



CATOLICA
ESCOLA SUPERIOR DE BIOTECNOLOGIA

PORTO

**THE VALUE OF LIPID BIOMARKERS IN CORONARY
HEART DISEASE – SYSTEMATIC REVIEW**

by

Beatriz de Castro Frias

January 2023



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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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Abstract

According to Instituto Nacional de Estatística, in 2017 in Portugal, 32 336 people die of cardiovascular diseases ¹. The field of lipidomics has been gaining interest over the years to identify the feature of lipids that are associated with cardiovascular diseases, due to the ability of lipidomics to provide a detailed lipid profile, to characterize this type of pathologies ². This review focus on lipidomic studies conducted to determine the link between lipid markers and coronary artery disease (CAD), including stable angina pectoris and, acute coronary syndromes (ACS) such as myocardial infarction, coronary calcification, and major adverse cardiac events. To determine these links between lipids and cardiovascular diseases, different articles were selected after researching in MedLine Databases from March to June 2020. The chosen articles had to mention a lipidomic comparison between cardiovascular pathologies and control groups. Based on the analysis of the different selected articles, the main mass spectrometry techniques used were HPLC and UHPLC. Through the analysis of different blood samples of healthy individuals, and from individuals that suffer from different pathologies, such as CAD and ACS, different lipid groups presented different levels between healthy individuals and patients that suffer from different pathologies. Sphingolipids and glycerophospholipids were the main lipid classes detected in abnormal levels in individuals that suffer from the previously mentioned pathologies. The relationship between sphingolipids and lipoproteins are a significant risk factor for CAD ^{3,4}. Elevated levels of ceramides (Cer) and sphingomyelins (SM) can be linked to a higher risk of cardiovascular diseases and the development of atherosclerosis ^{3,4}. Phosphatidylcholine (PC) is one of the most prominent glycerophospholipids. CAD and CAC have been linked to dysregulation of PC levels as well as increased activity and mass of PLA2, and the bioactive lipid products of various PLA2 isoforms have been linked to anti- and pro-atherosclerotic effects ⁵⁻⁷. Different lipid molecules of Cer, SM, and PC were also found in the selected articles, which can be used to detect cardiovascular diseases, such as CAD and ACS. To conclude, different sphingolipids and glycerophospholipids can be used to determine cardiovascular diseases, such as CAD and ACS. Despite that, the field lipidomics still needs to be more explored to analyze and give a proper diagnosis of these pathologies.

Key words: Lipidomics, Lipids, Ceramides, Sphingomyelins, Phosphatidylcholine, HPLC, UHPLC, Coronary Artery Disease, Acute Coronary Syndrome.

Resumo

Segundo o Instituto Nacional de Estatística, em Portugal em 2017, 32 336 pessoas morreram devido a doenças cardiovasculares ¹. O ramo da lipidómica tem vindo a ganhar interesse ao longo dos últimos anos, para identificar características lipídicas associadas a doenças cardiovasculares, devido à sua capacidade de fornecer uma análise detalhada do perfil lipídico, o que auxilia na caracterização deste tipo de patologias². O foco desta revisão, serão estudos lipidómicos focados na relação entre marcadores lipídicos e doenças cardiovasculares, como doenças arteriais coronárias, incluindo angina de peito estável, e síndromes coronárias agudas, como enfarte do miocárdio, calcificação coronária, e eventos cardiovasculares adversos maiores. Para determinar essa ligação vários artigos foram selecionados após a pesquisa na MedLine Databases desde março a junho de 2020. Os artigos selecionados, comparam os resultados obtidos através de uma análise lipidómica, de doentes que sofrem de doenças cardiovasculares e respetivos grupos controlo. Pela análise dos diferentes artigos, as principais técnicas de espectrometria de massa utilizadas foram, HPLC e UHPLC. Através da análise das diferentes amostras de sangue de indivíduos saudáveis, e de indivíduos com diferentes patologias, tais como CAD e ACS, diferentes grupos lipídicos foram detetados em diferentes níveis, entre indivíduos saudáveis e indivíduos que sofrem de diferentes patologias. Esfingolípidos e glicerofosfolípidos foram as principais classes de lípidos detetadas em níveis anormais nos indivíduos que sofrem das patologias supramencionadas. A relação entre esfingolípidos com lipoproteínas é um fator significativo para CAD ^{3,4}. Níveis elevados de ceramidas (Cer) e esfingomielinas (SM), podem estar relacionados com o aumento do risco de doenças cardiovasculares e o desenvolvimento de aterosclerose ^{3,4}. Fosfatidilcolinas (PC) são um dos mais proeminentes glicerofosfolípidos. CAD e CAC estão relacionadas com a desregulação dos níveis de PC, com o aumento da atividade e massa de PLA2, e com os produtos lipídicos bioactivos derivados de várias isoformas de PLA2, que estão relacionadas com anti e pró efeitos ateroscleróticos ⁵⁻⁷. Diferentes moléculas lipídicas de Cer, SM, e PC foram também detetadas ao longo dos vários artigos selecionados, lípidos esses que podem ser utilizados de forma a detetar doenças cardiovasculares, tais como CAD e ACS. Em suma, diferentes esfingolípidos e glicerofosfolípidos podem ser utilizados para a deteção de doenças cardiovasculares. Apesar disso, o ramo da lipidómica ainda necessita de ser mais explorado, de forma que possa analisar e dar um diagnóstico fidedigno das diferentes patologias.

Palavras-chave: Lipidómica, Lípidos, Ceramidas, Esfingomielinas, Fosfatidilcolinas, HPLC, UHPLC, Doença Arterial Coronária, Síndrome Coronária Aguda.

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List of abbreviation

ACS	Acute coronary syndrome
CAD	Coronary artery disease
CAC	Coronary artery calcification
CE	Cholesteryl esters
Cer	Ceramides
CHD	Coronary heart disease
CL	Cardiolipins
CVD	Cardiovascular disease
DG	Diglycerides
DiHETrE	Dihydroxyeicosatrienoic acid
ESI	Electrospray ionization
FA	Fatty acids
FIA	Flow Injection Analysis
GL	Glycerolipids
GP	Glycerophospholipids
HC	Healthy controls
HDL-C	High density lipoproteins cholesterol
HDoHE	Hydroxydocosaheptaenoic acid
HEPE	Hydroxyeicosapentaenoic acid
HETE	Hydroxyeicosatetraenoic
HPLC	High Performance Liquid Chromatography
HODE	Hydroxyoctadecadienoic acids
LacCer	Lactosylceramides

LC	Liquid Chromatography
LDL-C	Low density lipoproteins cholesterol
lysoPC	lysophosphatidylcholine
MACE	Major adverse cardiovascular events
MC	Mild calcification
MI	Myocardial infarction
M/MS	Tandem mass spectrometry analyzer
NC	Non calcification
Non-STEMI	non-ST-segment elevation myocardial infarction
oxCE	Oxidized cholesteryl esters
PA	Phosphatidic acid
PAD	Peripheral arterial disease
PC	Phosphatidylcholine
PE	Phosphatidylethanolamine
PG	Phosphatidylglycerol
PI	Phosphatidylinositol
PR	Prenol Lipids
PS	Phosphatidylserine
PUFA	Polyunsaturated fatty acids
Q	Quadrupole
QqQ	Triple quadrupole analyzers
QTOF	Quadrupole analyzer coupled with a time of flight analyzer
QTrap	Triple quadrupole analyzer coupled with an ion trap
SAP	Stable angina pectoris

SC	Severe calcification
SM	Sphingomyelins
SP	Sphingolipids
ST	Sterol Lipids
STEMI	ST-segment elevation myocardial infarction
TG	Triglycerides
TOF	Time of flight
UHPLC	Ultra - High Performance Liquid Chromatography
UPLC	Ultra Performance Liquid Chromatography

1. Introduction

Nowadays the fields of genomics, metabolomics, proteomics, and transcriptomics are extremely important for scientific research ^{2,8}. Different types of biological molecules, such as lipids, compose the human body ². The study of lipids on large scale is called lipidomics and is a subfield of metabolomics, where the lipids are analyzed and characterized in detail according to their interactions and influence on biological systems ². Due to the ability of lipidomics to provide a detailed lipid profile, the use of this technique has been gaining interest over the years to identify the feature of lipids that are associated with cardiovascular diseases (CVD) ⁹. According to the European Society of Cardiology ¹⁰, cardiovascular diseases were the leading cause of death in Europe in 2017, killing about 3.9 million people. According to Statistics Portugal (INE – *Instituto Nacional de Estatística*) the leading cause of death in 2017 in Portugal, was cardiovascular disease, causing a total of 32 336 deaths. The majority of these deaths were caused by strokes, resulting in 11 270 deaths, followed by 7 134 deaths caused by diseases related to heart ischemic disease ¹.

This review will be focused on lipidomic studies conducted to determine the link between lipid markers and coronary artery disease, including stable angina pectoris and, acute coronary syndromes such as, myocardial infarction, coronary calcification, and major adverse cardiac events.

1.1. Blood and plasma composition

The adult human, in average has five to six liters of blood, where half of this volume is composed by three distinct types of blood cells: erythrocytes, which are filled with hemoglobin and are specialized in transporting O₂ and CO₂; leukocytes, of multiple forms such as lymphocytes, which are vital to the immune system; and platelets that helps to mediate blood clotting ¹¹. The liquid portion of the blood is made up of blood plasma, which is about 90% water and 10% solutes ¹¹. That 10% of solutes is constituted by 70% of plasma proteins, 20% of organic metabolites and waste products, and 10% of inorganic components ¹¹. The Figure 1 shows a representation of the blood and plasma components.

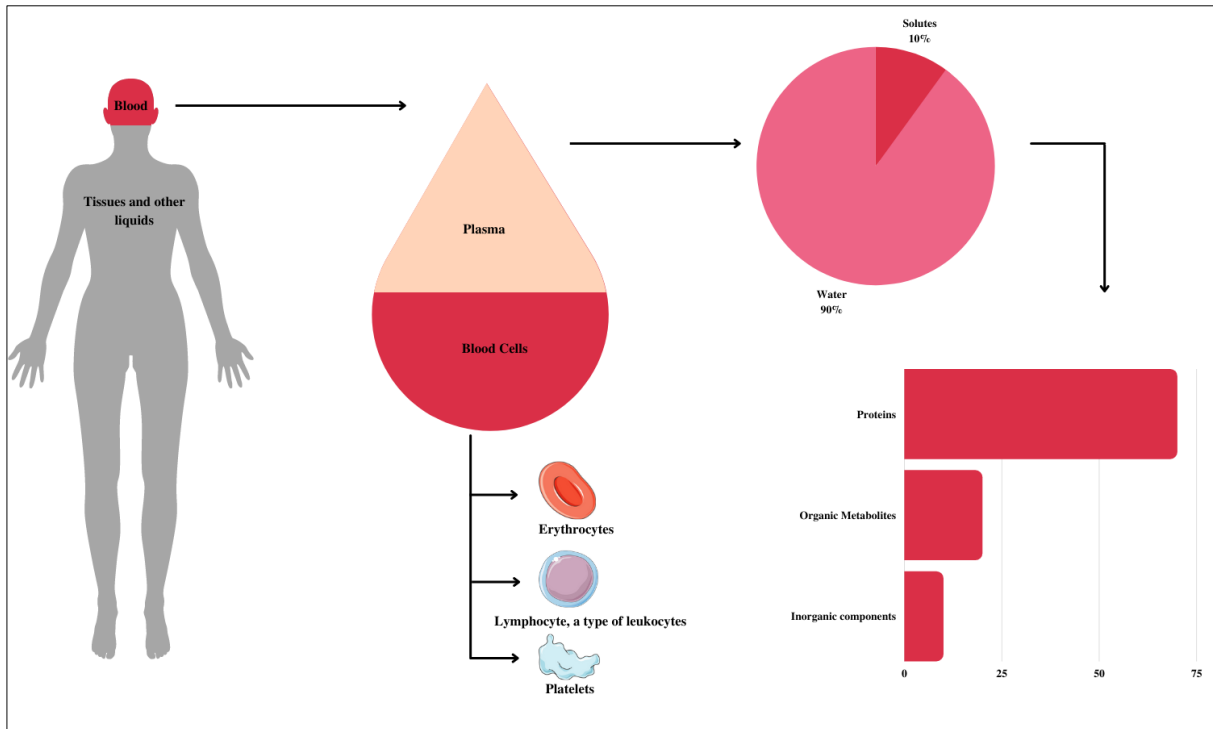


Figure 1 - Blood and plasma components.

In human plasma, there are several distinct lipid molecular species that can be classified into six classes: fatty acids, glycerolipids, glycerophospholipids, sphingolipids, sterol lipids, and prenol lipids ¹². Figure 2 shows the various lipids classes in human plasma and their respective representations.

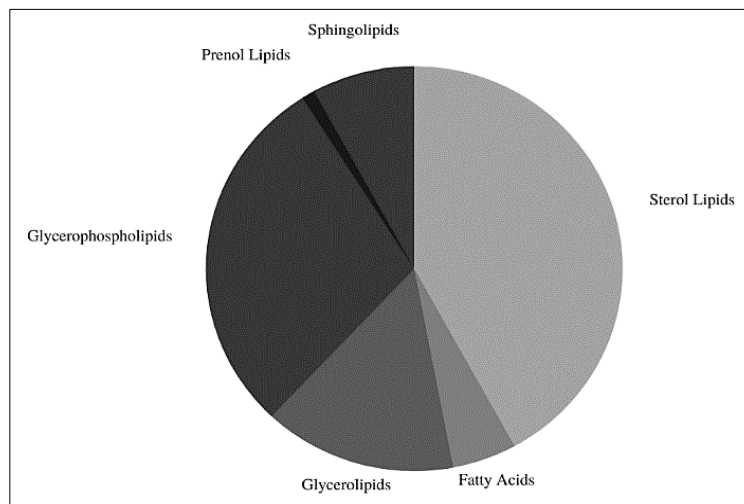


Figure 2 - Representation of the different lipid classes in the human blood.

1.2. Lipids

Lipids are a diverse group of compounds where the common and defining characteristic is their insolubility in water, and their solubility in nonpolar solvents, such as ethers, alcohols, benzene, and chloroform^{11,13,14}. Due to their diversity, lipids can be broadly classified as “simple”, they yield at most two types of products on hydrolysis (e.g., fatty acids, and sterol); and “complex”, if three or more types of products result from hydrolysis (e.g., glycerophospholipids)¹³. The diversity of lipids compounds is also reflected in their functions¹⁵. Fatty acids and oils, for example, have a role in storing energy, while sterols and phospholipids are important structural components of biological membranes¹¹. Other lipids that are found in smaller concentrations in the organism play essential roles such as electron carriers, enzyme cofactors, hydrophobic protein anchors, and they also function as "chaperones" to assist in the folding of membrane proteins.¹¹

According to the chemical backbone, lipids can be categorized into eight main classes: fatty acids (FA), glycerolipids (GL), glycerophospholipids (GP), sphingolipids (SP), sterol lipids (ST), prenol lipids (PR), saccharolipids (SL), polyketides (PK)¹³. The classes can be further subdivided into subclasses¹³. This review will only include the lipids present in the human plasma lipidome, mentioned before.

The nomenclature of lipids follows a specific pattern, such as:

- i. To describe glycerolipids and glycerophospholipids is used the method of stereospecific numbering (sn), where the glycerol group is usually acylated or alkylated at the sn1 and/or sn2 position except the lipids that have more than one glycerol group^{13,15};
- ii. Sphinganine and sphing-4-enine are the core structure of the sphingolipid category^{13,15};
- iii. For the sterol lipids, is used the core names like, cholestane, androstane, and estrane^{13,15};
- iv. In glycerolipids and glycerophospholipids, the term ‘lyso’ refers to the lack of a radyl group^{13,15};
- v. The abbreviations are used to refer to species with one or two radyl side-chains, where the structures of the side chains are indicated within parentheses follow the ‘Head-group(sn1/sn2)’ format (e.g. PC(16:0/18:1))^{13,15}.

1.2.1. Lipid classes and subclasses

1.2.1.1. Fatty Acids

Fatty acids are one of the most important category of biological lipids¹³. This category includes not only fatty acids but also several other variants such like alcohols, aldehydes, amines, and esters¹⁵. Chain elongation of an acetyl-CoA primer with malonyl-CoA or methylmalonyl-CoA groups is used to synthesize this lipid category¹⁵.

Fatty acids are carboxylic acids with hydrocarbon chains with a range of 4 to 36 carbons long and a carboxyl group^{11,13}. In some fatty acids, the hydrocarbon chain is fully saturated (meaning that does not have double), and in some other fatty acids, the hydrocarbon chain is unsaturated (meaning that does have one or more double bonds)¹¹. The follow figure (Figure 3) shows two different structures of two different fatty acids.

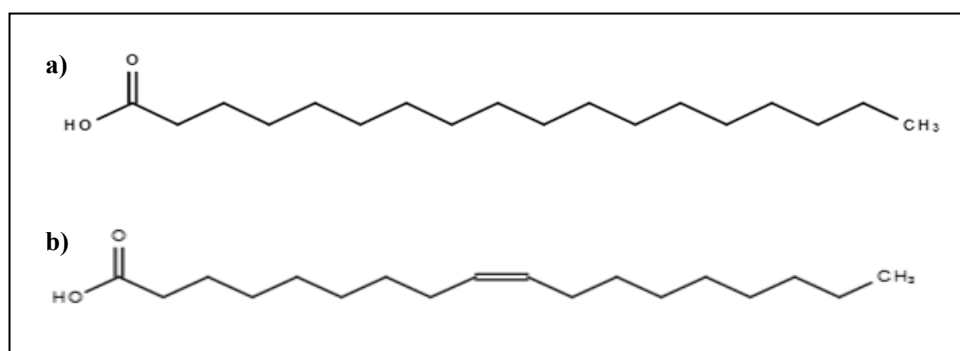


Figure 3 - Two examples of structure of fatty acids. a) Stearic acid (18:0); b) Oleic acid (18:1).

The degree of unsaturation and the length of the hydrocarbon chain determine the physical properties of fatty acids and the compounds that contain them. The fatty acid's solubility in water decreases as the fatty acyl chain lengthens and the number of double bonds increases. Short-chain fatty acids have a slight solubility in water due to the carboxyl group of the fatty acids¹¹. The length and the degree of unsaturation of the hydrocarbon chain, also influence the melting point of this type of lipids¹¹. Variations in fatty acid content and head groups determine the heterogeneity of complex lipids within a category¹².

Polyunsaturated fatty acids (PUFAs) are a type of fatty acid in which the third and fourth carbons from the methyl end of the chain have a double bond. PUFAs' physiological role is determined by the position of the first double bond closest to the methyl end of the chain rather than the carboxyl end¹¹.

1.2.1.2. Glycerolipids

Glycerolipids, GL are composed of a 3-carbon glycerol backbone, where each carbon is numbered by sn-1, sn-2, and sn-3 ^{13,14}, as can be observed in Figure. Monoglycerides (MG), diglycerides (DG), and triglycerides (TG) form this class ^{13,14}.

Diglycerides (DG), or diacylglycerols, are a component of the lipid bilayer that helps to keep the cell membrane stable ^{13,14}. This type of molecule not only helps to keep the cell membrane stable, but it also acts as a second messenger, regulating the activation of protein kinase C, which is involved in T cell activation ¹⁴. The accumulation of DG can cause lipotoxicity, which can result in cell dysfunction, and consequently cell apoptosis. This accumulation can lead to health issues such as diabetes, carcinoma, and coronary artery disease ¹⁴.

Triglycerides (TG), or triacylglycerols, are composed of three fatty acids in ester linkage with one glycerol, as seen in Figure 5 ¹¹. Inflammation and insulin resistance are linked to the metabolism of TG ¹⁴. The inflammatory condition of adipose tissues affects lipid metabolism, causing TG synthesis to decrease and lipolysis (catabolic process that breaks the TG into free fatty acids and glycerol) to increase ¹⁴. Increased levels of free fatty acids resulting from this process, can lead to the accumulation of TG and activated lipids, particularly long-chain fatty acyl-CoA esters in skeletal muscle, liver, and β -cells ¹⁴.

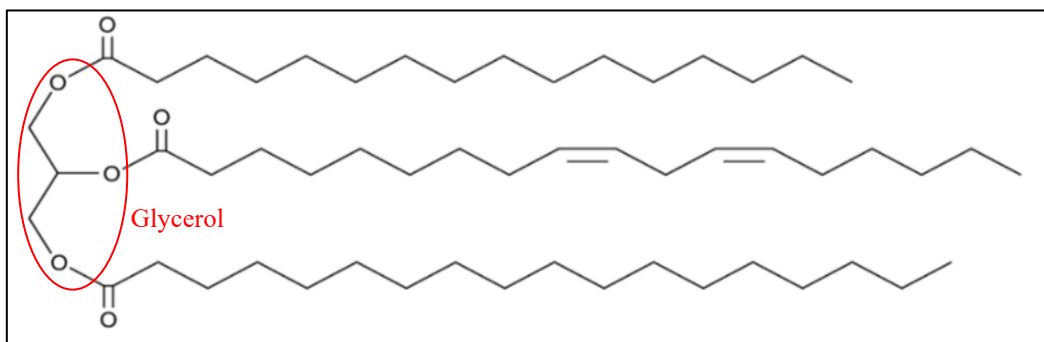


Figure 4 - 1-stearoyl, 2-linoleoyl, 3-palmitoyl glycerol; an example of triglycerides.

1.2.1.3. Glycerophospholipids

Glycerophospholipids (GP) are a major structural component of cell membrane¹⁴. This type of membrane lipids are constituted by two fatty acids, bounded by ester linkage to the first and second carbons of glycerol, and a highly polar or charged group is attached through a phosphodiester linkage to the third group¹¹. According to the nature of the head group, GP class can be divided in various subclasses^{11,14}. Figure 5 illustrate two structures from two different subclasses of GP.

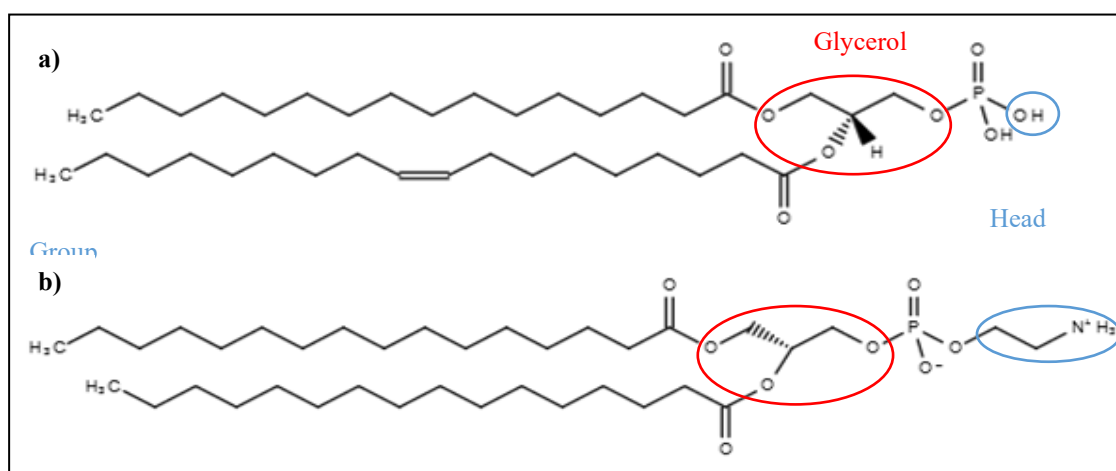


Figure 5 - Example of GP structures. a) Phosphatidic Acid; b) Phosphatidylethanolamine.

GP class can be divided into seven subclasses: phosphatidic acid (PA), phosphatidylglycerol (PG), phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidylethanolamine (PE), and cardiolipin (CL).

One of the most important subclasses of the GP class is phosphatidylcholines (PC)¹⁶. In this subclass, a choline molecule constitutes the head group. PC and lysophosphatidylcholines (lysoPC) can affect cell signaling by interacting with specific proteins and changing the lipid acyl chain composition, which controls downstream signaling¹⁴. PC also play a major structural role in cell membranes and blood proteins¹⁶. Fatty acids, which are precursors to lipid-derived signaling molecules, are stored in PC¹⁶. LysoPC is a bioactive lipid that is produced by partial hydrolysis of a PC molecule, and it is involved in monocyte recruitment, vascular smooth muscle cell proliferation, and endothelial dysfunction¹⁶. PC and lysoPC molecules differ in their functional properties and susceptibility to oxidative stress due to the different composition and distribution of fatty acids on the glycerol backbone⁶. The

dysregulation of PC levels has been associated to coronary artery disease and coronary artery calcification ⁶.

The phosphatidylethanolamine (PE) subclass is the second most abundant GP subclass, and it is found on the inner leaflet of the cell membrane ⁶. This type of lipids that have ethanolamine in the head group is associated with protein biogenesis, oxidative phosphorylation, membrane fusion, mitochondrial stability, and autophagy (a mechanism that breakdown cellular components of lysosomes) ⁶.

1.2.1.4. Sphingolipids

Sphingolipids (SP) are constituted by one molecule of the long-chain amino alcohol sphingosine or one of its derivatives, one molecule of a long-chain fatty acid, and polar head group linked by a glycosidic linkage in some cases and a phosphodiester in others ^{11,13}. Unlike GP, SP does not include glycerol in its composition ¹³. Ceramides (Cer) and sphingomyelins (SM) are examples of major subclasses of the SP class ^{11,13}. Figure 6 shows two different types of SP, one of which is a ceramide and the other a sphingomyelin.

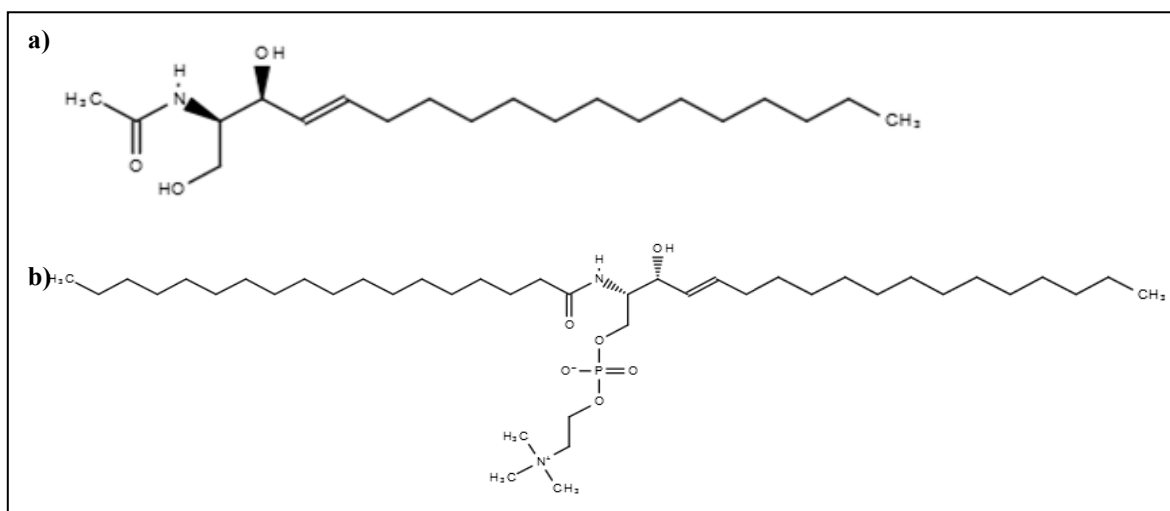


Figure 6 - Example of SP structures. a) Ceramide; b) Sphingomyelin.

Ceramides (Cer) are composed of a fatty acid connected to the $-NH_2$ molecule by an amide bond ¹¹. This subclass has been associated to atherosclerotic mechanisms such as lipoprotein aggregation, cholesterol accumulation within macrophages, nitric oxide synthesis regulation, superoxide anions production, and cytokine expression ^{4,17}. Lactosylceramide (LacCer) affects the atherogenic process in atherosclerotic plaques by suppressing macrophage apolipoprotein

E production, causing cholesterol to accumulate in macrophage foam cells¹⁸. As a result, higher LacCer levels are linked to a clinical cardiovascular outcome¹⁸.

Sphingomyelins (SM) have as head group phosphocholine or phosphoethanolamine¹¹. In terms of properties and three-dimensional structure, this subclass is similar to phosphatidylcholines¹¹. The atherogenic potential of lipoproteins is increased when SM synthase is overexpressed⁴.

Cer and SM levels in atherosclerotic plaques have been shown to contribute to smooth muscle cell inflammation in coronary arteries.⁴

1.2.1.5. Sterol Lipids

The main characteristic of the structure of sterol lipids (ST) is the steroid nucleus consisting of four fused rings, three with six carbons and one with five¹¹.

As seen in Figure 7, cholesterol is an amphipathic molecule with a polar head group and a non-polar hydrocarbon body composed of the steroid nucleus and the hydrocarbon side chain¹¹.

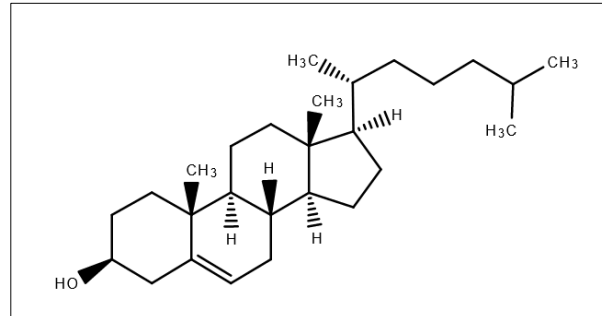


Figure 7 - Cholesterol chemical structure.

Cholesterol is found naturally in the human body and plays an important role in how each cell functions¹⁹. Cholesterol can be converted into vitamin D and a variety of hormones by the human body, and it is also the starting source for bile acids, which the body uses to aid fat digestion¹⁹. Regardless, elevated cholesterol levels in the body will increase the risk of heart disease¹⁹. This type of lipid is transported around the body in the form of lipoproteins. The main two lipoproteins used to transport cholesterol are low density lipoprotein (LDL), and high density lipoproteins (HDL)¹⁹. LDL cholesterol (LDL-C) is transported from the liver to the cells via the bloodstream, and HDL cholesterol (HDL-C) is transported from the body cells back to the liver via the bloodstream. If the levels of LDL-C are too high, too much cholesterol

reaches the artery walls, and if the levels of HDL-C, which carries away the excess cholesterol, are insufficient, cholesterol continues to build up on the artery walls, resulting in coronary artery disease as the artery walls become less stable and thicker¹⁹.

1.2.1.6. Prenol lipids

Prenol lipids (PR) are synthesized from the five carbon precursors isopentenyl diphosphate and dimethylallyl diphosphate that are mainly produced by the mevalonic acid pathway¹³. This form of lipid is found in lower amounts in the human body¹².

1.3. Lipidomics

The word lipidomic was first introduced in PubMed in 2003, and since then, the search for it has escalated⁸. Despite being a relatively new area of study, a number of analytic techniques, especially mass spectrometry (MS), have seen rapid advances, due to its ability to analyze a sample's entire lipid profile^{8,13}. Lipidomics is a method for fully analyzing and characterizing the lipidome, which entails a detailed analysis of lipid molecules, their interactions, and their influence in an organism^{2,11}. Lipidomic techniques provide a comprehensive analysis of an organism's lipidome, which is critical for understanding various pathologies¹³.

1.4. Cardiovascular diseases

The cardiovascular system consists of the heart and blood vessels. The blood vessels are classified into three types: arteries, which carry blood away from the heart; veins, which carry blood toward the heart; and capillaries, which bind the arteries and veins and serve as the exchange points for oxygen, nutrients, and waste within the tissues²⁰.

Cardiovascular disease (CVD) is a group of diseases that affect the heart and blood vessels, including coronary heart disease (CHD) and, coronary artery disease (CAD), among others²¹.

Coronary artery disease (CAD) is a form of cardiovascular disease caused by atherosclerosis or occlusions of the coronary arteries caused by atherosclerosis, which results in insufficient oxygenation of a region of the myocardium, that could lead to cell death²⁰. The magnitude of the condition can be determined by the size and location of the artery in question,

whether or not the blockage is complete, and whether or not there are collateral vessels that provide perfusion of the area from other vessels ²⁰. Obstructive and nonobstructive artery lesions can cause major adverse cardiovascular events (MACE), such as myocardial infarction (MI), and cardiovascular death. Usually, these events occur as a result of atherothrombosis, which occurs when an atheroma is disrupted, resulting in the development of a thrombus that may block blood supply locally or embolize ²². Since atherothrombosis is a systemic condition, patients with CAD can potentially experience a stroke due to known or occult cerebrovascular artery disease, or an acute limb ischemia due to known or occult peripheral artery disease (PAD) ²². Figure 8 illustrates the development of atherothrombotic vascular diseases, including the progression of CAD, the various manifestations of ACS, and the possibility of thrombosis at the plaque rupture location.

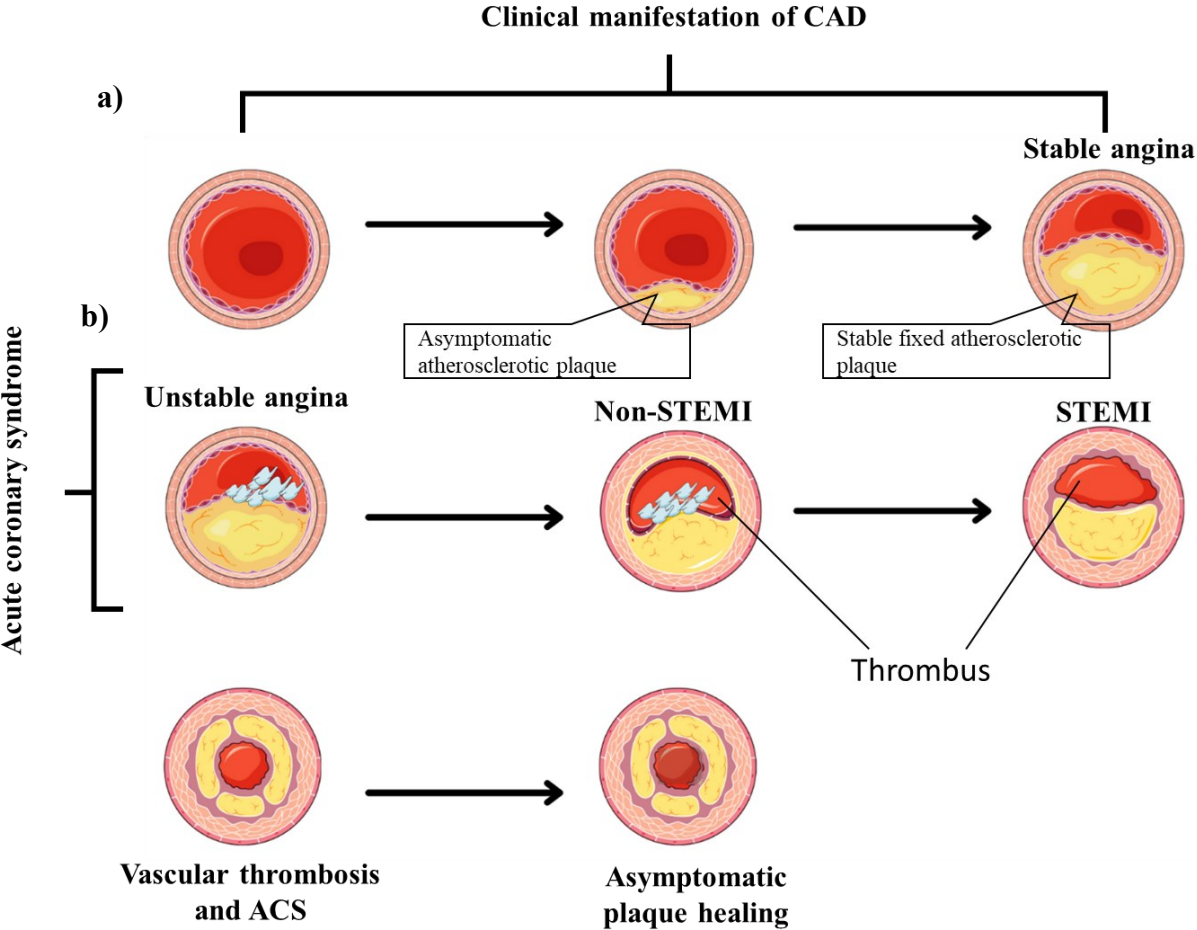


Figure 8 - Progression of atherothrombotic vascular disease. a) Clinical manifestation of CAD; b) risk of thrombosis at the site of plaque rupture. Adapted from Fox, et al. ²²

Clinical manifestations of CAD include angina pectoris, acute coronary syndrome and sudden cardiac death ²³.

Acute coronary syndrome (ACS) is characterized by a variety of signs and symptoms related to decreased blood supply across the coronary tree and the resulting acute myocardial ischemia ²⁴. The term ACS usually include unstable angina, non-ST-segment elevation myocardial infarction (Non-STEMI), and ST-segment elevation myocardial infarction (STEMI) ^{22,24}. Unstable angina is a condition that occurs between angina pectoris and acute myocardial infarction, and is characterized by ischemic pain ²². Stable angina, also known as stable angina pectoris (SAP), is described as a transient chest pain or tightness triggered by physical exertion or emotional stress, and is most often caused by obstructive CAD ²². Non-STEMI is characterized by the myocardial necrosis without acute ST-segment elevation showing on the electrocardiogram, and STEMI is characterized by the myocardial necrosis with acute ST-segment elevation showing on the electrocardiogram ²¹.

Myocardial infarction, also known as acute myocardial infarction, is a result of myocardial necrosis that occurs due to a sudden decrease in coronary blood flow of the myocardial. Despite being a region of possibly reversible ischemia adjacent to infarcted tissue, the tissues damaged by necrosis are permanently inactive ²¹.

2. Methods

2.1. Literature search and selection criteria

The list of studies reviewed in this paper was searched in MedLine Databases from March to June 2020. This search strategy was developed to maximize the sensitivity of articles identification using a not only controlled vocabulary (e.g., Medical Subject Headings [MeSH]) but also the keywords such as coronary disease or acute coronary syndrome or myocardial infarction or myocardial ischemia or heart attack and lipidomics, yielding a total of 68 articles. For this review, the articles included had to present a lipidomic comparison between coronary pathologies and control groups. Review articles, editorials, articles focus on other pathologies such as diabetes, genetic studies, intervention studies, articles with poor information about lipidomic analysis, analyzes of the role of a specific lipid in coronary heart disease were excluded. After analyzing all the 68 articles to see if they fulfill the criteria mentioned before, only 19 articles were selected for this review, present in Table 1. Figure 9 shows the percentage of included and excluded articles. Appendix 1 shows the flowchart representative of the research done.

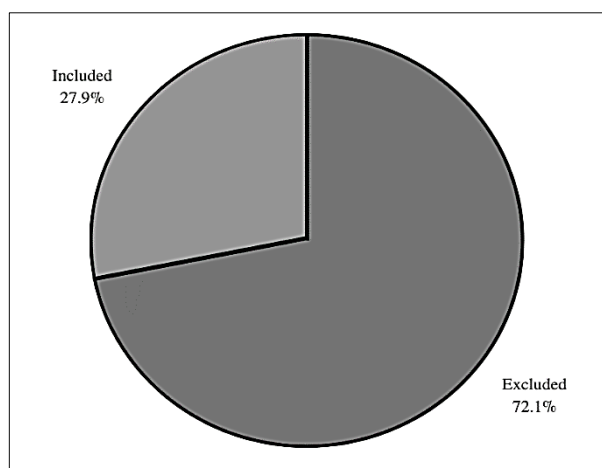


Figure 9 - Representative chart of the percentage of included and excluded articles for the review.

To evaluate the quality of the selected research papers was used the portal *SCImago Journal & Country Rank* that assess the quality of the journal, measuring the amount of citations received as well as the credibility of the journal from which the citation originated. In addition

to calculating the number of citations received by the journal, the SCImago Journal Rank (SJR) indicator reflects the scientific impact of the journal, but also the credibility of the journal from which the citations derive. The set of journals is classified into four equal quartiles according to this indicator, where Q1 contains the journals with the highest values for scientific impact factor and Q4 contains the journals with the lowest values. With the exception of three journals (Lipids in Health and Disease, Canadian Journal of Physiology and Pharmacology, Vascular Health and Risk Management) that had the evaluation belonging in the Q2, all the other journals where the papers reviewed published belong in the Q1.

Appendix 2 shows all the selected articles with the respective author, publication date, journal, and journal evaluation.

Table 1 - Selected articles and respective scientific journal where they were published.

Article	Scientific Journal
Garcia, et al. ²⁵	Journal of Thrombosis and Haemostasis
Havulinna, et al. ¹⁷	American Heart Association, Inc.
Lu, et al. ⁹	Redox Biology
Rached, et al. ²⁶	Biochimica et Biophysica Acta
Guo, et al. ²⁷	Redox Biology
Meikle, et al. ²⁸	American Heart Association, Inc.
Paapstel, et al. ¹⁶	Nutrition, Metabolism and Cardiovascular Diseases
Poss, et al. ²⁹	The Journal of Clinical Investigation
Hancock-Cerutti, et al. ³⁰	Journal of Clinical Lipidology
Vorkas, et al. ⁷	International Journal of Cardiology
Sutter, et al. ³¹	Atherosclerosis
Cheng, et al. ³	Atherosclerosis
Anroedh, et al. ¹⁸	Journal of Lipid Research
Sun, et al. ³²	Journal of Lipid Research
Chatterjee, et al. ³³	European Heart Journal
Djekic, et al. ⁵	International Journal of Cardiology
Djekic, et al. ⁶	Dove Press journal: Vascular Health and Risk Management
Caligiuri, et al. ³⁴	Canadian Journal of Physiology and Pharmacology
Zalloua, et al. ⁴	Lipids in Health and Disease

3. Results

3.1. Studied Population

Every article selected for this review had different main goals but all of them had the goal to identify lipids in different forms of coronary heart diseases. Appendix 3 shows the aim of each individual article.

The selected articles to be analyzed in this review were divided into four groups according to the populations studied:

- **Group 1: comparison between healthy individuals and patients with coronary heart disease.**
- **Group 2: comparison among patients with stable coronary heart disease, patients with acute coronary syndrome and healthy individuals.**
- **Group 3: comparison between patients with stable coronary heart disease and patients with acute coronary syndrome.**
- **Group 4: coronary calcification.**

In order to select the study papers for this review, participants had to provide relevant information about demographics and comorbidities, such as age, gender, body mass index (BMI), smoking, hypertension, diabetes, total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglycerides (TG). In the case of groups involved in Group 4, instead of providing information on total cholesterol, HDL-C, LDL-C, and triglycerides, they offer calcification score details (Appendix 4).

Table 2 - Selected article for each group as well as the study type of the respective article.

Group	Article	Study type
Group 1	Caligiuri, et al. ³⁴	Prospective cohort
	Garcia, et al. ²⁵	Case control
	Hancock-Cerutti, et al. ³⁰	
	Havulinna, et al. ¹⁷	Prospective cohort
	Paapstel, et al. ¹⁶	Case control
	Poss, et al. ²⁹	
	Rached, et al. ²⁶	
	Sun, et al. ³²	
	Zalloua, et al. ⁴	
Group 2	Guo, et al. ²⁷	Case control
	Lu, et al. ⁹	
	Sutter, et al. ³¹	
Group 3	Anroedh, et al. ¹⁸	Prospective cohort
	Chatterjee, et al. ³³	Case control
	Cheng, et al. ³	Prospective cohort
	Meikle, et al. ²⁸	
Group 4	Djekic, et al. ⁵	Prospective cohort
	Djekic, et al. ⁶	
	Vorkas, et al. ⁷	

Studies included in this systematic review were either case control or prospective cohort studies (Table 2).

The word cohort is characterized as a group of individuals with common features who are tracked to assess, for example, the prevalence of a particular disease. A disease-free population or an outcome is identified in a cohort study and tracked for a time before the disease or consequence of interest emerges. The key value of this method of analysis is that it is possible to analyze the various effects of one study, to gather data in despite of the sequence of events, and to investigate unusual diseases as well as to assess the occurrence of a specific disease.³⁵ The key disadvantages are the amount of subjects needed to study, how costly this form of study can be, the long duration of cohort studies, because of the loss of follow-up or withdrawals of the sampled group, the preservation of this type of research can be incredibly difficult.³⁵ The descriptions of the selected group of participants by exposure status at the beginning of the

investigation are the hallmark of the cohort studies and the crucial aspect of the subject selection is that all exposed and unexposed groups have been selected from the same population. Due to the long follow-up periods resulting in missed results, the lack of communication with the population under the study contributes to the battle against the general rule that the loss of follow-up incidence must not reach 20% of the survey. In order to minimize that loss, subjects who are more likely to be missed during the recruiting process are excluded, such as persons who are preparing to move, so gathering records on subjects that allow the investigator to observe them in the future and establish frequent communication during the follow-up period.³⁵ In the case of the studies used in this review, they are prospective cohort studies, which means that, unlike retrospective cohort research carried out from the present to the past, the studies are carried out from the present to the future.

Case-control studies are similar to a patient evaluation, the patient is questioned and examined and elements from his past are taken and combined to reveal traits or attributes that have predisposed a patient to a disease.³⁵ In this sort of analysis, at the beginning of the investigation, the subject is identified by outcome status, the outcome of interest may be whether the patient underwent any kind of surgery or operation, was diagnosed with some disease, or suffered some health problems. They are categorized into two groups, cases and controls, after the result is established and the analyzed population is described. The key advantages of this type of research are the quick way to perform the study, how inexpensive these studies compared with cohort studies, how is possible to use prior reports of patient health conditions, and how few subjects are needed to achieve a statistically valid study. The key disadvantages are the difficulty in validating the information concerning the participants past, the difficulty in selecting the relevant reference sample, and the rate of exposure and non-exposure of patients to the disease.³⁵ Case-control trials are simpler, inexpensive, and require fewer patients compared to cohort studies. The explicit definition of the inclusion and exclusion criteria of participants assigned to case selection is an imperative consideration in case-control studies, since certain criteria guarantee that all cases are homogeneous. It is very important to choose the right control group in this type of study so both the control and case group should come from the same source population as the study validation depends on their comparability. Due to the comparability of both groups, is important to use a matching method to attempt to ensure that and minimize the variability and systemic differences in background variables that are not interesting to the investigation. Due to the elimination of measurable confounders such as age and sex, this matching method is not only important to use, but also helps eliminate the impact of confounders that are difficult to measure. Despite this method may improve the

efficiency of the study because can make the population selection process easier, this may be time-consuming and expensive and requires a matched analysis and some matched variables cannot be examined in this studies ³⁵. Table 3 shows the difference between the case-control and prospective cohort studies.

Table 3 - Differences between Prospective cohort studies and case control studies.

	Prospective Cohort	Case-Control Studies
Long study	√	×
Presence of a control, or unexposed, group	×	√
High cost	√	×
Subjects from the same population	√	×
Small group	×	√
Selection Criteria	√	√

Group 1 compared patients with coronary heart disease and healthy patients. In this group, Havulinna, et al. ¹⁷, and Caligiuri, et al. ³⁴ were the only prospective cohort studies. Havulinna, et al. ¹⁷ studied for 13 years, 8101 individuals from the general population from FINRISK 2002, where 813 individuals of that population suffer from MACE, which 116 of them suffer from fatal MACE. Caligiuri, et al. ³⁴, consisted of 98 patients follow during a six-month period, where 16 suffer from PAD, 10 patients suffer TIA, 16 suffering a cerebrovascular accident, and 24 patients suffer from Stable Angina and CAD. Rached, et al. ²⁶ study, consisted of 26 individuals to compare the 16 of them that suffer from STEMI with the 10 healthy individuals that work as a control group. The study conducted by Poss, et al. ²⁹, compared 462 patients that suffer from CAD with 212 healthy individuals that were used as a control group. Hancock-Cerutti, et al. ³⁰, divided the 60 patients into three groups: group one, consisted of 25 patients that suffer from CAD and HDL-C higher than 90th (HCAD); group two, consisted of 25 patients that do not suffer from CAD and HDL-C lower than 90th (HHDL); and a third group, consisted with 10 patients with HDL-C between 25-75th and does not suffer from CAD (NHDL). Sun, et al. ³² study, is a case control study that studied the population included in a follow-up study that lasted five years. This study was included 1454 individuals that were divided into two groups with 727 individuals each was one of the groups contain individuals that suffer from MI and one group with their matched controls. The study Zalloua, et al. ⁴, compared 109 patients,

divided into two groups, one with 47 patients that suffer from CAD and the other with 62 healthy controls. Garcia, et al. ²⁵, compared 30 patients who suffer from ACS with 30 patients that have a high risk of CVD but do not suffer from CAD. The article Paapstel, et al. ¹⁶, consisted of patients divided into three groups, one with 32 patients that suffer from PAD, the other group with 52 patients that suffer from CAD, and the last one with 40 healthy controls.

Group 2 compared patients with stable coronary heart disease and patients with acute coronary syndrome with healthy individuals. Lu, et al. ⁹, consisted of 28 patients, where 10 of them suffer from stable angina; eight patients suffer from MI and 10 healthy patients. The article Guo, et al. ²⁷, divided 49 patients into four groups, one with 10 patients that suffer from MI, the other with 15 patients that suffer from CAD and Cerebrovascular Disease, the third one with 14 patients that suffer from CAD, and the last one with 10 healthy patients. Sutter, et al. ³¹ study, consisted of 67 patients where 23 of them suffer from CAD, 22 that suffer from ACS, and 22 healthy controls.

Group 3 compared patients with acute coronary syndrome with patients with stable coronary heart disease. The article Cheng, et al. ³, follow 581 patients for three years, divided into two groups, one with 313 patients who suffer ACS, where 162 of them suffer from STEMI and 151 suffer from Non-ST-elevation ACS; and the other with 261 patients with stable coronary heart disease. Anroedh, et al. ¹⁸, follow 574 patients for three years where 313 patients suffer from ACS and 261 patients suffer from SAP. In the study Meikle, et al. ²⁸, consisted of 130 patients, where 47 patients suffer from ACS, and of those 47, 36 suffer from STEMI and 11 from Non-STEMI, and 83 patients suffering from CAD. Chatterjee, et al. ³³ study, included 175 patients where 94 suffer from CAD, 46 suffer from Non-STEMI and 12 from STEMI, and 15 healthy individuals.

Lastly, Group 4 compared patients that suffer from different stages of coronary calcification. All the articles that belong in this group, Vorkas, et al. ⁷, Djekic, et al. ⁵, and Djekic, et al. ⁶, used the same methodology. They divided 70 patients into three groups, one group with 26 patients that do not have coronary calcification, the other with 27 patients that suffer from mild calcification, and the last group with 17 patients that suffer from severe coronary calcification.

3.2. Analyzed blood products in the different research articles

The analyzed blood product was either plasma or serum in all the articles included in this study, as can be seen in Table 4.

Table 4 - Analyzed blood product and respective research articles that used that same tissue

Analyzed tissue	
Plasma	Serum
Lu, et al. ⁹	Garcia, et al. ²⁵
Rached, et al. ²⁶	Havulinna, et al. ¹⁷
Guo, et al. ²⁷	Paapstel, et al. ¹⁶
Meikle, et al. ²⁸	Poss, et al. ²⁹
Hancock-Cerutti, et al. ³⁰	Vorkas, et al. ⁷
Sutter, et al. ³¹	Chatterjee, et al. ³³
Cheng, et al. ³	Djekic, et al. ⁵
Anroedh, et al. ¹⁸	Djekic, et al. ⁶
Sun, et al. ³²	Zalloua, et al. ⁴
Caligiuri, et al. ³⁴	

The first step was always to extract blood from all the patients selected for the study in sterile tubes, regardless of the blood product used for analysis. In the studies presented in table 8 that analyzed plasma, plasma was prepared by centrifugation after the blood was collected and stored at -80 °C until analysis ^{30,18, 9, 27} because these conditions showed that any minor variations that might occur during the stored time at this temperature would be consistent for all the samples.²⁸ Before the lipidomic analysis, the lipids had to be extracted from all the plasma using chloroform, benzene, ether, or ethyl because these solvents do not permit lipid clustering driven by hydrophobic interactions.¹¹ The solution were mixed, and then dried in a stream of nitrogen gas. The dried lipid extracts were reconstituted (e.g. isopropanol/hexane/water, 10:5:2 v/v), the internal standards were added - such as phosphatidylserine (PS) (17:0/17:0), phosphatidylglycerol (PG) (17:0/17:0), phosphatidylethanolamine (PE) (17:0/17:0), monoacylglycerol (MG) (17:0/0:0/0:0), diglycerides (DGs) (17:0/17:0/0:0), triglycerides (TG) (17:0/17:0/17:0), TG(16:0/16:0/16:0), phosphatidylcholine (PC) (17:0/0:0), PC(19:0/19:0), ceramide (Cer) (16:0) ⁶ - and analyzed in the different forms mentioned in the next subchapter. As seen in Table 8, some studies used serum as there analyzed blood product. In such studies, the sterile tube was kept at room temperature for a brief amount of time to coagulate after the blood had been extracted. ^{7, 6} The samples were centrifuged after clotting and the serum was extracted and stored at -80 °C before analysis. Before analysis, the lipids had to be extracted using the same methodology as in the studies that used plasma as tissue to analyze.

3.3. Methods of analysis – Lipidomic tools

Due to its sensitivity and selectivity, the method of choice for qualitative and quantitative lipidomic analysis is mass spectrometry (MS).³⁶ Although the chemical properties of identical lipids are very similar, the components of a lipid mixture can be isolated and classified simultaneously in mass spectrometry by their distinctive fragmentation pattern.¹¹ The higher the resolution of MS, the higher is the possibility to identify individual lipids in a complex mixture.¹¹ A mass spectrometer (Figure 10) consists of an inlet system to inject the sample into the ion source, such as a liquid chromatograph; an ion source, to produce ions from the sample; one or more mass analyzers, to separate the ion generated from the ion source according to their mass to charge ratio (m/z); a detector, to register the number of ions coming from the last analyzer, and a computer to process the data and produce the mass spectrum.³⁷

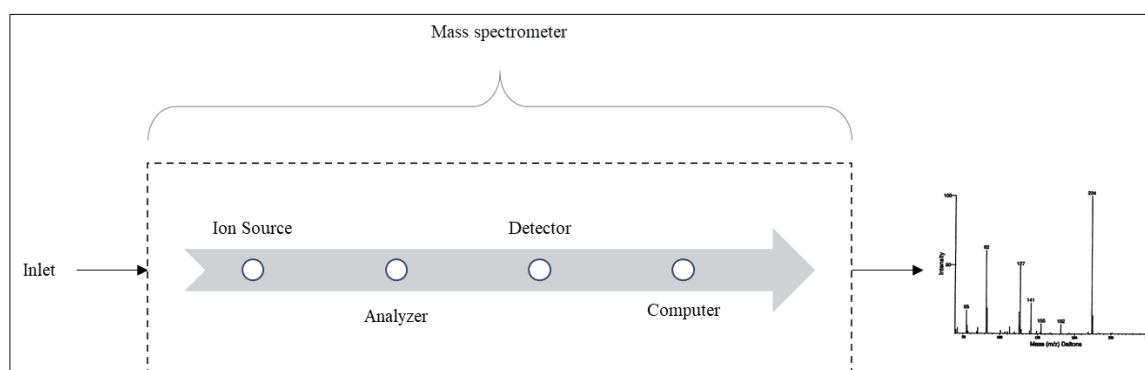


Figure 10 - Representation of a mass spectrometer coupled with an inlet that could be a liquid chromatograph and an example of a spectrum obtained.

As table 5 show, all the studies used in this review use MS as tool for lipidomic analysis.

Table 5 - Article and respective lipidomic tool used.

Article (reference)	Lipidomic Tools Inlet – Ion Source – Analyzer/MS
<i>Garcia, et al.</i> ²⁵	HPLC – ESI – QqQ/MS
<i>Havulinna, et al.</i> ¹⁷	UHPLC –ESI - QTrap/MS
<i>Lu, et al.</i> ⁹	UHPLC – ESI - QTOF/MS/MS
<i>Rached, et al.</i> ²⁶	HPLC – ESI – QTrap/MS/MS
<i>Guo, et al.</i> ²⁷	LC – ESI – QqQ/MS
<i>Meikle, et al.</i> ²⁸	LC – ESI – QqQ/MS/MS
<i>Paapstel, et al.</i> ¹⁶	FIA – MS/MS
<i>Poss, et al.</i> ²⁹	LC - ESI– QqQ/MS/MS
<i>Hancock-Cerutti, et al.</i> ³⁰	HPLC – ESI - Qtrap/MS/MS
<i>Vorkas, et al.</i> ⁷	UPLC – ESI – QTOF/MS
<i>Sutter, et al.</i> ³¹	HPLC – ESI/MS/MS
<i>Cheng, et al.</i> ³	UHPLC – ESI - QTrap/MS
<i>Anroedh, et al.</i> ¹⁸	UHPLC – ESI - QTrap/MS
<i>Sun, et al.</i> ³²	UPLC – ESI – QqQ/MS
<i>Chatterjee, et al.</i> ³³	UHPLC – ESI – QTOF/MS/MS
<i>Djekic, et al.</i> ⁵	UHPLC – ESI – QTOF/MS
<i>Djekic, et al.</i> ⁶	UHPLC – ESI – QTOF/MS
<i>Caligiuri, et al.</i> ³⁴	HPLC – ESI - Qtrap/MS/MS
<i>Zalloua, et al.</i> ⁴	UPLC - ESI – Qtrap/MS

Legend: ESI - Electrospray ionization; FIA - Flow Injection Analysis; HPLC - High Performance Liquid Chromatography; QqQ - Triple quadrupole analyzers; QTrap - Triple quadrupole analyzer coupled with an ion trap; QTOF - Quadrupole analyzer coupled with a time of flight analyzer; UHPLC - Ultra - High Performance Liquid Chromatography; UPLC - Ultra Performance Liquid Chromatography.

Different studies used different mass spectrometer inlets. As table 5 show, there were used as inlet: Liquid Chromatography (LC), High Performance Liquid Chromatography (HPLC), Ultra Performance Liquid Chromatography (UPLC), Ultra - High Performance Liquid Chromatography (UHPLC), and Flow Injection Analysis (FIA). Liquid Chromatography is a technique used for the separation, identification, and quantification of different components in a mixture. The main principle of this technique is the separation of a mixture based on the interaction of the components with the stationary phase and the mobile phase when it passes through the separation column.³⁸ In FIA, the sample is injected into a moving, nonreactive carrier stream. The sample is pushed down the tube to the junction and the reagent is

continuously pumped in another tube and pushed to the same junction.³⁹ The carrier stream that contains the sample is merged with the reagent creating a chemical reaction or an analyte concentration gradient followed by MS detection, in the case of FIA – MS.⁴⁰ High Performance Liquid Chromatography is one of the most versatile chromatography techniques. In these analyses, the stationary phase consists of small particles achieving more efficiency in separation than in LC.⁴¹ Ultra Performance Liquid Chromatography and Ultra - High Performance Liquid Chromatography are essentially the same technique. The main characteristic of UPLC and UHPLC is that they require higher pressure to function. This pressure should be higher than 6000 psi, which is usually the upper limit of HPLC.⁴²

With exception of the Paapstel, et al.¹⁶ that uses flow injection analysis mass spectrometry (FIA – MS), as the Table 5 shows, all the other studies used electrospray ionization (ESI). In ESI, the sample is dissolved in a solvent mixture and then injected into a capillary held with a potential of three to four kV, creating a solvent droplets spray containing the ion forms of the sample components. Due to the evaporation of the solvent, multiply charged ions produced during this step would then enter the mass analyzer to be separated according to their m/z .³⁷ The different studies selected for this review used different types of analyzers such as quadrupole (Q), time of flight (TOF), and ion trap analyzer. As mentioned before, a mass spectrometer can have more than one analyzer. As the table 9 shows, the studies used more than one analyzer, for instance, triple quadrupole analyzers (QqQ), triple quadrupole analyzer coupled with an ion trap (QTrap), a quadrupole analyzer coupled with a time of flight analyzer (QTOF), and a tandem mass spectrometry analyzer (M/MS) coupled with other analyzers mentioned before.

The quadrupole mass analyzer consists of four evenly spaced parallel rods around the central axis, where one set of parallel rods is positive, and the other set is negative. In a continuous beam along the central axis between the poles, ions coming from the ion source are introduced and filtered according to their m/z . The m/z ratio of ions passing through the Q is proportional to the voltage applied to the rod, where the greater the voltage, the greater the m/z ratio.⁴³ QqQ analyzer consists of three Q analyzers configured together where the first and the third analyzer are used for scanning; the second analyzer is used as a collision cell to fragment the ions by collision-activated dissociation.⁴³ Ion trap analyzer can be visualized as a quadrupole bent in turn to create a closed loop. All m/z ions enter the closed loop that acts as a trap, held inside and subjected to oscillating frequencies aligned with their m/z frequency. Their trajectory expands as a function of time, as the ions repel each other. Whereas oscillating frequencies of the ions are in function of their mass, ions of different m/z will exit the trap at different times

and voltages.⁴³ TOF analyzers use an electric field to pulse through a flight tube the ions of the sample toward the detector. The m/z ratio is determined by the time that the ion takes to pass from the pulser to the detector because the ions with smaller m/z move faster than the ions with higher m/z .^{43,44} Tandem mass spectrometry consists of two mass analyses sequentially carried out to maximize the limits of selectivity and analytical identification.⁴⁴

3.4. Lipidomic results

3.4.1. Low and High Density Lipoprotein Cholesterol, and Triglycerides levels measured in the participants of the studies

To characterize the different blood samples collected in the different studies, were measure the levels of LDL – C, HDL – C, and TG. The levels of these lipids were measured by traditional methods, not using MS to be analyzed.

As previously mentioned, the levels of low and high density lipoproteins cholesterol were measured at the beginning of all studies, with the except those in Group 4, which measured the calcium score instead. Caligiuri, et al.³⁴, and Cheng, et al.³ did not measure the levels of LDL-C and HDL-C. Appendix 4 contains all the results obtained regarding the levels of LDL-C and HDL-C from all the studies mentioned in the subchapter.

As seen in Appendix 4, the levels of LDL-C, except for the studies conducted by Chatterjee, et al.³³, Rached, et al.²⁶, Sutter, et al.³¹, and Paapstel, et al.¹⁶, that determined that the levels of LDL – C were lower in the disease group when compared with healthy patients, the remaining studies, presented higher levels of LDL – C in the disease group than the healthy one. Like the levels of LDL – C, the levels of HDL – C also presented to be higher in the disease group when compared with healthy patients, except of Havulinna, et al.¹⁷, Rached, et al.²⁶, Chatterjee, et al.³³, Sutter, et al.³¹, and Paapstel, et al.¹⁶, that presented lower levels of HDL – C in the disease group.

When compared the different disease groups of patients with healthy individuals, the level of TG presented to be higher in the disease patients, when compared with healthy individuals, with exception of the studies conducted by Lu, et al.⁹, and Guo, et al.²⁷, where healthy individuals presented higher TGs levels than SA and CAD patients, respectively.

Vorkas, et al. ⁷ and Djekic, et al. ⁶ analyzed specific TG that suffer alterations between the different stages of coronary calcification. Vorkas, et al. ⁷ determinate that TG(16:0/18:1/22:5), TG(18:1/18:1/20:4), and TG(16:0/18:1/18:1) were higher in patients that suffer from severe calcification than patients that had no coronary calcification. Djekic, et al. ⁶ determinate that the levels of TG(49:2), TG(51:1), TG(54:5), and TG(56:8) were lower in patients that suffer from severe calcification when compared with patients that suffer from mild calcification. TG(60:5) was higher in patients that suffer from mild calcification when compared with non-calcification patients.

3.4.2. Lipidomic subclasses detected

The major lipid subclasses obtain as relevant in the lipidomic analysis conducted in all the studies selected for the review, were: Cer, LacCer, SM, PC, lysoPC, PI, PE, lysoPE, PS, lysoPS, PG, PA, lysoPA, CE, oxCE, and oxylipins.

Table 6 - Lipid subclasses studied in the articles belonging to Group 1.

Lipid subclasses	Group 1																					
	Article																					
	Havulinna, et al. 17		Rached, et al. 26		Poss, et al. 29		Hancock-Cerutti, et al. 30			Sun, et al. 32		Zalloua, et al. 4		Caligiuri, et al. 34				Garcia, et al. 25		Paapstel, et al. 16		
	MACE	ASYM	STEMI	HC	CAD	HC	HCAD	HHDL	NHDL	MI	HC	CAD	HC	ACS	TIA	CAD	SAP	ACS	Non-CAD	PAD	CAD	HC
Cer	↑	↓	↓	↑	↑	↓	↓	↑	↑↑	-	-	↑	↓	-	-	-	-	-	-	-	-	-
SM	-	-	→	→	↑	↓	↑↑	↑	↓	-	-	↑	↓	-	--	-	-	-	-	-	-	-
PC	-	-	↓	↑	-	-	↓	↑	↑↑	-	-	-	-	-	-	-	-	-	-	↓	↑	↑ ↑
lysoPC	-	-	↑	↓	-	-	↑	↑↑	↓	-	-	-	-	-	-	-	-	-	-	-	-	-
PI	-	-	→	→	-	-	↓	↑	↑↑	-	-	-	-	-	-	-	-	-	-	-	-	-
PE	-	-	→	→	-	-	↑	↓	↑↑	-	-	-	-	-	-	-	-	-	-	-	-	-
lysoPE	-	-	-	-	-	-	↑	↑↑	↓	-	-	-	-	-	-	-	-	-	-	-	-	-
PS	-	-	→	→	-	-	↑	↓	↑↑	-	-	-	-	-	-	-	-	-	-	-	-	-
PG	-	-	→	→	-	-	↑	↓	↑↑	-	-	-	-	-	-	-	-	-	-	-	-	-
PA	-	-	↑	↓	-	-	↑	↓	↑↑	-	-	-	-	-	-	-	-	-	-	-	-	-

5-HETE	-	-	-	-	-	-	-	-	-	-	→	→	-	-	-	-	-	-	-	-	-	-	-
12-HETE	-	-	-	-	-	-	-	-	-	-	↓	↑	-	-	-	-	-	-	-	-	-	-	-
16-HETE	-	-	-	-	-	-	-	-	-	-	-	-	-	-	↓	↓	↑	↑↑	-	-	-	-	-
9-HODE	-	-	-	-	-	-	-	-	-	-	→	→	-	-	-	-	-	-	↑	↓	-	-	-
13-HODE	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	↑	↓	-	-	-
18-HEPE	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	↑	↓	-	-	-
17-HDoHE	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	↑	↓	-	-	-
14-HDoHE	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	↑	↓	-	-	-
8,9-DiHETrE	-	-	-	-	-	-	-	-	-	-	-	-	-	-	↑↑	↓	↑	↑	-	-	-	-	-

Legend: ↑ Higher concentration, ↓ Lower concentration, ↑↑ the higher concentration in a group, → Without difference in the different groups

Table 7 - Lipid subclasses studied in the articles belonging to Group 2.

Lipid subclasses	Group 2								
	Articles								
	Lu, et al. ⁹			Guo, et al. ²⁷			Sutter, et al. ³¹		
	SAP	MI	HC	CAD	MI	HC	CAD	ACS	HC
SM	↑	-	↓	-	-	-	↑	↓	↑↑
PC	↑↑	↑	↓	-	-	-	↑	↓	↑↑
lysoPC	↑↑	↑	↓	-	-	-	-	-	-
lysoPS	↑	↑↑	↓	-	-	-	-	-	-
lysoPA	↑	↑↑	↓	-	-	-	-	-	-
5-HETE	↑	↑↑	↓	-	-	-	-	-	-
9-HODE	↑	↑↑	↓	↓	↑↑	↓	-	-	-
oxCE	-	-	-	↑	↑↑	↓	-	-	-

Legend: ↑ Higher concentration, ↓ Lower concentration, ↑↑ the higher concentration in a group, → Without difference in the different groups.

Table 8 - Lipid subclasses studied in the articles belonging to Group 3.

Lipid subclasses	Group 3								
	Articles								
	Cheng, et al. ³		Anroedh, et al. ¹⁸		Meikle, et al. ²⁸		Chatterjee, et al. ³³		
	ACS	CAD	ACS	SAP	CAD	ACS	STEMI vs HC	SAP vs HC	CAD vs HC
Cer	↑	↓	↑	↓	↓	↑	↑	↑	↑
LacCer	↑	↓	↑	↓	-	-	-	-	-
SM	-	-	-	-	-	-	↑↑	↑	-
CE	↑	↓	↑	↓	-	-	↑	↑	-
DG	-	-	-	-	↓	↑	↑↑	↑	-
PC	-	-	-	-	↑	↓	↑↑	↑	-
lysoPC	-	-	-	-	↑	↓	↑	↑	-
PE	-	-	-	-	↓	↑	-	-	-

Legend: ↑ Higher concentration, ↓ Lower concentration, ↑↑ the higher concentration in a group, → Without difference in the different groups.

Table 9 - Lipid subclasses studied in the articles belonging to Group 4.

Lipid subclasses	Group 4								
	Article								
	Vorkas, et al. ⁷			Djekic, et al. ⁵			Djekic, et al. ⁶		
	SC	MC	NC	SC	MC	NC	SC	MC	NC
SM	↓	↓	↑	↓	↓	↑	↓	↓	↑
PC	↔	↔	↔	↔	↔	↔	↔	↔	↔
lysoPC	↑	↑	↓	-	-	-	-	-	-
PI	↓	↓	↑	-	-	-	-	-	-
PE	-	-	-	-	-	-	↓	↓	↑
DG	-	-	-	-	-	-	↓	↓	↑

Legend: ↑ Higher concentration, ↓ Lower concentration, ↔ unregulated lipid subclass.

3.4.3. Specific lipid species present in the studies

3.4.3.1. Ceramides

Different specific ceramides were detected in different studies, as Appendix 5 shows. Those ceramides may be used as key markers in specific cardiovascular diseases.

Havulinna, et al. ¹⁷ determine that in patients that suffer from MACE, when compared with a group of individuals that do not suffer from MACE, the levels of Cer(d18:1/16:0), Cer(d18:1/18:0), Cer(d18:1/24:1), and the ratios Cer(d18:1/18:0)/ Cer(d18:1/24:0), and Cer(d18:1/24:1)/ Cer(d18:1/24:0) were higher. In that study, the levels of Cer(d18:1/18:0) showed the strongest association with the MACE patients, where Cer(d18:1/16:0), and Cer(d18:1/24:1) showed the higher association with MACE death.

The study conducted by Rached, et al. ²⁶, detected different specific ceramides that can be seen in Appendix 5. The ceramides detected - Cer(d18:1/22:0), Cer(d18:1/23:0), Cer(d18:1/24:0), Cer(d18:1/24:1) – appear to be lower in patients that suffer from STEMI when compared with a healthy control group.

Cer(d18:1/16:0), Cer(d18:1/18:0), Cer(d18:1/24:0), and Cer(d18:1/24:0), in Poss, et al.²⁹, appear to be higher in patients that suffer from CAD, when compared with healthy controls.

Cheng, et al.³ obtain that the levels of Cer(d18:1/16:0), Cer(d18:1/24:0), Cer(d18:1/16:0)/ Cer(d18:1/24:0), Cer(d18:1/20:0)/ Cer(d18:1/24:0), and Cer(d18:1/24:1)/ Cer(d18:1/24:0) were higher in patients that suffer from ACS than in patients that suffer from CAD. They also determinate that the levels of Cer(d18:1/16:0) was more associated with MACE and death.

Anroedh, et al.¹⁸ determinate that the concentration of Cer(d18:1/16:0), and Cer(d18:1/24:1) were higher in patients that suffer from MACE. Cer(d18:1/16:0), Cer(d18:1/20:0), Cer(d18:1/24:1), Cer(d18:1/16:0)/ Cer(d18:1/24:0), Cer(d18:1/20:0)/ Cer(d18:1/24:0), and Cer(d18:1/24:1)/ Cer(d18:1/24:0), were also associated to death and nonfatal ACS.

In the study done by Meikle, et al.²⁸, the levels of Cer(d18:1/18:0) appear to be higher in patients that suffer from ACS when compared with patients that suffer from CAD.

At last, Chatterjee, et al.³³ obtained that Cer(d18:1/16:0), Cer(d18:1/18:0), Cer(d18:1/24:0), and Cer(d18:1/24:1) were significantly high in patients that suffer from CAD comparatively with healthy individuals. These lipid species were also associated with cardiovascular mortality.

3.4.3.2. Sphingomyelins

Like ceramides, different sphingomyelins were detected in some studies, as can be observed in Appendix 6. The SM species detected were: SM(d42:2), SM(d34:1), SM(d42:1), SM(d36:2), SM(d18:0/18:0), SM(d18:1/16:0), SM(d18:1/22:0), SM(d18:1/23:0), SM(d18:2/24:1).

Rached, et al.²⁶, determined that the levels of SM(d42:2), and SM(d34:1) were higher in patients that suffer from STEMI when compared with healthy individuals.

SM(d18:0/18:0), according to Poss, et al.²⁹, has a higher concentration in patients that suffer from CAD than in healthy individuals.

In the study conducted by Sutter, et al.³¹, the levels of SM(d42:1) presented to be lower in patients that suffer from ACS than in healthy individuals and CAD patients.

Djekic, et al. ⁵, obtained that SM(d18:1/16:0), SM(d18:1/22:0), and SM(d18:1/23:0), had a lower concentration in patients that had severe coronary calcification than in individuals that had no coronary calcification.

Djekic, et al. ⁶, determinate that SM(d34:1), were lower in patients that suffer from severe coronary calcification than in patients that do not suffer from coronary calcification. When compared patients that suffer from severe with patients that suffer from mild coronary calcification, the levels of SM(d36:2), and SM(d18:2/24:1) were lower in patients with severe coronary calcification.

3.4.3.3. Phosphatidylcholines

Specific phosphatidylcholines (PC), just like Cer and SM, were detected in different studies selected for this review.

In the study performed by Rached, et al. ²⁶, the specific PC detected were: PC(34:2), PC(36:2), and PC(36:4). Those PC detected, appear to have a small difference between both groups of individuals analyzed, where the patients that suffer from STEMI had lower levels of those lipid species.

Hancock-Cerutti, et al. ³⁰, detected that PC(34:2), PC(36:2), and PC(36:3) were lower in patients that suffer from CAD, while PC(36:4), and PC(38:4) tended to be higher in that patients.

The study conducted by Sutter, et al. ³¹, obtained that PC(33:3), PC(34:3), and PC(35:2) were significantly lower in patients that suffer from CAD when compared with healthy individuals. PC(33:3), PC(35:2), and PC(35:3), and PC(35:5) were lower in patients that suffer from ACS. They also determinate that the levels of PC(38:4) were significantly higher in patients that suffer from CAD when compared with healthy individuals. Finally, PC(34:2) was shown to have a strong relationship with ACS and CAD patients, who had lower levels of these specific lipid than the healthy group.

After the analysis conducted by Paapstel, et al. ¹⁶, were obtained that the levels of PC(28:1), PC(30:0), and PC(34:2) had lower levels in patients that suffer from PAD when compared with healthy individuals. PC(32:2) presented to be lower in patients that suffer from CAD than in the healthy control group.

Vorkas, et al. ⁷, detected that the levels of PC(16:0/20:4) were higher in patients that suffer from severe coronary, while the levels of PC(18:2/18:0), and PC(18:2/18:2) were lower in those patients than in patients that had no coronary calcification. In patients that suffer from mild coronary calcification, they presented the same pattern of PC levels.

Djekic, et al. ⁵, determinate that the levels of PC(16:0/24:0) were higher in patients that suffer from severe coronary calcification when compared with patients that had no calcification. They also determinate that the levels of PC(18:2/18:0) and PC(18:2/18:2) were lower in patients that had severe calcification than in patients that had none.

Finally, in the study performed by Djekic, et al. ⁶, the levels of PC(16:0/20:4) were higher in patients that suffer from severe coronary calcification, when compared with patients that suffer from mild and non-coronary calcification. PC(18:2/18:2), and PC(36:3) were lower in patients that suffer from severe calcification when compare with those who do not, and with those who suffer from mild calcification. When compared patients that suffer from mild calcification with individuals that had no coronary calcification, the levels of PC(30:0), PC(32:1), PC(33:0), PC(34:3), PC(35:1), and PC(38:4) were higher in the group of patients that suffer from mild coronary calcification.

All the results mentioned can be observed in Appendix 7.

4. Discussion

The field of lipidomics has gained interest over the years due to the possibility of analyzed lipids could work as biomarkers for different diseases. Different lipids that may be used as biomarkers for cardiovascular diseases like CAD including, SAP, and ACS, have been identified in different studies using mass spectrometry techniques. Cer, SM, and PC were the most detected lipids group. Different specific lipid species that belong to these groups, were also detected in abnormal levels in the disease group of patients when they were compared with the levels of the same molecules in healthy individuals.

The majority of the selected publications used HPLC and UHPLC methods, which are promising approaches for diagnosing and validating numerous CVD diseases. HPLC and similar techniques separate different molecules in a solution based on their furthest affinity for the material in the column or the mobile phase, which causes the molecules to travel at different velocities, resulting in different retention times (time that the solute stays in the column) and separate them^{41,45}. Separation by HPLC and UHPLC is influenced by a variety of mobile phase variables such as polarity, flow rate, pH, composition, and sample matrix characteristics, the type and nature of the stationary phase, and some environmental variables such as detector type and defined settings^{41,45}. HPLC is extremely fast and efficient when compared to other chromatographic techniques, and it provides accurate results^{41,45}. HPLC is also consistent, and it can produce high-resolution results in 10 to 30 minutes^{41,45}. Despite the numerous advantages of HPLC and similar techniques, their expense, and the requirement for vast amounts of costly chemical molecules are the main disadvantages⁴⁶. Roche's Cobas c311 is an example of a device used in industrial laboratories for professional medical diagnosis. Ion-selective electrode detection of sodium, potassium, and chloride in human bodily fluids is achievable with the Cobas c311 analyzer⁴⁷. When compared to HPLC and other similar techniques, Cobas c311 fails to detect lipid species like Cer, SM, and PC⁴⁷. As a result, methods like HPLC and UHPLC are useful in analyzing blood, plasma, and serum in order to diagnose various pathologies, including CVD.

Triglycerides were measured at the beginning of the majority of experiments, just like LDL-C and HDL-C, to provide information about the participants' health status. The recommended TG levels are not to exceed 3 mmol/L (300 mg/dL) for a person to be healthy⁴⁸. The levels of TG did not exceed the recommended levels, according to the results present on Appendix 4. The articles belonging to the Group 4 do not measure TG in the beginning of the

experiments but measure their levels by using UPLC in Vorkas, et al. ⁷, and UHPLC in Djekic, et al. ⁶ and Djekic, et al. ⁵ techniques. The specific TG detected by Vorkas, et al. ⁷, were TG(16:0/18:1/22:5), TG(18:1/18:1/20:4), and TG(16:0/18:1/18:1), and Djekic, et al. ⁶ detected TG(49:2), TG(51:1), TG(54:5), TG(56:8), and TG(60:5). CAC is defined by the development of calcium in one or more layers of the coronary wall ⁴⁹. It is also a measure of calcification of atherosclerotic lesions in the vascular intima, which is linked to inflammatory mediators and high lipid contents, and a measure of calcification in the vascular media, which is associated with increased arterial stiffness, advanced age, diabetes, and chronic kidney disease ^{6,49}. This pathology is considered to be a marker for intermediate-risk subclinical CAD and has been demonstrated to reduce the likelihood of CAD manifestations, even in patients with low coronary calcium scores ^{6,7}. The calcium score is used to determine CAC, with patients with NC receiving a score of zero, patients with MC receiving a score between one and 250, and patients with SC receiving a score more than 250 ^{5,7}. Even though these triglycerides have been detected in patients with severe and mild coronary calcification, it is unclear if TG levels are simply a marker of proatherogenic lipoprotein dynamics and structure or are causally linked to lipoprotein preservation in the vessel, plaque stabilization, and thrombogenicity ⁵⁰.

Sphingolipids are a sort of lipid that can act as intracellular messengers, assisting in cell wall integrity, cell growth, and differentiation, and signal transduction ⁴. Cer and SM are protein kinase regulators, and ceramides or their derivatives are implicated in cell division, differentiation, migration, and apoptosis control ^{4,11}. The relationship between sphingolipids and lipoproteins is a significant risk factor for CAD, with elevated Cer and SM being linked to a higher risk of cardiovascular disease, and with the development of atherosclerosis ^{3,4}.

Cer are one of the most frequently observed lipid subclasses in patients that participated in the selected studies. These types of lipids have the potential to act as signaling molecules, regulating cell responses and functions such as cell division, proliferation, apoptosis, and gene expression ¹⁸. Being composed of sphingosine and an amide-linked fatty acid, Cer belongs to the waxy molecule family ^{3,18}. Cer is primarily produced by the sphingomyelinase (SMase) pathway, which releases Cer by breaking down SM in the cell membrane ^{3,18}. Different cardiovascular risk factors, such as homocysteine ¹⁸, which induces lipid peroxidation, free radical development, inflammation, and endothelial dysfunction ⁵¹ and oxidized-LDL ¹⁸ which is a major component of atherosclerotic lesions, may stimulate these pathways. Inflammatory cytokines can also stimulate the SMase pathway, resulting in an increase in Cer production due to an increase in reactive oxygen species (ROS), such as H₂O₂, superoxide, and hydroxyl radicals. Despite being a minor component of circulating lipoproteins, Cer levels can increase

once the lipoproteins are held in atherosclerotic lesions, where Cer are found predominantly in aggregated lipoproteins, most particularly LDL^{52,53}. The development of arterial extracellular sphingomyelinase, which binds SM on the surface of lipoproteins and generates phosphorylcholine and Cer, is likely to be the cause of the elevated Cer levels⁵³. Cer aggregation in lipoprotein particles makes them unstable, causing conformational changes in apoB-100 and stimulating the formation of very large LDL aggregates⁵³. Due to the features explained about the metabolism of Cer, the levels of these types of lipids are normally high in patients who suffer from the cardiovascular diseases studied in the different articles selected for this review. Apart from Rached, et al.²⁶ (present in Table 6), all the articles analyzed found that the levels of Cer were higher in the patients' populations, which is to be expected given the features of the diseases that the patients suffer from. Both Rached, et al.²⁶ and Chatterjee, et al.³³ compared patients that suffer from STEMI with healthy patients. Contrary to the expectations, in the study conducted by Rached, et al.²⁶, the levels of Cer were lower in patients that suffer from STEMI. These low levels of Cer, according to the researchers, are caused by inflammatory proteins including Serum Amyloid A (SAA), complement C3 and C9, and apolipoprotein (apo) C-III causing physicochemical changes in the STEMI HDL-C.

LacCer (present in Table 8) is an example of a sphingolipid that accumulates in atherosclerotic plaques and affects the atherogenic process^{3,18}. This form of lipid inhibits macrophage apolipoprotein E formation, causing cholesterol to build up in macrophage foam cells¹⁸. LacCer also increases the activity of nicotinamide adenine dinucleotide phosphate oxidase, which produces superoxide radicals, that also activates p44MAPK, where it increases nuclear transcription factor expression and stimulates smooth muscle cell proliferation, leading to atherosclerosis¹⁸. In brief, as LacCer levels increase, atherosclerotic plaque increases with them, so these links between LacCer and CVDs like ACS and CAD are to be predicted^{3,18}.

Another type of sphingolipids most detected in the mentioned studies was SM. These lipids were found in 10 of the 19 articles, making them one of the most found species. SM is one of the main components of HDL particles, influencing their metabolic activity^{4,30,33}. The presence of SM on HDL can impact HDL's capacity to link with cholesterol, as well as reduce HDL fluidity^{4,30}. This reduction in fluidity has been linked to an increase in the cholesterol efflux ability of reconstituted HDL containing unsaturated fatty acids⁴. Furthermore, HDL SM content inhibits plasma enzymes involved in HDL metabolism, such as phospholipid transfer protein or lecithin-cholesterol acyltransferase (LCAT), affecting reverse cholesterol transport^{4,33}. SM has also been shown to influence LDL functions in tissue, with the combination of LDL and SM affecting macrophage aggregation and accumulation⁴. This occurs as a result of

SMase's activity in coronary plaques, which converts SM to Cer, which aids in LDL aggregation and absorption ⁴. Patients with the studied pathologies were expected to have greater SM levels due to the metabolism described, which appears to be the case in almost all studies that identified these lipid species, except patients in Group 4. The SM levels in patients with CAC were found to be lower than in those who did not have coronary calcification. These findings might be explained by the Cer pathway described previously, which also occurs in CAC and could be associated with cell apoptosis as a possible mechanism for ectopic calcification in later and more severe forms of the illness ⁷. The results obtained by Sutter, et al. ³¹ regarding SM levels do not correspond with those reported by other research that identified these lipid species. This might be explained by the fact that serum and growth factors were added to the participants' plasma to cause cell death in order to assess HDL's anti-apoptotic activity, which was measured through endothelial cell apoptosis.

Glycerophospholipids were another prominent lipid class reported across several studies. Phospholipids can affect signal transduction in two ways: by interacting with specific proteins and by changing the lipid acyl chain composition, which controls downstream signaling ¹⁴.

PC was the most common subclass of glycerophospholipids identified. The glycerol backbone of a PC molecule can be linked to a variety of fatty acid combinations of various lengths and saturation, although the 16-, 18-, and 20-carbon fatty acids are the most frequent ¹⁶. PC are important components of cell membranes, as well as cell metabolism and signaling ⁶. They can carry fatty acids, providing crucial information about disease manifestation's dysregulated cellular systems ⁷. CAD and CAC have been linked to dysregulation of PC levels as well as increased activity and mass of PLA₂, an enzyme that breaks down PC into fatty acid and a lysophosphatidic acid ^{6,7}. Since PC and PI, particularly those with 18-carbon chains, are converted into arachidonic acid during the fatty acid elongation and desaturation process, they are detected in lower levels in individuals with CVD ⁷. Glycerophospholipid components such as fatty acids, phosphate, glycerol, and choline are stored and transported by PC and lysoPC ¹⁶. Decreased levels of choline, a key nutrient for humans, might result from lower amounts of these lipids ¹⁶. Meikle, et al. ²⁸ conducted research that compared different disease groups, discovering that individuals suffering from CAD had higher levels of PC. These results might be explained by alipoprotein A-I (apoA-I) deficiency in ACS patients ³⁰. The dominant lipoprotein in HDL particles is ApoA-I ^{28,54}, and overexpression of this alipoprotein can result in a decrease in arterial wall fat formation ⁵⁴. As a result, those with lower levels of these lipoproteins have a higher fatty content in their artery walls, which can contribute to CVD, such as ACS ⁵⁴. Paapstel, et al. ¹⁶, Rached, et al. ²⁶, and Hancock-Cerutti, et al. ³⁰ found lower PC

levels in the illness group than in the healthy group, as predicted. PC species with short to mild chain length and a moderate degree of unsaturation, which means two or three double bonds, tended to be lower in the disease group, whereas PC species with longer chain length and higher degrees of unsaturation, which means more than four double bonds, tended to increase in this group, according to Hancock-Cerutti, et al. ³⁰. The results obtained regarding PC levels by Sutter, et al. ³¹, showed agreement with the three articles mentioned previously. The results obtained in Chatterjee, et al. ³³ concerning PC levels do not match with the results of the previously stated articles. The oxidative stress caused by lipid peroxidation (LPO) products may trigger the enzymatic products of arachidonic acid and linoleic acid, resulting in these outcomes ³³. LPO can oxidize phospholipids in the context of different CVD and originate a variety of oxidation products that can affect the levels of PC ³³. The results of Lu, et al. ⁹ study on PC levels could be explained by the researchers' method of incubating blood platelets in vitro with fluorochrome-labeled oxidized LDL-LDL in the presence or absence of recombinant CXCL12 and blocking anti-CXCR-4/-7 monoclonal antibodies in the presence or absence of recombinant CXCL12 ⁹. The levels of PC do not differ between the several phases of coronary calcification analyzed in articles where atherosclerosis was the major emphasis, such as those in Group 4. LysoPC was also detected in a few of the research examined. LysoPC is a result of PC hydrolysis, as previously stated. Besides being produced via the hydrolysis of PC, lysoPC may also be produced by the PLA2 family of enzymes ^{7,28}. The activity of these enzymes is complex, and the bioactive lipid products of various PLA2 isoforms have been linked to anti- and pro-atherosclerotic effects ²⁸. Lower lysoPC levels have been linked to sepsis, obesity, diabetes, and atherosclerosis-related vascular damage ²⁸. The lower levels of these lipids might be linked to inflammation, which could be the more probable explanation of the difference in lysoPC levels ¹⁶. Lower lysoPC levels can also occur as a result of effective blood removal into tissues, either as oxidized LDL or albumin ¹⁶. Only Paapstel, et al. ¹⁶, and Meikle, et al. ²⁸ of the articles that detected lysoPC, agreed with the preceding statement that the higher the inflammation, the lower the levels of lysoPC might be. The results of the remaining articles that identified these types of lipids were unexpected and might be explained by the same justifications given for the contradictory results for PC levels.

Aside from PC and lysoPC, other types of glycerophospholipids such as PE, lysoPE, PI, PS, lysoPS, PG, PA, and lysoPA were detected. PE is a glycerophospholipid present on the inner leaflet of cell membranes and is the second most abundant glycerophospholipid in mammalian cells ^{6,14}. Protein synthesis, oxidative phosphorylation, membrane fusion, mitochondrial stability, and autophagy, a procedure that includes lysosomes breaking down

cellular components, are all linked to these lipids ^{6,14}. Macroautophagy is the most common subtype of autophagy, in which cytoplasmic material is sequestered into a double membrane vesicle that merges with a lysosome for destruction and recycling ⁶. Autophagosomes are present in higher quantities in macrophages in atherosclerotic plaques and are believed that play a protective role ⁶. Intracellular PE deficiency has been linked with autophagy malfunction and the production of reactive oxygen species ⁶. This indicates that calcification is associated to autophagy failure and the production of reactive oxygen species ⁶. PE levels are increased in individuals with CVD, and they are generally connected to excessive visceral fat, which disrupts the tissues' normal metabolism ⁵⁵. The results obtained in the articles reviewed in this study both PE and lysoPE levels were consistent with the results predicted according to the metabolism described. Due to the fatty acid elongation and desaturation process, PC and PI, particularly those with 18-carbon chains, are detected in lower amounts in people with CVD ⁷. PS is one of the most common lipids on the inner leaflet of the plasma membrane ¹⁴. In apoptotic cells, PS can be exposed to the exterior leaflet, resulting in the loss of phospholipid symmetry ¹⁴. PS on apoptotic cells is recognized by phagocytes, which remove them via receptors like oxidized LDL, decreasing the amount of these lipids in the blood plasma of CVD patients ¹⁴. The results obtained by Lu, et al. ⁹ do not match those predicted, which might be explained by the previously described method of incubating blood platelets. PA is a potent activator of phosphatidylinositol 4-phosphate 5-kinase, which leads to an increase in phosphatidylinositol 4,5-phosphate levels and the formation of a positive feedback loop, resulting in the accumulation of large amounts of PA and lysoPA, which explains the research results of the articles that detected these lipids ¹⁴.

DG was the only glycerolipid found in addition to TG. These glycerolipids are generated by enzymatic hydrolysis of phosphatidylinositol 4,5-bisphosphate on the plasma membrane and can function as intracellular signaling lipids that drive proliferation, differentiation, migration, survival, and apoptosis ⁶. Protein kinase C is activated by DG, which prevents vascular calcification ⁶. Lower levels of DG were expected in the articles selected due to the reasons mentioned. Djekic, et al. ⁶ detected lower DG levels in patients that suffer from SC which goes according to the expected. Meikle, et al. ²⁸ observed that ACS patients had higher DG levels than CAD patients, which was unexpected given that ACS patients could have more coronary calcification than CAD patients. Chatterjee, et al. ³³ results were likewise not following what was expected. As previously stated, these outcomes might potentially be due to oxidative stress caused by LPO.

Cholesterol is regulated in a homeostatic condition by balancing the *de novo synthesis* ⁵⁶. The esterification of amphipathic cholesterol molecules to fatty acids and the tight packing of the resultant hydrophobic CEs in the core of intracellular lipid droplets or circulating molecules is how they are stored and transported ^{56,57}. CE levels are high in macrophage foam cells, which are commonly found in atherosclerotic plaque ⁵⁷. In the studies that identified CE, higher levels were found in individuals who, as a result of their pathology, have more atherosclerotic plaque, which was to be expected. Cholesteryl esters transfer protein does not differentiate between CE and oxCE, and it facilitates the exchange of oxCE between HDL and LDL at the same rate as CE ⁵⁶. In the fatty acyl chain, oxCE is a combination of reactive and/or functional oxidation moieties ⁵⁶. The presence of oxCE in high concentrations in atherosclerotic lesions may have an atherogenic effect ⁵⁶. The results obtained by Guo, et al. ²⁷ go according to the expected due to the atherosclerotic lesions present in patients that suffer from MI and CAD.

Oxylipins have the ability to regulate inflammation, vasoconstriction, cell adhesion, cell apoptosis, and blood clotting ^{32,34}. These molecules are oxidized PUFA metabolites that are processed by a variety of enzyme mechanisms ^{32,34}. Eicosanoids, for example, are the most common oxylipins derived from arachidonic acid. They can also derivate from fatty acids ²⁷ using the enzymes cyclooxygenase enzyme (COX), lipoxygenase enzyme (LOX) and cytochrome P450 ⁹. Eicosanoids produced from arachidonic acid are the most widely researched oxylipins. Cells may take them up and integrate them into phospholipids ⁵⁸. Eicosanoids are linked to the activity of phospholipase A2, which releases arachidonic acid from phospholipids' sn-2 position ⁵⁸. HETEs are formed when free arachidonic acid is oxidized at the double bonds. Oxylipins, which are derived from arachidonic acid and produced through the COX, and LOX pathways, are pro-inflammatory mediators that contribute to inflammation ⁵⁹. Different pathological and physiological processes, such as pro-inflammatory effects, are regulated by bioactive lipids like HETE. 12-HETE, for example, can enhance thrombogenesis by activating platelet NADPH oxidase, which produces superoxide, which slows ADP metabolism and scavenges nitric oxide ⁶⁰. By stimulating endothelial cells, 12-HETE can potentially induce metastasis ⁶⁰. HODE is a product of linoleic acid oxidation, and their levels rise as oxidative stress rises, like in atherosclerosis ⁶¹. Increased levels of these lipid mediators have been linked to the development of atherosclerosis and the risk of clinical events including MI, ACS, and CAD ⁶¹. Lastly, collagen-related peptide-induced platelet aggregation in HEPE and HDoHE could be an indication of atherosclerosis ²⁵. The levels of both HEPE and HDoHE are higher in patients with ACS when compared to those who have a high risk of CVD but do not have CAD, according to the articles used for this review.

Aside from the various lipid groups, particular lipid compounds from the CER, SM, and PC groups were detected. These molecules might be utilized as markers in the future to contribute to the identification of the various pathologies discussed in the articles. Appendix 4, 5, and 6 contain examples of good compounds that can be used as markers in the diagnosis of CVD. These markers can be used to confirm an inconclusive diagnosis as well as identify CVD such as CAD, ACS, and CAC.

5. Conclusions

This review aimed to determine the link between lipid markers and coronary artery disease, and acute coronary syndromes. To determine these links, different articles were selected after researching in MedLine Databases from March to June 2020. The chosen articles had to mention a lipidomic comparison between coronary pathologies and control groups. Based on the analysis of the different selected articles, the main mass spectrometry techniques used were HPL and UHPLC. Through the analysis of different blood samples of healthy individuals, and from individuals that suffer from different pathologies, such as CAD and ACS, it can be concluded that different lipid groups presented different levels between healthy individuals and patients that suffer from different pathologies. Sphingolipids and glycerophospholipids were the main lipid classes detected in abnormal levels in individuals that suffer from the previously mentioned pathologies. Due to the relationship between sphingolipids and lipoproteins being a significant risk factor for CAD, elevated levels of Cer and SM can be linked to a higher risk of cardiovascular diseases and the development of atherosclerosis. PC is one of the most prominent glycerophospholipids. CAD and CAC have been linked to dysregulation of PC levels as well as increased activity and mass of PLA₂, and the bioactive lipid products of various PLA₂ isoforms have been linked to anti- and pro-atherosclerotic effects. The use of mass spectrometry to analyze the lipidomic profile of different cardiovascular diseases is something that needs more research to be used to detect these types of pathologies without room for doubt. SM, Cer, and PC are lipids groups that can be used in future studies to detect different cardiovascular pathologies. These studies should focus on these groups of lipids, using a lipidomic target analysis. To conclude, different SM, Cer, and PC can be used to determine cardiovascular diseases, such as CAD and ACS. Lipids, like Cer, SM, and PC, can be used in the future, to differentiate and classify patients, according to their lipid levels. This information can be useful to give the patient a more personalized treatment, based on the lipid profile and the pathology that they suffer.

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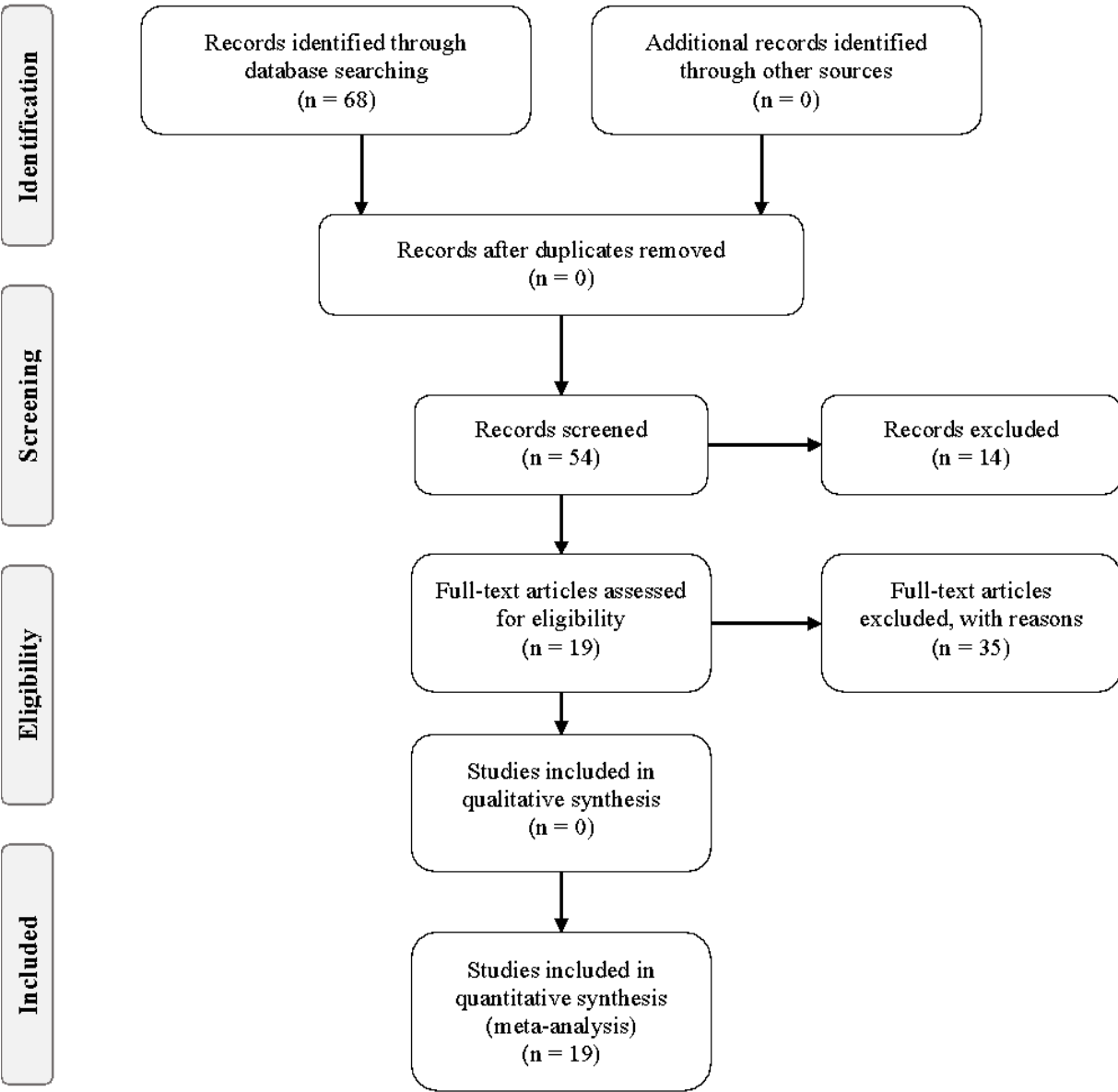
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7. Appendix

Appendix 1 - Flowchart of the research done for the development of the systemic review ⁶².



Appendix 2 - Selected articles for the systemic review and respective authors, publication date, journal, and journal evaluation.

Article	Authors	Publication date	Journal	Journal Evaluation
Acute coronary syndrome remodels the antiplatelet aggregation properties of HDL particle subclasses	C. Garcia; n. Mont ee; <i>et.al</i>	mar.2018	Journal of Thrombosis and Haemostasis	Q1
Circulating ceramides predict cardiovascular outcomes in the population-based FINRISK 2002 cohort	Aki S. Havulinna; Marko Sysi-Aho; <i>et.al</i>	nov.2016	American Heart Association, Inc.	Q1
Comprehensive metabolomics identified lipid peroxidation as a prominent feature in human plasma of patients with coronary heart diseases	Jianhong Lu, Buxing Chen, <i>et.al</i>	apr.2017	Redox Biology	Q1
Defective functionality of small, dense HDL3 subpopulations in ST segment elevation myocardial infarction: Relevance of enrichment in lysophosphatidylcholine, phosphatidic acid and serum amyloid A	Fabiana Rached; Marie Lhomme; <i>et.al</i>	may.2015	Biochimica et Biophysica Acta	Q1
Endogenous cholesterol ester hydroperoxides modulate cholesterol levels and inhibit cholesterol uptake in hepatocytes and macrophages	Guo Shuyuan; Lu Jianhong; <i>et.al</i>	nov.2018	Redox Biology	Q1
HDL phospholipids, but not cholesterol distinguish acute coronary syndrome from stable coronary artery disease	Peter J. Meikle; Melissa F. Formosa; <i>et.al</i>	jul.2019	American Heart Association, Inc.	Q1
Inverse relations of serum phosphatidylcholines and lysophosphatidylcholines with	K. Paapstel, J. Kals; <i>et.al</i>	jun.2017	Nutrition, Metabolism and	Q1

vascular damage and heart rate in patients with atherosclerosis			Cardiovascular Diseases	
Machine learning reveals serum sphingolipids as cholesterol-independent biomarkers of coronary artery disease	Annelise M. Poss; <i>et.al</i>	jan.2020	The Journal of Clinical Investigation	Q1
Paradoxical coronary artery disease in humans with hyperalphalipoproteinemia is associated with distinct differences in the HDL phosphosphingolipidome	William Hancock-Cerutti; Marie Lhomme; <i>et.al</i>	jun.2017	Journal of Clinical Lipidology	Q1
Perturbations in fatty acid metabolism and apoptosis are manifested in calcific coronary artery disease: An exploratory lipidomic study	Panagiotis A. Vorkas; Giorgis Isaac; <i>et.al</i>	jun.2015	International Journal of Cardiology	Q1
Plasmalogens of high-density lipoproteins (HDL) are associated with coronary artery disease and anti-apoptotic activity of HDL	Iryna Sutter; Srividya Velagapudi; <i>et.al</i>	jun.2015	Atherosclerosis	Q1
Plasma concentrations of molecular lipid species in relation to coronary plaque characteristics and cardiovascular outcome: Results of the ATHEROREMO-IVUS study	Jin M. Cheng;Matti Suoniemi; <i>et.al</i>	oct.2015	Atherosclerosis	Q1
Plasma concentrations of molecular lipid species predict long-term clinical outcome in coronary artery disease patients	Sharda Anroedh; Mika Hilvo; <i>et.al</i>	jun.2018	Journal of Lipid Research	Q1

Plasma fatty acids, oxylipins, and risk of myocardial infarction: the Singapore Chinese Health Study	Ye Sun; Hiromi W. L. Koh; <i>et.al</i>	may.2015	Journal of Lipid Research	Q1
Regulation of oxidized platelet lipidome: implications for coronary artery disease	Madhumita Chatterjee; Dominik Rath; <i>et.al</i>	mar.2017	European Heart Journal	Q1
Replication of LC-MS untargeted lipidomics results in patients with calcific coronary disease: an interlaboratory reproducibility study	Demir Djekic; Michael Henein; <i>et.al</i>	jul.2016	International Journal of Cardiology	Q1
Serum untargeted lipidomic profiling reveals dysfunction of phospholipid metabolism in subclinical coronary artery disease	Demir Djekic; Rui Pinto; <i>et.al</i>	jul.2019	Dove Press journal: Vascular Health and Risk Management	Q2
Specific plasma oxylipins increase the odds of cardiovascular and cerebrovascular events in patients with peripheral artery disease	Stephanie P.B. Caligiuri; Harold M. Aukema; <i>et.al</i>	jul.2017	Canadian Journal of Physiology and Pharmacology	Q2
Untargeted mass spectrometry lipidomics identifies correlation between serum sphingomyelins and plasma cholesterol	Pierre Zalloua; Hanane Kadar; <i>et.al</i>	feb.2019	Lipids in Health and Disease	Q2

Appendix 3 - Aim of each article selected for these review

Every article selected for this review had different main goals but all of them had the goal to identify lipids in different forms of coronary heart diseases.

Garcia, et al. ²⁵ had as the main goal to quantify with target lipidomic PUFAs in HDL subfractions, from patients with ACS and Non-CAD patients that work as a control group.

Havulinna, et al. ¹⁷ had the main goal to determine the ceramide behavior in a long-term study, to validate the value of ceramides at a population level.

The aim of the study done by Lu, et al. ⁹, was to identify different metabolites with a targeted lipidomic analysis, in three different groups of population (SA, MI, and healthy controls).

Rached, et al. ²⁶, had the aim of analyzing the lipidome of total HDL and major plasma HDL particles in patients that suffer from STEMI, and compare them to a control group.

The investigation conducted in Guo, et al. ²⁷, had the aim to detect and quantify major oxCE in human plasma using target lipidomic analysis and compare healthy controls, patients that suffer from CAD, patients that suffer from CAD, and Cerebrovascular Disease, and patients that suffer from MI.

Meikle, et al. ²⁸, had the aim to determine the lipid signatures that could discriminate between patients that suffer from CAD and patients that suffer from ACS, STEMI, and Non-STEMI, using a lipidomic analysis.

The aim of the study realized by Paapstel, et al. ¹⁶, was to examine serum PC and lysoPC species using a lipidomic approach, and compare patients that suffer from CAD, PAD, and healthy subjects.

The study performed by Poss, et al. ²⁹, had the aim to compare SL analyzed by a lipidomic approach, of patients that suffer from CAD with a healthy control group.

Hancock-Cerutti, et al. ³⁰, had the aim of study the alterations in HDL phosphosphingolipidome in patients that suffer from CAD and had an HDL-C level higher than 90th, patients that do not suffer from CAD and had the HDL-C lower than 90th, patients, with HDL-C between 25-75th but doesn't suffer from CAD.

Vorkas, et al. ⁷, had the aim to differentiate individuals that suffer from coronary calcification, using a lipidomic tool to identify different species that could work as diagnostic biomarkers for coronary artery calcification.

The aim of the study realized by Sutter, et al.³¹, was to identify PLs that could be identified in patients that suffer from CAD, ACS, and healthy patients, using a lipidomic approach.

Cheng, et al.³, conducted a study that had the aim to investigate molecular lipids using lipidomic tools and compare patients that suffer from ACS and patients that suffer from CAD.

The study conducted by Anroedh, et al.¹⁸, had the aim to compare patients that suffer from ACS and patients that suffer from SAP, using a lipidomic approach to identify molecular species that could be associated with those pathologies.

Sun, et al.³², had the aim to associate the plasma concentration of fatty acids and oxylipins in patients that suffer from MI and control groups, using a lipidomic approach.

Chatterjee, et al.³³ performed a study that had the aim to explore the lipid metabolism using a lipidomic tool in patients that suffer from CAD, Non-STEMI, STEMI, and health patients.

The study of Djekic, et al.⁵, had the aim of evaluating the reproducibility of lipidomic in patients that suffer from coronary artery calcification and their corresponding controls.

The aim of the study realized by Djekic, et al.⁶, had aim to identify the lipids that more likely to be involved in coronary artery calcification.

Caligiuri, et al.³⁴, performed a study that had the aim to assess the relationship between plasma fatty acids and oxylipins concentration in patients that suffer from PAD, transient ischemic attack, cerebrovascular accident, and patients that suffer from Stable Angina and CAD.

Lastly, Zalloua, et al.⁴ conducted a study that had the aim to identify metabolic features associated with CAD, using a lipidomic approach and comparing the lipidomic profile of the patients that suffer from CAD and a healthy control group.

Appendix 4 - Analyzed parameters of all the participants of the studies reviewed.

Analyzed parameters	Garcia, et al. ²⁵		Havulinna, et al. ¹⁷		Lu, et al. ⁹			Rached, et al. ²⁶		Guo, et al. ²⁷		
	ACS	Non-CAD	MACE	Asymp	HC	SA	MI	HC	STEMI	HC	CAD	MI
Age, mean	57.6	61.4	48.5	48.5	54.9	68.5	52.9	54	57	56.2	69	58.66
Gender, % of men	70	50	47	47	70	70	87.5	100	100	70	69	90
BMI, mean	-	-	-	-	26.18	24.92	23.11	24.4	26.8	25.79	24.47	26.67
% of smokers	36.7	10.3	25.7	25.7	30	30	87.5	0	0	50	37.5	70
% of hypertension	36.7	82	-	-	70	90	37.5	0	53	100	85.7	30
% of diabetes	20	24.1	5.5	5.5	30	20	12.5	0	19	0	42.9	20
% of statin treatment	33.3	37.9	7.4		-			-		80	64.2	40
Total cholesterol	197.3 mg/dL	193.0 mg/dL	5.94 mmol/L	5.48 mmol/L	3.82 mmol/L	4.06 mmol/L	4.01 mmol/L	186 mg/dL	182 mg/dL	3.45 mmol/L	3.77 mmol/L	4.65 mmol/L
LDL-C	118.9 mg/dL	114.3 mg/dL	3.7 mmol/L	3.3 mmol/L	2.23 mmol/L	2.41 mmol/L	2.35 mmol/L	117 mg/dL	115 mg/dL	1.86 mmol/L	2.19 mmol/L	3.65 mmol/L
HDL-C	52.3 mg/dL	51.2 mg/dL	1.28 mmol/L	1.46 mmol/L	1.08 mmol/L	0.99 mmol/L	0.88 mmol/L	52 mg/dL	36 mg/dL	1.03 mmol/L	0.99 mmol/L	1.03 mmol/L
TG	129.7 g/L	140.5 g/L	1.5 mmol/L	1.16 mmol/L	1.7 mg/dL	1.62 mg/dL	3.03 mg/dL	82 mg/dL	133 mg/dL	1.76 mg/dL	1.3 mg/dL	2.33 mg/dL
LDL/HDL ratio	2.27	2.23	2.89	2.26	2.06	2.43	2.67	2.25	3.19	1.81	2.21	3.54
Calcium Score	-	-	-	-	-	-	-	-	-	-	-	-

Analyzed parameters	Meikle, et al. ²⁸		Paapstel, et al. ¹⁶			Poss, et al. ²⁹		Hancock-Cerutti, et al. ³⁰		
	CAD	ACS	PAD	CAD	HC	HC	CAD	HCAD	HHDL	NHDL
Age, mean	65	61	61.7	63.2	60.3	53.5	55.6	61	63	64
Gender, % of men	72.3	87.2	-	-	-	43	77	68	84	60
BMI, mean	29	28.4	25.8	27.9	26	28.3	29.1	23.8	23	25.9
% of smokers	19.3	38.3	78	25	13	20	45	-	-	-
% of hypertension	77.1	57.5	-	-	-	26	57	72	20	30
% of diabetes	28.9	31.9	-	-	-	5	23	12	4	10
% of statin treatment	72.3	55.3	22	58	0	6	10	76	8	30
Total cholesterol	4.30 mmol/L	4.70 mmol/L	5.3 mmol/L	5.1 mmol/L	5.5 mmol/L	189.5 mg/dL	209.2 mg/dL	183 mg/dL	224 mg/dL	194 mg/dL
LDL-C	2.40 mmol/L	2.90 mmol/L	3.4 mmol/L	3.2 mmol/L	3.6 mmol/L	103.1 mg/dL	128.7 mg/dL	73 mg/dL	109 mg/dL	120 mg/dL
HDL-C	1.10 mmol/L	1.00 mmol/L	1.3 mmol/L	1.5 mmol/L	1.6 mmol/L	46.7 mg/dL	40.9 mg/dL	96 mg/dL	110 mg/dL	46 mg/dL
TG	1.30 mmol/L	1.60 mmol/L	1.6 mmol/L	1.2 mmol/L	1.0 mmol/L	178 mg/dL	202.7 mg/dL	58 mg/dL	67 mg/dL	97 mg/dL
LDL/HDL ratio	2.18	2.9	262	2.13	2.25	2.21	3.15	0.76	0.99	2.61
Calcium Score	-	-	-	-	-	-	-	-	-	-

Analyzed parameters	Vorkas, et al. ⁷			Sutter, et al. ³¹			Cheng, et al. ³	Anroedh, et al. ¹⁸		Sun, et al. ³²	
	NC	MC	SC	HC	CAD	ACS	Total patients	ACS	SAP	MI	HC
Age, mean	61	67	67	56.5	63	56.5	61.5	59.7	63.6	66.1	66
Gender, % of men	27	41	65	86	100	86	75.3	74	77	64.79	64.79
BMI, mean	26.4	26.4	27.8	25.3	28.05	26.9	-	-	-	23.2	22.9
% of smokers	4	11	12	0	13	41	28.9	37	19	31.09	22.28
% of hypertension	-	-	-	-	-	-	51.9	44	62	46.77	36.73
% of diabetes	15	11	29	-	-	-	16.9	13	22	24.76	12.1
% of statin treatment	38	78	88	0	91	41	62.2	98	90	-	-
Total cholesterol	-	-	-	5.5 mmol/L	4.35 mmol/L	4.65 mmol/L	-	-	-	-	-
LDL-C	-	-	-	3.55 mmol/L	2.3 mmol/L	2.9 mmol/L	-	3.1 mmol/L	2.37 mmol/L	3.34 mmol/L	3.19 mmol/L
HDL-C	-	-	-	1.39 mmol/L	1.32 mmol/L	1 mmol/L	-	1.05 mmol/L	1.03 mmol/L	1.29 mmol/L	1.35 mmol/L
TG	-	-	-	0.88 mmol/L	1.25 mmol/L	1.52 mmol/L	-	1.15 mmol/L	1.41 mmol/L	1.68 mmol/L	1.57 mmol/L
LDL/HDL ratio	-	-	-	2.55	1.74	2.9	-	2.95	2.30	2.59	2.36
Calcium Score	0	50	801	-	-	-	-	-	-	-	-
Analyzed parameters	Chatterjee, et al. ³³			Djekic, et al. ⁵			Djekic, et al. ⁶			Caligiuri, et al. ³⁴	Zalloua, et al. ⁴

	CAD	Non-STEMI	STEMI	NC	MC	SC	NC	MC	SC	-	HC	CAD
Age, mean	69.7	71.7	62.2	61	67	67	60.8	66.5	67.1	-	53.66	53
Gender, % of men	68.1	65.2	75	27	41	67	26.6	40.7	64.7	-	58.06	78.7
BMI, mean	28.3	27.6	32.6	-	-	-	27.2	27.2	28.2	-	27.1	28.6
% of smokers	12.8	8.7	41.7	12	7	12	57.7	55.5	70.6	-	-	-
% of hypertension	93.6	82.6	58.3	62	74	88	61.5	74.1	88.2	-	16.12	25.35
% of diabetes	38.3	37	25	-	-	-	14.8	11.1	29.4	-	16.12	19.14
% of statin treatment	63.8	60.9	0	38	78	88	39	78	88	-	-	-
Total cholesterol	167.7 mg/mL	166.7 mg/mL	153.6 mg/mL	-	-	-	-	-	-	-	185.9 mg/dL	195.1 mg/dL
LDL-C	96.6 mg/mL	94.1 mg/mL	91.4 mg/mL	-	-	-	-	-	-	-	111.9 mg/dL	122.4 mg/dL
HDL-C	47.0 mg/mL	43.6 mg/mL	40.4 mg/mL	-	-	-	-	-	-	-	41.2 mg/dL	38.3 mg/dL
TG	162.8 mg/mL	137.6 mg/mL	109.4 mg/mL	-	-	-	-	-	-	-	187.5 mg/dL	208.9 mg/dL
LDL/HDL ratio	2.05	2.16	2.26	-	-	-	-	-	-	-	2.72	3.20
Calcium Score	-	-	-	0	50	801	0	50	801	-	-	-

Appendix 5 - Specific Cer detected in different studies

Cer and Cer ratios	Articles that analyzed different specific Cer						
	Havulinna, et al. ¹⁷	Rached, et al. ²⁶	Poss, et al. ²⁹	Cheng, et al. ³	Anroedh, et al. ¹⁸	Meikle, et al. ²⁸	Chatterjee, et al. ³³
Cer(d18:1/16:0)	X		X	X	X		X
Cer(d18:1/18:0)	X		X			X	X
Cer(d18:1/20:0)					X		
Cer(d18:1/22:0)		X					
Cer(d18:1/23:0)		X					
Cer(d18:1/24:0)	X	X	X	X	X		X
Cer(d18:1/24:1)	X	X	X		X		X
Cer(d18:1/16:0)/ Cer(d18:1/24:0)	X			X	X		
Cer(d18:1/18:0)/ Cer(d18:1/24:0)	X						
Cer(d18:1/20:0)/ Cer(d18:1/24:0)				X	X		
Cer(d18:1/24:1)/ Cer(d18:1/24:0)	X			X	X		

Appendix 6 - Specific SM detected in different studies

SM	Articles that analyzed different specific SM				
	Rached, et al. ²⁶	Poss, et al. ²⁹	Sutter, et al. ³¹	Djekic, et al. ⁶	Djekic, et al. ⁵
SM(d18:0/18:0)		X			
SM(d18:1/16:0)				X	
SM(d18:1/22:0)				X	
SM(d18:1/23:0)				X	
SM(d18:2/24:1)					X
SM(d34:1)	X				X
SM(d36:2)					X
SM(d42:1)			X		
SM(d42:2)	X				

Appendix 7 - Specific phosphatidylcholine detected in different studies

PC	Articles that analyzed different specific phosphatidylcholine						
	Rached, et al. ²⁶	Hancock-Cerutti, et al. ³⁰	Sutter, et al. ³¹	Paapstel, et al. ¹⁶	Vorkas, et al. ⁷	Djekic, et al. ⁵	Djekic, et al. ⁶
PC(28:1)				X			
PC(30:0)				X			X
PC(32:1)							X
PC(32:2)				X			
PC(33:0)							X
PC(33:3)			X				
PC(34:2)	X	X	X	X			
PC(34:3)			X				X
PC(35:1)							X
PC(35:2)			X				
PC(35:3)			X				
PC(35:5)			X				
PC(36:2)	X	X					
PC(36:3)		X					X
PC(36:4)	X	X					
PC(38:4)		X	X				X
PC(16:0/20:4)					X	X	X
PC(18:0/18:0)						X	
PC(18:2/18:0)					X		
PC(18:2/18:2)					X	X	X