction
Human// Animal	Human	Animal
Studied Population	Lipidomic research was performed on 640 subjects, 389 of whom were normoglycemic and 251 of whom had prediabetes, with 147 having impaired fasting glucose and 183 having impaired glucose tolerance; 79 individuals had both IFG and IGT	Atherogenesis was induced in male Golden Syrian hamsters (n=15–16 per group); The aorta was detached, and the descending aorta was separated from the aortic arch; To investigate dietary control of atheromatosis, another group followed the same diet as the atherogenic group but added 0.2 percent grape seed extract
Targeted// Untargeted	Untargeted	Untargeted
Follow-up// No Follow-up	No	No
Study	Lipidomic study	Lipidomic analysis and Metabolomic analysis
Analysis done in	Plasma	Descending aorta and Plasma samples; Plasma metabolites
Study Disease	Risk of cardiometabolic disease	Atherosclerosis
Technique used	LC-MS	LC-MS
Statistical Analysis	Matlab 2013a or R (3.4.0)	SPSS software
Results	It was characterized in detail the associations among plasma lipid species and prediabetes, which included several atypical lipid species and several uncommon pathways; 328 lipid species were found to be significantly associated with either FBG, 2h-PLG, or both, demonstrating the extensive dysregulation of lipid homeostasis that occurs before the start of T2D	The findings revealed previously unknown changes in lipid and amino acid metabolism, the peroxisome proliferator-activated receptor γ pathway, and oxidative and endoplasmic reticulum stress, as well as cell senescence; Cer(d18:1/24:1) and the PUFA DHA were found to be higher in atherogenic plasma and aorta
Conclusions	The results support a precision medicine strategy in which IFG and IGT are identified early and treated appropriately	Discovery of new pathways in atherogenesis, as well as new possible plasma biomarkers, which may allow for disease prediction and measurement in its early stages

Table A2 – Data from the PAD folder's articles (cont).

Reference	Jung *et al.* (2018) [75]	Kain, Prabhu & Halade (2014) [76]
Hypothesis	Metabolites that expressed differently in plaque-containing and control plaque-free human aortic tissues were investigated; In addition, in vitro testing was performed to demonstrate the biological significance of the metabolites discovered in the study	REVIEW
Human// Animal	Human	
Studied Population	At first, 60 samples (from 44 patients) were collected and 42 samples (from 33 patients) were suitable to and used in quantitative analysis; 25 of the 42 samples (from 22 patients) were evaluated with global profiling; The average patient age was 65, and 58% were men. Only two of the 33 participants had a background in coronary artery disease	
Targeted// Untargeted	Untargeted	
Follow-up// No Follow-up	1 year follow-up	
Study	Lipidomics	
Analysis done in	Aortic tissue samples	
Study Disease	Atherosclerosis	
Technique used	LC-MS	
Statistical Analysis	SPSS software	
Results	The metabolic profiles of atherosclerotic and non-atherosclerotic vessels differed significantly; In plaques, oxidative stress dysregulation was demonstrated by metabolites in the purine and glutathione pathways, and levels of GlcCer, tryptophan, and kynurenine were altered, all of which are correlated with inflammation; it was found a rise in quinic acid levels in plaques, as well as an inhibitory effect of quinic acid on inflammatory activation and oxidative stress in macrophages	
Conclusions	It was discovered that altered patterns of metabolites in human aortic tissues are mechanistically related to the proatherogenic method; The discovery of higher quinic acid levels, as well as evidence of anti-inflammatory activity, contributes to the understanding of the metabolic component of plaque biology	

Table A2 – Data from the PAD folder's articles (cont).

Reference	Karjalainen *et al.* (2019) [77]	Koop *et al.* (2019) [78]
Hypothesis	The effects of apoE polymorphism on plasma ceramides, with a focus on CAD risk prediction	Determine the changes in RV lipid content during chronic pressure load and their relationship to RV operation, remodeling, and metabolism over time
Human// Animal	Human	Animal
Studied Population	In 2007, 2,200 randomly chosen subjects aged 30-45 years old took part in the 27-year follow-up	Male Wistar WU rats were randomly assigned to either pressure load via pulmonary artery banding (PAB, n = 25) or sham surgery (control, n = 13), and clinical signs of RV failure were monitored daily; Animals were euthanized at 2 (n = 5 vs. 4), 5 (n = 11 vs. 4), and 12 weeks (n = 9 vs. 5) after surgery
Targeted// Untargeted	Targeted	Targeted
Follow-up// No Follow-up	27 years follow-up	No
Study	ApoE	semi-quantitative measurements of lipids like as TG, DG, Cer, cardiolipin (CL), PC, PE, PI, PG and phosphatidic acid (PA)
Analysis done in	Blood (serum)	Plasma
Study Disease	CAD	Right ventricular (RV) failure
Technique used	MS	
Statistical Analysis	R program	R program
Results	6 Cer species were found to be associated with apoE genotypes; Sex-stratified studies, the high-risk CAD Cer(d18:1/16:0) was linked to apoE genotypes both in women and men; Cer(d18:1/18:0) was only observed in women	Lipidomics showed substantial decreases in myocardial DG and cardiolipins following 12 weeks of pressure load, induced by (poly-)unsaturated types; Cardiolipins diminished due to the most frequent form, tetralinoleoyl cardiolipin; Mitochondrial ability for fatty acid oxidation was maintained, but glucose oxidation ability increased
Conclusions	Focused on quantified molecular Cer species from plasma, apoE polymorphism is correlated with the likelihood of developing CAD; There is a possible correlation among Cer metabolism, apoE polymorphism, plasma LDL metabolism and atherogenesis	RV dysfunction caused by pressure load is linked to lower intracardiac unsaturated lipid levels, particularly tetralinoleoylcardiolipin; This was followed by retained mitochondrial capacity for fatty acid oxidation, increased mitochondrial ability for glucose oxidation, and early oxidative stress activation.

Table A2 – Data from the PAD folder's articles (cont).

Reference	Kujala & Nevalainen (2015) [79]	Kujala, Nevalainen, Marz, Laaksonen & Datta (2015) [80]
Hypothesis	Two case studies were used to examine the actions of regularized regression methods for binary classification and to discuss related statistical data preprocessing techniques; the main goal is to develop an efficient statistical methodology for lipidomics, particularly for identifying comprehensible and reliable biomarkers based on a small number of lipids	Compare differential network connectivities and modular structures of two LURIC patient subgroups, cases and controls, and classify lipids associated with an increased risk of CVD-related death
Human// Animal		Human
Studied Population		Among 1997 and 2002, patients were recruited when presenting to one of the study hospitals with symptoms resembling CVD; Lipidomic profiles were assessed in a subgroup of 445 males, 258 of whom were cases and 187 of whom were controls
Targeted// Untargeted		Untargeted
Follow-up// No Follow-up		5 years follow-up
Study		Lipidomic study
Analysis done in		Venous blood (serum)
Study Disease		CVD
Technique used		MS
Statistical Analysis		Gill, Datta, and Datta (PLS-scores)
Results		A total of 237 lipids were found in the lipidomic profiles of 445 CVD patients; 86 lipids were found in at least 60% of the patients and were used in the studies
Conclusions		A network with adaptive elastic nets was built, following a differential network analysis with a permutation test, that produced results similar to the PLS-based connectivity scores

Table A2 – Data from the PAD folder's articles (cont).

Reference	Laaksonen, Janis & Oresic (2008) [81]	Lam *et al.* (2014) [82]
Hypothesis		Patients with SIVD (Subcortical ischemic vascular dementia) and MixD (mixed dementia) had a lipidomics analysis of white and gray matter from the temporal cortex, which was compared to age-matched, nondemented controls
Human// Animal		Human
Studied Population		SIVD, MixD, and age-matched control subjects' postmortem frozen brain tissues; Gray and white matter samples from the temporal lobe; There was no clinical evidence of dementia, neurologic, or psychiatric disorder in the control subjects
Targeted// Untargeted		Untargeted
Follow-up// No Follow-up		No
Study		Lipidomic study
Analysis done in		Brain tissue
Study Disease		SIVD and MixD
Technique used		HPLC-MS
Statistical Analysis		One-way analysis of variance with post hoc Tukey test; Student t test
Results		A total of 334 different lipid species from 17 lipid subclasses were studied, such as neutral lipids like CEs, free cholesterols, DG, TG; sphingolipids like Cer, GalCer, GlcCer, SM, ganglioside mannoside 3 and sulfatides; and phospholipid subclasses like PC, PE, PG, PI, PS, phosphatidic acids, and lysobisphosphatidic acids
Conclusions		A reference frame of lipids was created to help advance existing diagnostic and therapeutic efforts of SIVD and Mix; Lipidomic findings indicate that Cer synthase 2 may be a potentially valid model for human SIVD, warranting further study

Table A2 – Data from the PAD folder's articles (cont).

Reference	Lankinen *et al.* (2009) [83]	Lee *et al.* (2017) [37]
Hypothesis	A particular fish diet high in polyunsaturated fatty acids influences not only serum fatty acid composition and bioactive lipid concentrations; The study aims to see how eating fatty or lean fish affects serum lipidomic profiles in CAD patients	To understand better cardiac metabolism in an atherogenic diet (AD)-induced atherosclerosis mouse model, an integrated metabolomic and lipidomic study was applied using multi-platform analytical methods such as high-resolution magic-angle-spinning (HR-MAS) NMR, solution NMR, and LC-MS
Human// Animal	Human	Animal
Studied Population	8-week monitored; The subjects were assigned to one of three groups: fatty fish (n= 11), lean fish (n= 12), or control (n= 10); Subjects in the fatty fish and lean fish groups were instructed to eat 100–150 g of fish per meal at least four meals per week	5-week-old male C57BL/6 J mice; The mice were divided into ND (n = 38) and AD (n = 36) groups after a 7-day adaptation phase; 1.25% (w/w) cholesterol and 0.5% cholate (w/w) were added to the AD; Each group included at least 10 mice (n = 10–13)
Targeted// Untargeted	Untargeted	Untargeted
Follow-up// No Follow-up	No	No
Study	Lipidomic study	Metabolomic and lipidomic analysis
Analysis done in	Blood samples	Sera and hearts
Study Disease	CAD	Atherosclerosis
Technique used	UPLC-ESI-MS and GC	HR-MAS, NMR and LC-MS.
Statistical Analysis	SPSS	SPSS
Results	Total of 307 lipids; At first, it was determined that there were no major lipid differences between the groups at baseline; Following the intervention, there were differences in 240 lipids between the three groups; When analyzing within-person differences in the three intervention groups, the control group had no major changes, although the fatty and lean fish groups had 63 and 5 lipid differences, respectively	The AD group's heart weight was slightly lower than the ND group's; The AD group had lower levels of serum TG and HDL-C than the ND group; The AD group had higher levels of LDL-C and total cholesterol than the ND group; Cer, GlcCer, DG, FFA, LysoPC, LysoPE, PC, PE, SM, and TG were classified as markedly different lipid classes
Conclusions	The 8-week intake of fatty fish reduced lipids, that are potential mediators of lipid-induced insulin resistance and inflammation	It was demonstrated that an AD induces major metabolic perturbations in heart and serum samples from a mouse model of atherosclerosis

Table A2 – Data from the PAD folder's articles (cont).

Reference	Li *et al.* (2014) [33]	Li *et al.* (2019) [84]
Hypothesis	Since lipid profiling seeks to quantify various types of lipids to examine and understand their function in a physio- or pathophysiological pathway of clinical significance, there is an increasing interest in the study of atherosclerosis	LC-MS-based metabolomics and lipidomics were used to perform detailed profiling of rat plasma and heart in reaction to acute myocardial ischemia and Danqi Tongmai tablet
Human// Animal	Human	Animal
Studied Population	1) subjects aged 40–75 years with carotid artery atherosclerosis shown by ultrasound; and 2) subjects undergoing stroke prevention management therapy, with a focus on blood pressure and lipid metabolism in laboratory testing. So, six controls and six subjects with atherosclerosis were selected	36 male Wistar rats were assigned to one of three groups: the sham-operating group (S), the AMI model group (A), and the DQTM group (D)
Targeted// Untargeted	Untargeted	Untargeted
Follow-up// No Follow-up	No	No
Study	Lipidomic study	Metabolic and Lipidomic analysis
Analysis done in	Human plasma	Plasma and heart
Study Disease	Atherosclerosis	Acute myocardial ischemia
Technique used	NP/RP 2D LC-QToF/MS	UHPLC-QTOF/MS
Statistical Analysis	PCA	MetaboAnalyst
Results	540 endogenous lipid species were identified, including FFA, PI, PG, LysoPG, PS, PE, LysoPE, PC, LysoPC, SM, Cer, LacCer, GalCer, GlcCer, MG, DG, and TG; After successfully separating GalCer and GlcCer, it was discovered that only the levels of GalCer in atherosclerosis patients were substantially increasing, instead of GlcCer, when compared to controls	The total amount of metabolites in the heart was significantly greater than in the plasma; The ischemia region's metabolites were dramatically altered due to a lack of blood and oxygen-induced by ligating the left anterior descending coronary artery, however, the metabolites that might be affected by DQTM were restricted; The ischemia area was affected more by AMI and less by DQTM
Conclusions	So, this NP/RP 2D LC-QToF/MS system detected 540 endogenous lipid species from 17 lipid classes in human plasma	Established a comprehensive and integrated approach to investigating the pathogenesis of AMI and investigating the beneficial effects of DQTM

Table A2 – Data from the PAD folder's articles (cont).

Reference	Lietz *et al.* (2013) [85]	Lim *et al.* (2018) [86]
Hypothesis	It aimed to look into the effects of quitting smoking on plaque formation and aortic arch content of numerous lipid molecular classes and species	This study aimed to look into the utility of consumer-grade wearables in cardiovascular and lipidomics study. To that end, multidimensional data was produced from 233 normal subjects selected for a longitudinal study
Human// Animal	Animal	Human
Studied Population	ApoE-/- mice fed a standard laboratory diet containing 0.003% cholesterol and 4.0% fat and were subjected to filtered fresh air (sham group) or mainstream CS for 3 and 6 months (continuous smoking group, CS) or 3 months only, followed by 3 months of sham exposure (cessation group); 7 to 8 mice were utilized for lipid measurements in the plasma and aorta, sixteen mice for plaque size measurements, and six to eight mice for lipidomics	The 233 volunteers were followed for a median duration of 4 days (range 2±6 days) per subject
Targeted// Untargeted	Untargeted	
Follow-up// No Follow-up	No	
Study	Lipidomic study	
Analysis done in		
Study Disease		
Technique used		
Statistical Analysis		
Results		
Conclusions		

Table A2 – Data from the PAD folder's articles (cont).

Reference	Lu *et al.* (2017) [87]	Ma *et al.* (2019) [88]
Hypothesis	Untargeted metabolomics analysis in plasma of SA (stable angina), MI (myocardial infarction), and HC (health controls) and defined specific metabolite characteristics that could be used to distinguish these three groups	HPS proteins are involved in lipid metabolism in hepatocytes, influencing plasma lipid material; In the current research, plasma lipidomics was used to describe the plasma lipid profiles of HPS mutant mice, and lipid droplet accumulation in hepatocytes was reported after an overnight starvation
Human// Animal	Human	Animal
Studied Population	28 subjects with stable angina (SA), myocardial infarction (MI), and healthy controls (HC)	Tpa, ru, and ep mutant mice (pa, HPS9 deficient; ru, HPS6 deficient; ep, HPS1 deficient) and the WT C57BL/6J mice
Targeted// Untargeted	Untargeted	Untargeted
Follow-up// No Follow-up	No	No
Study	Metabolomic analysis	Lipidomic study
Analysis done in	Human plasma	Plasma
Study Disease	CAD	Atherosclerosis
Technique used	LC-MS	UPLC-QTRAP
Statistical Analysis	Principal component analysis; Partial least-squares discriminant analysis; one-way ANOVA	Kruskal–Wallis test and Student's t tests
Results	In all paired comparisons, disruption of GPL metabolism was listed first among the most significantly impacted pathways, supporting the notion that dyslipidemia is a prominent characteristic in all stages of atherosclerosis; LPO and oxidative stress also play a significant role in atherosclerosis, and LPO metabolites can be used to distinguish MI from SA and HC	Demonstrate that the various HPS proteins have different roles in the lipid metabolism process; Hepatocytes are the primary site of lipid metabolism, with lipids primarily contained in lipid droplets, which are individual organelles with a lipid-rich center and surrounding phospholipid monolayers
Conclusions	To differentiate SA from HC, MI from SA and MI from HC groups, a total of 18, 37, and 36 different metabolites were found, respectively; LPO tended to be a common feature in CAD and could be used as a biomarker to distinguish MI from SA and HC	The anti-atherosclerotic effects of the pa mutant are similar to those of the ru mutant, but the ep mutant is atherogenic; The results could help explain why different HPS mutant mice have different anti-atherosclerotic or atherogenic effects after being fed high-cholesterol diets

Table A2 – Data from the PAD folder's articles (cont).

Reference	Mazereeuw *et al.* (2015) [89]	McManus *et al.* (2016) [90]
Hypothesis	Given the proposed mechanistic importance of Platelet-activating factors (PAFs), use cutting-edge lipidomics technology to produce hypotheses about the relationship between the PAF lipidome and depressive symptoms in CAD patients	Analyze the effects of EPA and DHA on postprandial vascular function as measured by blood pressure, pulse wave velocity (PWV), reactive hyperemia index (RHI), and Augmentation Index (AIx
Human// Animal	Human	Human
Studied Population	The researchers looked into the links between different PAF species and depressive symptoms in 26 CAD patients (age: 60.69.2 years, 69% male)	26 men between the ages of 35 and 55; Meals including EPA-rich oil (ERO), DHA-rich oil (DRO), or control oil (CO) were used on three different study days at least four weeks apart; Subjects were required to observe a restricted diet for three days prior to their clinical appointments
Targeted// Untargeted	Targeted	Targeted
Follow-up// No Follow-up	No	No
Study	PAFs	Impact of EPA vs. DHA
Analysis done in	Fasting blood	Blood samples
Study Disease	CAD	CVD
Technique used	HPLC electrospray ionization MS	MS
Statistical Analysis	SPSS, version 13.0	SPSS
Results	PAFs were found in 20 species, with PC(O-12:0/2:0), PC(O-14:1/2:0), PC(O-17:3/2:0), and PC(O-18:3/2:0) identified as main species with possible relevance to depressive symptoms in CAD patients	The ERO-meal elevated plasma concentrations of 15S-HEPE, 14,15-EpETE, 17,18-EpETE, 14,15-DiHETE, and 17,18-DiHETE, while the DRO raised concentrations of the EPA-derived metabolites 14,15-DiHETE, 17,18-EpETE, and 17,18-DiHETE, as well as the DHA-derived metabolite 19,20-DiHDPA; Following the intake of the control meal, no changes were observed
Conclusions	Further research into PAF species, specifically the PAFs PC(O-12:0/2:0), PC(O-14:1/2:0), PC(O-17:3/2:0), and PC(O-18:3/2:0), as markers of depressive symptoms in CAD patients is needed	A single dose of DHA enhanced postprandial arterial stiffness as measured by AIx, if maintained, would be correlated with a substantial reduction in CVD risk; The detected oxylipin increases give mechanistic insight into the AIx effect

Table A2 – Data from the PAD folder's articles (cont).

Reference	Meisenbichler, Doppler, Bernhard & Muller (2019) [91]	Mendez, Dasilva, Taltavull, Romeu & Medina (2017) [92]
Hypothesis	Based on the matrix deposition/ recrystallization technique, a succinct sample preparation procedure for MALDI MSI to analyze the histological distribution of lipids in aortic tissue was described	REVIEW
Human// Animal	Human	
Studied Population	Frozen human aortic tissue	
Targeted// Untargeted		
Follow-up// No Follow-up		
Study		
Analysis done in		
Study Disease		
Technique used		
Statistical Analysis		
Results		
Conclusions		

Table A2 – Data from the PAD folder's articles (cont).

Reference	Mishra *et al.* (2019) [27]	Moren *et al.* (2016) [93]
Hypothesis	Conduct a system-level review of lipidomics data in order to recognize networks of lipid species that were correlated with subclinical markers of both osteoporosis and atherosclerosis	The structural and compositional features of PON1-containing HDL particles were investigated to identify what characteristics differentiate them from total HDL and to identify possible functional consequences
Human// Animal	Human	Human
Studied Population	The research began in 1980 with 3,596 children and adolescents aged 3 to 18 years; The current research is focused on a 2007 follow-up of 1,494 participants aged 30 to 45, with four atherosclerotic and six osteoporotic markers	Plasma was collected from healthy subjects and treated with beta hydroxytoluene and an antiprotease cocktail; Nonbond samples were also obtained and immunoblotted; Samples obtained from six subjects; Proteomic tests were performed on three subjects' samples
Targeted// Untargeted	Untargeted	Targeted
Follow-up// No Follow-up	27 years follow-up	No
Study	Lipidomic study	Structural and compositional characteristics of PON1 containing HDL particles
Analysis done in	Serum samples	Plasma samples
Study Disease	Osteoporosis and Atherosclerosis	Atherosclerosis
Technique used	UHPLC-QTRAP	LC/MS/MS
Statistical Analysis	R statistical software	Western blot analysis
Results	Of the twelve analyzed molecular lipid modules, one module with 105 lipid species was found to be substantially and jointly correlated with both subclinical markers of osteoporosis and atherosclerosis; Most of the lipid species in the module is identified as glycerolipids, glycerophospholipids, and sphingolipids; Cer were also presented in the module	Lower levels of unsaturated LysoPC in P-HDL; Proteomes showed variations as well. When compared to T-HDL, P-HDL was higher in the anticoagulant, vitamin K-activated protein S (prot S), and alpha2 macroglobulin; Procoagulant proteins kininogen 1 and histidine-rich glycoprotein, on the other hand, were absent from P-HDL; PON1 immunoadsorption from plasma decreased prot S anti-coagulant function
Conclusions	New lipid module that was linked to surrogate markers of both subclinical osteoporosis and subclinical atherosclerosis; Changes in the metabolism of the defined lipid module, and more precisely, the TG related molecular lipids within the module, could provide possible new biomarkers	The lipidome and proteome of P-HDL differed significantly from those of T-HDL; Anti-coagulation protein enrichment suggests complementary features within P-HDL particles and emphasizes their anti-atherosclerotic ability

Table A2 – Data from the PAD folder's articles (cont).

Reference	Nam, Jung, Ryu & Hwang (2017) [94]	Ni, Milic & Fedorova (2015) [95]
Hypothesis	After ligation of the left anterior descending coronary artery, myocardial metabolism could be changed in a time-dependent manner, and tissue alterations occurring after ligation could be correlated with the mechanisms influencing MI	A novel process for the simultaneous detection of low and high molecular weight-carbonylated compounds derived from the oxidation of PUFAs and PC has been developed, and it has been expanded to the detection and identification of lipid peroxidation products obtained from six different PL classes
Human// Animal	Animal	Animal
Studied Population	6-week-old male Sprague-Dawley rats (250–330 g) were allocated to one of four groups; The rats were euthanized at various time intervals following ligation (sham [n = 8], 1 hour [n = 8], 1 day [n = 8], and 10 days [n = 8])	Each class of phospholipids (PL = PC, PE, glycerophosphatidic acids, PG, PS, or phosphatidylinositiolphosphates) was used to make lipid vesicles separately; Primary rat cardiomyocytes were cultured at 37 °C until 80 percent confluence in DMEM/F12 medium supplemented with fetal bovine serum, horse serum, L-glutamine, non-essential amino acids, sodium pyruvate, and antibiotics
Targeted// Untargeted	Untargeted	Targeted
Follow-up// No Follow-up	No	No
Study	Metabolic and lipidomic	Oxidized lipids
Analysis done in	Heart tissue	Rat cardiomyocytes
Study Disease	Myocardial infarction	
Technique used	UPLC/QTOF MS	LC-MS
Statistical Analysis	SPSS 21.0	
Results	Transcriptome analysis showed that levels of coenzyme Q (Coq)-3 and Coq5, both SAM-dependent methyltransferases, were reduced in the MI groups; These findings indicated that SAM dysregulation could be linked to a down-regulated COQ biosynthetic pathway	
Conclusions	The robust integration of multiple omics data sets offers a novel approach to understanding the underlying pathophysiological mechanisms of MI	

Table A2 – Data from the PAD folder's articles (cont).

Reference	Paapstel *et al.* (2018) [25]	Padro *et al.* (2017) [96]
Hypothesis	The current study's main goal was to look at serum PC and LysoPC species in comparison to arterial stiffness, hemodynamics, and endothelial dysfunction in CAD patients, PAD patients and healthy subjects	With the same animal model, this study attempted to examine if the observed impairment of HDL cardioprotective function was correlated with changes in HDL remodeling and functionality
Human// Animal	Human	Animal
Studied Population	There were 124 male subjects in total, with 32 PAD patients, 52 CAD patients, and 40 healthy subjects	Four-month-old pigs (n=14) were fed either daily normocholesterolemic chow (NC) (n=7) or a Western-style hyperlipidemic diet (HL) (n=7) for ten days
Targeted// Untargeted	Targeted	Targeted
Follow-up// No Follow-up	No	No
Study	PC and LysoPC	HDL lipidomic
Analysis done in	Peripheral venous blood samples	Plasma
Study Disease	Atherosclerosis	Symptomatic CVD
Technique used	Flow injection analysis tandem MS and LC	UPLC-MS coupled to time-of-flight-MS
Statistical Analysis	SPSS	Statview and R version 3.3.0 software
Results	When compared to healthy subjects, the patient groups had lower serum levels of many individual PC and LysoPC species; In addition, a significant number of PC and LysoPC were found to be inversely linked to carotid-femoral pulse wave velocity, heart rate, asymmetric dimethylarginine (ADMA), or ADMA/arginine in patients with symptomatic atherosclerosis but not in controls	CEs were substantially higher in HL-HDL relative to NC-HDL; 90 of the 255 molecular lipid species found in HL-HDL is consistently distinct from those found in NC-HDL; Some PC with elevated HL-HDL levels often had fatty acids with or without one double bond
Conclusions	Changes in PC and lysoPC profiles across the three sample groups; Patients with PAD and CAD can vary in how their lipid profiles contribute to other biochemical and functional variables; When comparing atherosclerotic patients to healthy subjects, researchers discovered altered relationships among PC and lysoPC profiles, inflammation, and arterial function	Hyperlipidemia caused quantitative and qualitative alterations in the HDL lipidome and proteome, causing HDL particles to become unstable

Table A2 – Data from the PAD folder's articles (cont).

Reference	Paul, Lydic, Hogan & Goo (2019) [97]	Pechlaner *et al.* (2017) [48]
Hypothesis	Lipid profiling of hi-oxLDL-induced macrophage foam cells was performed, and the effect of cholesterol acceptors on this lipid profile was studied	MS is used to quantify apolipoproteins and compare their interactions with incident CVD, as well as to achieve a system-level of apolipoprotein correlations with the plasma lipidome and proteome
Human// Animal	Animal	Human
Studied Population	Male C57BL/6J wild-type mice; Mouse peritoneal macrophages were collected of the peritoneal cavity 5 days after aged with 3% thioglycolate injection into the mice	702 subjects; 6.4% has a history of CVD, and 9% were prescribed statins
Targeted// Untargeted	Targeted	Targeted
Follow-up// No Follow-up	No	10-year follow-up
Study	hi-oxLDL-induced	Apolipoproteins
Analysis done in	Peritoneal cavity	Plasma samples
Study Disease	Atherosclerosis	If 13 apolipoproteins, 135 lipid species, and 211 other plasma proteins are linked to incident CVD (91 events)
Technique used	LC-MS	MRM-MS
Statistical Analysis	One-way ANOVA with Turkey's multiple	R 3.2.0
Results	Role of lipid-poor apoA-I is not restricted to cholesterol and phospholipid efflux, but that apoA-I is a major regulator of the foam cell lipidome and may play a significant role in reducing multiple lipid species involved in the pathogenesis of atherosclerosis	apoC-II, apoC-III, and apoE were found to have the strongest correlations with incident CVD, followed by apoL-I, apoB-100, and apoH. ApoL-I was found to have a strong correlation with stroke; ApoC-II, ApoC-III, and ApoE were found to have strong direct associations with CEs, PC, PE, and, in particular, TG
Conclusions	The study's unique features provide the analysis of over 500 lipid species among untreated macrophages and hi-oxLDL-induced foam cells; Cholesterol acceptors, particularly apoA-I, have been described as inverse regulators of various pro-atherogenic lipid species	very-low-density lipoprotein-associated apolipoproteins have good associations with incident CVD supporting the idea of reducing CVD risk by targeting triacylglycerol-rich lipoproteins

Table A2 – Data from the PAD folder's articles (cont).

Reference	Poss *et al.* (2020) [98]	Puri, Duong, Uno, Kataoka & Nicholls (2012) [99]
Hypothesis	Serum ceramides are validated as candidate biomarkers of CVD, and detailed sphingolipid panels should be viewed as CVD indicators; Ceramides are sphingolipids that perform causal roles in diabetes and heart disease, and their serum levels are clinically measured as CVD biomarkers	REVIEW
Human// Animal	Human	
Studied Population	A targeted lipidomics study was conducted on serum samples from individuals with familial CAD (n = 462) and population-based controls (n = 212) to investigate the relationship among serum sphingolipids and CAD, with objective machine learning used to classify sphingolipid species positively correlated with CAD; The patients ranged in age from 30 to 75 years old	
Targeted// Untargeted	Targeted	
Follow-up// No Follow-up	10-year follow-up	
Study	Sphingolipids	
Analysis done in	Serum samples	
Study Disease	CAD	
Technique used	LC-MS/MS	
Statistical Analysis	R 3.5.1	
Results	Almost every sphingolipid analyzed (n = 30 of 32) was significantly higher in subjects with CAD as opposed to measurements in the controls; A new sphingolipid-inclusive CAD risk score, known as SIC, was created that distinguishes patients with CAD independently and more accurately than traditional clinical CVD biomarkers such as serum LDL-C and TG	
Conclusions	This research validates serum Cer as CVD candidate biomarkers and recommends that extensive sphingolipid panels be considered as CVD indicators	

Table A2 – Data from the PAD folder's articles (cont).

Reference	Rabiei, Bigdeli, Rasoulian, Ghassempour & Mirzajani (2012) [100]	Rabiei, Bigdeli & Rasoulian (2013) [101]
Hypothesis	Determine the effect of dietary olive leaf extract (OLE) on brain infarct length, neurological dysfunction, and brain lipidomics in rats following middle cerebral artery occlusion (MCAO)	Can't find it online and doesn't suit what we're looking for
Human// Animal	Animal	
Studied Population	For 30 days, four groups of 12 animals each received a dietary intervention; A control group was given gastric gavage (g.g.) with distilled water regularly; The other 3 groups got 50, 75, and 100 mg/kg/day of OLE gastric gavage	
Targeted// Untargeted	Untargeted	
Follow-up// No Follow-up	No	
Study	Brain lipidomics	
Analysis done in	Brain	
Study Disease	Brain ischemia	
Technique used	HPTLC	
Statistical Analysis	One Way ANOVA (spss16.0 post hoc LSD)	
Results	In doses of 50, 75, and 100 mg/kg/day, OLE increased brain CE, cholesterol, cerebroside, and PC levels; In contrast to the control group, OLE increased brain TG levels at doses of 75 and 100 mg/kg/day and decreased brain Cer levels at doses of 50, 75, and 100 mg/kg/day	
Conclusions	Although more research is required, it appears that the mechanism of OLE-induced ischemic tolerance in rats is linked to changes in brain lipid levels	

Table A2 – Data from the PAD folder's articles (cont).

Reference	Rached *et al.* (2015) [102]	Rasmiena *et al.* (2015) [103]
Hypothesis	Integrated analyses of the lipidome, proteome and functionality of total HDL and the major plasma HDL particle subpopulations from ST-elevation myocardial infarction (STEMI) patients displaying low HDL-C levels in the acute phase	Modulation of plasmalogen concentration by Batyl alcohol will slow the progression of atherosclerosis; As a proof of concept that plasmalogen may have an atheroprotective function, it is examined the impact of plasmalogen enrichment in murine models of atherosclerosis with varying levels of oxidative stress
Human// Animal	Human	Animal
Studied Population	Patients with ST-segment elevation MI (n=16) were selected within 24 hours of diagnosis and had low HDL-C and acute-phase inflammation as opposed to age- and gender-matched controls (n=10)	Six-week-old male C57/BL6, ApoE-\- and ApoE-\-GPx1-\- mice, were put on high fat diet, with either 0% or 2% 1-O-octadecyl-rac-glycerol (batyl alcohol) for 12 weeks (N=10/group)
Targeted// Untargeted	Targeted	Targeted
Follow-up// No Follow-up	No	No
Study	Plasma HDL particles	Plasmalogen
Analysis done in	Plasma	Plasma
Study Disease	STEMI patients	Atherosclerosis
Technique used		HPLC-Q/TRAP-MS
Statistical Analysis		Mann-Whitney U test
Results	Individual HDL subpopulation plasma concentrations did not vary among STEMI patients and control group; Total HDL were constant in STEMI patients compared to controls, while CEs were reduced and PL enhanced; Plasma levels of HDL apoA-I in HDL subpopulations were lower in comparison to controls; In HDL particles, 9 PL and sphingolipid subclasses were quantified; Plasma levels of PC remained constant in STEMI HDLs	Giving batyl alcohol to ApoE- and ApoE/GPx1-deficient mice caused higher levels of plasmalogen in both plasma and heart; Atherosclerotic plaques in the aorta were decreased in ApoE- and ApoE/GPx1-deficient mice; ApoE/GPx1-deficient mice showed a 40% reduction in plaque in the aortic sinus; ApoE/GPx1-deficient mice who were treated showed a reduction in VCAM-1 staining in the aortic sinus and nitro tyrosine staining in the aorta
Conclusions	Adjustments in the molecular structure and functionality of HDL particle subpopulations are revealed and modifications in lipidome and proteome lead to functional deficiencies in cholesterol efflux and antioxidative activities of dense HDL3	Plasmalogen enrichment via batyl alcohol supplementation reduced atherosclerosis in ApoE- and ApoE/GPx1-deficient mice, with the latter group benefiting the most

Table A2 – Data from the PAD folder's articles (cont).

Reference	Razquin *et al.* (2018) [104]	Ruuth *et al.* (2019) [105]
Hypothesis	In the PREDIMED trial, the associations of baseline lipidomic patterns or their 1-year shifts with the risk of CVD were investigated	Since LDL aggregation has previously been linked to unique characteristics of the LDL lipidome, it was presumed that such a difference in LDL aggregation will be mirrored by variations in LDL lipid composition among South Asians and white Caucasians
Human// Animal	Human	Human
Studied Population	983 participants: 230 incident cases and 790 subcohort participants (including 37 overlapping cases). Also, 907 participants (777 in the subcohort and 160 cases, including 30 overlapping cases) had plasma samples accessible after a year of follow-up and were put in the lipidome shift study	12 healthy Dutch South Asian and 12 Dutch white Caucasian men were paired for age (18-32 years) and BMI (18-27 kg/m2); South Asian subjects were liable if they were born and raised in the Netherlands and had four South Asian grandparents
Targeted// Untargeted	Untargeted	Targeted
Follow-up// No Follow-up	1-year follow-up	No
Study	Lipidomic	LDL aggregation
Analysis done in	Plasma	Plasma samples
Study Disease	CVD	Atherosclerotic CVD
Technique used	HPLC	MS
Statistical Analysis	PCA	GraphPad Prism
Results	2 groups: lipid group A - were inversely correlated with CVD and lipid group B - metabolites directly correlated with CVD; Lipid group A is made up of three metabolite families: 1) PC family, which includes PC, LysoPC, and PC-plasmalogens, 2) CE with N3 double bounds, and 3) long-chained TG; 4 families of metabolites were reported; in lipid group B: 1) all MG and DG, 2) all short-chained TGs, 3) PE minus those containing saturated fatty acids, and 4) all hydroxyPC	When compared to white Caucasians, LDL from South Asians was significantly more likely to aggregate; SM 24:0 and TG 56:8 had the strongest positive association with LDL aggregation; LDL from South Asians was also higher in AA containing PC 38:4 and lower in PC and CEs with monounsaturated fatty acids; Body fat percentage was positively associated with LDL aggregation and strongly positively associated with TG 56:8, SM 24:0, and total SM in South Asians
Conclusions	Suggests that polyunsaturated PC and CEs can offer CVD security; MG, DG, TG and PE, on the other hand, tended to be linked to an increased risk of CVD	Stable young South Asians have a higher risk of LDL aggregation; This can be explained in part by South Asians' higher body fat percentage, which leads to SM-enrichment of LDL

Table A2 – Data from the PAD folder's articles (cont).

Reference	Salatzki *et al.* (2018) [106]	Saleem *et al.* (2017) [107]
Hypothesis	Using an untargeted shotgun lipidomics strategy, it was discovered that cardiac lipidome variations were strongly correlated with LV failure and that adipose triglyceride lipase (ATGL) deficiency in adipose tissue prevented this	Changes in sphingolipid plasma concentrations were linked to improvements in verbal memory capacity and other cognitive domains in CAD patients undergoing a 6-month cardiac rehabilitation (CR) program
Human// Animal	Both	Human
Studied Population	Male adipose tissue specific ATGL deficient mice were developed, as well as control littermates; Mice were assigned to sham or transverse aortic constriction (TAC) groups; Human fasting plasma samples were obtained from healthy subjects and systolic heart failure patients	CAD patients (n = 120, mean age = 64±6 y, 84% male) underwent CR with neuropsychological tests and blood drawn at baseline, 3-, and 6-months
Targeted// Untargeted	Untargeted	Targeted
Follow-up// No Follow-up	No	No
Study	Lipidomics	Sphingolipids
Analysis done in	Mouse LV samples and Human plasma	Plasma
Study Disease	LV failure	CAD
Technique used	HPLC-MS/MS	LC/MS/MS
Statistical Analysis	ANOVA	MIXED procedure
Results	In LV samples, 225 lipid species were examined; PC, PE, and CLs were the most prevalent lipid groups in mouse hearts; The analysis of lipid classes showed substantial differences in levels of low-abundant classes of Cers, LysoPI, PC O-s, and PI in failing control-hearts vs. sham controls	Over 6 months of CR, lower Cer 18:0 concentrations were significantly correlated with improvements in verbal memory capacity, visuospatial memory, processing speed and global cognition
Conclusions	Disrupting adipose tissue lipolysis by deleting AT-specific ATGL improves pressure-induced heart failure; The production of LV failure was linked to a distinct lipidomic profile in human plasma and mouse hearts, implying a mechanism of cardiac FA partitioning into separate cardiac lipid classes	Cer 18:0 decreased as fitness increased over CR; These results indicate that Cer 18:0 (and probably other associated sphingolipids) may be modulated by lifestyle changes such as exercise

Table A2 – Data from the PAD folder's articles (cont).

Reference	Schlotterbeck, Chatterjee, Gawaz & Lammerhofer (2019) [108]	Shi, Lin, Wang, Zou & Li (2018) [109]
Hypothesis	A workflow for a detailed lipidomics analysis of platelets involved in CAD is presented, which makes use of recent advances in data-independent acquisition (DIA) techniques	The effect of Astaxanthin (ASX) on cardiac fibrosis following MI in mice is being studied; A systematic and objective lipidomic approach was used to explain the mechanisms of action of ASX
Human// Animal	Human	Animal
Studied Population	Plasma was obtained from CAD patients and healthy donors; Washed platelets were extracted from healthy subjects (n=10), patients with stable angina pectoris (SAP) (n=10), and patients with acute coronary syndrome (ACS) with ST-elevation myocardial infarction (STEMI) (n=13)	C57BL/6 J male mice (wild type, 8-week-old, 20-22 g); Three groups of mice were formed: (1) MI þ ASX (Wild type of MI and ASX treatment), (2) MI þ vehicle (Wild type, MI and 0.9% saline) (3) Sham þ vehicle (Wild type with sham surgery and with 0.9% saline treatment); each group had eight mice
Targeted// Untargeted	Untargeted	Targeted
Follow-up// No Follow-up	No	No
Study	DIA techniques for a comprehensive lipidomics study	Effects of ASX
Analysis done in	Platelets	Heart tissue
Study Disease	CAD	Myocardial infarction
Technique used	UHPLC-ESI-QTOF-MS/MS	LC-MS/MS
Statistical Analysis	PCA	GraphPad Prism 7.0
Results	In total, 1971 molecular features were found in the samples, with 611 of them being identified; The acquired data's accuracy was checked using embedded quality control samples (n=11)	ASX therapy decreased cardiac fibrosis and enhanced heart function after MI, as measured by a lower collagen I/III ratio, hydroxyproline content, and left ventricular end-diastolic pressure; Lipidomic research showed an increase in myocardial Cerin mice with cardiac fibrosis, which was reversed by ASX therapy
Conclusions	The study demonstrates that lipid profiles vary significantly between patient groups, suggesting that this approach may be useful in clinical analysis in the sense of personalized medicine in cardiology	ASX therapy suppresses cardiac fibrosis and enhances heart function after MI, according to the findings; ASX prevents Smpd1 and reduces Cer metabolism, which alleviates cardiac fibrosis by reducing TGF-b1/Smad signaling indirectly

Table A2 – Data from the PAD folder's articles (cont).

Reference	Siegel *et al.* (2015) [110]	Stegemann *et al.* (2011) [35]
Hypothesis	Test the theory that HIV infection and/or HIV therapy cause qualitative and quantitative alterations in HDL protein and lipid composition, impairing HDL functionality and possibly explaining the enhanced risk of atherosclerosis in infected subjects	Radial arteries were compared, as were endarterectomy samples from symptomatic and asymptomatic patients, as well as stable and unstable regions in the same symptomatic lesion
Human// Animal	Human	Human
Studied Population	HIV-infected subjects were divided into three groups: those who had never received anti-retroviral therapy (ART) or who had been off ART for more than a year, those who had been on ritonavir-based ART for more than a year, and those who had been on non-nucleoside reverse transcriptase inhibitors (NNRTI)-based ART for more than a year; These three groups of subjects' samples were compared to HIV-negative subjects' HDL samples	Surgical samples were taken from carotid and femoral endarterectomies, as well as the radial arteries; This research involved a total of 26 patients; The control radial arteries were carefully selected to be free of macroscopically visible vascular pathology, such as atherosclerosis
Targeted// Untargeted	Targeted	Untargeted
Follow-up// No Follow-up	No	No
Study	HDL protein	Lipidomics
Analysis done in	Plasma samples	Endarterectomies and control radial arteries
Study Disease	Atherosclerosis	Atherosclerotic Plaques
Technique used	Electrospray ionization-MS/MS	QqQ-MS
Statistical Analysis	PCA and ANOVA	Student t test or ANOVA and Scheffe´ post hoc test and PCA
Results	Lipidomic profiling revealed a negative association between CD4 T cell counts and particle SM, LysoPC, and ether-linked PS material in HIV-infected subjects who were not on ART; In the ART-naive HIV-infected group, functional analysis revealed a negative association among HDL cholesterol efflux ability and viral load	Identification of 150 lipid species from 9 different classes, 24 of which were found only in endarterectomies; Polyunsaturated CEs with long-chain fatty acids and specific SM species had the highest relative enrichment in plaques relative to plasma and formed part of a lipid signature for vulnerable and stable plaque areas
Conclusions	HIV infection is associated with a variety of protein and lipid compositional alterations in HDL particles; HIV infection impairs HDL's cholesterol efflux activity, leading to an increased risk of atherosclerosis in this group of patients	This extensive lipidomics study of plaque lipids shows the power of lipidomics in revealing lipid heterogeneity inside atherosclerotic lesions

Table A2 – Data from the PAD folder's articles (cont).

Reference	Stegemann *et al.* (2014) [26]	Stubiger *et al.* (2012) [111]
Hypothesis	Lipidomics profiling was carried out in the prospective population-based Bruneck Study, and the correlation of 135 distinct lipid species with CVD risk was examined over a 10-year observation period	It centered on the identification and quantification of particular PL-subsets thought to be associated with lipoprotein alteration and endothelial inflammation
Human// Animal	Human	Human
Studied Population	The research population consisted of an age- and sex-stratified random sample of all Bruneck residents at the time of the 1990 baseline assessment; In the year 2000, 702 subjects still were alive and take part of the study; 685 people had plasma samples available for lipidomics studies	Plasma was obtained from clinically well-documented children and adolescents (n = 13) with familial hypercholesterolemia (FH), familial combined hyperlipidemia (FCH) and non-FH normolipidemic subjects (n = 7) as controls
Targeted// Untargeted	Untargeted	Targeted
Follow-up// No Follow-up	10 years follow-up	No
Study	Lipidomics	PL-subsets
Analysis done in	Plasma	Blood
Study Disease	CVD	CVD
Technique used	QqQ-MS	LC-ESI-MS
Statistical Analysis	Cox regression	SPSS 17.0
Results	A total of 135 lipid species from eight different lipid categories were identified; Over 10 years, levels of individual CE, LysoPC, PC, PE, SM, and TGs were linked to cardiovascular disease; TGs and CEs with a low carbon number and double-bond content, such as TG(54:2) and CE(16:1), as well as PE(36:5), were among the lipid species with the highest predictive value	In comparison to the other research groups, FH had the most characteristically important variations in SM/PC and PC/LysoPC ratios, as well as favorable associations between SM vs. LDL-C and LPC vs. VLDL-C; OxPC had a positive association with IMT and HDL-C, but a negative association with OxLDL
Conclusions	This research used mass spectrometry-based lipidomics profiling to identify molecular lipid signatures for CVD in population-based cohorts; Molecular lipid species are promising new biomarkers that outperform the existing biochemical measurements of lipid classes in clinics	supported intriguing insights into the relationship between novel PL-biomarkers and conventional atherosclerosis risk factors (e.g., LDL-C, HDL-C) as well as oxidative stress and vascular dysfunction parameters

Table A2 – Data from the PAD folder's articles (cont).

Reference	Su, Han, Mancuso, Abendschein & Gross (2005) [112]	Sun *et al.* (2016) [113]
Hypothesis	Using shotgun lipidomics, researchers discovered a 4-fold rise in acylcarnitines in diabetic myocardium that was reversible with insulin therapy	Investigate the relationship among plasma FAs, oxylipins, and the risk of acute myocardial infarction (AMI) in a Singapore Chinese population
Human// Animal	Animal	Human
Studied Population	Diabetes was induced in male Sprague Dawley rats (350-450 g) by a single intravenous injection	A nested case-control analysis of 744 events AMI cases and 744 matched controls was conducted; Systolic and diastolic blood pressures were determined and blood samples were obtained in heparin tubes from 1994 to 2005
Targeted// Untargeted	Targeted	Targeted
Follow-up// No Follow-up	No	11 years follow-up
Study	4-fold increase in acylcarnitines	Plasma FAs and oxylipins
Analysis done in	Heart tissue (myocardium)	Blood components
Study Disease	Diabetic myocardium	AMI
Technique used	ESI-MS	GC-MS/MS and LC-MS
Statistical Analysis		R version 3.0.3
Results	Diabetes increased both mRNA of calcium-independent phospholipase A2 (iPLA2) and iPLA2 activity in rat myocardium; Cardiac ischemia in myocardium genetically modified to overexpress iPLA2 increased the number of acylcarnitine in the myocardium significantly; inactivating iPLA2 via a mechanism in either wild-type or transgenic myocardium prevented a significant portion of the acylcarnitine increase	The cross-sectional multivariable-adjusted relationship among these two FAs and cardiovascular risk factors in the control group was investigated
Conclusions	The accumulation of LCACs in diabetic myocardium most likely causes alterations in membrane physical properties and electrophysiologic activity, which can contribute to ischemia- and diabetes-induced arrhythmias	TXB2 plasma concentration was found to be inversely related to the risk of AMI; Plasma long-chain n-3 PUFAs and stearic acid were linked to a lower risk of AMI, whereas plasma total AA was linked to a higher risk

Table A2 – Data from the PAD folder's articles (cont).

Reference	Sutter *et al.* (2015) [114]	Syme *et al.* (2016) [115]
Hypothesis	Low HDL-C levels and loss of HDL's atheroprotective functions are linked to CAD; The interactions of HDL phospholipids with acute and stable CAD, and also HDL anti-apoptotic activity, were investigated in this study	In puberty, researchers looked at a new panel of LysoPC and platelet-activating factors (PAFs) for their correlation with classic CVD risk factors, such as excess visceral fat (VF), elevated blood pressure (BP), insulin resistance, and atherogenic dyslipidemia
Human// Animal	Human	Human
Studied Population	HDL samples were collected from 23 patients with stable CAD and 22 patients with acute coronary syndrome (ACS), and also 22 healthy subjects	Participants in their adolescence (n=1028; ages 12–18 years); Targeted serum lipidomics was achieved in 990 of the 1028 participants (479 men and 511 women)
Targeted// Untargeted	Targeted	Targeted
Follow-up// No Follow-up	No	No
Study	HDL lipidome	Glycerophosphocholine species
Analysis done in	Plasma samples	Blood samples
Study Disease	CAD	CVD
Technique used	LC-MS/MS	LC-ESI-MS
Statistical Analysis	Orthogonal partial least square - discriminant analysis	Linear regression
Results	Quantification of 29 PC (plasmalogens) species, 4 LysoPC species, and 16 SM species; three S1P species in HDL that vary in length or sphingoid base desaturation; Relevant PL species associations and correlations with the existence of acute or stable CAD, as well as HDL's ability to inhibit apoptosis; The plasmalogens PC33:3, PC35:2, and PC34:2 were found to have consistent associations and correlations with CAD, and also anti-apoptotic activity	Numerous new GPCs were discovered and linked to a variety of CVD risk factors; PC18:2/0:0, a GPC is newly shown to predict incident CAD in older adults, was correlated with many CVD risk factors in adolescents
Conclusions	The inverse relationship between HDL-plasmalogen levels and both stable and acute CAD may be due to plasmalogens' direct anti-apoptotic effects on endothelial cells	New GPCs were discovered to be closely correlated with several CVD risk factors in adolescents; These GPCs could be responsive markers of obesity-related risk for CVD outcomes in adults, and they could help us understand the biology of CVD risk

Table A2 – Data from the PAD folder's articles (cont).

Reference	Sysi-Aho *et al.* (2011) [116]	Tabassum *et al.* (2019) [117]
Hypothesis	To investigate the effects of Lamin A/C gene mutations on serum lipid profiles, researchers performed global serum lipidomics in the same Lamin A/C gene carriers and controls; Second, a new visualization model was developed to forecast the potential variations in these lipid parameters on left ventricular function as measured by cardiac MRI	(1) determine lipid species heritability and genetic correlations; (2) identify genetic variations affecting lipid species plasma levels; (3) investigate the connection between known lipid–species-associated variants and CVD manifestations; (4) gain mechanistic insights into well-known lipid variants
Human// Animal	Human	Human
Studied Population		Lipidomic analysis was conducted on 1142 randomly chosen participants from the FINRISK 2012 study
Targeted// Untargeted		
Follow-up// No Follow-up		
Study		
Analysis done in		
Study Disease		
Technique used		
Statistical Analysis		
Results		
Conclusions		

Table A2 – Data from the PAD folder's articles (cont).

Reference	Takeda *et al.* (2015) [118]	Takeda *et al.* (2018) [119]
Hypothesis	Lipidomic analysis of plasma and lipoprotein components in Watanabe heritable hyperlipidemic (WHHLMI) rabbits at risk of myocardial infarction	Practical methodology for wide-ranging quantitative lipidome research using SFC/ QqQMS and theoretically measured lipid MRM library
Human// Animal	Animal	Animal
Studied Population	5 WHHLMI rabbits and 3 Japanese white (JW) rabbits	
Targeted// Untargeted	Targeted	
Follow-up// No Follow-up	No	
Study	Lipidome analysis	
Analysis done in	Plasma lipoprotein	
Study Disease	MI	
Technique used	SFC/Q-Orbitrap-MS	
Statistical Analysis		
Results	WHHLMI rabbits had higher plasma levels of functional lipids like alkyl phosphatidylcholines, phospholipids, plasmalogens, and CEs; LysoPC, SM, and CE were elevated in the LDL fractions than in the VLDL fractions; The concentration of neutral lipids such as DGs and TGs in the LDL fractions was lower than in the VLDL fractions; Increased levels of PE plasmalogens were found in the LDL fractions than in the VLDL fractions	
Conclusions	In WHHLMI rabbits, 11 PE plasmalogens were significantly increased; Phospholipids containing u-6 FA were also found to be significantly higher; PLA2 catalyzes PC in LDL, which are then transformed to LysoPC; In atherosclerotic lesions, the degree of LysoPC rises, resulting in atherogenesis	

Table A2 – Data from the PAD folder's articles (cont).

Reference	Talib *et al.* (2019) [120]	Tang *et al.* (2018) [121]
Hypothesis	A single small arterial extract of <5 mg from the aortic root of an Apoe+/+ or Apoe-/- mouse was used for simultaneous proteomic, lipidomic, and metabolomic research	This study aimed to use high-throughput liquid chromatography time-of-flight mass spectrometry (LC-TOF-MS) to characterize the lipid profiles of HF erythrocytes
Human// Animal	Animal	Human
Studied Population	C57BL/6J WT male mice and Apoe–/– mice; The blood was collected via cardiac puncture and the animal was then perfused with phosphate-buffered saline under physiological pressure; Before excising the hearts, the underlying adipose and connective tissue is removed; The aortic roots were removed from the hearts	Patients with HF were divided into: A - were high risk and asymptomatic, but did not have structural heart disease; B - had structural heart disease but were asymptomatic; C - had been treated for acute or decompensated chronic HF; Patients aged 39 to 85 years old
Targeted// Untargeted	Untargeted	Targeted
Follow-up// No Follow-up	No	No
Study	Proteomic, lipidomic, and metabolomic	Lipidomics
Analysis done in	Blood	Blood samples
Study Disease	Atherosclerosis	Heart failure
Technique used	LC-MS	LC-TOF-MS
Statistical Analysis	PCA	SIMCA-P software
Results	Abundance of 60 lipids (PC, SM, and TG), as well as two species of oxidized TG, in lesion-containing arteries; It was also discovered that 15 lipid features were substantially increased in lesion tissues; In lesion-containing tissue, the concentration of 52 lipid molecules increased (SM, phospholipids, and CL); 17 lipid features had a higher abundance and no structural assignment	Sterols, phospholipids, and Cer are essential differentiators between normal controls and stage C patients; LysoPL (lysoPC and lysoPE), Cer, and oxysterols (7-ketocholesterol (7KCh)) were found to be higher in HF erythrocytes than in control erythrocytes; The levels of phospholipids (PC, PE, and SM) in HF erythrocytes were lower than in normal cells
Conclusions	Along with the lipids that are typically increased in lesion-containing tissues (TG, CEs, phospholipids, and sphingolipids), several new lipid features were established without structural elucidation	In HF patients, blood 7KCh is concentrated in erythrocytes; The 7KCh-laden erythrocyte ghost causes cardiomyocyte death, implying a role in the pathogenesis of HF; The results confirm the significance of erythrocyte 7KCh as a risk factor for heart failure

Table A2 – Data from the PAD folder's articles (cont).

Reference	Venturini *et al.* (2019) [122]	Vorkas *et al.* (2015) [123]
Hypothesis	To understand additional pathways modulated in ECs under atheroprone and atheroprotective flow, researchers combined stable isotope labeling by amino acids, proteomics, and metabolomics	Identify metabolic markers of vascular calcification that can help understand the disease, monitor its progression, and generate hypotheses about its pathophysiology
Human// Animal	Human	Human
Studied Population	Human umbilical vein endothelial cells were cultured in an endothelial basal medium from a single newborn male donor	70 patients (48–83 years old) with exertional angina; No indication of coronary artery calcification in 26 patients, 27 had a coronary calcium score within 1-250 (mild calcification group; MC), and 17 had a calcium score of >250 (severe calcification group; SC)
Targeted// Untargeted		Untargeted
Follow-up// No Follow-up		No
Study		Metabolic markers
Analysis done in		Serum samples
Study Disease		Calcific CAD
Technique used		UPLC–MS
Statistical Analysis		MVDA methods and univariate statistical tests and PCA
Results		The disease states, PC levels were found to be substantially altered; 18-carbon fatty acyl chain (FAC) PC were found in lower concentrations in the SC group, whereas 20:4 FAC lipid species were found in higher concentrations; A pattern with PC lipids was found in the MC group; Numerous SM signals were also found in SC at lower intensities as compared to the NC or MC groups
Conclusions		It shows dysregulations of PC lipid species, implying changes in fatty acid elongation/desaturation; The changed levels of 18-carbon and 20:4 FAC lipids may indicate a disruption in inflammation homeostasis; The significant SM dysregulation in SC is consistent with profound apoptosis as a possible mechanism of Calcific CAD

Table A2 – Data from the PAD folder's articles (cont).

Reference	Vorkas *et al.* (2015) [32]	Vorkas *et al.* (2016) [31]
Hypothesis	Investigate the metabolic changes that occur during the development from intimal thickening (INT) to stenosing plaque formation in human carotid (CAR) and femoral (FEM) endarterectomy specimens	Depending on the patient's symptomatic status, stenosing carotid plaque tissue will have a distinct metabolic signature.
Human// Animal	Human	Human
Studied Population	78 patients; Carotid endarterectomy was performed on 52 patients and femoral endarterectomy on 26 patients; Areas of intimal thickening (INT) were extracted from the stenosing plaque section (9 samples from carotid and 7 samples from femoral tissue); INT tissue located at the proximal and distal extents of stenosing atheroma samples serves as control tissue	Carotid plaque tissue samples were collected from patients with carotid-related cerebrovascular symptoms (n = 5) and from asymptomatic patients (n = 5). From each tissue, two adjacent biological replicates were collected.
Targeted// Untargeted	Untargeted	Untargeted
Follow-up// No Follow-up	No	No
Study	Metabolic profiling	Metabolic phenotyping
Analysis done in	Plaque tissue samples	Three transverse segments of stenosing carotid plaque tissue
Study Disease	Atherogenesis	stroke risk in stenosing carotid plaque
Technique used	UPLC-MS	UPLC-MS
Statistical Analysis	PCA and OPLS-DA, ANOVA	Principal components analysis
Results	It discovered changes in many metabolite species such as the cholesterol, purine, pyrimidine, and Cer pathways; Demonstrate differences in the levels of lipids, namely PE-Cer; These molecules seem to be bridging two pathways known to be involved in atherosclerosis: Cer and cholesterol	The two research groups revealed distinct plaque phenotypes and metabolites that distinguished the two groups. Metabolites of the eicosanoid pathway and three acylcarnitine species, intermediates of b-oxidation, were found in higher concentrations in symptomatic patients
Conclusions	The use of INT tissue as the control group, as well as metabolites detected with patterns consistent with the published literature and well-known risk factors – specifically Cho and oxCEs, purines and pyrimidines, and the Cer pathways – adds belief in the findings	Metabolic profiling technologies are used to distinguish between symptomatic and asymptomatic carotid plaque tissue; Two biological pathways were identified as being involved in distinguishing symptomatic from asymptomatic patients and will be studied further

Table A2 – Data from the PAD folder's articles (cont).

Reference	Voros *et al.* (2014) [124]	Wang, Cheng & Liu (2018) [125]
Hypothesis	A large number of biologically relevant factors will be collected to investigate biological interactions and causality among genomic, proteomic, metabolomic, lipidomic, and phenotypic features	Create a simplified plasma critical amino acid-based profile by measuring only two amino acids to risk-stratify patients after acute/decompensated HF; The metabolism information provided by the profiles was tested to see if it has prognostic value in addition to conventional risk factors and B-type natriuretic peptide
Human// Animal	Human	Human
Studied Population		Patients hospitalized for acute or decompensated chronic HF with a left ventricular ejection fraction (LVEF) of < 50% and aged 20–85 years; Normal controls from 20-85 years old and had no major systemic disease
Targeted// Untargeted		Untargeted
Follow-up// No Follow-up		3 years follow-up
Study		Metabolomic analysis
Analysis done in		Plasma
Study Disease		Heart failure
Technique used		UPLC
Statistical Analysis		ANOVA
Results		HF patients at low risk of incidents had higher levels of long-chain and short-chain acylcarnitines than standard controls; In comparison to the low-risk group, high-risk type 1 was correlated with higher levels of long-chain, medium-chain and short-chain acylcarnitines; High-risk type 2 patients had comparable levels of long-, medium-, and short-chain acylcarnitines
Conclusions		Patients with high-risk type 1 have significant metabolism disruption and an accumulation of circulating waste of incompletely metabolized lipids; Patients with high-risk type 2 are marked by extreme malnutrition and a number of clinical factors indicating a poor prognosis

Table A2 – Data from the PAD folder's articles (cont).

Reference	Wheelock *et al.* (2009) [126]	Wong, Chan,Kingwell, Leckie & Meikle (2014) [127]
Hypothesis	REVIEW	?
Human// Animal		
Studied Population		
Targeted// Untargeted		
Follow-up// No Follow-up		
Study		
Analysis done in		
Study Disease		
Technique used		
Statistical Analysis		
Results		
Conclusions		

Table A2 – Data from the PAD folder's articles (cont).

Reference	Wu *et al.* (2017) [128]	Wu *et al.* (2018) [129]
Hypothesis	Identify the lipidome of LVTA induced by either MI or myocardial ion channel diseases and to see if their lipidomes shared any characteristics; The researchers also wanted to look for any widely deregulated lipid species that could be used as biomarkers	An aconitine-induced LVTA-SCD rat model was developed in this study; Lipidomes in sera before and after LVTA were investigated and compared using UPLC-MS-based lipidomics
Human// Animal	Animal	Animal
Studied Population	There were 13 ACO-LVTA rats and 7 ACO-N (control) rats created; also, 12 CAL-LVTA rats and 12 CAL-N (control) rats were created	Adult male Sprague-Dawley rats
Targeted// Untargeted	Untargeted	Untargeted
Follow-up// No Follow-up	No	No
Study	Lipid features of LVTA	Lipidomics
Analysis done in	Serum samples	Caval vein blood samples; tail vein blood samples were drawn 3 days before the experiment to serve as the controls of 7post-LVTA samples.
Study Disease	Lethal ventricular tachyarrhythmia (LVTA)	LVTA
Technique used	UPLC-MS	UPLC-MS
Statistical Analysis	PLS-DA	PLS-DA
Results	A total of 1,010 myocardial lipids and 746 serum lipids of 23 lipid groups were reported; LysoPC, PE, PI, and TG were all disrupted at the same time in both myocardial and serum specimens, but with separate molecular species and opposite transition patterns	188 lipids were found to be substantially different among post-LVTA sera and controls; SM, LPC, and PC were found to be in the middle of the associated network, indicating a central role in the pathophysiological processes of LVTA-SCD
Conclusions	CL 70:5, CL 74:9, and Cer d34:2 were reported in both models' myocardia as possible lipid biomarkers of LVTA; In the models, these three lipid species had high diagnostic potential and were closely linked to the subjects' electrophysiological parameters	Following LVTA-SCD, the serum lipidome was disrupted; The majority of the lipids that were disrupted interacted; During the LVTASCD phase, 10 lipid-related metabolic pathways were activated; The key lipid classes involved in these pathways included Cer, SM, PC, PE, and PS. PC (O-40:4), PE (40:4), and SM (d46:5) were evaluated in serum as possible lipid biomarkers of LVTA-SCD

Table A2 – Data from the PAD folder's articles (cont).

Reference	Yan *et al.* (2019) [36]	Yang *et al.* (2017) [40]
Hypothesis	Investigate Lyso-PL profiles in apolipoprotein E-deficient (ApoE-/-) model group and wild type control group at various time points	Possible lipid biomarkers for the diagnosis of lacunar infarction, as well as providing insight into disrupted pathways of the underlying pathophysiology
Human// Animal	Animal	Human
Studied Population	12 ApoE−/−mice and 20 C57BL/6 J wild-type littermates; The C57BL/6 J mice were then divided into two control classes, NDC (normal diet control) and HFDC (high-fat diet control); ApoE-/- mice were used as the model group and were fed a high-fat diet	6 Lacunar Infarction patients and 6 control subjects - identify biomarker candidates: 29 Lacunar Infarction patients and 15 control subjects - plasma lipid biomarkers
Targeted// Untargeted	Targeted	Untargeted
Follow-up// No Follow-up	No	No
Study	Lyso-PL profile	Lipid biomarkers
Analysis done in	Plasma samples	plasma samples
Study Disease	Atherosclerosis	Lacunar infarction
Technique used	UPLC-QTRAP-MS/MS	NP/RP 2D LC-QToF/MS
Statistical Analysis	OPLS-DA	IBM SPSS Statistics software, Version 20
Results	58 differential Lyso-PLs were established, with 18 Lyso-PLs down-regulated and 40 Lyso-PLs up-regulated; Lyso-PLs with the same acyl groups exhibited similar alteration patterns	Total of 90 lipids were chosen as biomarker candidates; 3 of these lipids species were down-regulated, while the other ten, were up-regulated in the patient group; these lipids were suggested as potential lipid biomarker
Conclusions	Changes in Lyso-PL profiles were discovered to be caused by genes and diet; The concentration of different types of Lyso-PLs was influenced by gene status, whereas the fatty acid composition of Lyso-PLs was more sensitive to dietary influences; 12 Lyso-PLs changed with the progression of atherosclerosis, and hierarchical clustering analysis based on these Lyso-PLs may distinguish the 3 animal groups, making them reliable biomarkers	Following a thorough screening and validation process, 13 lipid species were identified as potential lipid biomarkers

Table A2 – Data from the PAD folder's articles (cont).

Reference	Yang *et al.* (2018) [50]	Zalloua *et al.* (2019) [130]
Hypothesis	Isosteviol sodium treatment was used to examine the lipid profiles of stroke rats	Classify metabolic features associated with CAD risk factors as well as improvements in clinical and intermediate biochemical phenotypes in patients who had been screened for signs of CAD
Human// Animal	Animal	Human
Studied Population	Sprague-Dawley male rats were assigned to one of 4 groups: 1) sham group (n=10); 2) model group (n=10); 3) STV-Na group (n=10); 4) EDA group (n=10). TTC staining was examined using additional rats (n = 24, six rats in each group)	The presence (for cases) or absence (for controls) of coronary artery stenosis was used to select 109 subjects aged 17–81 (73 males and 36 females) recruited around 2006 and 2009 for participation in the FGENTCARD patient set for this research
Targeted// Untargeted	Untargeted	Untargeted
Follow-up// No Follow-up	No	3 years follow-up
Study	Lipid profiles	Lipidomics analysis
Analysis done in	Brain samples	Serum samples
Study Disease	Stroke rats with STV-Na treatment	CAD
Technique used	UHSFC-IT-TOF/MS	UPLC-MS
Statistical Analysis	PLS-DA	SPSS
Results	Following STV-Na treatment, 15 lipids were found to be trending towards normal levels. STV-Na dramatically reversed the levels of 6 lipids to normal. STV-Na protective effects may be linked to the regulation of AA metabolism, sphingolipid metabolism and glycerophospholipid metabolism	1328 metabolic features were significantly associated with at least one of the clinical or biochemical phenotypes; There was evidence of a significant relationship between 34 metabolite signals, which corresponded to a collection of SM, and serum HDL-C; Many of these metabolite interactions were also found with serum LDL and total cholesterol levels, but not with serum TG levels
Conclusions	This approach proved to be a quick and efficient way to investigate the protective effects of STV-Na against stroke and it could be applied to other lipidomics studies	Sphingolipids in the form of SM are directly linked to serum lipoprotein and total cholesterol levels

Table A2 – Data from the PAD folder's articles (cont).

Reference	Zhang *et al.* (2014) [131]
Hypothesis	The investigator showed a beneficial effect of metformin relative to glipizide in type 2 diabetic patients for 3-year cardiovascular outcomes in the SPREAD-DIMCAD trial; So, in the research, a systematic lipidomics study is conducted to determine the different lipid metabolites in serum samples collected from study participants
Human// Animal	Human
Studied Population	There were 21 patients in the glipizide group and 23 patients in the metformin group
Targeted// Untargeted	Untargeted
Follow-up// No Follow-up	5 years follow-up
Study	Lipid metabolites
Analysis done in	Serum samples
Study Disease	Type 2 Diabetes and CAD
Technique used	LC-Q-TOF-MS
Statistical Analysis	Student t test or the Mann-Whitney test and a one-way ANOVA with the two-sided Dunnett post hoc test
Results	A total of 118 molecular species of serum lipids were described and quantified; When compared to glipizide, metformin caused a significantly greater improvement in serum lipid species during treatment; 3 lipid metabolites were related to long-term composite cardiovascular events among the significantly modified lipid species
Conclusions	Metformin and glipizide had different clinical effects on systematic lipidomics, which were similar to their different long-term effects on cardiovascular outcomes

Appendix 3 – Chosen articles

Table A3 – Chosen Articles.

Groups		Articles
a) Atherosclerosis (in general)	Prospective studies in humans	*Lipidomic architecture shared by subclinical markers of osteoporosis and atherosclerosis: The Cardiovascular Risk in Young Finns Study*, Binisha H. Mishra
		Lipidomics Profiling and Risk of Cardiovascular Disease in the Prospective Population-Based Bruneck Study, Christin Stegemann
	Diet-induced atherosclerosis – animal studies	*Atherosclerotic dyslipidemia revealed by plasma lipidomics on ApoE$^{-/-}$ mice fed a high-fat diet*, Yanyan Chen
		Lipidomic and metabolomic analyses reveal potential plasma biomarkers of early atheromatous plaque formation in hamsters, Mariona Jové
		Myocardial metabolic alterations in mice with diet-induced atherosclerosis: linking sulfur amino acid and lipid metabolism, Jueun Lee
		Lysophospholipid profiles of apolipoprotein E-deficient mice reveal potential lipid biomarkers associated with atherosclerosis progression using validated UPLC-QTRAP-MS/MS-based lipidomics approach, Yingfei Yan
b) Cerebrovascular Disease	Plasma from patients with atherosclerosis in the carotid	*Circulating inflammation-resolving lipid mediators RvD1 and DHA are decreased in patients with acutely symptomatic carotid disease*, Hernan A. Bazan
		A not-stop-flow online normal-/reversed-phase two-dimensional liquid chromatography–quadrupole time-of-flight mass spectrometry method for comprehensive lipid profiling of human plasma from atherosclerosis patients, Min Li

	Plasma from patients who suffer a stroke	*Lipidomic analysis of plasma in patients with lacunar infarction using normal-phase/reversed-phase two-dimensional liquid chromatography–quadrupole time-of-flight mass spectrometry*, Li Yang
c) Peripheral Arterial Disease	Plasma	*Serum phosphatidylcholines and lysophosphatidylcholines are inversely related to vascular damage and heart rate in patients with atherosclerosis*, K Paapstel
	Adipose tissue	*Diversity of lipid mediators in human adipose tissue depots*, Joan Clària
d) Cerebrovascular Disease and Peripheral Arterial Disease	Plaques from carotid arteries versus femoral arteries	*Comparative Lipidomics Profiling of Human Atherosclerotic Plaques*, Christin Stegemann
		Metabolic phenotyping of atherosclerotic plaques reveals latent associations between free cholesterol and ceramide metabolism in atherogenesis, Panagiotis Andrea Vorkas
		Metabolic Phenotypes of Carotid Atherosclerotic Plaques Relate to Stroke Risk: An Exploratory Study, P.A. Vorkas
		Specific Plasma Oxylipins Increase the Odds of Cardiovascular and Cerebrovascular Events in Patients with Peripheral Artery Disease, Stephanie P B Caligiuri

Appendix 4 – Questionnaire of The Newcastle – Ottawa Scale

Newcastle - Ottawa Quality Assessment Scale – Case-Control Studies [132]

Selection:

1) Is the case definition adequate?
   a. yes, with independent validation ★
   b. yes, e.g., record linkage or based on self-reports
   c. no description
2) Representativeness of the cases
   a. consecutive or obviously representative series of cases ★
   b. potential for selection biases or not stated
3) Selection of Controls
   a. community controls ★
   b. hospital controls
   c. no description
4) Definition of Controls
   a. no history of disease (endpoint) ★
   b. no description of source

Comparability:

1) Comparability of cases and controls based on the design or analysis
   a. study controls for _____ (Select the most important factor) ★
   b. study controls for any additional factor ★ (These criteria could be modified to indicate specific control for a second important factor)

Exposure:

1) Ascertainment of exposure
   a. secure record (e.g., surgical records) ★
   b. structured interview where blind to case/control status ★
   c. interview not blinded to case/control status
   d. written self-report or medical record only
   e. no description
2) Same method of ascertainment for cases and controls
   a. yes ★
   b. no
3) Non-Response rate
   a. same rate for both groups ★
   b. non respondents described
   c. rate different and no designation

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