SUMMARY

Cardiovascular Diseases (CVDs) are a group of disorders of the heart and blood vessels including both diseases of the blood vessels supplying the heart muscle (coronary heart disease) and damages of heart muscle and valves due to different factors, such as streptococcal bacteria which cause rheumatic heart disease. Build-up of fat deposits on the inner walls of the blood vessels (atherosclerotic plaques) that supply the heart is one of the main factor of coronary heart disease. Atherosclerosis is a well known inflammatory condition in which the artery wall thickens due to the accumulation of fatty materials, mainly cholesterol, caused by the accumulation of macrophage white blood cells and promoted by low-density lipoproteins. Various anatomic, physiological and behavioral risk factors for atherosclerosis, such as obesity, are known. Body fat is stored in various depots; fat stored subcutaneously reach around 85% of total and the remaining 10% is stored in the viscera area. Fat is also localized in other different depots and is known as pericardial, epicardial, intracellular, buccal and ectopic fat. It has been recognized that the correlation between CVDs and increased body weight/obesity is more linked to body fat distribution rather than to the total amount of body fat. Epicardial adipose tissue (EAT) might function as a lipid-storing depot, as an endocrine organ secreting hormones and as an inflammatory tissue secreting cytokines and chemokines. Due to its proximity to the adventitia of the coronary arteries and myocardium it is possible that it could play a role in the pathogenesis of coronary atherosclerosis (CAD). During era of genomic, trascriptomics, metabolomics, lipidomics, lipids composition is highly studied to better understand the lipid molecular profile in different diseases. Goal of our study is monitoring lipid alteration in biological samples obtained from CAD patients to better understand lipid involvement in cardiovascular disease and found potential biomarkers involved in this pathology. Patients who underwent coronary artery bypass grafting (CABG) showed a decrease concentration in polyunsaturated fatty acids (PUFA) compared to patients undergoing valvular replacement and utilized as negative-CAD controls. PUFA/saturated fatty acid (SFA) ratio was statistically lower in CABG patient compared to valvular patients. Percentage of oleic acid (18:1) was higher in

CABG compared to valvular replacement patients while percentage of linoleic (18:2) was lower. No significant differences have been observed relative to other lipids between CABG and valvular patients. Lipid analysis was quantified by electrospray ionization tandem mass spectrometry (ESI-MS/MS) in positive ion mod. Glycerophospholipid analysis revealed lower levels of phosphatidylethanolamine (PE), phosphatidylcholine (PC) and phosphatidylserine (PS) in CABG patients compared to valvular patients. Lower statistically significant lipid concentration was visible in CABG compared to valvular for PC species, such as PC 36:2, 34:3, 36:3, 36:4, 38:4, 40:4, 36:5, 38:5, 38:6 and 40:6. Similarly lipid composition for PE species was for PE 34:1, 36:1, 36:2, 36:3, 36:4, 38:4, 40:4, 38:5, 40:5 and 40:6 statistically lower in CABG compared to valvular. While for PS class only one lipid specie, PS 36:1 was statistically lower in CABG compared to valvular. Epicardial tissue lipid species analysis, also after subdivision for BMI, waist, waist-to-hip ratio (WHR), showed a decrease of lipid classes of PC, PE and PS in CABG patients compared to valvular patient. Obesity is a pre-disease condition that induces pathological angiogenesis and impaired vascular functions. These changes lead to the outset, development and progression of many diseases such as, cancer, CVD, diabetic complication and chronic inflammation. Transcriptomic data from epicardial tissue, showed an up- or down-regulation of genes involved in angiogenesis, both pro- and anti-genetic, such as matrix metalloproteinase 2 and 9 (MMP-2 and MMP-9), vascular endothelial growth factor (VEGF), fibroblast growth factors (FGF), angiogenin (ANG) and tissue inhibitor of metalloproteinases (TIMP). MMP-2 and MMP-9 were highly expressed in CABG compared to valvular (129 and 73 folds respectively). The same was observed for hepatocyte growth factor (HGF), VEGFA, FGF-2, hypoxia-inducible factors-1 (HIF-1) and TIMP-1. For these genes fold increase values were 170.55, 34.25, 28, 46.3, and 138.74 respectively. Otherwise, ANG and endostatin (COL18A-1) genes had lower expression in CABG compared to valvular (-13.41 and -5.2 folds respectively). Trascriptomic data showed, for gene involved in adipocyte differentiation, that peroxisome proliferator-activated receptor gamma (PPAR gamma; -5.8 folds), delta-like 1

homolog (DLK; -36.08 folds), adiponectin (adipoQ; -19.19 folds), activin A receptor, type I (ACVR1; -45.37 folds), fatty acid binding protein 4 (FABP4; -33.58 folds), cytoplasmic polyadenylation element binding protein 1 (CEBP; -25.20 folds), lipin 1 (LPIN1; -42,94 folds), lipoprotein lipase (LPL; -18.86 folds) and phosphoenolpyruvate carboxykinase 1 (PCK1; -44.95) were down regulate in CABG compared to valvular. While leptin (LEP; 27 folds), GATA binding protein 2 (7.7 folds), GATA binding protein 3 (75 folds) uncoupling protein (UCP-1; 112.05, UCP-2; 246.35 and UCP-3; 43.29 folds), complement factor D preproprotein (CFD; 47.88 folds), proteoglycan 4 (PRG4; 142.26 folds), solute carrier family 2 (facilitated glucose transporter), member 4 (SLC2a4; -22.24 folds), Glyceraldehyde 3 phosphate dehydrogenase 1 (GAPDH; 46.44 folds), resistin (RETN; 152.36 folds) and mesoderm specific transcript homolog (MEST; 71.77 folds) were highly expressed in CABG compared to valvular. A great increase in the expression of all of the genes evaluated was observed in CABG compared to valvular patients. Decrease in PC species and reductions in percentage of PUFA are associated whit CVDs, a down or up expression of studied genes also are connected with heart disease. Our data therefore emphasize a possible greater involvement of lipids in patients with CABG compared to valvular patients

INTRODUCTION

ADIPOSE TISSUE

Adipose tissue is a highly specialized organ able to regulate process of storage and energy release trough a complex network to meet the energy requirements of the body. In times of a positive energy balance, excess energy is stored as fat while in times of a negative energy balance, fat resources are mobilized (Fruhbeck, Gomez-Ambrosi et al. 2001). These processes are regulated and work in a highly efficient to prevent wasting of energy and to ensure survival of the organism (Loucks 2004). Adipose tissue increasing fat and number cells store a large amount of fat. There are two different forms of adipose tissue: white adipose tissue (WAT), the most part in the body and brown adipose tissue (BAT).

White Adipose Tissue

The white adipose tissue (WAT) is composed of spherical cells, called adipocytes, with a diameter ranging from 15 to 150 µm. This enormous variability in size is due to the ability of the cell to accumulate different amounts of lipids such as triglycerides that form a single vacuole in the jaloplasm. Their surface in contact with the jaloplasm is devoid of any unitary membrane. Mitochondria are elongated and show short and randomly oriented cristae. Rough endoplasmic reticulum is organized in short cisternae, but sometimes it forms stacks of variable size. Smooth endoplasmic reticulum is always well visible. Pinocytotic vesicles are present at the level of the plasma membrane. On the external side of the plasma membrane a distinct external lamina is always visible. The amount of the organelles as well as their size and extension are variable in relationship to the cell's functional and developmental stages. WAT's functions are important for body survival and can be summarized in: heat insulation, mechanical cushion and as source of energy. Approximately 60 to 85% of the weight of white adipose tissue is lipid, with 90-99% being triglyceride, are also present, in small amounts free fatty acids, diglyceride, cholesterol, phospholipids, cholesterol ester and monoglyceride. Approximately 90% of the total this lipid is

composed of six fatty acids: myristic, plamitic, palmitoleic, stearic, oleic, and linoleic. Varying the composition of your diet can vary the fatty acid profile in adipose tissue. The remaining weight of white adipose tissue is composed of water (5 to 30%) and protein (2 to 3%). WAT is not an organ richly vascularised but each adipocyte is in contact whit act least one capillary (Cinti and Vettor, 2010).

Brown Adipose Tissue

Brown adipose tissue (BAT), which it is colour derives from rich vascularisation and densely backed mitochondria, is found in various locations, depending upon the species and/or age of the animal. The mitochondria of this tissue has vary size and may be round, oval, or filamentous in shape, particularity of this mitochondria is a specific carrier called uncoupling protein, UCP-1 able to transfers protons from outside to inside without subsequent production of ATP. BAT is important for regulation body temperature via non-shivering thermogenesis. The UCP-1 to brown adipocytes is central to uncoupling of brown fat mitochondrial respiratory chain, the mechanism of heat production in this tissue. Cell in BAT has homogeneous diameter (60 microns) than WAT and the lipid droplet within the cell may reach 25 microns in diameter (Sinard, 2005) (Figure 1).

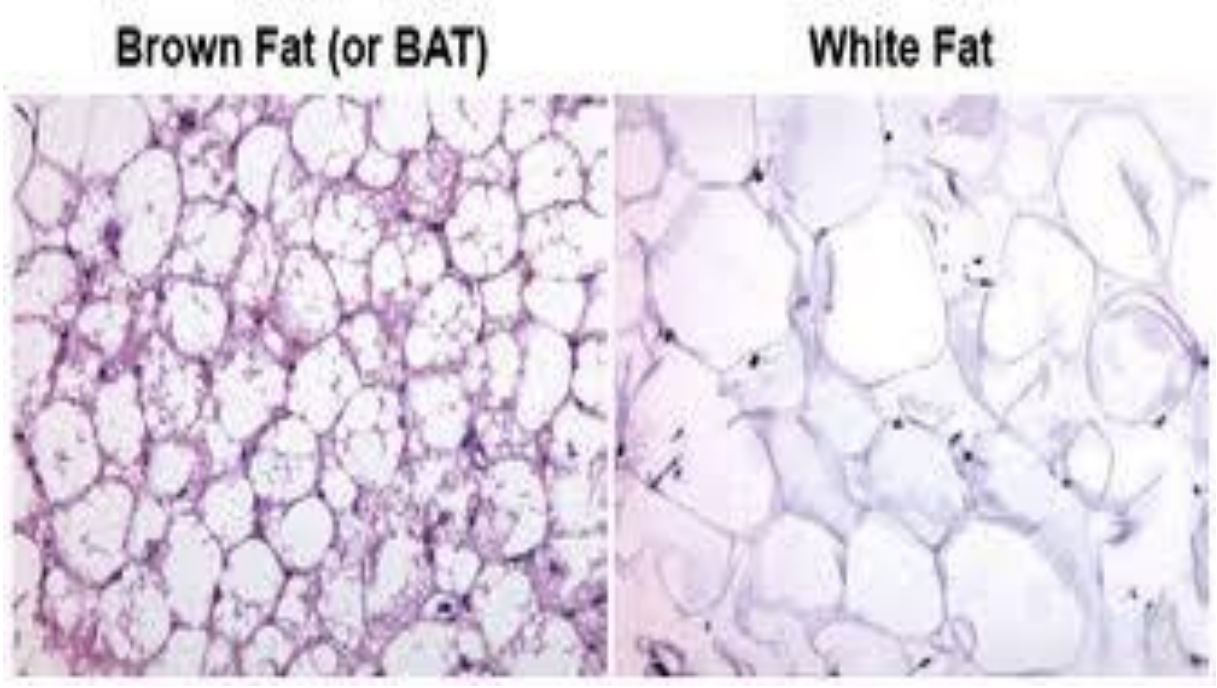

FIGURE 1: White fat cell and brown fat cell. Note the single large lipid vacuole in the white fat cell and the numerous smaller lipid vacuoles in the brown fat cell.

Metabolism of adipose tissue

Lipogenesis is the deposition of fat (Figure 2). This process occurs in adipose tissue and in the liver at cytoplasmic and mitochondrial sites. Energy ingested as fat beyond that needed for current energy demands is stored in adipose tissue (Albright and Stern, 1998). In addition, carbohydrate and protein consumed in the diet can be converted to fat. Energy ingested as carbohydrate can be stored as glycogen in the liver and muscle. Carbohydrate can also be converted to triglycerides primarily in the liver and transferred to adipose tissue for storage. Amino acids from ingested proteins are used for new protein synthesis or they can be converted to carbohydrate and fat. Fatty acids, in the form of triglycerides or free fatty acids bound to albumin, are ingested in the diet or synthesized by the liver. Very little synthesis of free fatty acids occurs in the adipocytes. Triglycerides are the most significant source of fatty acids, because this is the form in which dietary lipids are assembled by the gut and liver. Triglycerides made up of long chain fatty acids, in the form of chylomicrons (from intestinal absorption) or

lipoproteins (from hepatic synthesis), are hydrolyzed to glycerol and free fatty acids by an enzyme called lipoprotein lipase (LPL). Lipoprotein lipase is synthesized in adipocytes and secreted into adjacent endothelial cells. Chylomicrons and lipoproteins (very low density lipoproteins) contain C-ll apoprotein, which activates LPL. Free fatty acids are taken up by adipocytes in a concentration-dependent manner by a transmembrane transport protein. Once inside the adipocyte, fatty acids enter a common pool made up of both incoming and outgoing fatty acids. Fatty acids that are stored in the adipose tissue must first combine with coenzyme A to form a thioester and then they are re-esterified in a stepwise manner to triglycerides. Glucose is the primary source of glycerol for this re-esterification process. Only a small amount of glycerol released, when triglycerides are hydrolyzed by LPL, can be reused by adipocytes to form alpha glycerol phosphate to be used for triglyceride assembly. Most glycerol is returned to the circulation. Insulin, a hormone secreted by the beta cells of the pancreas, plays a predominant role in the lipogenic process. The net effect of insulin is to enhance storage and block mobilization and oxidation of fatty acids. Insulin exerts its effect by stimulating LPL formation, so that circulating triglycerides are hydrolyzed and free fatty acids can enter the adipocyte. Insulin is also required for the transport of glucose, which is needed for re-esterification of the triglycerides once inside the adipocyte. Finally, the conversion of glucose to fatty acids is accomplished by insulin's activation of several enzymes (Chong, Hodson et al. 2008). Lipolysis is the chemical decomposition and release of fat from adipose tissue. This process predominates over lipogenesis when additional energy is required (Figure 2). The triglycerides within the adipocyte are acted upon by a multi-enzyme complex called hormone sensitive lipase (HSL), which hydrolyzes the triglyceride into free fatty acids and glycerol. These lipases act consecutively on triglycerides, diglycerides, and monoglycerides. Triglyceride lipase regulates the rate of lipolysis, because its activity is low. Once triglycerides are hydrolyzed to fatty acids and glycerol, fatty acids enter the common free fatty acid pool where they may be re-esterified, undergo beta-oxidation (metabolic degradation), or be released into the circulation as substrates

for skeletal muscle, cardiac muscle, and liver. If the fatty acids are to undergo beta-oxidation for ATP production, fatty acids move from the adipocytes into the blood and are carried to the tissues that can use them as an energy source. Long-chain fatty acids enter the cells of these tissues by passive diffusion, and their rate of uptake is proportional to their difference in concentration inside and outside of the cell. Once inside the cells, beta-oxidation begins with "activation", that is, the formation of thioesters with coenzyme A. This activation step converts the fatty acids to a form that is more amenable to the successive biochemical changes that ultimately result in ATP formation. Insulin reduces mobilization of fatty acids from adipose tissue by inhibiting triglyceride lipase. The mechanism of this inhibition may be through a decrease in cyclic AMP which in turn results in an inhibition of cyclic-AMP-dependent protein kinase. This suppression of lipolysis lowers the rate of fatty acid delivery to the liver and to peripheral tissues. The consequence of fewer fatty acids to the liver is a reduction in the formation of ketoacids (e.g., ketones). Insulin also stimulates the use of ketoacids by peripheral tissues, preventing an accumulation of these acids in the blood (Lei, Xie et al. 2004).

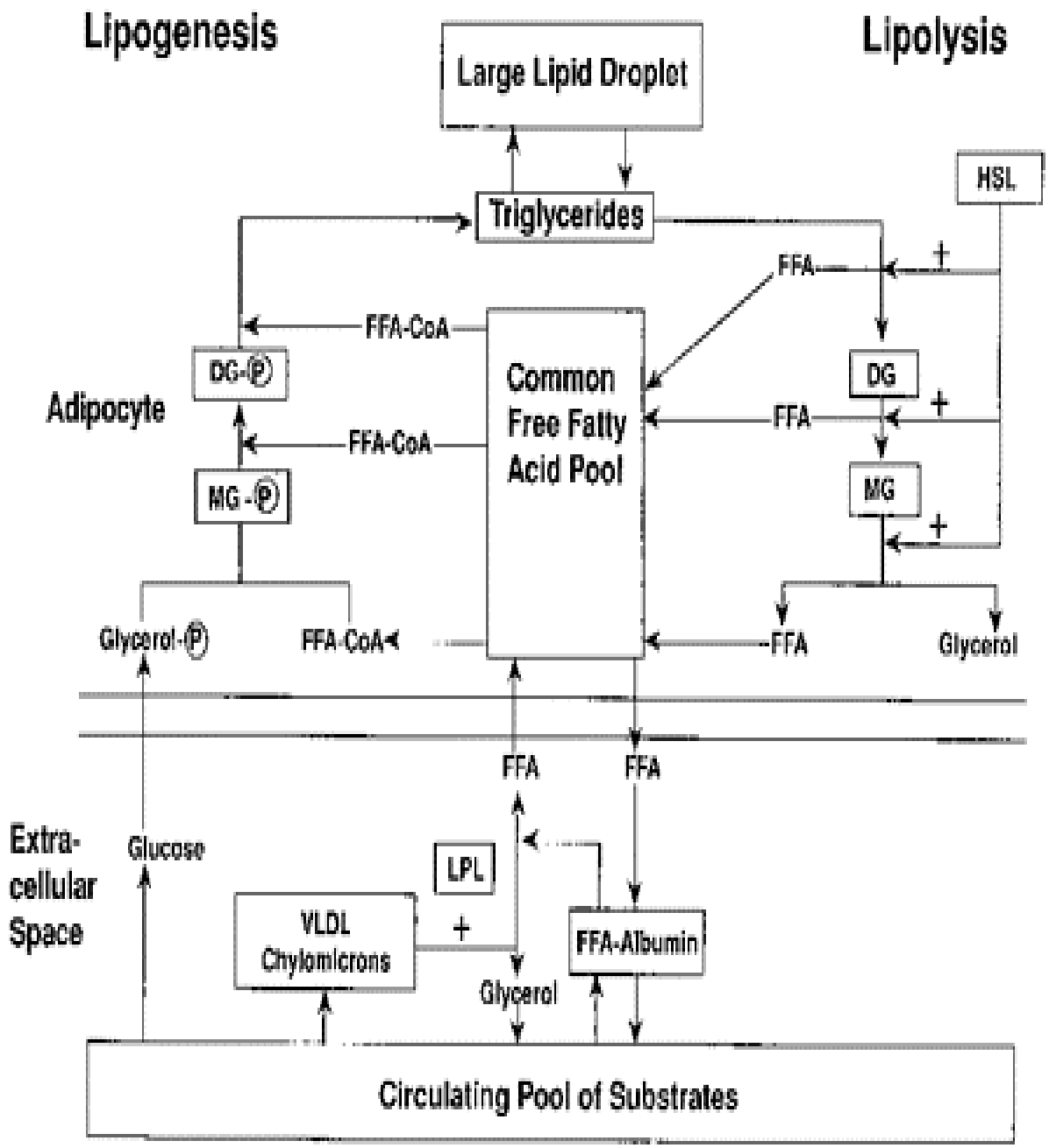

FIGURE 2: Diagrammatic representation of triglyceride storage (lipogenesis) and breakdown (lipolysis) in adipocytes. FFA: free fatty acid; FFA-CoA: thioester; HSL: hormone sensitive lipase; LPL: lipoprotein lipase; DG: diglyceride; MG: monoglyceride; DG-P: phosphatidic acid; MG-P: lysophosphatidic acid; VLDL: very low density lipoproteins. Adapted from Leibel, Berry, and Hirsch, 1983.

Fat distribution

Excess of fat stored in a body has been suggested as being responsible for myriad of metabolic consequences of obesity such as cardiovascular disease. Body fat is stored in various depots, with over 85% of fat stored subcutaneously, the subcutaneous fat (SAT) that is found beneath the epidermis and is the protective wrap over the body's surface, and around 10% stored in the viscera, the visceral fat (VAT) also known as organ fat that is located inside the peritoneal cavity (Figure 3) Peritoneal cavity is the serous membrane that invests viscera (Canoy). Other fat depot that may have relevance to atherosclerotic disease include heart resides in three distinct depots (pericardial, epicardial and intracellular fat), buccal fat and ectopic fat. It has been recognized for more than 60 years that the cardiovascular risk of obesity and increased body weight are related more to body fat distribution rather than total body fat (Ball, Wilson et al. 1993). Compared with SAT, visceral fat depot have higher lipolytic rate, are less responsive to antilipolytic effect of insulin, shows increased expression of inflammatory markers and secretion of other adipokines and exhibits an enhanced activity of intravascular coagulation factors. Inflammatory cells (macrophages) are more prevalent in VAT compared to SAT (Bruun, Helge et al. 2006).

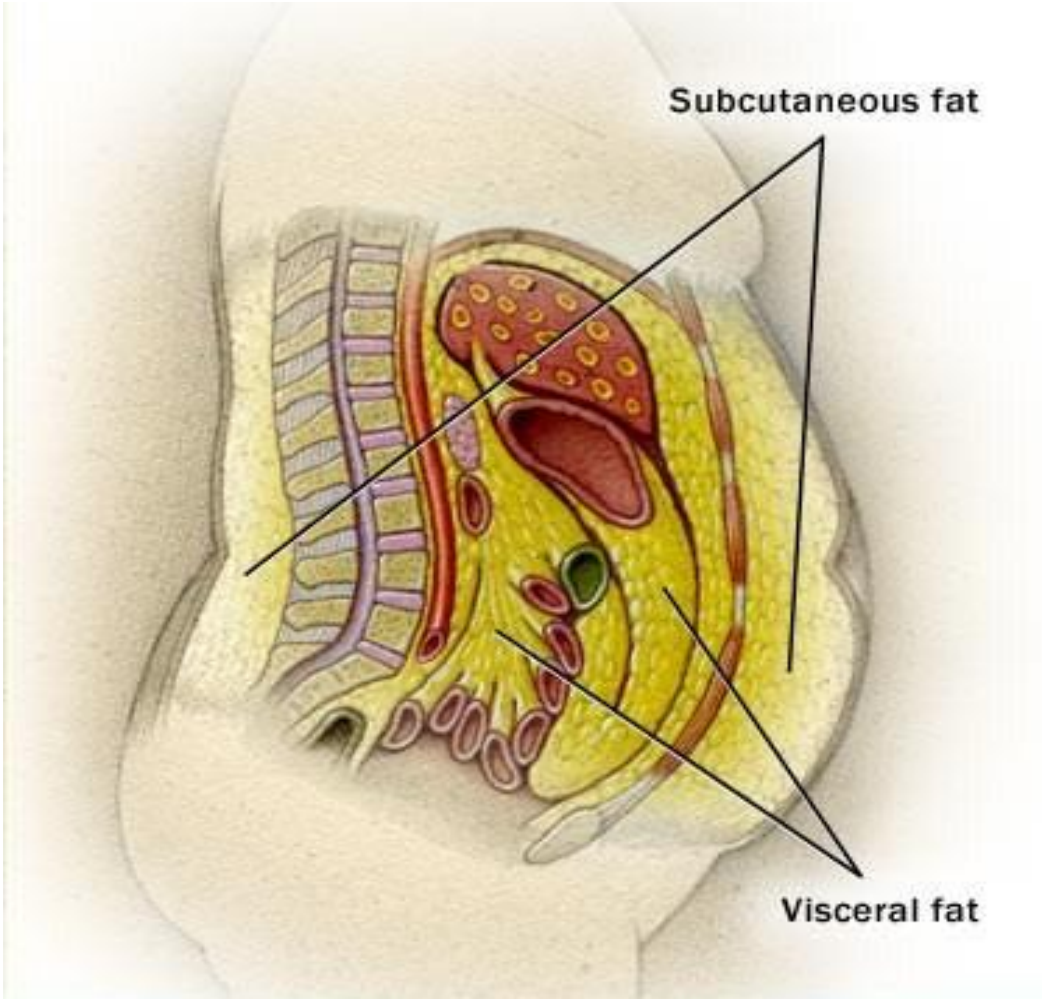

FIGURE 3 Subcutaneous and visceral adipose tissue distribution

SAT vs. VAT

The adipose tissue is made from of a large number of adipocytes, other non fat-cells, connective tissue matrix, vascular tissues, neural tissues, inflammatory cells, immune cells, preadipocytes and fibroblasts. Adipocytes, their endocrine function, lipolytic activity, response to insulin and other hormones differ between SAT and VAT (Ibrahim). Adipocytes constitute the main cellular component of adipose tissue and are the chief storage depots of the energy in form of triglycerides (TG) droplets. New smaller adipocytes act a cleavage or powerful buffers, which avidly absorb free fatty acids (FFAs) and TGs in the postprandial period (Prattes, Horl et al.

2000). Large adipocytes are insulin-resistant, hyperlipolytic and resistant to anti-lipolytic effect of insulin, while, small adipocytes are more insulin-sensitive and have high avidity for FFAs and TGs uptake preventing their deposition in non-adipose tissue (Marin, Andersson et al. 1992). When storage capacity of SAT is exceed or its ability to generate new adipocytes is impaired fat begin to accumulate in areas outside the SAT (Misra and Vikram 2003). Chronic stress leads to elevated cortesol levels that may leads to accumulation of VAT. VAT contains Greater number of large adipocytes is contained in VAT in contrast with SAT. Mature adipocytes act as an active endocrine and paracrine organ and through a communication network with other tissue, sympathetic nervous system and brain can influence appetite, energy balance, immunity, insulin sensitivity, angiogenesis, blood pressure, lipid metabolism and homeostasis (Trayhurn and Wood 2004),(Matsuzawa 2006), (Tritos and Mantzoros 1997) . Adipocytes contribute to the raised proinflammatory state in obesity and diabetes. They are capable of synthesizing pro-inflammatory and anti-inflammatory proteins. They secrete monocyte chemoattract protein-1 that can induce macrophages infiltration and activation in adipose tissue. Macrophages are important source of inflammatory cytokines such as tumour necrosis factor α (TNF- α) and IL-6. There are differences between VAT and SCAT regarding the capacity to synthesize and release adipokines. VAT is more infiltrated with inflammatory cells and is more capable of generating those proteins than SCAT (Weisberg, McCann et al. 2003), (Lemieux, Pascot et al. 2001), (Hirschfield and Pepys 2003). Abdominal obesity increases levels of inflammatory markers. Also at vascular and nerve level exists differences between VAT and SAT showing a SAT more vascular, rich in blood supply and more heavily innervated than SCAT VAT accumulation is associated with tendency to hyperglycaemia, hyperinsulinemia, hypertriglyceridemia, impaired glucose tolerance, increased apolipoproteins, which are features of the insulin resistance syndrome. Increased risk of developing diabetes is greater in individuals with excess VAT. Furthermore,VAT has been identified as an independent risk factor for cardiovascular disease, hypertension and stroke (Dobbelsteyn, Joffres et al. 2001). Increase in circulating FFAs in

abdominal obesity is associated with increase in cardiovascular risk. Elevation in FFA levels promotes endothelial dysfunction.

Adipose tissue and inflammation

Different studies performed in obese and not obese patients showed different expression and secretion of adipocytokynes in VAT compared to SAT. VAT expresses more pro-inflammatory molecules, such as tumor necrosis factor-α (TNF- α), monocytes chemoattractive proteine-1 (MCP-1), Interleucyne-8 and Interleucyne-6 (IL-8/6), and pro-angiogenetic factors, such as vascular endothelia growth factor (VEGF) than SAT. Instead, SAT express principally leptin and adiponectin. FFA secreted in VAT reduces the anti-lipolytic answer to insulin increasing catecolamines, sensibility to glucocorticoids and activating the lipoprotein lipase (Pou, Massaro et al. 2007). For these reasons increase of VAT is considered a marker risk of cardiovascular disease.

Interest from researchers in other fat depots

In the last years literature suggests that regional fat distribution plays an important part in the development of metabolic disorders and cardiovascular risk profile. Despite their similar qualitative properties, different types of adipose tissue, particularly subcutaneous and visceral adipose depots, are now recognized as having distinct quantitative characteristics (Dusserre, Moulin et al. 2000; Wajchenberg 2000). While much of the interest has focused on the importance of intra-abdominal visceral fat, some extra-abdominal visceral fat depots, including mediastinal and epicardial fat, have also been studied (Sharma 2004). In particular the interest of researcher is focused on epicardial adipose tissue (EAT) and his possible role as cardiovascular risk marker. Fat in the heart resides in three distinct depots: pericardial (adipose tissue in the visceral pericardium), intracellular (the microscopic lipid accumulation within the cytoplasm of

cardiac muscle and can be the result of myocardial ischemia, cell damage or cell death (1)) and epicardial fat. EAT is located on the surface of the heart especially around the epicardial coronary vessels and can extend into the heart so as to be interspersed with myocardial muscle fibres. EAT covers about 80% (range 56–100%) of the surface area of the human heart and constitute approximately 20% of heart weight. Fat is present over the base of the heart, the atrioventricular groove, bases of the great vessels, along the distribution of the coronary arteries, over the right ventricle especially over the free wall – along the right border, anterior surface and at the apex (Figure 4). The greatest amount of epicardial fat is seen over the lateral right ventricular wall followed by the anterior wall with little present posteriorly (Rabkin 2007). Epicardial fat is concentrated along the acute margin, atrioventricular groove and intraventricular groove, so that the coronary arteries and their main epicardial branches are embedded in epicardial fat (de Jonge, van Ooijen et al. 2008).

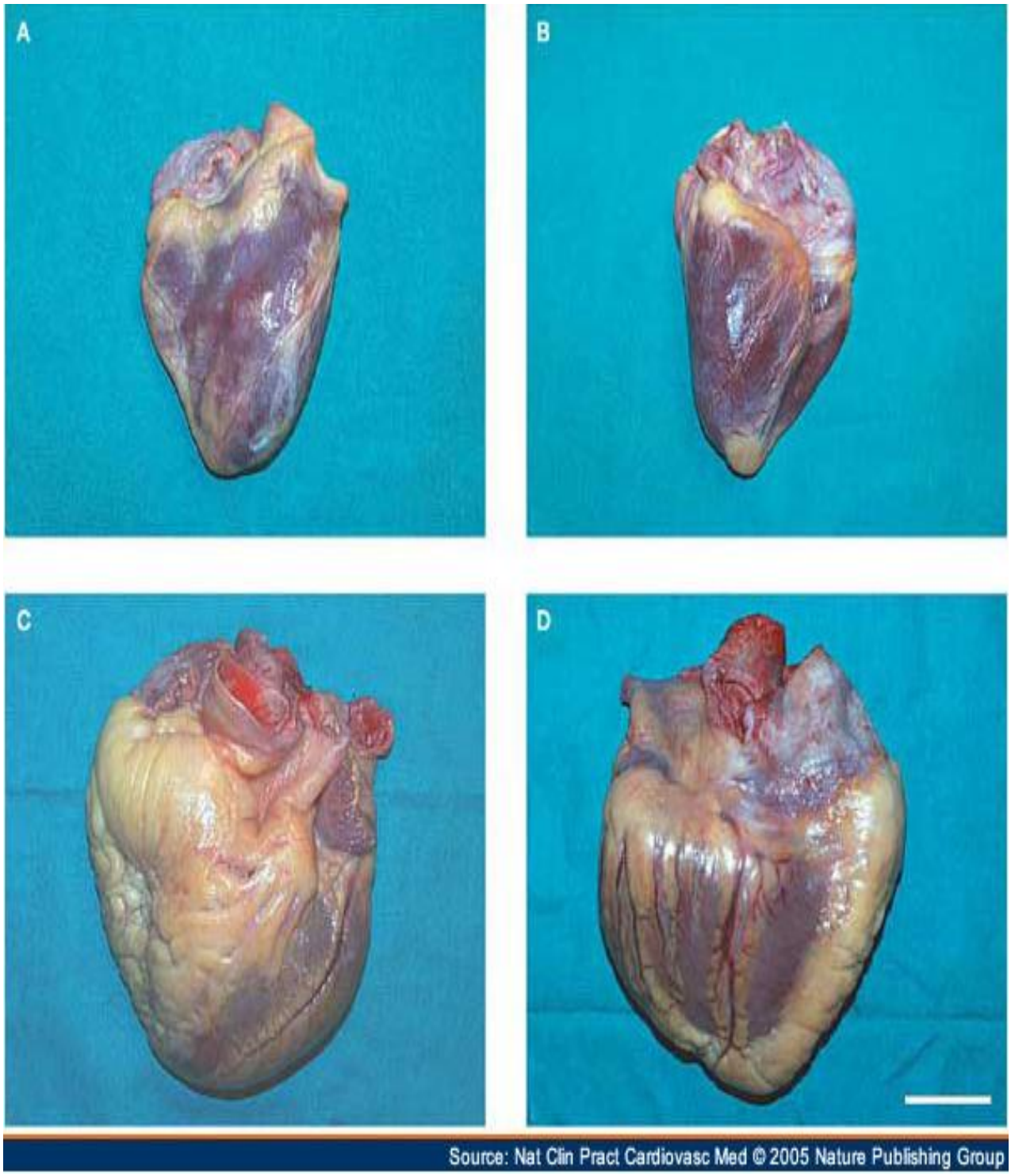

FIGURE 4: Macroscopic appearance of epicardial fat. (A) Anterior view of a normal (210 g) heart. (B) Posterior view of a normal (210 g) heart. (C) Anterior view of a hypertrophic (900 g) heart. (D) Posterior view of a hypertrophic (900 g) heart. In the normal heart, the fat distribution is limited to the atrioventricular and interventricular grooves, and along the major coronary branches (A, B). In the hypertrophic heart—the hypertrophy is mainly on the right-hand side—the adipose tissue also fills the epicardial spaces between these sites. Scale bar = 4 cm.

Putative physiologic function of epicardial fat

To epicardial fat, thanks to its location, have been attributed several functions, both structural than metabolic. Most of the putative physiologic functions are: buffer the coronary artery against the torsion induced by the arterial pulse wave and cardiac contraction or to offset rapid changes in the width of the blood vessels with arterial pulse limiting the motion of the coronary arteries perhaps reducing the potential extremes of coronary artery velocity (Keegan, Gatehouse et al. 2004). EAT and the myocardium share the same blood supply so another putative function is maintenance of fatty acid homeostasis in the coronary microcirculation (Marchington and Pond 1990) bufferuring the heart against an expose to excessively high circulating levels of fatty acids by its ability to rapidly take up and incorporate fatty acids. A third role, based on the high rate of fatty acid breakdown in EAT, is that this fat depot serves as a local energy source for cardiac muscle in times of high demand(Marchington, Mattacks et al. 1989). Epicardial fat is a source of cytokines that can induce monocytes in the intimae to differentiate into macrophages, which is a critical step in the development of atherosclerosis then EAT could be considered also as endocrine organ. Several lines of evidence support a role for EAT in the pathogenesis of coronary artery disease. First, there is close anatomic relationship between epicardial fat and coronary arteries. Second, there is a positive correlation between epicardial fat and presence of coronary atherosclerosis. Third, there are several potential pathophysiologic linkages between epicardial fat and atherosclerosis.

CARDIOVASCULAR DISEASE

Cardiovascular Diseases (CVDs) are a group of disorders of the heart and blood vessels and include: disease of the blood vessels supplying the heart muscle (coronary heart disease), disease of the blood vessels supplying the brain (cerebrovascular disease) and damage to the heart muscle and heart valves from rheumatic fever, caused by streptococcal bacteria (rheumatic heart disease). CVDs, the most deadly disease in the world, are mainly caused by a build-up of fatty deposits on the inner walls of the blood vessels (atherosclerotic plaques) that supply the heart or brain (US Department of Health; The World Health Organization). Vascular disease begins as a malfunction of specialized cells, called endothelial cells that line our arteries. These cells are the key to atherosclerosis and underlying endothelial dysfunction is the central feature of this dreaded disease. The cause and progression of vascular disease is intimately related to the health of the inner arterial wall. Blood vessels are composed of three layers: the external layer is mostly composed to connective tissue and provides structure to the layers beneath, the middle layer is formed by smooth muscle that contracts and dilates to control blood flow and maintain blood pressure, and the inner one consists of a thin layer of endothelial cells (the endothelium), which provides a smooth, protective surface. Endothelial cells prevent toxic, blood-borne substances from penetrating the smooth muscle of the blood vessel. If endothelial cells are damaged lipids and toxins can penetrate the endothelial layer and enter the smooth muscle cells resulting in the initiation of an oxidative and inflammatory cascade that culminates in the development of plaque deposits (Brott, Halperin et al.). For many years, lipids were considered to be intractable and uninteresting oily materials with two main functions such as source of energy and building blocks of membranes. They were certainly not considered to be appropriate candidates for such important molecular tasks as intracellular signalling or local hormonal regulation. Now lipids are considered a diverse and ubiquitous group of compounds which have many key biological functions, not only acting as structural components of cell membranes or serving as energy

storage sources but also important because able to participate in signaling pathways. Lipids may be divided into eight categories: Fatty Acyls, Glycerolipids, Glycerophospholipids, Sphingolipids, lipids derived from condensation of ketoacyl subunits and Lipids derived from condensation of isoprene subunits. Imbalance of these major lipids signaling pathways contribute to disease progression in chronic inflammations, atherosclerosis and heart disease. Previous studies have highlighted the potential importance of adipose tissue in relation to inflammatory burden in CVD, describing the expression and secretion of both pro-inflammatory and protective factors, collectively termed adipocytokines (Ahima and Flier 2000). These factors include tumour necrosis factor alpha (TNF-α), a pluripotent cytokine that is a key mediator of the acute phase response that also affects non-esterified fatty acid (NEFA) metabolism, as well as myocardial contractility (Ferrari 1999), resistin (Hotta, Funahashi et al. 2000; Steppan, Brown et al. 2001), adiponectin with his anti-inflammatory and anti-atherogenic properties with serum levels reduced coronary artery disease (CAD) . Also Plasminogen activator inhibitor-1 (PAI-1), angiotensin II (ANG II) and the active metabolite of angiotensinogen (AGT) has been studied in adipose tissue (Ridker, Gaboury et al. 1993) and in particular few studies have investigated the adipocytokines profile of epicardial adipose tissue. This depot, has been shown to have a high capacity for non-esterified fatty acid (NEFA) release and is proposed as a source of this preferred metabolite for the myocardium (Marchington, Mattacks et al. 1989). The adipocytes from epicardial fat are directly in contact with myocardial layer suggesting that factors secreted by the adipocytes would readily interact with the adjacent cardiomyocytes. Clinical studies have noted a strong correlation between epicardial adipose tissue, central abdominal fat and the associated risk of CVD (Iacobellis, Assael et al. 2003). These inflammatory status, when a blood clot (thrombus) forms on top of this plaque and the artery becomes completely blocked, cause a heart attack. When the atherosclerotic plaques partially or totally occlude the blood vessel (Figure 5) is necessary a surgical procedure called coronary artery bypass grafting (CABG). The cardiac surgeon makes an incision down the middle of the chest and then saws through the breastbone

(sternum). This procedure is called a median (middle) sternotomy (cutting of the sternum). The heart is cooled with iced salt water, while a preservative solution is injected into the heart arteries. This process minimizes damage caused by reduced blood flow during surgery and is referred to as "cardioplegia." Before bypass surgery can take place, a cardiopulmonary bypass must be established. Plastic tubes are placed in the right atrium to channel venous blood out of the body for passage through a plastic sheeting (membrane oxygenator) in the heart lung machine. The oxygenated blood is then returned to the body. The main aorta is clamped off (cross clamped) during CABG surgery to maintain a bloodless field and to allow bypasses to be connected to the aorta. The most commonly used vessel for the bypass is the saphenous vein from the leg. Bypass grafting involves sewing the graft vessels to the coronary arteries beyond the narrowing or blockage. The other end of this vein is attached to the aorta. Chest wall arteries, particularly the left internal mammary artery, have been increasingly used as bypass grafts. This artery is separated from the chest wall and usually connected to the left anterior descending artery and/or one of its major branches beyond the blockage. The major advantage of using internal mammary arteries is that they tend to remain open longer than venous grafts. Ten years after CABG surgery, only 66% of vein grafts are open compared to 90% of internal mammary arteries. However, artery grafts are of limited length, and can only be used to bypass diseases located near the beginning (proximal) of the coronary arteries. Using internal mammary arteries may prolong CABG surgery because of the extra time needed to separate them from the chest wall. Therefore, internal mammary arteries may not be used for emergency CABG surgery when time is critical to restore coronary artery blood flow. CABG surgery takes about four hours to complete. The aorta is clamped off for about 60 minutes and the body is supported by cardiopulmonary bypass for about 90 minutes. The use of 3 (triple), 4 (quadruple), or 5 (quintuple) bypasses are now routine. At the end of surgery, the sternum is wired together with stainless steel and the chest incision is sewn closed. Plastic tubes (chest tubes) are left in place to allow drainage of any remaining blood from the space around the heart (mediastinum). About

5% of patients require exploration within the first 24 hours because of continued bleeding after surgery. Chest tubes are usually removed the day after surgery. The breathing tube is usually removed shortly after surgery. Patients usually get out of bed and are transferred out of intensive care the day after surgery. Up to 25% of patients develop heart rhythm disturbances within the first three or four days after CABG surgery. These rhythm disturbances are usually temporary atrial fibrillation, and are felt to be related to surgical trauma to the heart. Most of these arrhythmias respond to standard medical therapy that can be weaned one month after surgery. The average length of stay in the hospital for CABG surgery has been reduced from as long as a week to only three to four days in most patients. Many young patients can even be discharged home after two days. A new advance for many patients is the ability to do CABG without going on cardiopulmonary bypass ("off pump"), with the heart still beating. This significantly minimizes the occasional memory defects and other complications that may be seen after CABG, and is a significant advance.

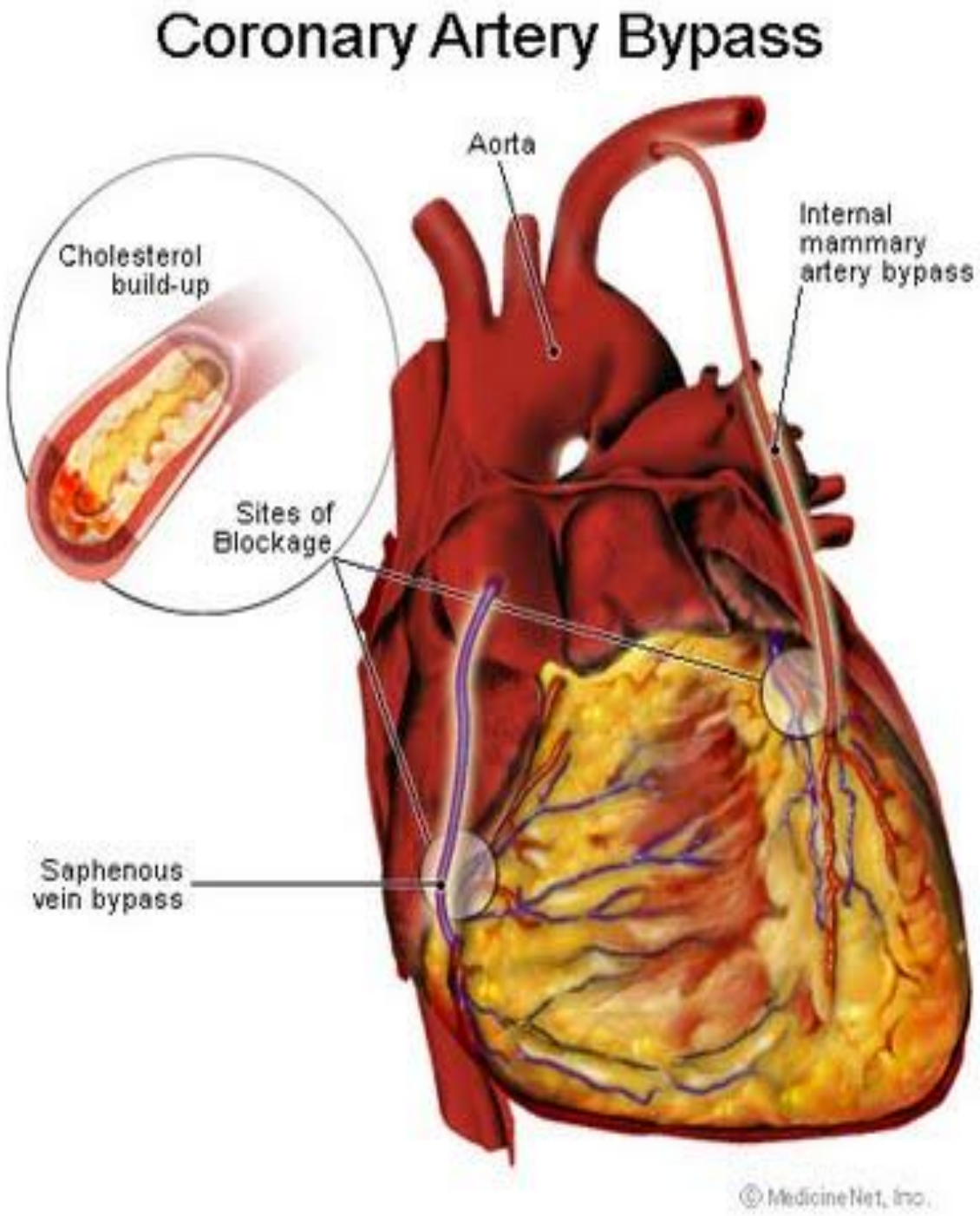

FIGURE 5: The heart, its sites of blockage and blood vessels used for grafting.

EAT: ROLE IN CAD

EAT thickness is associated whit VAT amount and other CVD's factors such as waist, fasting glucose, fasting insulin. Iacobellis *et al.*hypothesized that an increase in EAT could be a predictive risk factor for CVD (Iacobellis, Ribaudo et al. 2003). In which way EAT could lead CAD? A possible answer can be that atherogenesis is caused by transcitosis of lipoproteins Apo-B riches in cholesterol from plasma to intimae. These lipoproteins are fixed in sub-endothelial space where oxidized and gave an inflammation of intimae, adventitia and media that lead to atheroma. If atherosclerosis derived from lipid on lumen surface and produce an inflammatory answer, is possible that inflammation in EAT could be lead to CAD pathogenesis. Figure 6 shows that adipokines produced in EAT can cross the intimae plaques and give blood vessel inflammation leading to cardiovascular disorders in different ways: paracrine and vasocrine (Sacks and Fain 2007).

Paracrine signaling

Adipokines secreted from adipocytes and stromal-vascular cells in EAT overlying the lipid core of atherosclerotic plaques diffuse in interstitial fluid across the adventitia, media, and intimae and interact respectively with vasa vasora, vascular smooth muscle cells, endothelium, and cellular components of the plaque. Paracrine signaling may also occur between adipokines and FFA diffusing from epicardial fat into the underlying myocardium (not shown).

Vasocrine signaling

Adipokines secreted by epicardial adipocytes and stromal-vascular cells closely apposed to adventitial vasa vasorum traverse the vessel into its lumen and are transported downstream to react with cells in the media and the intimae around plaques. In this model, macrophages and lymphocytes can migrate alongside the vasa vasorum through breeches in the media

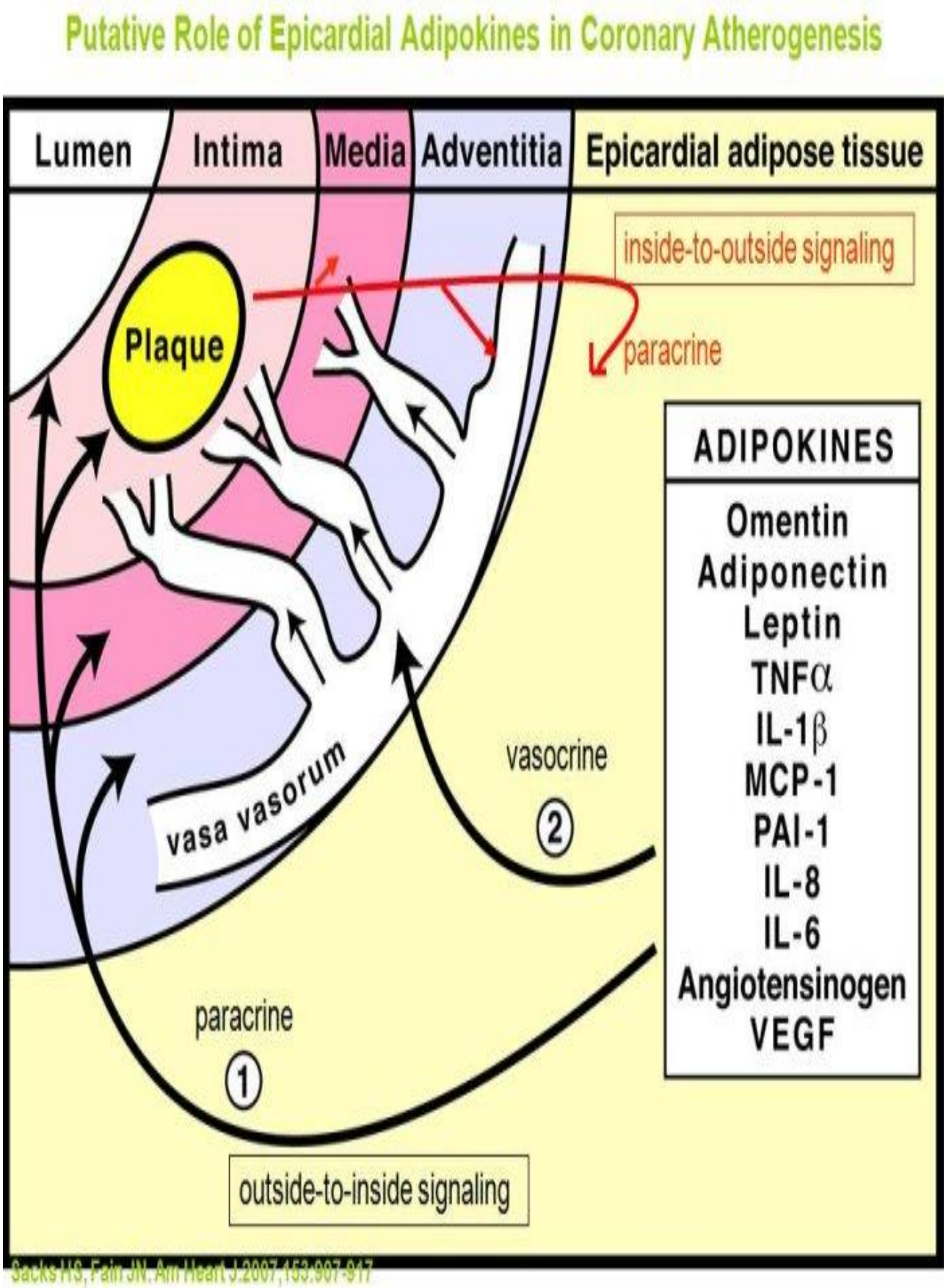

FIGURE 6: epicardial fat and adipokines. Sacks H.S., Fain JN Am Heart J. 2007, 153:907-917

MATERIALS AND METHODS

PATIENTS POPULATION

During 12 months, 41 patients, referred to IRCCS Policlinico San Donato for open-heart surgery were enrolled and divided into subgroups: obese and normalweight with coronary artery disease (CAD) underwent elective coronary artery bypass grafting (CABG) surgery; obese and normal weight patients without significant coronary stenosis who underwent to valvular replacement. All groups are age and sex matched. Exclusion criteria were: age <18 or >65 years, type 1 diabetes mellitus, recent acute myocardial infarction, malignant disease, prior major abdominal surgery, renal failure and more than 3% change in body weight in the previous 3 months. Smoking habits, alcohol intake, use of drugs and the duration of obesity, diabetes mellitus, hypertension and dyslipidemia were recorded. All the patients gave informed consent and the study submitted to local Ethics Committee approval. EAT biopsies (average 0.5-1g) were collected adjacent to the proximal right coronary artery. Biopsy samples from EAT, aliquots of peripheral blood, plasma EDTA were collected before cardiopulmonary bypass pumping. All patients underwent to M-mode colour Doppler echocardiography. EAT thickness measured on the free wall of the right ventricle from both parasternal long- and short-axis views. Left ventricular morphology and diastolic function (by pulsewave Doppler echocardiography) were evaluated. Angiographic coronary findings were examined and the angiographic coronary score was assessed by Gensini's method, by which the degree of each vessel stenosis is multiplied by a specific factor depending on the topographical and functional importance of the coronary vessel affected by atheroma.

CLINICAL PARAMETHERS

Age, body mass index (BMI), weight, height, fasting glucose, fasting insulin, homeostasis model assessment of insulin resistance (HOMA), total cholesterol, high density lipoproteins (HDL), triglycerides and blood pressure were measured by standard methods. C-reactive protein was assayed by immunoturbidimetric assay.

- Blood pressure: systolic blood pressure and diastolic blood pressure were measured on both harms and after 15 minutes of quite position. The blood pressure recorded was the media of three different reading. BMI is the result of ratio obtained from weight on two time height.
- Anthropometric measures: weight was performed by and approximation of 100g, while height whit 0.5 cm. The measure was recorded fasting, without clothes and after emptying of the bladder. Circumferences of waist measured 1 cm on umbilicus and hip a level of big trochanter. The waist hip ratio (WHR) was calculated as index of visceral fat distribution.
- Hematological parameters: insulin resistance (HOMA) was obtained from fasting glucose and fasting insulin measure.

ECHOCARDIOGRAPHIC STUDY

Each subject underwent transthoracic 2-dimensional guided M-mode echocardiography using commercially available equipment (Vingmed-System Five, General Electric, Milwaukee, Wisconsin). Standard parasternal and apical views were obtained in the left lateral decubitus position. All echocardiograms were recorded and analyzed off-line for epicardial fat thickness quantification, according to the method that we previously described and validated. Epicardial

fat thickness was measured by 2 readers on 2 different days and with no knowledge of previous readings. Epicardial fat was identified as echocardiographic-free space between the outer wall of the myocardium and the visceral layer of the pericardium. Epicardial fat thickness was measured perpendicularly on the free wall of the right ventricle at end-systole in 3 cardiac cycles. Parasternal long- and short-axis views allowed the most accurate measurement of EAT in the right ventricle, with optimal cursor beam orientation in each view. Maximum epicardial fat thickness was measured at the point on the free wall of the right ventricle along the midline of the ultrasound beam, perpendicular to the aortic annulus, and used as an anatomic landmark for this view. For midventricular parasternal short-axis assessment, maximum epicardial fat thickness was measured on the right ventricular free wall along the Midline of the ultrasound beam, perpendicular to the ventricular septum at the midchorda and tip of the papillary muscle level, as an anatomic landmark. The average value of 3 cardiac cycles from each echocardiographic view was considered. Concordance of long- and short-axis average epicardial fat thickness measurements was excellent. Integrand intraobserver variabilities of epicardial fat thickness were not statistically significant. Intra- and interobserver reproducibilities of this measurement were excellent (intraclass correlation coefficient 0.90 and 0.87, respectively) as was agreement. Left ventricular (LV) mass was estimated and adjusted by body surface area (indexed LV mass), as previously described.9 Right ventricular end-diastolic diameter and the left atrium were measured according to American Society of Echocardiography recommendations. LV diastolic function was evaluated using pulse-wave color Doppler echocardiography. E-wave deceleration time was calculated in 3 to 5 consecutive cardiac cycles.

GAS CHROMATOGRAPHY-MASS SPECTROMETRY *(GS-MS):*

GM-CS (Figure 7) is a method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample. Applications of GC-MS include drug detection, fire investigation, environmental analysis, explosives investigation, and identification of unknown samples. GM-CS founds application also in clinical medicine where help researcher to investigate molecules, such as lipids, involved in different pathology. The use of a mass spectrometer as the detector in gas chromatography was developed during the 1950s by Roland Gohlke and Fred McLafferty. These sensitive devices were bulky, fragile, and originally limited to laboratory settings. The development of affordable and miniaturized computers has helped in the simplification of the use of this instrument, as well as allowed great improvements in the amount of time it takes to analyze a sample. In 1964, Electronic Associates, Inc. (EAI), a leading U.S. supplier of analog computers, began development of a computer controlled quadruple mass spectrometer under the direction of Robert E. Finnigan. By1966 Finnigan and collaborator Mike Uthe's EAI division had sold over 500 quadrupole residual gas-analyzer instruments. In 1967, the Finnigan Instrument Corporation was formed and in early 1968, delivered the first prototype quadrapole GC/MS instruments to Stanford and Purdue University. FIC was eventually renamed Finnigan Corporation and went on to establish itself as the worldwide leader in GC/MS systems. In 1996 the top-of-the-line high-speed GC-MS units completed analysis of fire accelerants in less than 90 seconds, whereas first-generation GC-MS would have required at least 16 minutes. By the 2000s computerized GC/MS instruments using quadrupole technology had become both essential to chemical research and one of the foremost instruments used for organic analysis. Today computerized GC/MS instruments are widely used in environmental monitoring of water, air, and soil; in the regulation of agriculture and food safety; and in the discovery and production of medicine. The GC-MS is composed of two major building blocks: the gas chromatograph and the mass spectrometer. The gas chromatograph

utilizes a capillary column which depends on the column's dimensions (length, diameter, film thickness) as well as the phase properties (e.g. 5% phenyl polysiloxane). The difference in the chemical properties between different molecules in a mixture will separate the molecules as the sample travels the length of the column. The molecules take different amounts of time (called the retention time) to come out of (elute from) the gas chromatograph, and this allows the mass spectrometer downstream to capture, ionize, accelerate, deflect, and detect the ionized molecules separately. The mass spectrometer does this by breaking each molecule into ionized fragments and detecting these fragments using their mass to charge ratio. These two components, used together, allow a much finer degree of substance identification than either unit used separately. It is not possible to make an accurate identification of a particular molecule by gas chromatography or mass spectrometry alone. The mass spectrometry process normally requires a very pure sample while gas chromatography using a traditional detector (e.g. Flame Ionization Detector) detects multiple molecules that happen to take the same amount of time to travel through the column (*i.e.* have the same retention time) which results in two or more molecules to co-elute. Sometimes two different molecules can also have a similar pattern of ionized fragments in a mass spectrometer (mass spectrum). Combining the two processes reduces the possibility of error, as it is extremely unlikely that two different molecules will behave in the same way in both a gas chromatograph and a mass spectrometer. Therefore, when an identifying mass spectrum appears at a characteristic retention time in a GC-MS analysis, it typically lends to increased certainty that the analyte of interest is in the sample.

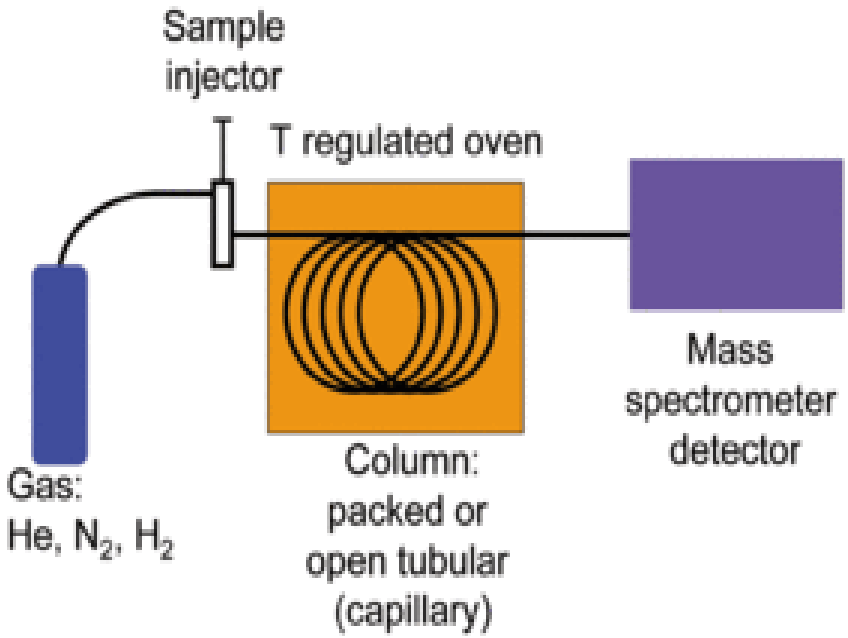

FIGURE 7: GM-CS schematic

Free fatty acids analysis by GC-MS

Fatty acid analysis was performed as describe previously (Ecker, Liebisch et al.). Fatty acid methyl esters (FAME) were generated with acetyl-chloride and methanol and extracted with hexane. Total FA analysis was carried out using a Shimadzu 2010 GC-MS system. FAMEs were separated by a BPX70 column (10 m length, 0.10 mm diameter, 0.20 µm film thickness) from SGE using helium as carrier gas. The initial oven temperature was 50°C, which was programmed to increase with 40°C per min to 155°C, with 6°C per min to 210°C, and with 15°C per min to finally reach 250°C. The FA species and their positional and *cis/trans* isomers were characterized in scan mode and quantified by single-ion monitoring (SIM) mode detecting the specific fragments of saturated and unsaturated FAs (saturated: *m/z* 74; monounsaturated: *m/z* 55; diunsaturated: *m/z* 67; polyunsaturated: *m/z* 79). As an internal standard, nonnaturally occurring C13:0 was used

MASS SPECTROMETRY

A mass spectrometer produces ions from the substance under investigation, separates them according to their mass-to-charge ratio (*m/z*), and records the relative abundance of each ionic species present. The instrument consist of three major components: an ion source for producing gaseous ions from the substance being studied, an analyzer for resolving the ions into their characteristics mass components according to their mass-to-charge ratio and a detector system for detecting the ions and recording the relative abundance of each of the resolved ionic species. Samples are introduced either as a gas to be ionized in the ion source, or by ejection of charged molecular species from a solid surface or solution. In some cases sample introduction and ionization take place in a single process, making a distinction between them somewhat artificial. Substances that are gases or liquids at room temperature and atmospheric pressure can be admitted to the source as a neutral beam via a controllable leak system. Volatilizable compound dissolved or absorbed in solids or liquids can be removed and concentrated with a headspace analyzer. Vapors are flushed from the solid or liquid matrix with a steam of carrier gas and trapped on an adsorbing column. The trapped vapors are subsequently desorbed by programmed heating of the trap and introduced into the mass spectrometer by a capillary connection. For Volatilizable solids, the most frequently used method of sample introduction is the direct insertion probe. Here, the sample is placed in a small crucible at the tip of the probe, which is heated under high vacuum in close proximity to the ion source. A variation of this technique involves desorption of samples inside the ionization chamber from a rapidly heated wire or with the aid of a laser beam. Such desorption techniques, in combination with electron, chemical, or field ionization, are preferred for the analysis of heat sensitive or poorly volatile samples. Sample introduction techniques that involve the ejection of charged molecules from the surface of solid samples include the field desorption method and various sputtering techniques, where the samples are bombarded by high energy photons, by a primary ion beam, or by a neutral

particle beam. Electrospray Ionization (ESI) technique involves spraying of a solution of the sample through a highly charged needle so-called capillary which is at atmospheric pressure (Figure 8). The spraying process can be streamlined by using a nebulizing gas. The charged droplets are produced in which the positive or negative ions are solvated with solvent molecules. Heat gas or a dry gas, usually called as desolvation gas is applied to the charged droplets to cause solvent evaporation. The desolvation process decreases the droplet size, leads to the columbic repulsion between the charges present in the droplet and further the droplet fission leads to the formation of individual gas phase analyte ions (that critical point known as the Rayleigh limit), that are guided through ion optics into the mass analyzer. ESI can produce singly or multiply charged ions. The number of charges retained by a particular analyte depends on several factors such as the size, chemical composition, and higher order structure of the analyte molecule, the solvent composition, the presence of co-solutes, and the instrument parameters. For small molecules (< 2000 Da) ESI typically generates singly, doubly, or triply charged ions, while for large molecules (> 2000 Da) ESI can produce a series of multiply charged ions. ESI is very suitable for a wide range of biochemical compounds including peptides and proteins, lipids, oligosaccharides, oligonucleotides, bio-inorganic compounds, synthetic polymers, and intact non-covalent complexes.

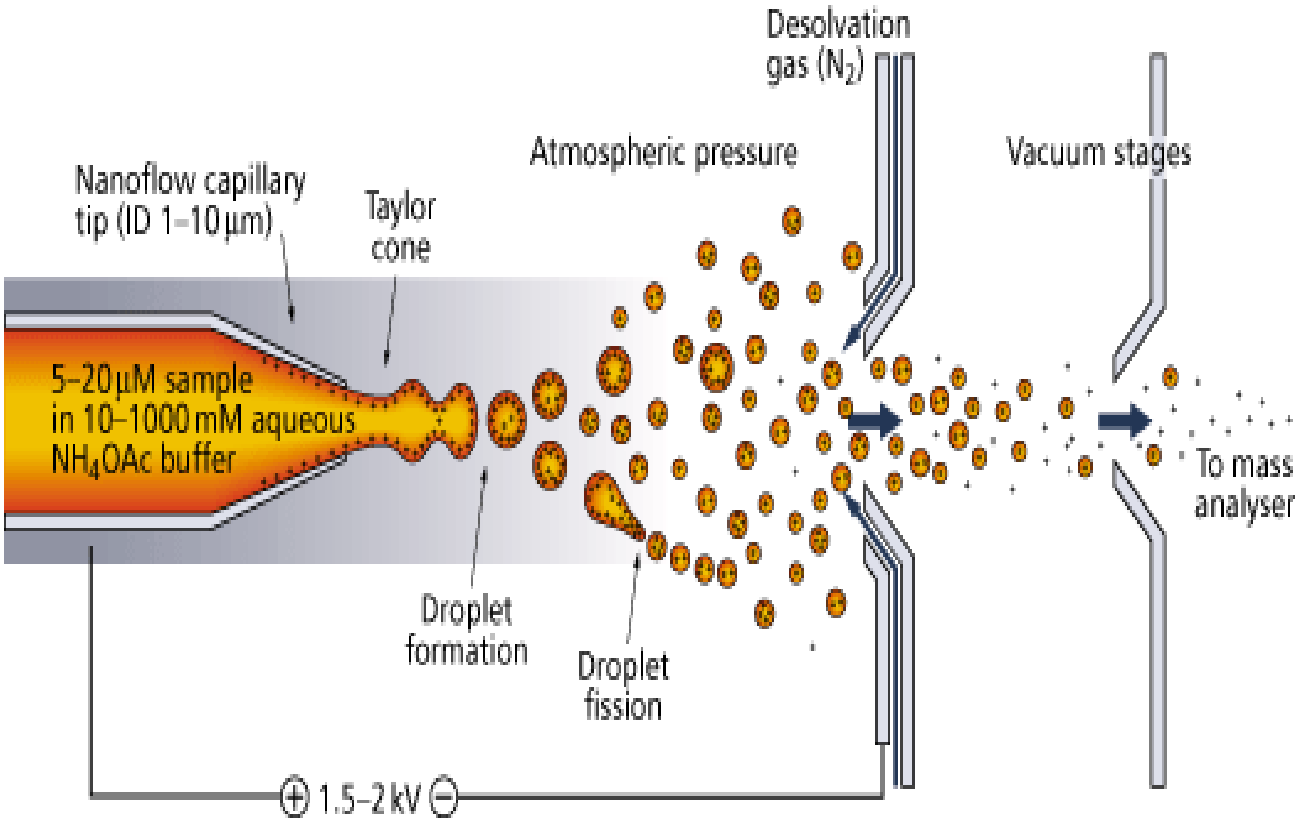

FIGURE 8: Schematic diagram of a typical ESIMS source phenomenon operating in positive mode

Mass analyzers separate the charged species in the ionized sample according to their *m/z* ratios, thus permitting the mass and abundance of each species to be determined. Commonly used analyzer is the quadrupole. The instrument is based on four parallel rods in a square array. The ion beam is focused down the axis of the array and an electrical potential of fixed (DC) and radio frequency (RF) components is applied to diagonally opposed rods. For a given combination of DC and RF components, ions of one specific *m/z* ratio have a stable path down the axis. All others are deflected to the sides and lost. Mass scanning is achieved by changing the DC and RF components of the voltage, while maintaining a fixed ratio. The quadrupole analyzer (Figure 9) is a mass filter because it separates ions on the basis of their *m/z* ratio.

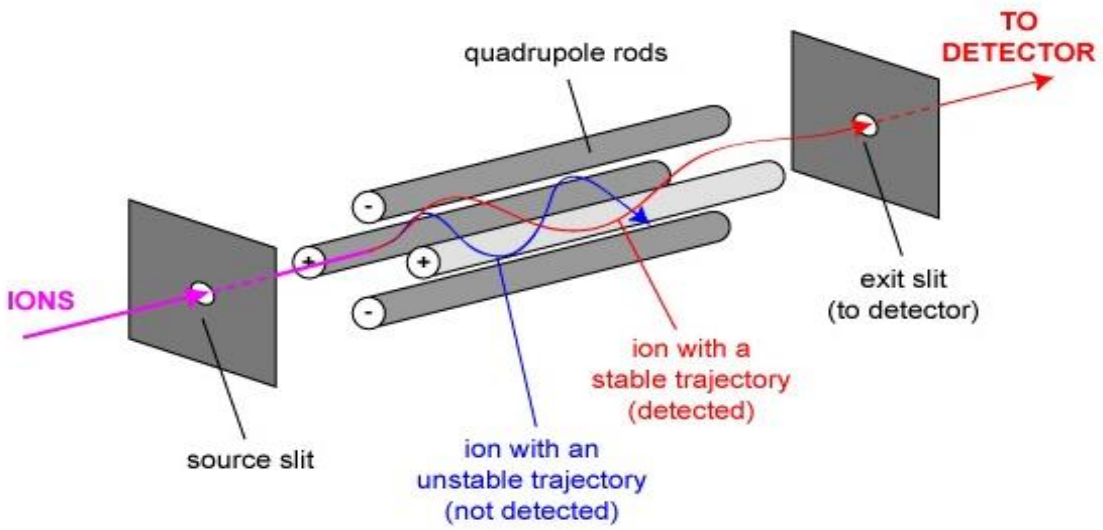

FIGURE 9.Schematic diagram of quadrupole analyzer

Two mass spectrometers connected in series (MS/MS), tandem mass spectrometry, refers to the use of two or more sequential mass analysis steps. In its simplest form MS/MS (Figure 10) consist of two mass spectrometers linked in such a way that ion preselected by the first mass analyzer (MS1) are chemically or energetically modified and the results analyzed by the second mass analyzer (MS2). The basic concept of MS/MS involves the ability to determine the mass relationship between a precursor ion in MS1 and a product ion in MS2. Different mass can be probed depending on how MS1 and MS2 are scanned. These include fragmentation of a precursor and measurement of all its fragments (a product scan), selection of multiple precursors and testing for a common fragment (a precursor scan), or scanning to see if a number of precursors all lose the same neutral species (a constant neutral loss scan). Fragmentation of the precursor ion can be induced by momentum transfer through collision with gas molecules and/or solid surfaces or by electronic excitation using lasers. These techniques are known as collision induced dissociation, surface-induced dissociation or laser-induced dissociation, respectively. Allowing the ion to fragment without additional activation is known as metastable

decomposition. Quantitative mass spectrometry involves measuring the abundance of a specific ion, or set of ions, and relating the response to a known standard. External or internal standards may be used, but the latter are preferred for greater accuracy. For mass spectrometry, internal standards may be either structural or stable isotope analogs.

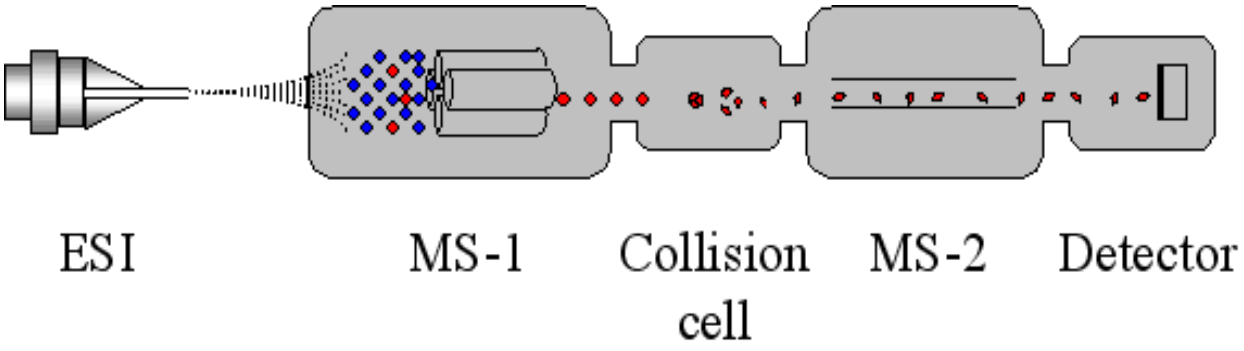

FIGURE 10: Schematic tandem MS/MS

Lipids analysis by ESI/ MS-MS

Total lipid extraction and purification was performed according with protocol described by Blight and Dyer (Bligh and Dyer 1959). Lipid species were quantified by ESI-MS/MS using methods validated and described previously (Wiesner, Leidl et al. 2009). In brief, samples were analyzed by direct flow injection on a Quattro Ultima triple-quadrupole mass spectrometer (Micromass, Manchester, UK) by direct-flow injection analysis using a HTS PAL autosampler (Zwingen, Switzerland) and an Agilent 1100 binary pump (Waldbronn, Germany) with a solvent mixture of methanol containing 10 mM ammonium acetate and chloroform (3:1, v/v). A flow gradient was performed starting with a flow of 55 µl/min for 6 s followed by 30 µl/min for 1.0 min and an increase to 250 µl/min for another 12 s. A precursor ion scan of m/z 184 specific for phosphocholine containing lipids was used for PC, SM, and lysophosphatidylcholine (LPC). A neutral loss scan of m/z 141 was used for phosphatidylethanolamine (PE), and PE-based plasmalogens (PE-pl) were analyzed according to the principles described by Zemski-Berry

(Zemski Berry and Murphy 2004). In brief, fragment ions of m/z 364, 380, and 382 were used for PE p16:0, p18:1, and p18:0 species, respectively. CER was analyzed similar to a previously described methodology using N-heptadecanoyl-sphingosine as internal standard. Free cholesterol (FC) and CE species were determined after selective acetylation of FC. Quantification was achieved by calibration lines generated by addition of naturally occurring lipid species (extraction of 20 µl 5-fold diluted plasma for single FPLC fractions or 20 µl undiluted plasma for pooled lipoprotein fractions). All lipid classes were quantified with internal standards belonging to the same lipid class, except SM (PC internal standards) and PE-pl (PE internal standards). Calibration lines were generated for the following naturally occurring species: PC 34:1, 36:2, 38:4, 40:0, and PC O 16:0/20:4; SM 34:2, 36:2, 36:1; LPC 16:0, 18:1, 18:0; PE 34:1, 36:2, 38:4, 40:6; and PE p16:0/20:4; Cer 16:0, 18:0, 20:0, 24:1, 24:0; FC, CE 16:0, 18:2, 18:1, 18:0. These calibration lines were also applied for not calibrated species, as follows: concentrations of saturated, monounsaturated, and polyunsaturated species were calculated using the closest related saturated, monounsaturated, and polyunsaturated calibration line slope, respectively. For example PE 36:2 calibration was used for PE 36:1, PE 36:3, and PE 36:4; PE 38:4 calibration was used for PE 38:3, PE 38:5, and so on. Ether-PC species were calibrated using PC O 16:0/20:4 and PE-pl were quantified independent from the length of the ether linked alkyl chain using PE p16:0/20:4.

GLOBAL GENE ANALYSIS

RNA extraction was performed by RNeasy mini kit (Qiagen, GmbH, Germany). EAT were pulverized by mortar in liquid nitrogen. The powder thus obtained was added to a solution formed with β-mercaptoethanol and RTL buffer and warmed for 10 minutes at 37°. Transferred in 2ml tubes and incubated for 1 hour, 900 rpm at 37°C. Centrifuge the homogenize 5 minutes at 2500rpm and transferred the second of three formed layers into the column. Centrifuged at

maximum speed and discard the column. Added 600 µl of ethanol to centrifuged in a new column. Centrifuged and discard the eluted. Added 80µl of DNase and RDD and incubated for 15 minutes at room temperature. Washed column and eluted RNA with RNA free water into a 1.5 ml tube. Read RNA concentration by NANO-drop. Perform quality control by Agilent 2100 bioanalyzer, which separated sample component electrophoretically, to obtain RNA integrity number (RIN). Gene expression analysis was performed by one colour microarray platform. The microarray has 41000 human gene and transcripts with one 60-mer oligonucleotide probe representing each sequence. 75 selected probes are replicated 10 times to allow for intra-array reproducibility measurement.

STATISTICAL ANALYSIS

Data are expressed as mean±SD and analyzed by Student's *t*-test. Differences were considered statistically significative at $p < 0.05$.

RESULTS

CLINICAL PARAMETERS

Between CABG and valvular patients (Table.1) there was no difference in age, BMI, weight, height, fasting glucose, fasting insulin, HOMA, total cholesterol, HDL, triglycerides and C-reactive protein.

TAB 1: Clinical Parameters

CABG or Valvular	CABG mean	CABG SD	valvular mean	Valvular SD
Age (years)	65,33	8,71	59,83	9,11
BMI	27,83	2,61	29,97	2,28
Weight (Kg)	77,86	7,64	87,17	15,51
Height (m)	1,70	0,08	1,70	0,12
Waist (cm)	103,24	5,69	103,67	8,89
fasting glucose (mg/dl)	96,29	50,36	91,50	20,22
fasting insulin (microU/ml)	9,45	6,46	8,96	2,88
HOMA	2,39	2,11	2,05	0,85
Cholesterol tot (mg/dl)	143,76	30,71	209,83	46,55
HDL (mg/dl)	35,81	7,87	41,83	7,19
Triglyceride (mg/dl)	142,70	83,86	156,67	88,70
CRP (mg/dl)	0,95	2,48	0,30	0,17
Systolic blood pressure (mmHg)	121,58	11,67	120,00	7,07
Diastolic blood pressure (mmHg)	72,11	6,31	70,00	0,40

BMI, body mass index; HOMA, homeostasis model assessment of insulin resistance; HDL, high density lipoproteins CRP, C-reactive protein; SD, standard deviation.

ECHOCARDIOGRAPHIC STUDY

EAT thickness (Figure 11) in CABG patients was not statistically significant higher (8.06 ± 0.11 mm) compared to valvular patients (6.03 ± 0.95 mm)

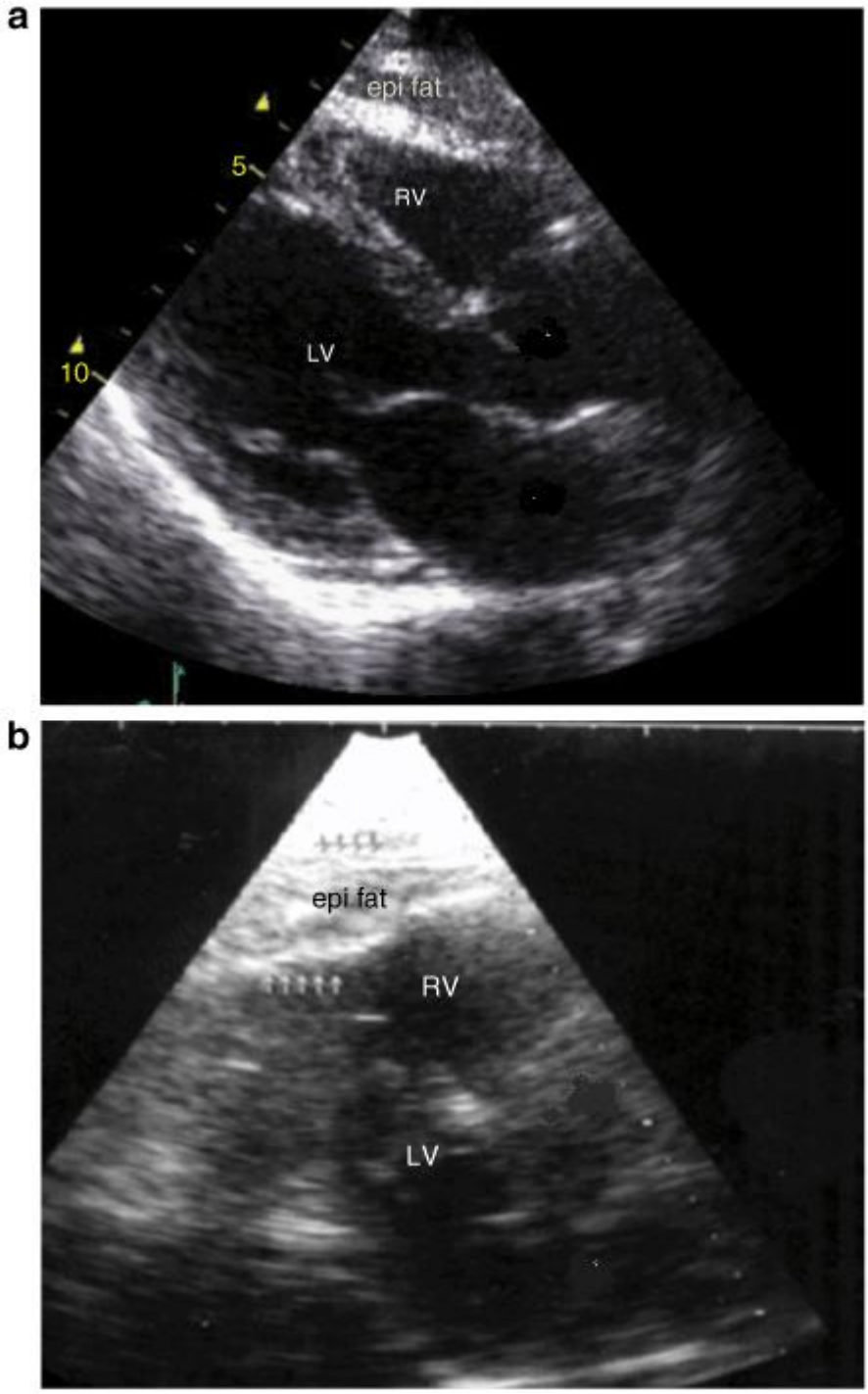

FIGURE 11: Echocardiographic epicardial fat thickness. Epicardial fat (epi fat) is identified as (a) the echo-free space between the outer wall of the myocardium and the visceral layer of pericardium in the parasternal long-axis view or (b) as hyperechoic space (epi fat, within white arrows), if in massive amounts (>15 mm). Epicardial fat thickness (white line in a, black line in b) is measured at the point on the free wall of the right ventricle along the midline of the ultrasound beam, with the best effort to be perpendicular to the aortic annulus, anatomical landmark. LV, left ventricle; RV, right ventricle

PLASMA FATTY ACIDS

Patients who underwent CABG showed (Fig.12) a decrease concentration in polyunsaturated fatty acids (PUFA) compared to valvular replacement patients (3.1 ± 0.27 vs. 4.5 ± 0.7 mmol/l p= 0.0031) while there was no significant difference for saturated fatty acids (SFA) and monounsaturated fatty acid (MUFA) between the two groups (p>0.05). PUFA/SFA ratio was statistically lower in CABG patient (0.87 ± 0.04) compared to valvular patients (1.08 ± 0.02 p<0.001). Percentage of oleic acid (18:1) was higher in CABG compared to valvular replacement patients (25.96± 3.93 vs. 21.76 ± 2.01 p=0.049) while percentage of linoleic (18:2) was lower (20.03 ± 3.84 vs. 24.77 ± 2.93 p=0.048). For the other fatty acids were not visible significant difference between CABG and valvular (Figure 13).

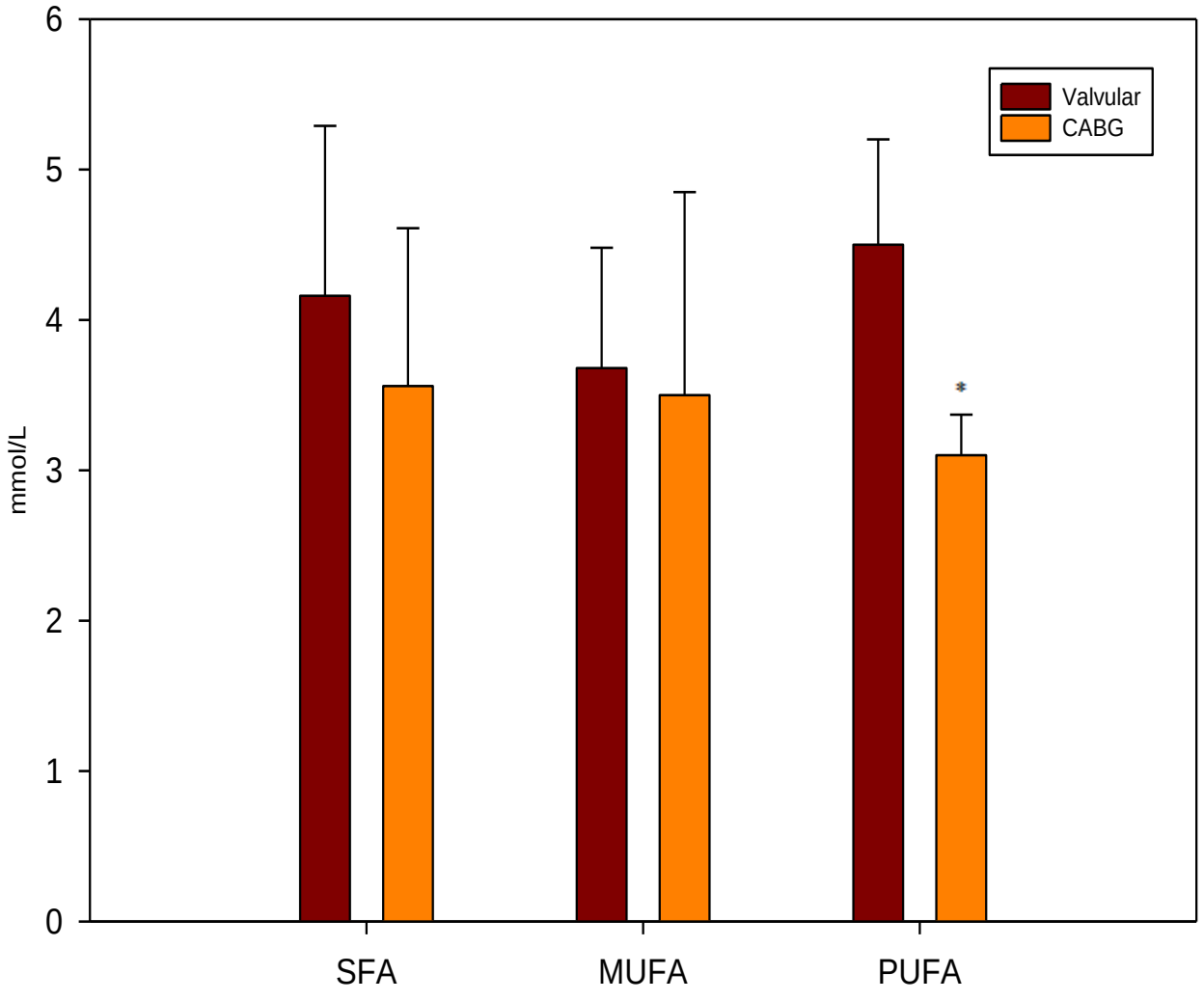

FIGURE 12: free fatty acids in epicardial adipose tissue

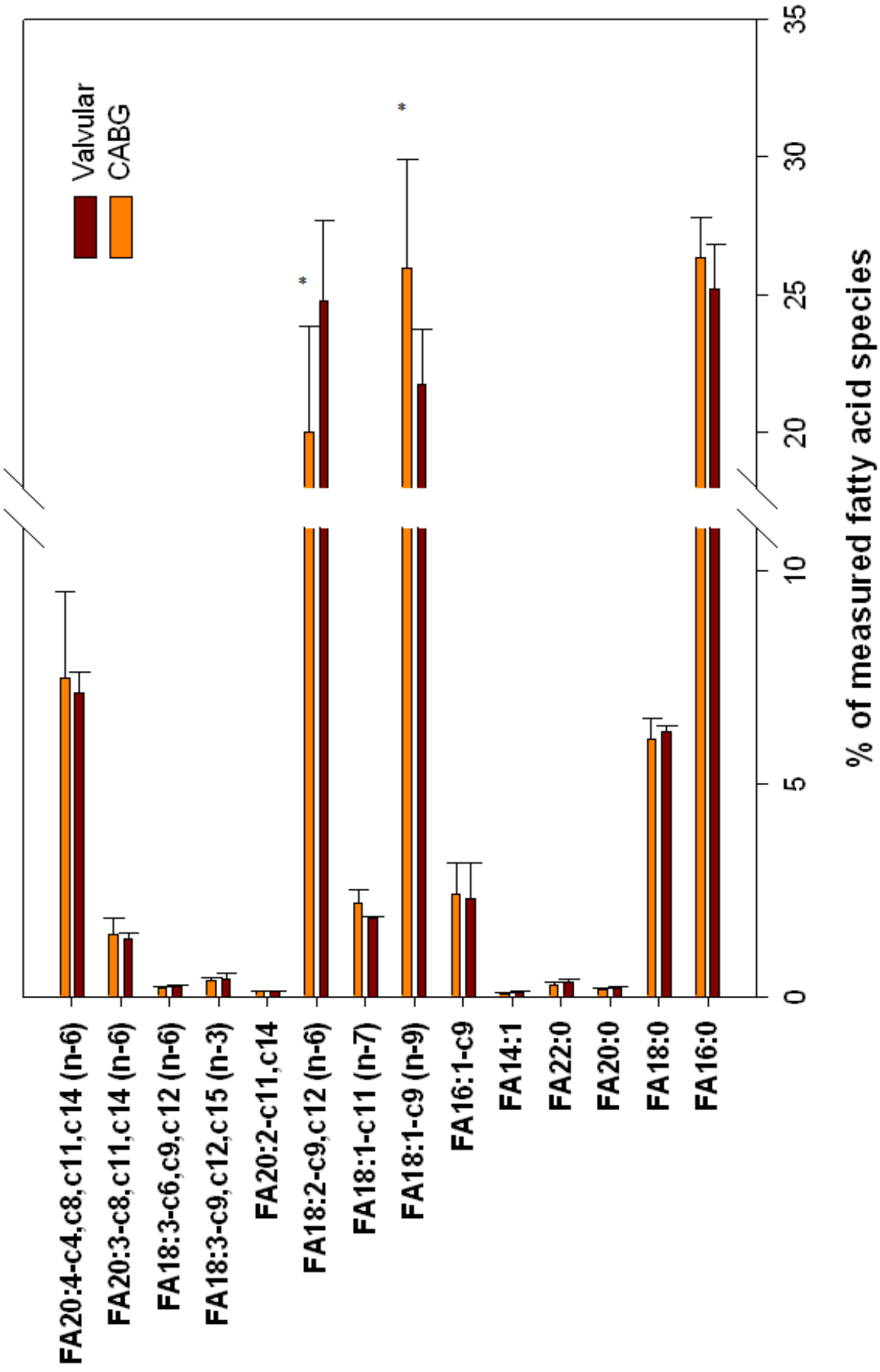

FIGURE 13: free fatty acids percentage in epicardial adipose tissue

GLYCEROPHOSPHOLIPIDS PROFOLE IN EPICARDIAL ADIPOSE TISSUE

Figure 14 summarized the percentage of glycerophospholipid classes analyzed in EAT from both surgery treatment (Figure 14 A).

Phosphatidylcholine (PC), Phosphatidylethanolamine (PE) and phosphatidylserine classes (PS) were statistically modified in the two different surgery.

Percentage of analyzed PC class was lower in CABG compared to valvular (39.34 ± 2.06 vs. 41.19 ± 1.56 p= 0.014), regarding PE class again CABG lipid levels was lower than valvular (5.28 ± 0.89 vs. 6.18 ± 0.93 p=0.02) and finally, even the percentage of PS class was lower in CABG than valvular patients (7.19 ± 0.82 vs. 8.64 ± 1.68 p=0.03).

Lower statistically significant lipid concentration was visible in CABG compared to valvular for PC species (Table 2), such as PC 36:2 (p=0.048), 34:3 (p=0.01), 36:3 (p=0.01), 36:4 (p=0.0004) 38:4 (p=0.002), 40:4 (p=0.002), 36:5 (p=0.03), 38:5 (p=0.00005), 38:6 (p=0.01) and 40:6 (p=0.0003). Similarly lipid composition for PE species was for PE 34:1 (p=0.01), 36:1 (p=0.03), 36:2 (p=0.02), 36:3 (p=0.02), 36:4 (p=0.0059 38:4 (p=0.007), 40:4 (p=0.01), 38:5 (p=0.005), 40:5 (p=0.02) and 40:6 (p=0.030) statistically lower in CABG compared to valvular. While for PS classes only one lipid specie, PS 36:1 was statistically lower in CABG compared to valvular (p=0.02). CABG and valvular samples if divided in subgroups after Body Mass Index (BMI) classification (Fig 14 B) showed for PC class no differences between both CABG and valvular normoweight and CABG and valvular obese. Regarding PE and PS class instead, there was no statistically difference between normoweight of both surgery but obese CABG patients appeared to be lower than valvular obese patients (p=0.01). Lipid species analyzed in epicardial adipose tissue after BMI classification (Table 2) showed more or less a statistically significant lower concentration of all PC species in CABG patients compared to valvular patients. Similarly all PE species after subgroup divisions showed lowered concentration values in CABG compared to

valvular. PS 36:1 specie, was also lower in CABG obese patients compared to valvular obese patients (p=0.001). CABG and valvular samples divided in subgroups after waist classification (Fig 14 C) showed for PC, PE and PS classes no differences between both CABG and waist <90 and CABG and valvular waist >90. Lipid species analyzed in epicardial adipose tissue after waist classification (Table 2) showed more or less a statistically significant lower concentration of all PC species in CABG patients compared to valvular patients with waist >90 ($p<0,05$). Same PE, 36:1, 36:2, 38:2, 36:3, 36:4, 38:5 and 42:2 ($p<0.05$) species after subgroup divisions showed lowered concentration values in CABG compared to valvular with waist >90. PS 36:1 and 38: 4 species, were also lower in CABG waist >90 patients compared to valvular waist >90 patients (p=0.01). CABG and valvular samples if divided in subgroups after WHR classification (Fig 14 D) showed for PC class differences between CABG and valvular WHR>94 ($p<0.05$). Regarding PE and PS classes instead, there was no statistically difference in both surgery between WHR < and > 94. Lipid species analyzed in epicardial adipose tissue after WHR classification (Table 2) showed more or less a statistically significant lower concentration of all PC species in CABG patients compared to valvular patients with WHR >94 ($p<0,05$). Same PE, 36:1, 36:2, 36:3, 36:4 and 38:5 ($p<0.05$) species after subgroup divisions showed lowered concentration values in CABG compared to valvular with WHR >94. PS 36:1 and 38: 4 species, were also lower in CABG WHR >94 patients compared to valvular WHR >94 patients (p=0.015).

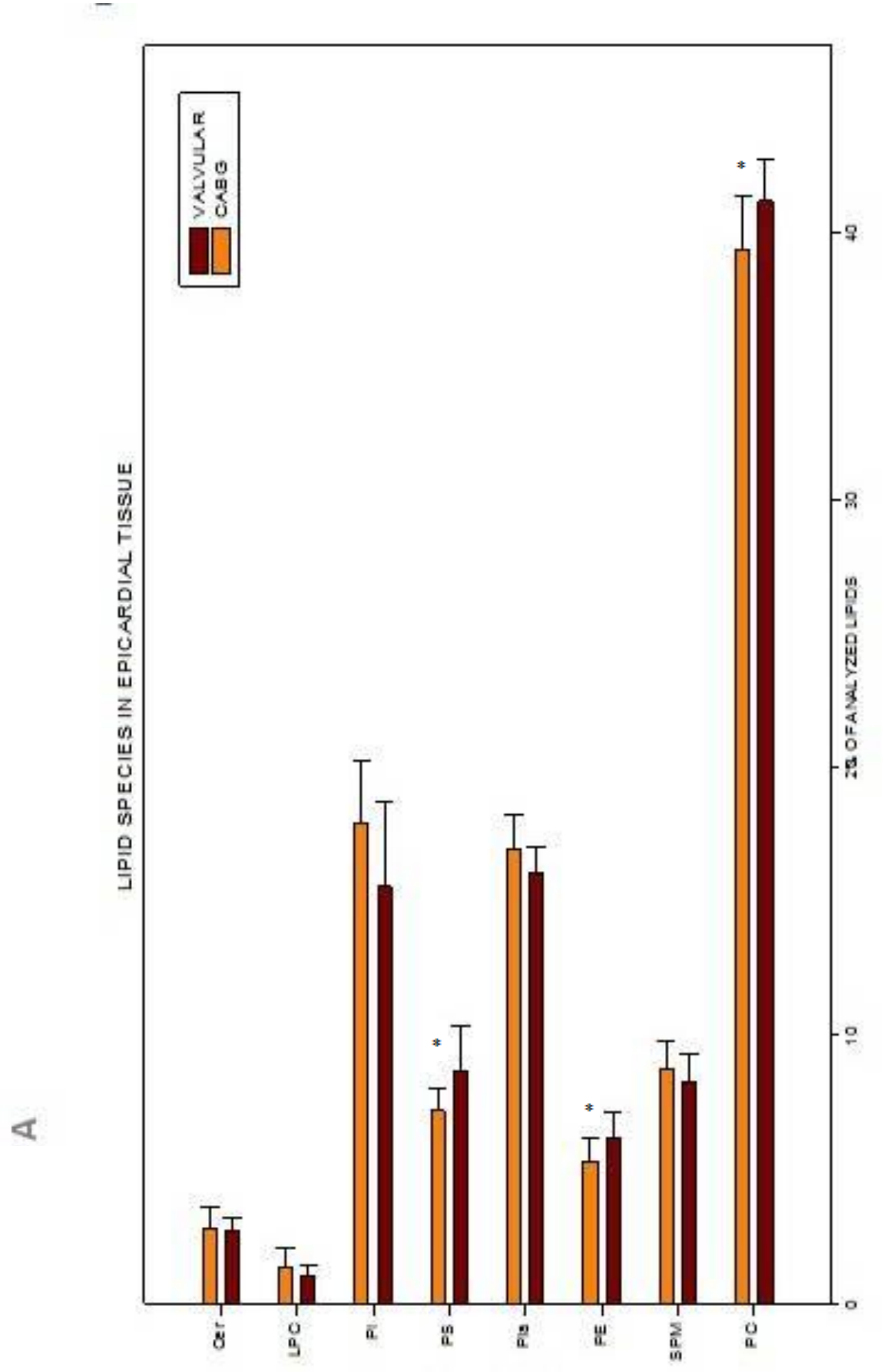
A
LIPID SPECIES IN EPICARDIAL TISSUE
VALVULAR
CABG
Cer
LPC
PI
PS
PIs
PE
SPM
PC
0
10
30
40
% OF ANALYZED LIPIDS

FIGURE 14 A

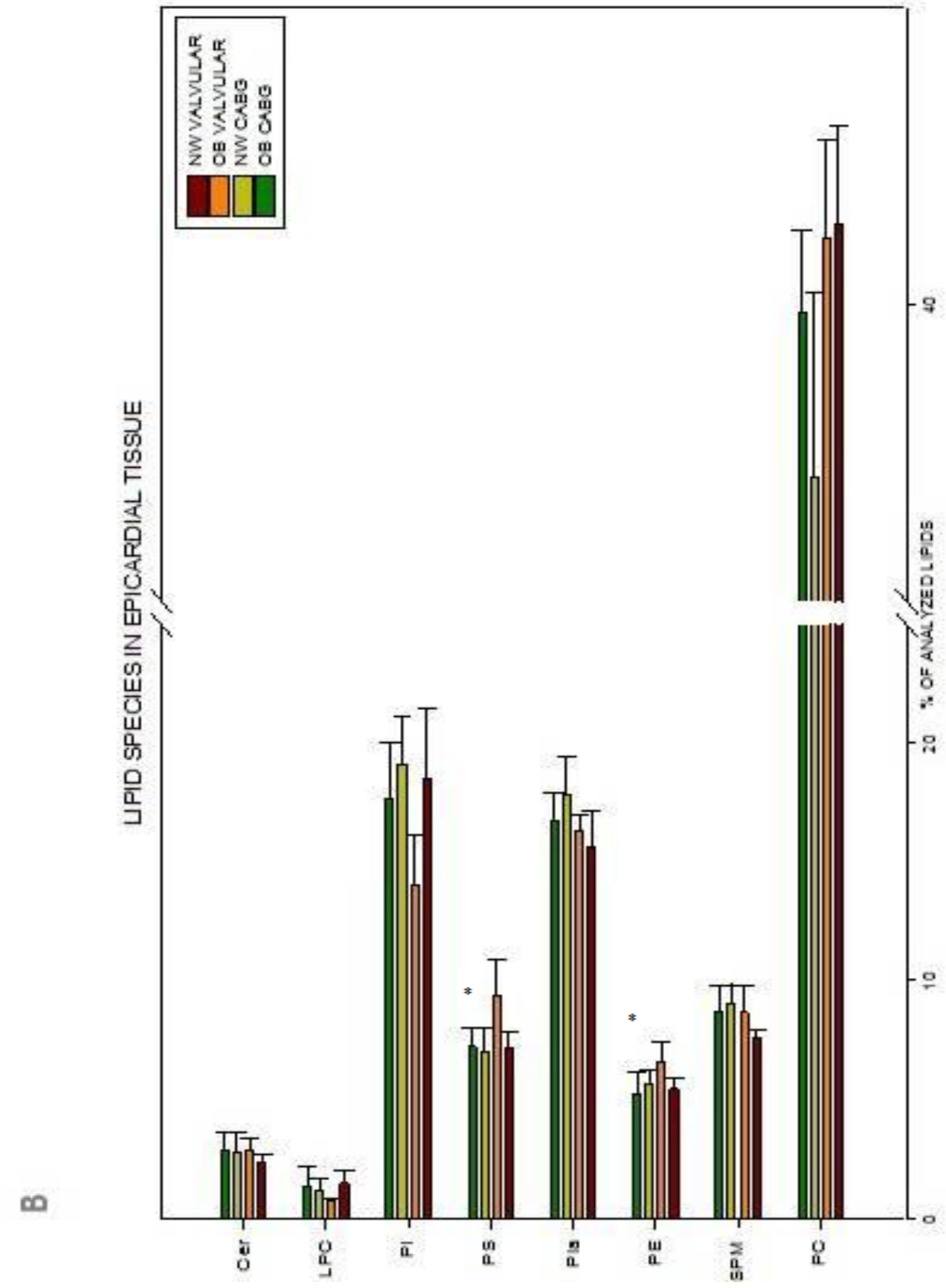
B
LIPID SPECIES IN EPICARDIAL TISSUE
NW VALVULAR
OB VALVULAR
NW CABG
OB CABG
% OF ANALYZED LIPIDS
0
10
20
40
Cer
LPC
PI
PS
Pls
PE
SPM
PC
*
*

FIGURE 14 B

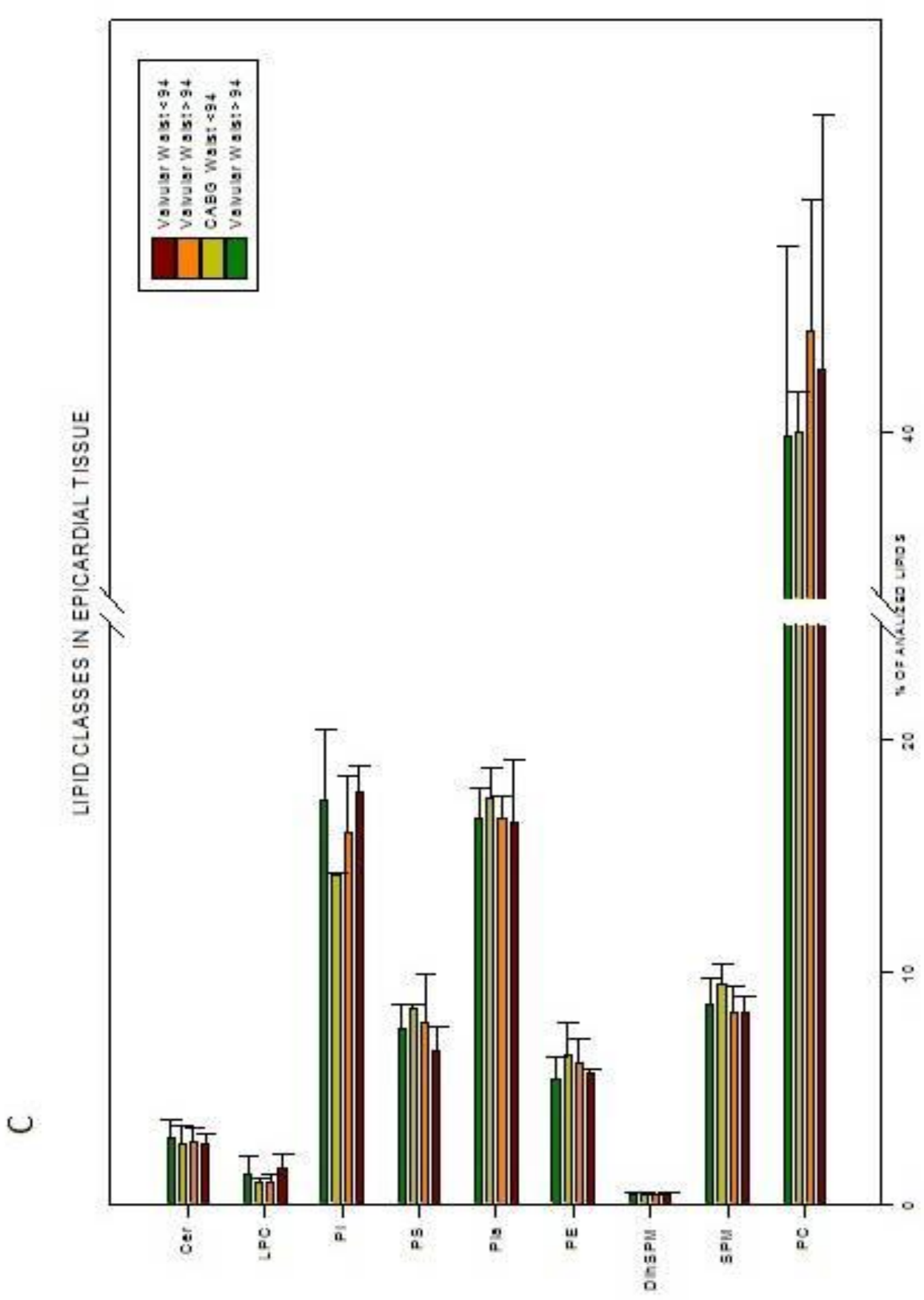
C
LIPID CLASSES IN EPICARDIAL TISSUE
Valvular Was:<94
Valvular Was:>94
CABG Was:<94
Valvular Was:>94
Cer
LPC
PI
PS
PE
DmSPM
SPM
PC
% OF ANALYZED LIPIDS
0
10
20
40

FIGURE 14 C

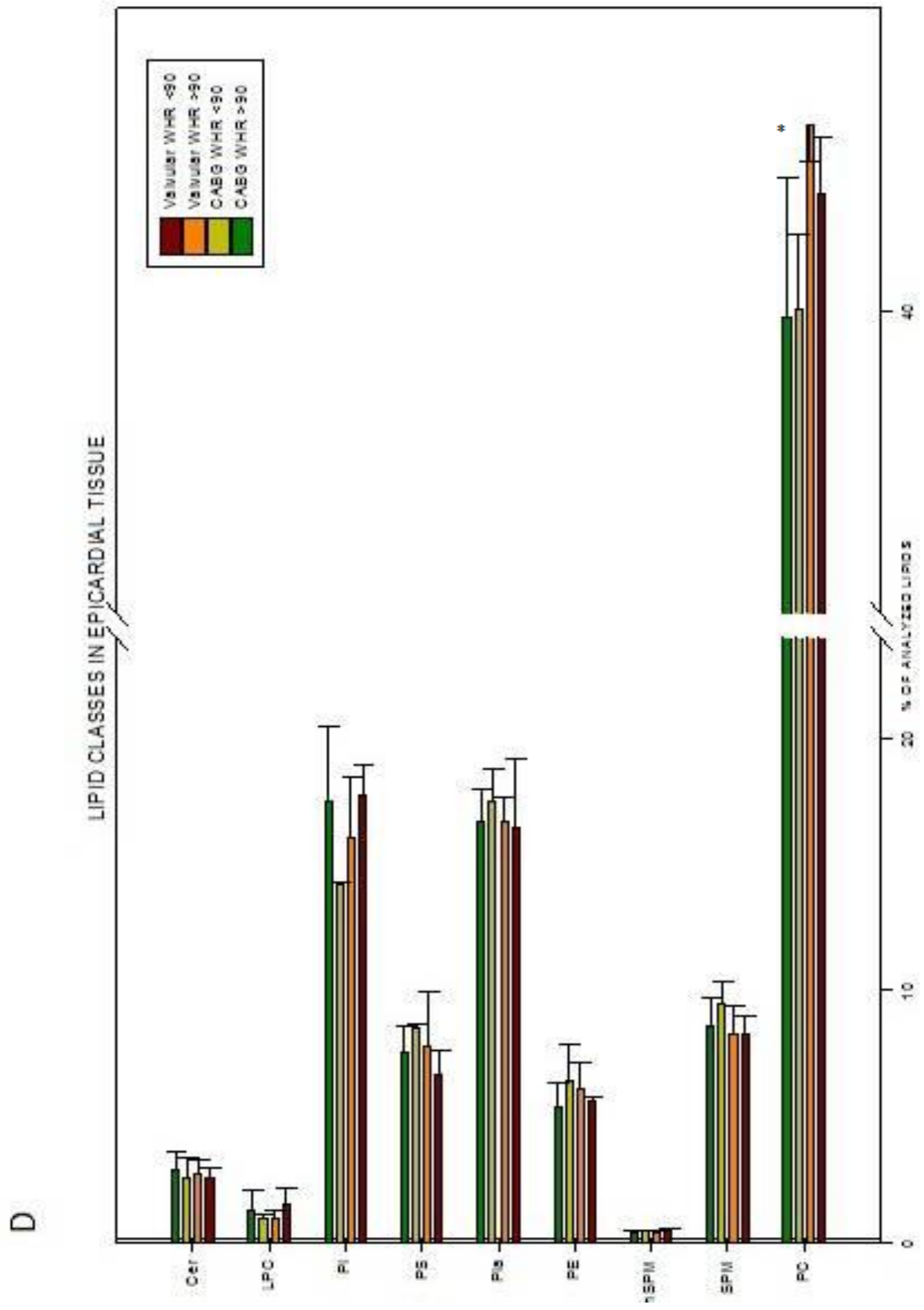
D
LIPID CLASSES IN EPICARDIAL TISSUE
Valvular WHR <90
Valvular WHR >90
CABG WHR <90
CABG WHR >90
Cer
LPC
PI
PS
PE
SPM
PC
*
0
10
20
40

FIGURE 14 D

LIPIDS SPECIES IN TISSUE (EPICARDIAL) VALVULAR VS CABG DIPENDENTE CON SOTTOGRUPPI

Species	BMI<30 Valvular	Standard Deviation	BMI>30 Valvular	Standard Deviation	BMI<30 CABG	Standard Deviation	BMI>30 CABG	Standard Deviation	Waist <94 Valvular	Standard Deviation	Waist >94 Valvular	Standard Deviation	Waist <94 CABG	Standard Deviation	Waist >94 CABG	Standard Deviation	WHR<90 Valvular	Standard Deviation	WHR>90 Valvular	Standard Deviation	WHR<90 CABG	Standard Deviation	WHR>90 CABG	Standard Deviation
PC 32:0	51,12	12,55	76,31	34,18	42,32	18,48	55,69	11,71	55,42	14,29	75,96	33,83	38,91	2,36	54,03	13,54	55,81	14,68	76,35	34,22	61,98	14,25	51,73	13,60
PC 32:1	35,75	7,90	28,64	7,65	21,52	0,62	25,70	10,63	40,30	0,67	28,64	7,65	24,66	4,86	25,02	9,85	40,69	1,06	28,03	8,04	21,21	1,33	25,36	9,83
PC 34:1	129,01	30,72	160,88	29,97	116,12	24,27	132,74	24,49	146,38	8,72	160,88	29,97	101,65	0,40	130,60	25,05	146,77	9,11	161,27	30,36	125,36	18,67	128,23	26,24
PC 38:1	1,26	1,14	2,78	3,05	3,21	0,47	1,66	1,60	1,11	1,57	2,78	3,05	1,81	2,56	1,95	1,50	1,50	1,96	3,17	3,44	3,42	0,99	1,79	1,52
PC 32:2	3,44	0,43	3,90	0,96	2,44	0,51	2,75	0,70	3,35	0,57	3,90	0,96	2,92	0,14	2,71	0,71	3,74	0,96	4,29	1,35	2,76	0,17	2,73	0,71
PC 34:2	110,74	22,14	143,86	30,31	99,94	48,66	111,93	25,17	113,97	30,30	143,86	30,31	124,14	27,95	107,93	29,45	114,36	30,69	144,25	30,70	119,75	45,89	108,37	28,46
PC 36:2	79,45	17,86	106,35	21,70	70,71	29,72	81,01	20,12	89,71	2,72	106,35	21,70	74,74	6,42	78,47	22,70	90,10	3,11	106,74	22,09	103,19	35,08	75,63	19,53
PC 38:2	0,00	0,00	3,50	1,70	3,21	0,99	1,77	1,83	0,00	0,00	3,50	1,70	0,63	0,89	2,08	1,82	0,39	0,39	3,89	2,09	4,34	0,73	1,71	1,69
PC 34:3	5,45	0,74	6,05	0,61	4,68	1,17	4,61	1,31	5,77	0,70	6,05	0,61	4,96	0,84	4,59	1,27	6,16	1,09	6,44	1,00	4,75	1,24	4,62	1,26
PC 36:3	39,20	12,66	42,36	7,86	27,47	9,16	33,80	7,08	46,48	1,59	42,36	7,86	36,50	2,09	31,80	7,94	46,87	1,98	42,75	8,25	34,85	5,07	31,97	7,96
PC 38:3	3,00	0,85	6,66	3,23	1,52	0,21	4,96	1,97	2,58	0,60	6,66	3,23	6,16	0,99	4,10	2,21	2,97	0,99	7,05	3,62	3,12	2,12	4,40	2,22
PC 36:4	58,97	5,46	92,19	26,31	43,48	16,95	52,57	14,60	62,13	0,07	92,19	26,31	43,18	5,12	51,69	15,19	62,52	0,46	92,58	26,70	40,87	7,54	51,93	14,98
PC 38:4	27,45	7,44	50,12	23,27	21,31	11,34	25,34	6,82	30,16	8,14	50,12	23,27	16,68	0,07	25,23	7,46	30,55	8,53	50,51	23,66	23,91	4,05	24,51	7,86
PC 40:4	1,43	0,23	2,19	0,73	0,95	1,13	0,97	0,61	1,31	0,14	2,19	0,73	0,43	0,61	1,06	0,70	1,70	0,53	2,58	1,12	1,62	0,14	0,94	0,70
PC 36:5	4,82	1,72	5,68	1,23	3,85	0,56	4,28	1,45	5,23	2,22	5,68	1,23	3,00	0,05	4,30	1,30	5,62	2,61	6,07	1,62	4,23	0,28	4,18	1,36
PC 38:5	17,97	4,62	24,19	7,06	11,99	4,45	13,59	3,24	20,19	3,59	24,19	7,06	9,83	1,87	13,64	3,29	20,58	3,98	24,58	7,45	12,47	1,79	13,38	3,49
PC 38:6	6,43	2,05	10,82	3,87	5,22	2,80	6,61	1,99	7,54	1,07	10,82	3,87	4,81	1,63	6,39	2,18	7,93	1,46	11,21	4,26	6,04	0,39	6,27	2,26
PC 40:6	3,15	0,77	4,17	1,44	1,78	0,58	2,53	0,58	2,92	0,93	4,17	1,44	1,80	0,57	2,50	0,66	3,31	1,32	4,56	1,83	2,31	0,87	2,45	0,68
PE 32:0	0,81	0,31	1,03	0,25	0,90	0,40	0,79	0,16	0,87	0,42	1,03	0,25	0,66	0,12	0,82	0,22	1,26	0,81	1,42	0,64	0,86	0,16	0,80	0,22
PE 34:1	7,94	1,51	10,06	2,85	8,29	1,79	6,83	1,78	8,74	0,82	10,06	2,85	6,30	0,52	7,15	1,86	9,13	1,21	10,45	3,24	6,34	0,30	7,15	1,87
PE 36:1	5,39	1,02	10,11	2,85	7,75	1,43	6,41	1,29	5,92	0,63	10,11	2,85	6,40	0,45	6,57	1,51	6,31	1,02	10,50	3,24	7,98	0,54	6,42	1,43
PE 36:2	8,98	1,82	14,81	3,17	10,80	3,33	9,49	2,75	9,90	1,22	14,81	3,17	11,55	0,30	9,43	2,88	10,29	1,61	15,20	3,56	10,94	0,68	9,49	2,92
PE 38:2	1,09	0,12	1,58	0,18	1,34	0,26	1,39	0,37	1,15	0,07	1,58	0,18	1,06	0,12	1,39	0,35	1,54	0,46	1,97	0,57	1,65	0,31	1,33	0,34
PE 36:3	2,78	0,67	3,68	0,62	2,69	1,00	2,54	0,89	3,15	0,29	3,68	0,62	3,21	0,12	2,48	0,89	3,54	0,68	4,07	1,01	2,95	0,21	2,50	0,91
PE 36:4	4,55	0,62	7,01	2,36	4,27	1,94	4,01	1,54	4,74	0,75	7,01	2,36	4,36	1,11	4,03	1,59	5,13	1,14	7,40	2,75	3,97	0,21	4,07	1,61
PE 38:4	24,56	5,75	51,27	26,58	23,68	11,91	24,79	9,04	27,76	2,16	51,27	26,58	23,12	3,59	24,40	9,63	28,15	2,55	51,66	26,97	20,34	4,90	24,68	9,51
PE 40:4	3,16	0,55	5,86	3,50	2,86	1,21	3,05	0,76	3,48	0,08	5,86	3,50	2,43	0,64	3,05	0,82	3,87	0,47	6,25	3,89	2,64	0,70	3,03	0,84
PE 36:5	0,72	0,17	1,01	0,27	0,73	0,15	0,79	0,18	0,69	0,23	1,01	0,27	0,82	0,32	0,77	0,16	1,08	0,62	1,40	0,66	0,78	0,17	0,78	0,17
PE 38:5	5,72	2,33	8,82	2,11	5,57	2,90	5,32	1,50	7,05	0,61	8,82	2,11	5,37	0,00	5,25	1,87	7,44	1,00	9,21	2,50	4,84	0,91	5,30	1,85
PE 40:5	2,80	0,76	5,06	2,47	3,10	1,57	2,75	0,85	3,21	0,36	5,06	2,47	2,12	1,18	2,85	0,96	3,60	0,75	5,45	2,86	3,37	1,32	2,72	0,96
PE 38:6	2,15	0,71	3,46	1,38	2,41	1,18	2,26	0,62	2,51	0,45	3,46	1,38	2,20	0,68	2,25	0,75	2,90	0,84	3,85	1,77	3,13	0,71	2,16	0,68
PE 40:6	3,41	1,44	5,88	2,97	2,63	1,40	3,54	1,31	3,99	1,44	5,88	2,97	2,74	1,03	3,37	1,37	4,38	1,83	6,27	3,36	4,44	1,48	3,20	1,31
PE 42:6	0,29	0,51	0,88	0,45	0,55	0,64	0,51	0,51	0,00	0,00	0,88	0,45	0,52	0,73	0,54	0,51	0,39	0,39	1,27	0,84	0,95	0,03	0,49	0,52
PE 42:7	0,99	0,21	1,36	0,08	1,14	0,17	1,17	0,14	0,94	0,26	1,36	0,08	1,10	0,05	1,17	0,15	1,33	0,65	1,75	0,47	1,18	0,10	1,16	0,15
PS 36:1	62,48	20,01	108,45	34,93	66,48	26,34	69,39	15,21	72,96	11,91	108,45	34,93	58,24	15,21	68,53	17,91	73,35	12,30	108,84	35,32	63,99	13,69	67,95	18,22
PS 38:4	5,30	4,72	15,61	5,16	7,76	9,32	7,90	6,95	4,53	6,41	15,61	5,16	5,38	7,60	8,07	7,12	4,92	6,80	16,00	5,55	3,80	5,38	8,23	7,15

TAB. 2 lipid species in epicardial adipose tissue BMI, Waist and WHR dependent.

GLICEROPHOSPHOLIPIDS PROFILE IN PLASMA

PC, PE, PS, Sphingomyelin (SPM), Dihydrosphingomyelin (DihSPM), PE-based Plasmalogens (Pla), Phosphatidylinositol (PI), Lysophosphatidylcholine (LPC) and Ceramide (Cer) were not statistically modified in CABG and valvular patients. Lipid species profile showed (Figure 15) a lower concentration of PC species, such as PC 32:0 ($p=0.04$), 34:2 ($p=0.01$) and 36:2 ($p=0.02$) in CABG compared to valvular, while, PE 36:1 was higher in CABG compared to valvular ($p=0.008$) (Figure 16). CABG and valvular plasma samples if divided in subgroups after BMI classification showed that PC, PE, PS, SPM, DihSPM, Pla, PI, LPC and Cer classes were not statistically modified in CABG and valvular patients (Figure 17). Lipid species analyzed in plasma after BMI classification showed no significant difference in PE 36:1 specie between both surgical treatment after subdivision in normalweight and obese. Similarly PC 34:2 and 36:2 species after subgroup divisions not showed difference in concentration values in CABG compared to valvular. While PC 32:0 specie showed lower concentration level in CABG obese compared to valvular obese patients ($p=0.03$).

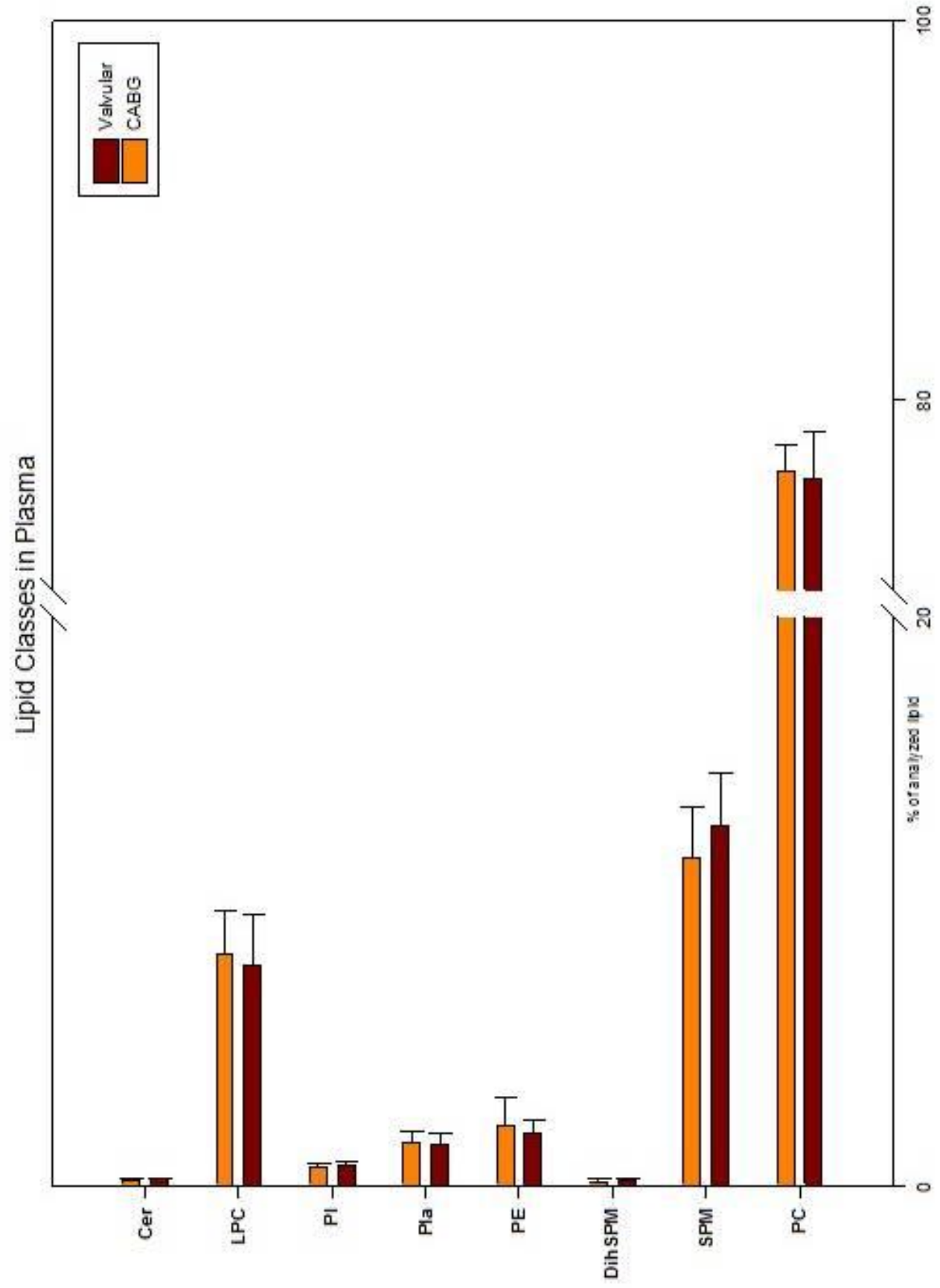

FIGURE 15: Lipids in plasma samples

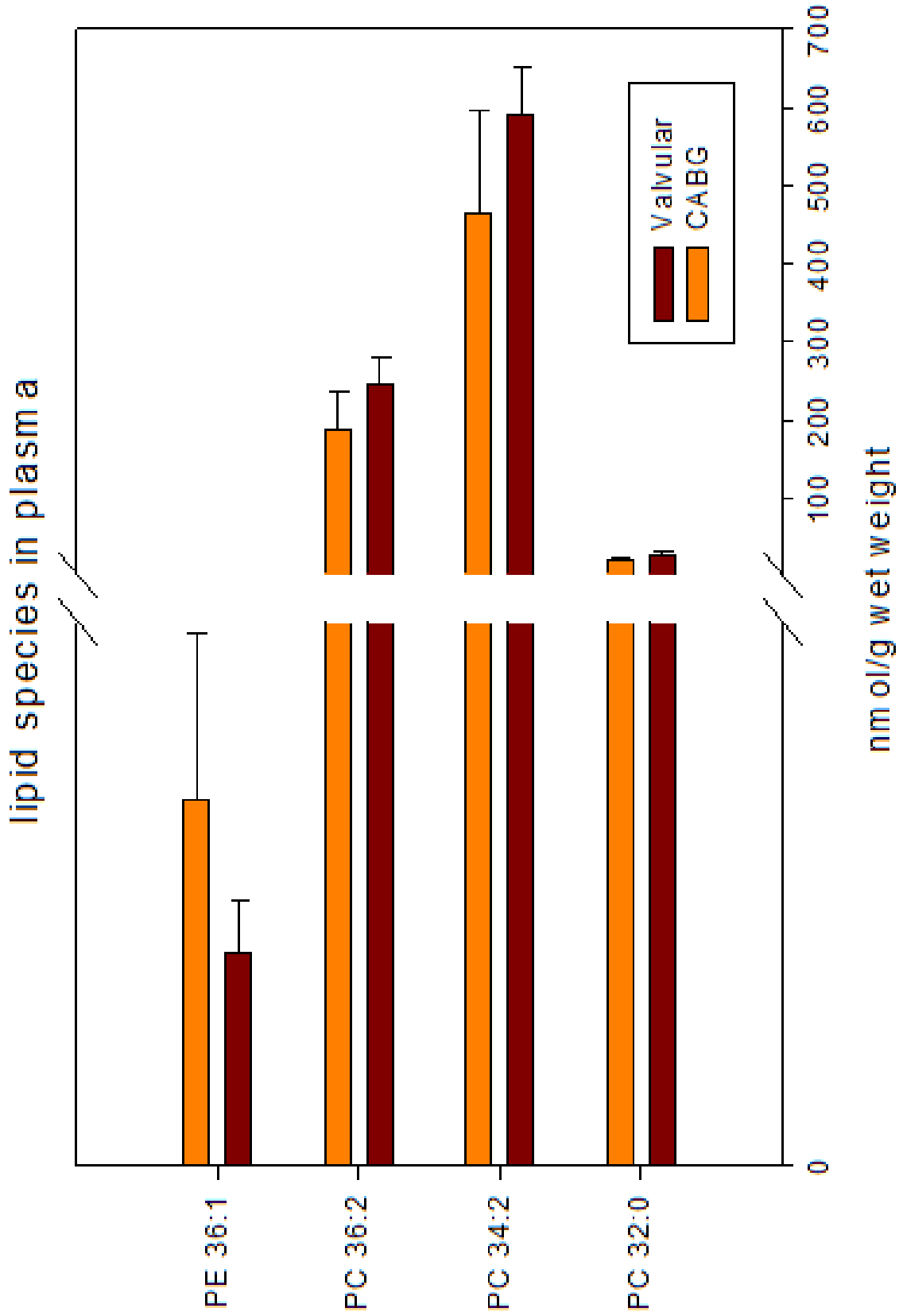

FIGURE 16: Lipid species in plasma samples

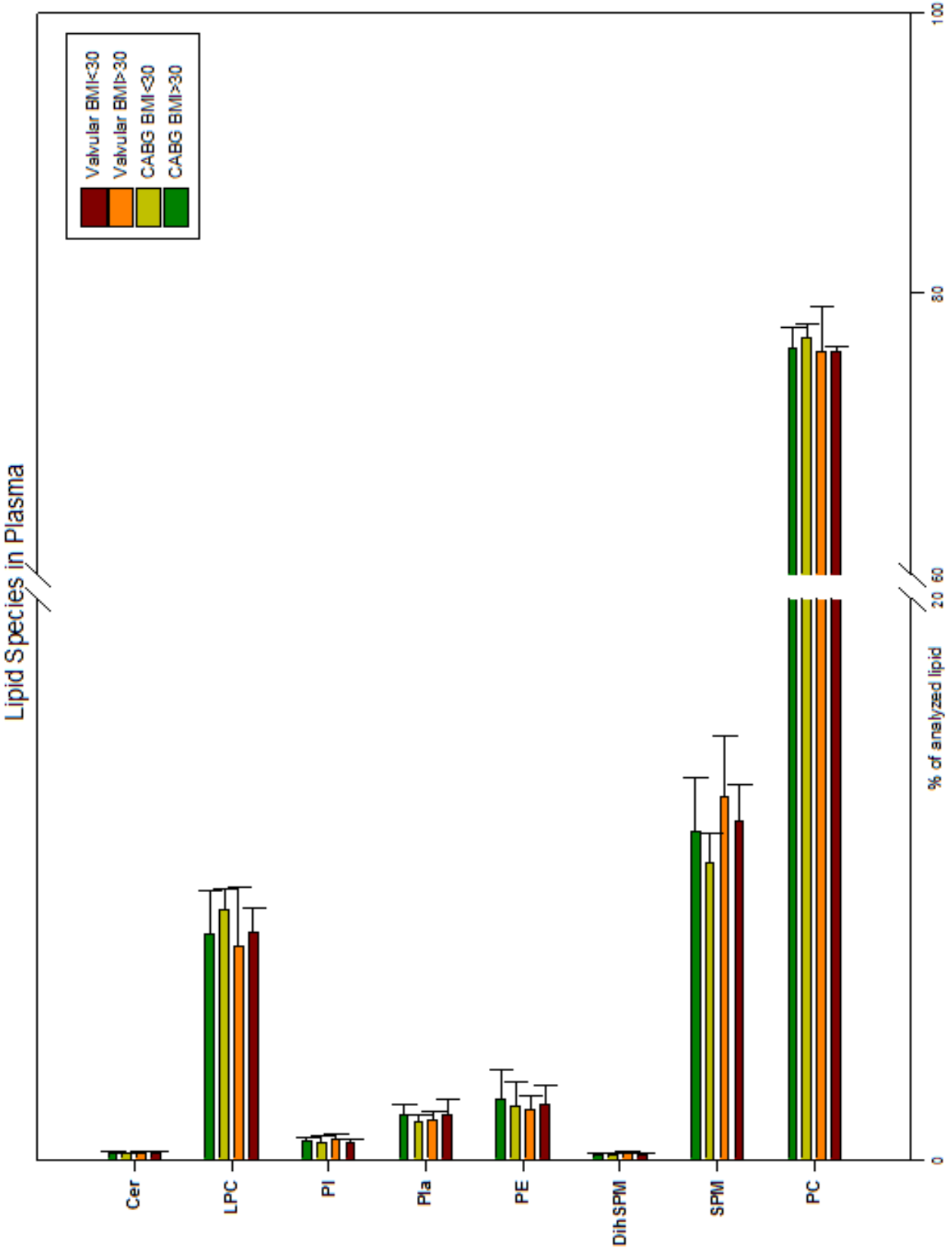

FIGURE 17: Lipid in plasma samples BMI dependent

ANGIOGENETIC GENES IN EPICARDIAL ADIPOSE TISSUE

Transcriptomic data from epicardial tissue (Table 3), showed an up- or down-regulation of genes involved in angiogenesis, both pro- and anti-genetic, such as matrix metalloproteinase 2 and 9 (MMP-2 and MMP-9), Vascular endothelial growth factor (VEGF), Fibroblast growth factors (FGF), angiogenin (ANG) and tissue inhibitor of metalloproteinases (TIMP).

MMP-2 and MMP-9 were highly expressed in CABG compared to valvular (129 and 73 folds respectively). The same for Hepatocyte growth factor (HGF), VEGFA, FGF-2, Hypoxia-inducible factors-1 (HIF-1) and TIMP-1 in which expression were 170.55, 34.25, 28, 46.3, and 138.74 folds respectively. While ANG and endostatin (COL18A-1) genes had lower expression in CABG compared to valvular (-13.41 and -5.2 folds respectively). For Delta-5 and delta-6 desaturases, FADS1 and FADS2, instead was in CABG down express (-15 and -35 respectively) compared to valvular patients.

TAB. 3 Angiogenetic Genes

GENES	FC CABG VS VALVULAR	PRO-ANGIOGENETIC	INHIBITOR OF ANGIOGENESIS
MMP-2	129	X	
MMP-9	73	X	
HGF	170,55	X	
VEGFA	34,25	X	
FGF-2	28	X	
ANG	-13,41		X
HIF-1	46,3	X	
TIMP-1	138,74		X
COL18A-1	-5,16108509		X

GENES INVOLVED IN CELL DIFFERENTIATION

Trascriptomic data (Table 4) showed that peroxisome proliferator-activated receptor gamma (PPAR gamma; -5.8 folds), delta-like 1 homolog (DLK; -36.08 folds), adiponectin (adipoQ; -19.19 folds), activin A receptor, type I (ACVR1; -45.37 folds), fatty acid binding protein 4 (FABP4; -33.58 folds), cytoplasmic polyadenylation element binding protein 1 (CEBP; -25.20 folds), lipin 1 (LPIN1; -42,94 folds), lipoprotein lipase (LPL; -18.86 folds) and phosphoenolpyruvate carboxykinase 1 (PCK1; -44.95) were down regulate in CABG compared to valvular. While leptin (LEP; 27 folds), GATA binding protein 2 (7.7 folds), GATA binding protein 3 (75 folds) uncoupling protein (UCP-1; 112.05, UCP-2; 246.35 and UCP-3; 43.29 folds), complement factor D preproprotein (CFD; 47.88 folds), proteoglycan 4 (PRG4; 142.26 folds), solute carrier family 2 (facilitated glucose transporter), member 4 (SLC2a4; -22.24 folds), Glyceraldehyde 3 phosphate dehydrogenase 1 (GAPDH; 46.44 folds), resistin (RETN; 152.36 folds) and mesoderm specific transcript homolog (MEST; 71.77 folds) were highly expressed in CABG compared to valvular

TAB. 4 Genes involved in cells differentiation

Genes	Tissues predominance
PPPARG	VALV
LEP	CABG
GATA2	CABG
GATA3	CABG
DLK1	VALV
UCP1	CABG
UCP2	CABG
UCP3	CABG
ADIPOQ	VALV
CFD	CABG
ACVR1C	VALV
FABP4	VALV
CEBPA	VALV
CEBPB	CABG
PRG4	CABG
SLC2a4	VALV
GAPDH	CABG
LPIN1	VALV
LPL	VALV
PCK1	VALV
RETN	CABG
MEST	CABG

IL-18, IL-18 R1 AND IL-18-RAP GENE EXPRESSION LEVELS IN EAT

A great increase in the expression of all of the genes evaluated was observed in CABG compared to valvular patients. In particular, a 77 fold increase was observed for IL-18 gene, a 31 for IL-18 R1 and a 242 for IL-18-RAP.

DISCUSSION

Patients who underwent CABG showed a decrease concentration in PUFA compared to patients undergoing valvular replacement and utilized as negative-CAD controls. PUFA/saturated fatty acid (SFA) ratio was statistically lower in CABG patient compared to valvular patients. In literature has been known that the omega-6 fatty acid, linoleic acid (LA) is the most abundant dietary PUFA. The results of prospective cohort studies, examining the relationships between PUFA intake and the risk of coronary heart disease (CHD), have been inconsistent (Kris-Etherton, Hecker et al. 2004). More other authors underlines that higher PUFA and LA intakes are associated with significant reductions in CHD risk and a higher ratio of PUFA to SFA intake was associated with lower CHD risk (Shekelle, Shryock et al. 1981; Ascherio, Rimm et al. 1996; Oh, Hu et al. 2005) or cardiovascular-related mortality (Laaksonen, Nyyssonen et al. 2005). In addiction our results showed that percentage of oleic acid (18:1) was higher in CABG compared to valvular replacement patients while percentage of linoleic (18:2) was lower. Results from our study showed a down regulation of fatty acid desaturase 1 and 2 (FADS1 and FADS2) genes in CABG compared to valvular patients. PUFAs can affect membrane fluidity and cholesterol content, and influence the generation of signaling molecules (Bazinet, Douglas et al. 2004). They are also processed to powerful promoters of inflammation called eicosanoids (Calder 2005). PUFA levels in phospholipids are determined by both nutrition and metabolism (Di Stasi, Bernasconi et al. 2004; Emken, Adlof et al. 2004; Calder 2005). The key enzymes in PUFA metabolism are Delta -5 desaturase (D5D) and D6D, which are encoded by FADS1 and FADS2 genes, respectively. They are enzymes that catalyze the conversion of linoleic acid into arachidonic acid and α-linoleic acid into eicosapentanoic acid (Narce, Asdrubal et al. 1994). Kwak et al showed that alteration in FADS1 and FADS2 genes contribute to increase level of oleic acid, fatty acid considered cardiovascular risk factor (Kwak, Paik et al.). In a study by Blankenhorn it was found that increased intake of oleic acid significantly increased the risk of new atherosclerotic lesion in human coronary arterias (Blankenhorn, Johnson et al. 1990; Topaz, Mackall et al. 1992).

Various FADS alleles were associated with high concentration of arachidonic acid and low concentration of linoleic acid. Differences in desaturase activity may affect the availability of eicosanoid precursor which may conditioned the vascular response to an inflammatory damage preparing vascular wall to atherosclerotic damage (Martinelli, Girelli et al. 2008). Deficiency of linoleic acid derived exclusively from the dietary source and may also contribute to the vascular disease. Several study underline that a reduction of this fatty acid is followed by an increase of atherosclerosis (Smith 1960; Djousse, Pankow et al. 2001; Baylin and Campos 2004) and its deficiency could promote excessive oxidative stress via mitochondria (Bernal-Mizrachi, Gates et al. 2005). Glycerophospholipid analysis revealed lower levels of phosphatidylethanolamine (PE), phosphatidylcholine (PC) and phosphatidylserine (PS) in CABG patients compared to valvular patients. Choline is an important nutrient throughout life. It is the precursor for the acetylcholine and for phosphatidylcholine (Zeisel and Blusztajn 1994). Several study presented that low choline diet could lead pathological problems in humans (Food and nutrition board, 2000). So phosphatidylcholine supplementation could decrease the risk to develop diseases such as cardiovascular pathology (Olthof, Brink et al. 2005). In animal tissues, PE is especially important in the sarcolemmal membranes of the heart during ischemia (Iliskovic, Panagia et al. 1997; Singal, Iliskovic et al. 1997), it is involved in secretion of the nascent very-low-density lipoproteins from liver (Agren, Kurvinen et al. 2005). In addition, it donates the ethanolamine component in the biosynthesis of the glycosylphosphatidylinositol anchors for many cell-surface proteins (Lehto and Sharom 1998). Lower statistically significant lipid concentration was visible in CABG compared to valvular for PC species, such as PC 36:2, 34:3, 36:3, 36:4, 38:4, 40:4, 36:5, 38:5, 38:6 and 40:6. Similarly lipid composition for PE species was for PE, 34:1, 36:1, 36:2, 36:3, 36:4, 38:4, 40:4, 38:5, 40:5 and 40:6 statistically lower in CABG compared to valvular. While, for PS, only one lipid species, PS 36:1 was statistically lower in CABG compared to valvular.

EAT lipid species analysis, also after subdivision for BMI, waist, waist-to-hip ratio (WHR), showed a decrease of lipid species of PC, PE and PS in CABG patients compared to valvular patient particularly with higher BMI, waist and WHR value. Change in lipid composition was studied from researchers in patients subdivide for BMI, the results obtained underline that, increasing BMI values increased cardiovascular risks and lipids profile (Aziz, Siddiqui et al. 2003). Obesity is a pre-disease condition that induces pathological angiogenesis and impaired vascular functions. These changes lead to the outset, development and progression of many diseases such as, cancer, CVD, diabetic complication and chronic inflammation (Cao). The role of angiogenesis in atherosclerosis and other cardiovascular diseases has emerged as a major unresolved issue. Angiogenesis has attracted interest from opposite perspectives. Angiogenic cytokine therapy has been widely regarded as an attractive approach both for treating ischemic heart disease and for enhancing arterioprotective functions of the endothelium (Zachary and Morgan; Isner 1996); conversely, a variety of studies suggest that neovascularization contributes to the growth of atherosclerotic lesions and is a key factor in plaque destabilization leading to rupture (Khurana, Simons et al. 2005). Transcriptomic data from EAT, showed an up- or down-regulation of genes involved in angiogenesis, both pro- and anti-genetic, such as matrix metalloproteinase 2 and 9 (MMP-2 and MMP-9), vascular endothelial growth factor (VEGF), fibroblast growth factors (FGF), angiogenin (ANG) and tissue inhibitor of metalloproteinases (TIMP). MMP-2 and MMP-9 were highly expressed in CABG compared to valvular (129 and 73 folds respectively). The same was observed for hepatocyte growth factor (HGF), VEGFA, FGF-2, hypoxia-inducible factors-1 (HIF-1) and TIMP-1, for these genes fold increase values were 170.55, 34.25, 28, 46.3, and 138.74 respectively. Otherwise, ANG and endostatin (COL18A-1) genes had lower expression in CABG compared to valvular (-13.41 and -5.2 folds respectively). Trascriptomic data showed, for gene involved in adipocyte differentiation that, peroxisome proliferator-activated receptor gamma (PPARγ; -5.8 folds), delta-like 1 homolog (DLK; -36.08 folds), adiponectin (adipoQ; -19.19 folds), activin A receptor, type I (ACVR1; -45.37 folds),

fatty acid binding protein 4 (FABP4; -33.58 folds), cytoplasmic polyadenylation element binding protein 1 (CEBP; -25.20 folds), lipin 1 (LPIN1; -42,94 folds), lipoprotein lipase (LPL; -18.86 folds) and phosphoenolpyruvate carboxykinase 1 (PCK1; -44.95) were down regulate in CABG compared to valvular. Most clinical studies suggested vascular protective effects of PPARγ ligands related to improved insulin-sensitivity, decreased vascular and systemic markers of inflammation, reduced carotid wall thickness and neointima formation (Verges 2004). PPARγ is expressed in all cell types of the arterial wall, including endothelial cells, monocytes/macrophages and vascular smooth muscle cells (SMCs) (Marx, Libby et al. 2001) and besides its activity on macrophage lipid homeostasis with direct consequences for atherosclerosis development (Chinetti, Lestavel et al. 2001), PPARγ modulates the earliest step of the atherosclerotic lesion by inhibiting the expression of certain cytokines involved in the recruitment of monocytes/macrophages by endothelial cells (Li, Binder et al. 2004). Through these properties, PPARγ may exert cardiopreventive activities by decreasing foam cell formation. Adipose tissue has emerged as a metabolically active participant in mediating vascular complications, serving as an active endocrine and paracrine organ secreting adipokines, which participate in diverse metabolic processes. Among these adipokines is adiponectin, which seems to possess antiatherogenic and anti-inflammatory effects and may be protective against cardiovascular disease development (Szmitko, Teoh et al. 2007). Leptin (LEP; 27 folds), GATA binding protein 2 (7.7 folds), GATA binding protein 3 (75 folds) uncoupling protein (UCP-1; 112.05, UCP-2; 246.35 and UCP-3; 43.29 folds), complement factor D preproprotein (CFD; 47.88 folds), proteoglycan 4 (PRG4; 142.26 folds), solute carrier family 2 (facilitated glucose transporter), member 4 (SLC2a4; -22.24 folds), Glyceraldehyde 3 phosphate dehydrogenase 1 (GAPDH; 46.44 folds), resistin (RETN; 152.36 folds) and mesoderm specific transcript homolog (MEST; 71.77 folds) were highly expressed in CABG compared to valvular. High leptin levels are independently associated with cardiovascular disease (Romero-Corral, Sierra-Johnson et al. 2008). Adipocyte size has been associated to increase in inflammatory cytokines expression that can be related to the

cardiovascular risk of obesity. Epicardial adipose tissue (EAT) was discovered to play a key role in cardiovascular diseases by producing several inflammatory adipokines (Eiras, Teijeira-Fernandez et al.). Larger adipocytes secrete molecules that cause insulin resistance, such as TNF-α and resistin (Flier 2001), and smaller adipocytes secrete molecules that increase insulin sensitivity, such as adiponectin (Yamauchi, Kamon et al. 2001). Previous studies indicated that IL-18 protein release by adipose tissue was about 1000 times lower than other interleukins, like IL-6 and IL-8 (Skurk, van Harmelen et al. 2004). These observations thus may suggest that adipose tissue-derived IL-18 is of minor or no importance to circulating IL-18 levels. However this not excludes the possibility for IL-18 to exert autocrine or paracrine pro-inflammatory effects within the adipose tissue or tissue closely apposed. For this reason we focused our attention on EAT to evaluate whether a different local production of IL-18 by EAT may be observed among the two groups of patients (Dogliotti, Dozio et al. 2011). In CABG patients, we observed not only an increased production of IL-18 but also of its receptor R1 and of IL-18-RAP, an accessory subunit of the heterodimeric receptor for IL-18 which enhances the IL-18 binding activity of IL-18 R1 and is required for the activation of NF-kappaB and MAPK8 (JNK) in response to IL-18 . Although presently we performed only a gene expression study and we did not directly quantify the amount of protein produced by EAT, it appears that in CABG patients a major autocrine paracrine effect of IL-18 may exist. This means that IL-18 may directly affect heart and blood vessels of the heart and may also exert a pro-inflammatory effect within the EAT by promoting the release of other molecules, as already previously indicate. In conclusion as previously reported in literature, decrease in PC species and reductions in percentage of PUFA are associated whit CVDs, down or up expression of studied genes also are connected with heart disease. Our data therefore emphasize a possible greater involvement of lipids in patients with CABG compared to valvular patients

Further investigation will be carried out to analyze the complete lipid profile in both types of surgically collected patients in a higher number of patients, also looking for a possible correlation among lipid composition and EAT thickness measured by echocardiography analysis.

LEGGEND TO FIGURES

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