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Atherosclerosis, statins and fibrates treatment benefits

(Diploma Thesis)

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I declare that this thesis is my original copyrighted work. All literature and other resources I used while processing are listed in the bibliography and properly cited.

Date: *Signature:*

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ABSTRAKT

Cílem diplomové práce je analýza aterosklerózy a její spojitost s kardiovaskulárním onemocněním. Také opatření proti rozvoji aterosklerotické léze a jejími následnými komplikacemi. Budou uvedeny aspekty, jako jsou diagnóza, patogeneze a změna endotelu, a s nimi spojené komplikace a klinické projevy.

Budou analyzovány farmakologické látky/léčiva bránící rozvoji tohoto onemocnění s látkami/léčivy proti dyslipidémii – která je jedním z hlavních rizikových faktorů rozvoje aterosklerózy - je centrem pozornosti tohoto příspěvku je.

V dalších kapitolách bude popsán nový pohled na prevenci a léčbu aterosklerózy používající statiny a fibráty, analýza výhod a toxicity jednotlivých statinů, a také možnosti přínosů a rizik při jejich kombinaci.

ABSTRACT

The goal of this Thesis is to analyze atherosclerosis and its relationship to Cardiovascular Disease, as well as the measures taken to oppose the development of an atherosclerotic lesion and its following complications. Aspects such as Diagnosis, Pathogenesis and alteration of endothelium, as well as complications and clinical manifestations associated to them will be stated.

Pharmacological agents opposing the development of this disease will be analyzed, with agents opposing dyslipidemia - one of the major risk factors of developing atherosclerosis – being the center of this paper's focus.

In the next chapters, new insight on preventing and treating atherosclerosis using statins and fibrates, analysis of individual statins benefits and toxicities as well as possibilities of their combination benefits and risks will be brought to light.

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1 INTRODUCTION

After the industrial revolution (18^{th} to 19^{th} century A.D.), an increase in many asymptomatic diseases was observed, primarily because of population lifestyle changes in the developed countries. Diabetes mellitus, arterial hypertension and atherosclerosis are some examples of diseases whose development is attributed to sedentary lifestyle in a major degree, although other risk factors that will be analyzed later on exist.

This Thesis is focused on atherosclerosis and its development, as well as the treatment benefits with statins and fibrates, two of the most widely used medications for the treatment of the abnormalities leading to this particular lesion.

Atherosclerosis is a chronic vascular disorder developing in the endothelial intima. Many different factors including LDL cholesterol, smooth muscle cells, inflammatory mediators, receptors and calcium promote the building of a waxy and fatty mass called atherosclerotic plaque.

Because of its nature of being an asymptomatic disease, atherosclerosis is usually in an advanced stage when diagnosed, and this is the reason why precaution measurements are the best way to counter this disease. Changes in lifestyle are of critical importance. Smoking cessation, increasing physical exercise, being cautious with lipid intake, body weight management, diabetes management and blood pressure control are some of the major modifiable risk factors that should be taken into account. (1)

Regarding Pharmacotherapy, many different agents may be used including statins, fibrates, vitamin B3, anion exchange resins and other agents that will be analyzed later on in this Thesis.

2 DEFINITION OF ATHEROSCLEROSIS

Atherosclerosis, a word having its origins in the Greek words “athere” (gruel), and “skleros” (hard), is the progressive buildup of cholesterol, inflammatory mediator cells, calcium, smooth muscle cells (SMC’s) and other substances in the endothelium of middle and large-sized arteries, causing a gradual hardening of the arteries, which in turn causes many different complications. These complications usually include Acute Myocardial Infarction, Ischemic Stroke and Peripheral Artery Disease. All of these complications arise from the complete occlusion of an artery due to elevated levels of the above-mentioned factors (lipids, SMC’s, calcium), which cause the formation of a thrombus. (2)

3 HISTORY OF ATHEROSCLEROSIS

Despite its popularity as a modern lifestyle disease, research has shown that Atherosclerosis was a common health issue for more than 4000 thousand years. Despite the fact that treatment for Atherosclerosis was available prior to statin discovery, there is no solid evidence of its effectiveness. Due to its nature, being an asymptomatic disease, the diagnosis usually took place after a complication had already taken place, or after the subject was dead, making it extremely inefficient. As Endo *et al* reported, atherosclerosis started to become clinically significant for modern medicine in 1910, when Windaus first connected elevated cholesterol to the development of atherosclerotic lesions. In 1913, Anitschkow conducted experiments on animals (rabbits), feeding them foods rich in cholesterol. The result was the development of fatty deposits in the aortal endothelium, namely atherosclerosis, which led to CVD morbidity and mortality on the rabbits. After that, the matter of atherosclerosis took years to be focused on again, and it was not until 1939 when Müller connected atherosclerotic lesion development risk to genetic predisposition. Later on, many scientists were involved into separating all these parameters following atherosclerosis (Low Density Lipoprotein, cholesterol biosynthesis, enzymatic involvement and drug development), and up to this date research continues to maximize drug efficacy and minimize involvement of risk. (3)

4 EPIDEMIOLOGY

Atherosclerosis usually takes place in the latter stages of life, but the changes in arterial and blood physiology from adolescence and even childhood. The male gender is proved to have a higher risk of developing some type of atherosclerosis during their lifetime compared to females and this is both related to their lifestyle (alcohol abuse) and hormonal physiology (testosterone vs. estrogen). (4)

5 ENDOTHELIUM

In 1865, Wilhem His first introduced the term "endothelium", defining it as the cellular lining covering blood vessels, lymphatic vessels and mesothelial cavities. Later on, by the time electron microscope was introduced, a distinction regarding the vascular bed lining became possible. It was stated that the cells lining individual vascular trees were different in both structure and function from organ to organ and from cavity to cavity. (5)

5.1 Endothelial physiology

The endothelial physiology plays an important role in many functions of an organism, such as inflammation, oxygen supply, synthesis and migration of cells into the tissue, as well as metabolism of many different substances, according to the stimulus it receives. Vasomotor functions (vasodilatation, vasoconstriction) are regulated by the endothelium, through the production of certain substances. Endothelium is also responsible for many hemodynamic effects, (blood pressure and blood flow) regulated through vasodilatation and vasoconstriction, as well as through oncotic and hydrostatic pressures. Regulation of coagulation can also be accomplished through the endothelium, since it is able to produce substances responsible for blood-clotting and blood-thinning. One of the most important parts of the endothelial physiology regarding atherosclerotic lesion development, though, is its ability to maintain an inhibitory activity towards oxidative stress, since, as we will see in the next chapters, is a major cause of its formation through Low Density Lipoprotein (LDL) β-oxidation. (5)

5.2 Endothelial alteration

When altered, this physiology has many effects primarily on cardiovascular disease development and together with other risk factors such as arterial hypertension, Diabetes Mellitus, Dyslipidemia, smoking and sedentary lifestyle, the process is accelerated. In 1973, Bevilacqua et al. suggested that endothelial cell dysfunction is because the endothelium becomes hyperadhesive to platelet cells. Focusing on the endothelial dysfunction relation to atherosclerosis, it is confirmed that its homeostatic functional alterations (vasoconstriction, coagulation, oxidation, inflammation), can cause disease complications such as Acute Myocardial Infarction, (A.M.I.), stroke and other general thromboembolic and metabolic disorders. These alterations start with accumulation of lipids, (primarily low density lipoprotein) and sensitivity to fat oxidation (a process known as oxidative stress). (6)

6 DIAGNOSIS OF ATHEROSCLEROTIC CHANGES

There are several ways to diagnose a potentially dysfunctional endothelium. National Heart, Lung and Blood Institute suggests several ways of diagnosing atherosclerosis. Angiography is a method in which a catheter is inserted in a patient's arm, thigh or neck, infusing a dye. This dye is able to show blood circulation through the vascular tree, and the examination takes place with a chest X-ray. By evaluating the X-ray, the physician can observe any arterial stenosis, of which the most common cause is atherosclerosis. Electrocardiogram (EKG) is another method of diagnosing changes occurring because of cardiac damage. In EKG, measurements of cardiac electrical activity, heart rate and heart rhythm are accomplished painlessly. Abnormal EKG results may indicate damage caused by atherosclerotic vessel stenosis.

Stress echocardiography is a diagnostic method that uses physical heart exercise, or if that is not possible a β1-receptor agonist (Dobutamine), to induce cardiac work. Through echocardiography, a physician can observe cardiac wall abnormalities in rhythm and contractility, as well as abnormal blood flow through the chambers, which may indicate cardiac damage because of stenosis or occlusion. Stress echocardiography is one of the most common methods of diagnosing cardiac abnormalities, because of its non-invasive nature. (7)

Computed Tomography scanning (CT-scan), is a method able to diagnose significantly stenosed or obstructed coronary arteries (see figure 1), resulting in the diagnosis of atherosclerotic lesion. (8)

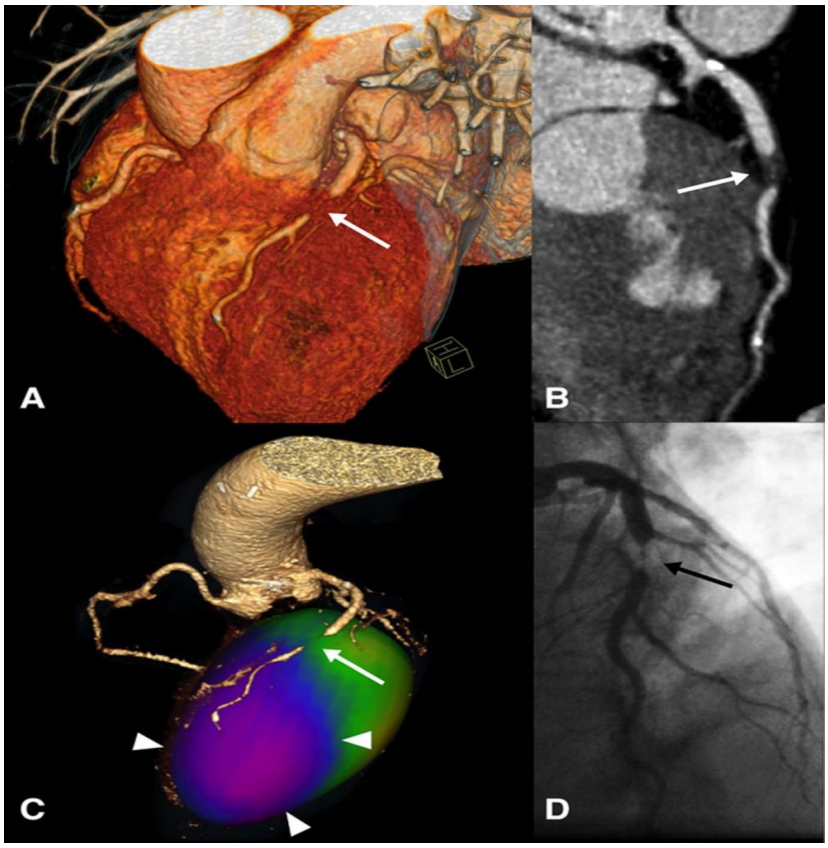

Figure 1: Coronary artery Computed Tomography scan, showing stenosis caused by non-calcified atheromatous plaque in the mid-left anterior descending coronary artery.

http://circ.ahajournals.org/content/129/12/1341/F2.large.jpg,11/4/2015

7 PATHOPHYSIOLOGY OF ATHEROSCLEROTIC PLAQUE DEVELOPMENT

Atherosclerosis is a multi-step disease and its development starts in the endothelium. Development of atherosclerotic changes occurs systematically, when abnormal endothelial functions accumulate.

The first step begins when monocytes adhere to the endothelium, causing their migration through the membrane into the tunica intima. (Figure 2,b) Monocytes then mature to become macrophages, which start storing lipids coming from β-oxidation of

Low Density Lipoprotein (LDL), forming Foam cells. This process slowly decreases the endothelial function, which combined with the inflammatory mediator dysfunction, namely macrophages, results in the formation of fatty streaks. (9)

The second step includes the migration of smooth muscle cells from tunica media, as well as an extended accumulation of smooth muscle cells already existing in intima. The accumulated smooth muscle cells then proliferate, with the concomitant accelerated production of collagen, elastin and proteoglycans. (Figure 2,c) Following these processes is the accumulation of fat derived from the apoptosis of macrophages and smooth muscle cells, creating a mass known as the lipid or necrotic core. (9)

The last step comprises of extended deposition of calcium, cholesterol and necrotic cells into the surface of the lipid core leading to the formation known as the atherosclerotic plaque. In the surface of this plaque, a continuous accumulation of collagen, elastin and smooth muscle cells form a layer called “fibromuscular cap”. The thickness of this layer is connected to the stability of the atherosclerotic plaque, meaning the thinner the cap is, the more susceptible it is to rupture and a potential vessel thromboembolism (Figure 2,d). (9)

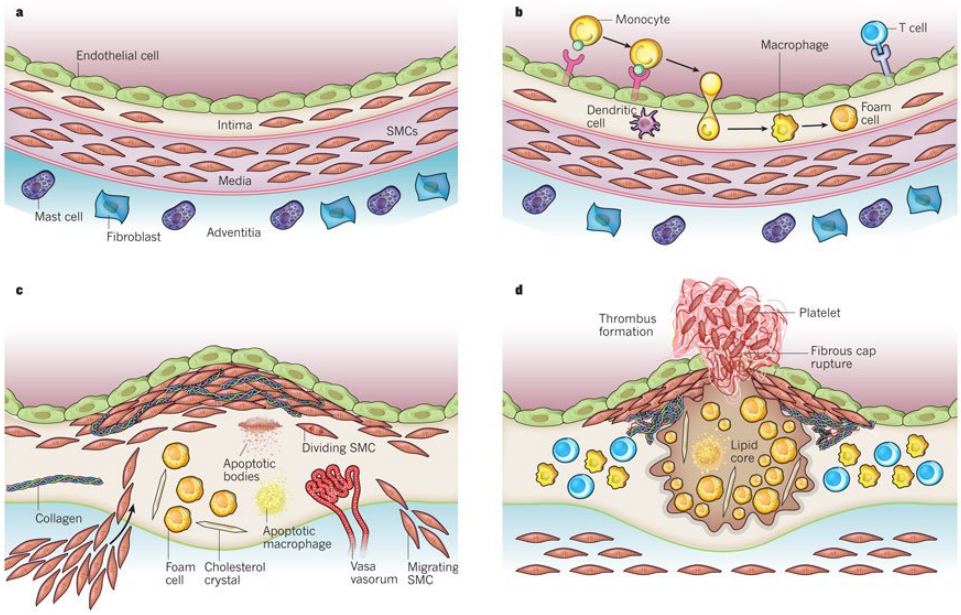

Figure 2: The process of atherosclerotic plaque formation and its rupture step by step.

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8 RISK FACTORS

Some conditions/states of the human organism tend to accelerate formation of any type of atherosclerotic lesion, such as Coronary or Peripheral Artery Disease. These states usually include arterial hypertension, Dyslipidemia, smoking, Diabetes Mellitus type II, age, gender and genetics and I. Risk factors can be categorized according to their modification potential in modifiable and non-modifiable. (10)

8.1 Modifiable risk factors

This type of risk factors is mostly present because of sedentary lifestyle, improper caloric quality and quantity consumption and lack of exercise.

Arterial Hypertension is one of the major causes of endothelial dysfunction, promoting vascular inflammation, oxidative stress and loss of vascular elasticity. Combined with a gradual drop in Nitric Oxide bioavailability, which is responsible for vascular dilatation, it results in functional change of the smooth muscle cells lining

the endothelium, causing vasoconstriction, LDL β-oxidation, inflammatory mediator arrival at the damaged lining, and the whole cascade forming the fibromuscular cap. (5)

Diabetes Mellitus is a pathological condition characterized by abnormal elevated glucose levels freely circulating the bloodstream, namely hyperglycemia. In Diabetes, lack of insulin (type I), or insulin resistance (type II), leads to a loss of endothelial functionality. Small blood vessels are affected first, which is why Diabetic Retinopathy and Nephropathy are common as complications of Diabetes. Damaged blood vessels are susceptible to inflammation, loss of elasticity and vascular tone, all of which are major risk factors of atherosclerosis. (5)

Smoking is a well-known risk factor for the development of many diseases, including atherosclerosis. Nicotine, a substance present in tobacco, infiltrates the blood stream, decreasing Nitric Oxide (NO) production and bioavailability, resulting in gradual vasoconstriction. In addition, nicotine is responsible for vascular spasm generation, as well as morphological and functional change in the endothelial lining. This results in elevated platelet adhesion on the endothelial surface, which may lead to formation of thrombus. (5)

Dyslipidemia, the last major modifiable risk factor for the development of atherosclerosis, describes a pathologic condition in which one or more lipoprotein is elevated or decreased. This can be attributed to genetic deficiencies or mutations caused by lack of exercise and saturated or trans-unsaturated fatty acid chronic intake. Dyslipidemia can be analyzed into several types, the most risky of which (in the development of atherosclerosis) is familial hypercholesterolemia. In this type of dyslipidemia, a decrease in the expression of LDL-R receptors leads to elevated circulating LDL, increasing the risk of β-oxidation and its following accumulation in the damaged vessel as foam cell. (11)

C-Reactive Protein (CRP) is a protein playing a role in inflammatory response. Its usual levels differ among different races and different health states, but should be in the range of 1-2.6 mg/L in a healthy individual. Elevated CRP suggests an atherogenic risk factor, since inflammatory response is one of the key factors for the pathogenesis of some atherosclerotic lesion. Other modifiable risk factors include obesity, sedentary lifestyle, lack of physical activity, stress and alcohol consumption.

Reducing or modifying these risk factors is critically importance to counter this disease. (12)

8.2 Non-modifiable risk factors

There are, however, risk factors for developing some type of atherosclerotic disease, which are not related to lifestyle. Age, gender and Genetic Predisposition are examples of risk factors that cannot be treated or manipulated by any means of pharmacotherapy or lifestyle change.

Aging is a natural process affecting each living organism, and hence, humans. As the age of a person increases, the endothelium becomes more and more susceptible to elasticity loss, NO bioavailability, oxidative stress and inflammation sensitivity, vasoconstriction and other vascular pathologic conditions. These abnormalities start to accumulate and at some point, irreversible endothelial damage leads to atherosclerosis, which in turn may affect every organ (kidney, brain, heart, lungs, eyes and bones). (5)

Gender is a risk factor by itself. Males are more susceptible to developing atherosclerotic lesion sometime in their life than females, and this can be explained by differences in hormonal nature and regulation between the two sexes. Premenopausal women are somewhat protected from Cardiovascular Disease (CVD) development, mainly because of the estrogen's effect on lipid profile and vasculature. Vasoconstrictors such as serotonin and angiotensin are also regulated through an estrogen-regulation pathway, leading to equilibrium between vasoconstrictor and vasodilator agents in women. After menopause, though, estrogen levels are highly depleted, leading to a multifold risk increase in developing CVD. In males, on the other hand, androgen levels decline with age, gradually and steadily decreasing as men grow older. Androgen depletion leads to numerous endothelial dysfunction effects, such as inflammation, disrupted lipid tolerance, changes in smooth muscle morphology as well as vasoconstriction and arterial hypertension. (13)

Genetic Predisposition plays a key role in the development of atherosclerosis and its complications. Mutations in physiological morphology and functionality of

endothelium as well as of some atherogenic substances (LDL) increase the probability of atherosclerotic lesion formation. There are types of dyslipidemias caused by genetic defects, such as familial hypercholesterolemia, causing an accelerated CVD development. History of atherosclerosis in the family usually suggests extra attention in these patients. Genetic predisposition is crucial to atherosclerosis prevention and care. (11)

9 CLINICAL MANIFESTATIONS AND COMPLICATIONS RELATED TO ATHEROSCLEROTIC LESION

Atherosclerosis is generally an asymptomatic disease especially in the early stages of development. However, if sufficient stenosis occurs, atherosclerosis is manifested as “angina pectoris”, a term originating from the Greek word □γχόνη *ankhonē* ("strangling"), and the Latin word *pectus* ("chest") which is used in medicine to describe deep, heavy and pressure generating chest pain. Updated on July 30, 2014, American Heart Association (AHA) describes angina as chest pain caused by cardiac muscle hypoxia, meaning that cardiac oxygen demands exceed oxygen supply. We must state that chest pain is a symptom, which means the underlying cause may be caused by some other disease like peptic ulcer.

AHA describes four types of angina pectoris with different clinical manifestations and risks for complications.

The most common type is the so-called stable or effort angina, in which the pain is manifested when a person is working out or during stress and/or anxiety.

Variant (also known as Prinzmetal or Vasospastic) angina is another type of angina pectoris clinically manifested as deep pain due to coronary vessel spasm. This spasm is usually related to exposure to cold, drug use and smoking and the pain attacks often take place during early hours.

The most dangerous type regarding risk of complication is the condition called unstable angina, and it can occur at any time, even when lying down or resting. In unstable angina, the patient feels extended discomfort, and the pain can lead to a series of thromboembolisms. The embolus is formed by the rupture of an unstable

atherosclerotic plaque, resulting in Acute Myocardial Infarction (AMI), stroke, or peripheral ischemia.

Peripheral artery disease (PAD) is also related to atherosclerosis and the place of the blood clot reflects the place of ischemia. This disease can lead to ischemia of many different organs (eyes, kidneys, intestines, sex organs, extremities etc.) causing blindness, kidney failure or erectile dysfunction. A series of complications due to PAD can result from Carotid artery disease. Carotid is the artery supplying the brain with oxygen and its stenosis or blockage can cause ischemic brain stroke, a common condition, especially in the elderly, causing disability in many patients. PAD is a risk factor of CVD development by itself, since a thrombus formation in the periphery can migrate and become an embolus in the coronary arteries, leading to Acute Myocardial Infarction (AMI). (14)

10 PREVENTION OF ATHEROSCLEROSIS

Primarily, health care providers tend to try to deal with asymptomatic diseases before a complication takes place. This is the main reason why prevention of atherosclerosis is critical at this point. In most people, the process of arterial hardening is already taking effect in their early adulthood. Generally speaking, there are three different approaches when discussing about prevention of atherosclerosis, namely Primary, Secondary and Tertiary.

10.1 Primary Prevention

In primary prevention, the goal is to control the modifiable risk factors of atherosclerosis development. Non-modifiable risk factors (family history, sex, genetics, age) can also be present, but as their name suggests, there is little to be done to affect them. Lifestyle changes can become the most effective treatment. Waking up early, quitting smoking, avoiding psychological stress, exercising, controlling lipid and carbohydrate consumption are some examples crucial in the primary prevention of atherosclerosis. As we will see in the next chapters, both pharmacological and non-pharmacological treatment can affect the levels of these factors (omega-3 fatty acids, antioxidant substances, drugs etc.) (15)

10.2 Secondary prevention

After the risk factors (hypertension, diabetes, dyslipidemia) appear, secondary prevention is on the table. In secondary prevention, risk factors of cardiovascular disease are present, but a complication has yet to appear. In addition to lifestyle changes, pharmacotherapy is usually very helpful in the treatment. Decreasing lipids, carbohydrates, thrombotic factors and inflammatory mediators circulating in the blood is of prior importance. Many different drugs affecting the levels of these factors are used to restore the healthy cardiovascular function. (14)

Arterial stress due to hypertension is a leading cause of atherosclerosis. Controlling arterial blood pressure, especially diastolic, being the pressure responsible for cardiac circulation and naturally affecting coronary vessels, is a good way to prevent the development of an arterial atherosclerotic lesion. To prevent atherosclerosis development, agents reducing blood pressure are advised. Obesity is another risk factor responsible for the disease development. In obese people, typical cardiovascular functions are burdened by the excess of fat tissue through the body and this stress can affect arterial hardening. Weight control is crucial at this point, before drug interventions occur. A change in diet and sedentary lifestyle can benefit a patient's lipid and carbohydrate profiles, greatly reducing the risk of developing atherosclerosis. Dyslipidemia, a general term including many lipid profile abnormality possibilities as we have already stated, can be controlled by many agents, as we will suggest in the next chapters. (15)

To oppose inflammatory response caused in atherosclerotic lesions, a number of anti-aggregation agents (e.g. acetylsalicylic acid, clopidogrel) are commonly used to protect cardiac function. (16)

11 GENERAL NON-PHARMACOLOGICAL TREATMENT

Atherosclerosis can be developed due to many risk factors such as hypertension, Diabetes Mellitus, dyslipidemias, cigarette smoking, sedentary lifestyle and quality of food consumed. However, the atherosclerotic changes in the endothelium might start occurring in late adolescence or early adulthood. This is why health care institutes and health care providers suggest that the best way to prevent and minimize the risk of developing atherosclerosis and its following complications is to follow a healthy lifestyle, which includes cessation of bad habits, exercising, and knowledge of daily caloric quality and quantity intake. (5)

To normalize lipid levels in the blood, omega-3 (ω-3) polyunsaturated fatty acid (PUFA) intake is recommended. Morphology of the fatty acids seems to play a major role in atherogenesis, as seemingly trans- fatty acids tend to be atherogenic, while cis-fatty acids do not. According to new insight on their mechanism of action, this type of PUFA decreases many atherogenic pathological conditions, such as hypertension, hypertriglyceridemia, cardiac arrhythmias, insulin sensitivity modulation, oxidative stress development as well as inflammatory expression in the endothelium. (5,17)

Alcohol has a positive lipid profile when consumed moderately (e.g. a glass of wine 5 times per week). Together with other constituents, like polyphenol compounds found in red wine, it has the ability to gradually normalize lipid profiles, by increasing HDL decreasing LDL. These effects are clinically significant only in red wine consumption (Huang et al). Antioxidant, antithrombotic and anti-inflammatory and angiogenic properties of alcohol may decrease the overall risk of atherosclerotic lesion development. Excessive alcohol intake, on the other hand, is associated with an increased risk of Cardiovascular and Hepatic disorders, because of its adverse effects (hypoglycemia, myopathy, gastrotoxicity, cardiac arrhythmias, hypovitaminosis). (5,18)

Antioxidant agents include Vitamins C (Ascorbic acid) and E (Alpha-Tocopherol), substances naturally occurring in some foods like Orange (Vitamin C) and wheat or sunflower oil (Vitamin E). Melatonin, an endogenous hormone naturally affecting sleep pattern, also has antioxidant, antihypertensive and anti-inflammatory effects. (5)

Regular physical activity is the key to prevent atherosclerosis. In combination with dietary changes, it can help reduce risk factor development (dyslipidemias, diabetes, hypertension) while keeping the cardiovascular system in a state of balance. The duration and intensity of the exercise should be managed according to a patient's co-morbidity states, age, gender and other risk factors. Chronic moderate exercise has evidence of reducing risk of cardiovascular disease, as it has positive effects on Peripheral Vascular Resistance (PVR) and physiological endothelial function restoration. (5)

According to World Health Organization (WHO), smoking is related to the development of at least 10% of all cardiovascular disease reported cases. Smoking has a complete negative effect on many levels. A gradual increase in LDL and decrease in HDL was first reported in 1989 (Craig et al). Further clinical studies up to the present day support these results. Nicotine and other carcinogenic substances contained in the smoke increase blood viscosity and thrombophilia by increasing fibrinogen and homocysteine levels. Smoking also causes vasoconstriction through a NO decreasing mechanism, which is very risky especially in patients already having a narrowed artery. One of the major controversial and ethical issues of non-smokers is the so-called passive smoking, which is as harmful as normal smoking, but can be even more harmful in groups like children and the elderly, or other groups suffering morbidities like asthma or Chronic Obstructive Pulmonary Disease (COPD). (5,19)

12 GENERAL PHARMACOLOGICAL TREATMENT

Many kinds of different pharmacological agents are used to treat risk factors of atherosclerosis and the complications that rise from this type of lesion. Agents acting against cholesterol and LDL formation, platelet aggregation and blood coagulation antagonists are often used.

Treatment of risk factors for atherosclerosis is the best way to minimize the risk of atherosclerotic lesion development, and that is, in fact, the main focus of pharmaceutical interventions regarding patients already diagnosed with one or more risk factors. Dyslipidemias are usually managed by lipid-lowering agents. Statins are agents inhibiting 3-hydroxy-3-methylglutaryl Coenzyme A, an enzyme responsible

for the last step of cholesterol synthesis. They are widely used to produce a cholesterol lowering effect and are often used in many heart disease and stroke prevention strategies. As we will further analyze, statins are usually the drug of choice in many anti-atherosclerotic measurements taken in patients at risk. (20)

In dyslipidemias, other kinds of drugs are used to lower and control cholesterol levels. One of these kinds are the Fibrates**,** a group of agents indirectly inducing LDL lipolysis through peroxisome proliferator activated receptor α (PPARα) agonism, and ultimately decreasing triglyceride levels circulating in the blood. (21)

Vitamin B3 or Niacin is an essential vitamin for the preservation of the human organism and responsible for some of its vital functions, being a precursor of Nicotinamide Adenine Dinucleotide Phosphate (NADP), and its protonated form (NADPH). These cofactors play a major role in catalyzing many biochemical metabolizing processes, such as fatty acid and cholesterol regulation. Elevated levels of niacin have evidence of lowering circulating LDL, but more importantly, elevating HDL levels, which is essential for better cholesterol utilization by the body. (22)

Drugs having the ability to bind bile acids in the intestinal tract, namely bile acid sequestering agents (Cholestyramine, Colesevelam, Colestipol), are sometimes used to lower circulating cholesterol in the blood, especially in combination with statins, fibrates and Niacin. Their ability to block enterohepatic bile acid recirculation has a negative feedback in hepatic cholesterol production. A homeostatic mechanism of the organism including HMG-CoA reductase tends to replenish the diminished cholesterol levels and therefore a combination of bile acid sequestrant drugs with HMG-CoA reductase inhibitors is usually advised to avoid this mechanism. Because of their high drug-drug interaction activity, these agents should be administered with time intervals before or after sequestering drug administration. (23,24)

Cholesterol is normally absorbed in the small intestine. Ezetimibe is an agent responsible for inhibiting the absorption of cholesterol by the small intestine and therefor increasing uptake and utilization of circulating cholesterol, resulting in an overall decrease in the cholesterol levels in the blood. (25)

Agents acting against platelet aggregation are a common action taken to prevent atherosclerosis formation and complications. Acetylsalicylic acid (Aspirin), is

commonly used in the low dose of 100mg once daily, to prevent atherosclerosis inducing aggregation and thromboembolism. The mechanism of action of aspirin is the acetylation of Cyclooxygenase-1 (COX-1), followed by inhibition of the substrate's (arachidonic acid) binding to cyclooxygenase. The result is the overall inhibition of the platelet aggregation pathway, followed by the inability of the blood to produce thromboxane. This results in a beneficial overall effect in the reduction of vasculopathies, especially if the patient is suffering with co-morbidities (Diabetes Mellitus, Hypertension, Dyslipidemias). As stated in ASTERIX and OBERON studies there is a 71% and 85% relative reduction in peptic ulcer formation respectively. Peptic ulcer develops due to a COX-1 inhibition mechanism, when aspirin is combined with a proton pump inhibitor agent (esomeprazole in ASTERIX and OBERON study). (26,27)

Clopidogrel is an agent affecting ADP (adenosine diphosphate) dependent response pathway, by producing an irreversible inhibition to its binding to the platelet receptor ($P2Y_{12}$). This causes a blockage in some essential functions resulting in the formation of fibrin. Fibrin is an essential component of the thrombus formation and its inhibition leads to a safe measurement taken against atherosclerotic lesions and the complications due to them. The strength of Clopidogrel is typically 75mg od, although it can be up to 325mg od. (26)

New $P2Y_{12}$ inhibitors include Prasugrel, a thienopyridine agent producing a more potent and faster effect, but at the same time a higher gastrotoxicity, as shown in the TRITON study. (26,28) Ticagrelor, another agent inhibiting $P2Y_{12}$ receptor, though a non-thienopyridine, shows a distinct faster response in onset of its action and potency when compared to Clopidogrel. According to the third phase of PLATO study, Ticagrelor shows much better benefit in mortality due to platelet aggregation related diseases such as atherosclerosis than Clopidogrel. (26,28) Elinogrel is a non-thienopyridine agent with the same mechanism of action as Clopidogrel, except the fact that Elinogrel's receptor inhibition is reversible and direct. It can be administered intravenously in addition to orally. (26) Cangrelor is the last new agent worth mentioning, because of its ability to resist enzymatic inactivation, and its usual intravenous application. (28)

Anticoagulant drugs are also used to deal with atherosclerotic lesion complications. They are widely used especially in the secondary and tertiary prevention of complications due to atherosclerosis (stroke, AMI). Warfarin is an oral anticoagulant that was firstly used as a pesticide before the 1950_s, but later on its anticoagulant activity was founded, making it the most common anticoagulant agent to this day. Commonly used in vasculopathies including thrombus formation like deep vein thrombosis and atherosclerosis, Warfarin is responsible for Vitamin K inhibition. Vitamin K is utilized by the human organism to produce many clotting factors, and its inhibition eventually leads to blood-thinning. Because of the increased risk of bleeding when administering warfarin, International Normalized Ratio (INR) is measured to ensure a safe course of treatment. (29,30)

New anticoagulant agents include different mechanisms of anticoagulant action. Direct thrombin inhibitors (Dabigatran) inhibit thrombin, a protease responsible for the formation of Fibrin through Fibrinogen, a component necessary for the formation of thrombus. Apixaban and Rivaroxaban are another class of new anticoagulant agents, and act by direct inhibition of factor Xa, the enzyme responsible for the formation of thrombin. (30)

13 DEFINITION OF STATINS

One of the major reasons why atherosclerosis develops is because of high levels of LDL cholesterol in the bloodstream. Cholesterol is produced by the liver, but before its production many different reactions with many substrates and enzymes must take place. Cholesterol is produced via a complex pathway including biotransformation of 3-Hydroxy-3-methylglutaryl Coenzyme A (HMG-CoA) into Mevalonate (a precursor of cholesterol) through the enzyme HMG-CoA Reductase. Statins are organic derivatives of fungi and act by inhibiting the enzyme HMG-CoA reductase. This happens through their binding to the enzyme, not letting the substrate (HMG-CoA) bind to its natural enzymatic binding site. At the same time, statins have the ability to increase the number of LDL-binding receptors in the liver. A modest decrease in the levels of LDL cholesterol is produced, while HDL levels are slightly heightened. (31) Statins are a drug category consisting of many different agents that have distinct

effects on LDL, while milder effects on HDL and TAG levels. A number of statins have been distinguished, with differences in pharmacokinetics, strength (5-80mg), albumin-binding degree, first-pass effect and potency, namely: Atorvastatin, Cerivastatin, Fluvastatin, Lovastatin, Pitavastatin, Pravastatin, Rosuvastatin and Simvastatin.

14 STATIN USAGE IN THE PREVENTION OF ATHEROSCLEROSIS

14.1 Statin usage in Primary Prevention of CVD

Statins are commonly used to prevent formation of atherosclerotic plaque in endothelial intima. In primary prevention of CVD, statins are commonly used. The JUPITER (Justification for the Use of statins in Prevention: an Intervention Trial Evaluating Rosuvastatin) study was conducted in people with elevated hs-CRP (high sensitivity C-Reactive Protein) and Low Density Lipoprotein levels that were lower than 7,2 mmol/l. The results were inconclusive regarding rosuvastatin's comparison to other medical interventions devoted to primary prevention of CVD. However, a general decrease in the risk of developing some kind of CVD complication was noted with rosuvastatin treatment versus the placebo groups. (12,32)

It is well-known nowadays that prescription of statins for primary prevention of CVD must be adjusted to each patient individually according to their degree of risk of developing CVD. Many organizations such as American Heart Association (AHA) approve the use of statins on patients at high risk of developing CVD. Generally speaking, the combination of statins with non-pharmacological treatment strategies such as lifestyle changes is crucial at this stage. (32,33)

14.2 Statin usage in Secondary Prevention of CVD

In secondary prevention of CVD, statin therapy is focused on the urgent necessity of lowering LDL cholesterol as well as CRP. Given to patients already suffering a risk factor of atherosclerotic plaque development, statins have shown promising results in reduction of LDL and CRP, having as a starting phase the results of the JUPITER study. Combined with their antithrombotic and anti-inflammatory effects, the resulting treatment is evident to lower morbidity and mortality due to atherosclerosis-

related complications (stroke, AMI). Patients suffering with Diabetes Mellitus have a significantly accelerated atherosclerotic lesion development since in Diabetes elevated glucose negatively affects the vascular system in many levels (elasticity, PVR, insulin receptor depletion, insulin resistance). Other morbidities like Peripheral Artery Disease are on the focus of secondary prevention of CVD and evidence including many clinical trials show benefit in statin usage. (5,32,34,35)

15 STATIN USAGE IN THE TREATMENT OF ATHEROSCLEROTIC ALTERATIONS

Treating a developed atherosclerotic lesion must be focused on management of pathophysiology that led to it. Management of hypercholesterolemia is crucial especially in the beginning of the treatment. Statins, as we have already described, have the ability to decrease cholesterol synthesis as well as increase LDL receptors in the liver, inducing LDL binding and utilization and at the same time decreasing LDL production. Despite their moderate LDL-lowering and mild HDL-increasing effects, research has shown that they help in the process of plaque dispersion and consequently gradual reversion of atherosclerosis. Their anti-inflammatory effect firstly became clinically important after the JUPITER study with hs-CRP. It is now known that the anti-inflammatory mechanism of statins begins in the cholesterol formation pathway. A process known as protein (iso)prenylation (see Figure 3), taking effect by products of mevalonate (a precursor of cholesterol), namely Farnesyl PyroPhosphate (FPP) and GeranylGeranylPyrophosphate (GGPP), is found to be responsible for many atherogenic processes including pro-inflammatory cytokines and signal transduction across the Cardiovascular System (CVS). Statins have the unique effect of inhibiting protein (iso)prenylation, which leads to a decrease in all these atherogenic inflammatory response processes across the CVS. In addition, statins are effective against oxidation stress caused by oxidized LDL (ox-LDL), as well as on NO synthesis in the vascular endothelium, which in turn helps in vasodilatation and alleviation of hypertension. A complete disease reversion has yet to be found but statins are the drug of choice at this point. (31,34,36)

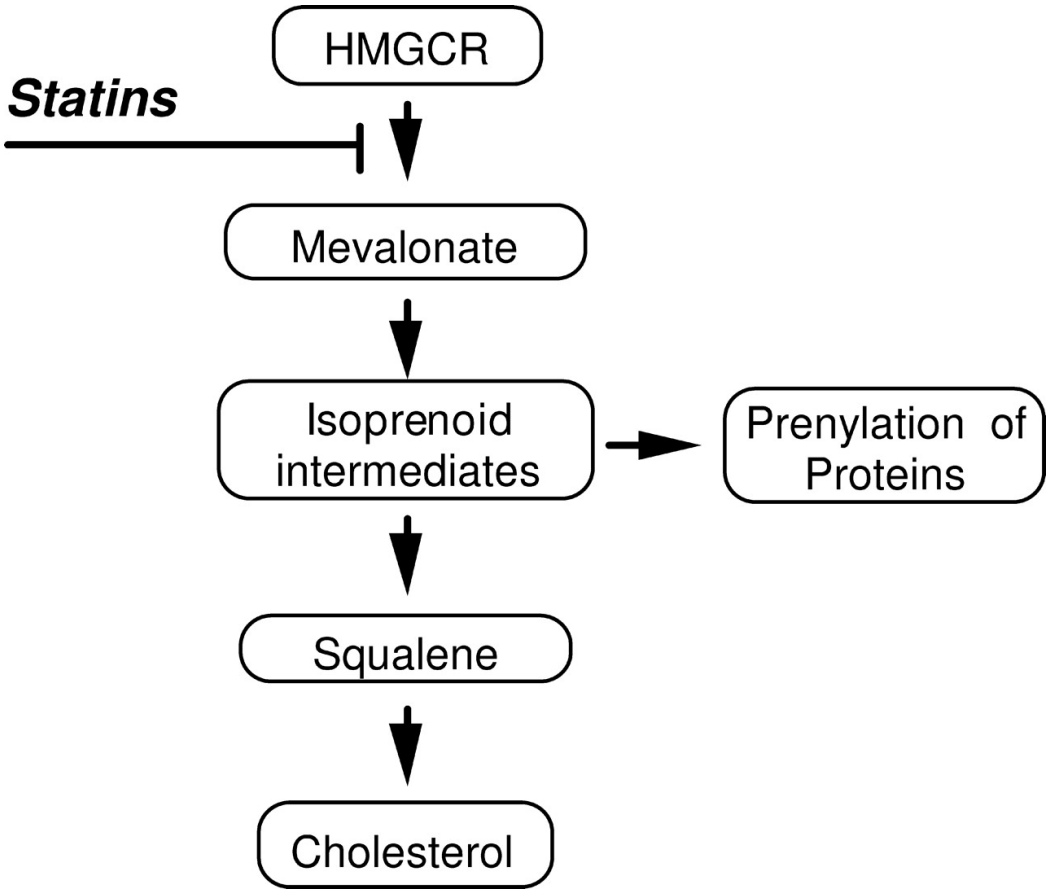

Figure 3: The mechanism of action of statins and how it affects protein prenylation.

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Leaving these effects aside, statins have mild endothelial activity. They are thought to reverse and restore endothelial physiological function and elasticity and reverse inflammation caused in tunica intima. When physiological endothelial function is restored, diminished levels of LDL in the liver cause an increase in the production of LDL receptors by the endothelium. The overall effect is increased uptake of LDL by the receptors, diminished LDL levels in the circulation, and an increase in quality of circulating agents. (34,36)

16 DIFFERENCES BETWEEN INDIVIDUAL STATIN MEMBERS

While they all have the same mode of action on cholesterol lowering, statins differ in potency, lipophilicity, degree of cholesterol decreasing effect, side effect intensity, drug-drug and drug-food interaction extent. Atorvastatin, Pitavastatin, Simvastatin, Fluvastatin and Lovastatin tend to be more lipophilic while Rosuvastatin and Pravastatin are hydrophilic. (37)

Their pharmacokinetics also play an important role in their absorption, distribution, metabolism and excretion from the body. Lovastatin and Simvastatin become active in their lactone form while Atorvastatin is active as a calcium trihydrate salt. There are also differences in their risk of side effect expression. For example, Cerivastatin has been withdrawn from the market in 2001 because of many cases of myositis and rhabdomyolysis in patients that received this agent. (37)

Regarding their potency, Atorvastatin and Rosuvastatin are the most potent agents that competitively inhibit HMG-CoA reductase, resulting in the most distinct LDL-lowering effect. Simvastatin and Pravastatin show moderate inhibiting potency, and moderate LDL-lowering effect. Correct agent and dose administration is possible, taking of course into account the individual patients' other risk factors, co-administered drugs and co-morbidities together with atherosclerotic disease. (33,37)

Leaving Pravastatin aside, all other statins extensively bind to albumin, making it less likely to displace other co-administered agents. Some of these agents (Simvastatin, Lovastatin) are prodrugs, and there is a delay in their starting time of action because they first need to be hydrolyzed to their active form. First-pass effect is of great significance to drug interactions that may arise, especially when taking into account the CYP-450 (Cytochrome P-450) metabolizing system, the metabolizing activity of which may be induced or inhibited by other agents. Pravastatin is the only statin that can produce active derivatives in the stomach and not metabolized by this system. (37,38)

17 PITAVASTATIN: A NEWER MEMBER WITH NEW BENEFITS

Focusing on its pharmacokinetics, Pitavastatin is greatly less affected by the CYP-450 metabolizing pathway in comparison with the rest of statin agents, making it less prone to develop drug-drug interaction induced toxicity and adverse effects. (37,39)

Many studies conducted in Europe and Asia showed the beneficial aspects of Pitavastatin over other statins. In Chiba study (Japan), a noticeable benefit of Pitavastatin in LDL cholesterol reduction resulted when compared to Atorvastatin. Many other comparative studies were conducted, and although some of them were inconclusive, most of them indicated benefits of Pitavastatin over other statins. Sasaki *et al* noted that these benefits included not only significant LDL reduction, but also significantly less adverse drug reactions mainly due to its non CYP-450 dependent metabolizing pathway. Despite its low dosage strength (1 to 4mg per dose), Pitavastatin has shown remarkable long-term improving outcomes regarding HDL cholesterol. Masana *et al* stated that as shown in the VOYAGER and PIAT studies there is a crucial benefit in patients suffering with Diabetes Mellitus, since there is a typical decrease in HDL due to impaired Lipoprotein Lipase activity in these patients. (39,40,41)

18 OTHER USES OF STATINS

Despite of their general usage for prevention and treatment of atherosclerosis, statins show promise when used to prevent complications of some other co-morbidities existing together with atherosclerotic lesions. High glucose levels in the bloodstream present in diabetic patients tend to damage blood vessels (especially the smaller ones), and this effects tends to accelerate the risk of developing an atherosclerotic lesion. Statins are helpful because of their ability to stop and even reverse inflammation of the endothelium to some degree.

Research has shown that statins may also be safely used to treat non-alcoholic fatty liver disease (NAFLD). This disease is characterized by excessive liver steatosis. Fat deposits replace the healthy hepatic tissue, and gradually decrease its functionality. This excess in fat deposits increases the risk of cardiovascular disease

because of plaque formation, and statins are beneficial at this point, reducing the risk of development of some complications because of atherosclerosis. Chatrath *et al* describes that according to a Greek study called GREACE (Greek Atorvastatin and Coronary heart disease Evaluation), there was a significant (68%) reduction in the risk of developing CVD in patients suspected of having NAFLD. (42)

A very interesting review has shown that beside their lipid-lowering ability, statins have systemic anti-inflammatory and immunity modulating effect. The mechanism of action includes immunomodulation of T-lymphocytes and endothelial progenitor stem cells, resulting in a positive endothelial effect. This effect takes place in hours, in contrast with the lipid-lowering effect showing results weeks after the first statin administration. Antonopoulos *et al* suggested that these effects are linked to a beneficial outcome when general inflammatory diseases such as Rheumatoid Arthritis (RA) are concerned. Data received from studies such as TARA (Trial of Atorvastatin on Rheumatoid Arthritis) suggest that there is an overall benefit of statin usage regarding inflammatory response and general immunomodulation throughout the whole body, and hence, the synovial fluid in which RA develops. This has a very positive impact on atherosclerosis, because patients suffering with RA have an increased risk of developing endothelial inflammation, and hence, atherogenesis. Combining this data with the results of JUPITER study in CRP reduction, we can conclude that the anti-inflammatory and immunity modulating effects of statins could be very beneficial to developing RA. (34)

Alzheimer's dementia (AD) is a disease characterized by gradual neuronal and synapse degeneration and that is why it is also stated as a neurodegenerative disorder. This gradual neuronal loss leads to memory loss and cognitive impairment. The link between this disease and statins is cholesterol. Shepardson *et al* linked observational studies and Randomized Control Trials to conclude that there was a reduced risk of AD development in patients receiving statins compared to those not. However, the results suggested that the higher the patient's age, the lesser the probability of a benefit from statin usage in memory loss because of AD. Despite the above mentioned facts, many of these studies were inconclusive and contained confusing results regarding statin benefit over AD complication development. (43,44,45)

19 STATINS SIDE EFFECTS AND CONTRAINDICATIONS

Drug safety is one of the most important measures taken into account before administration. There are manydifferent vulnerable groups of people that the physician needs to be cautious with when prescribing medicine. Regarding statins, side effects usually include liver damage, myopathy, cognitive impairment (rarely), and an increased risk of developing pre-diabetes and diabetes in comparison with patients that are not on statins.

Muscle pain is associated with their effect on Creatine Kinase (CK, also known as Creatine Phospokinase), an enzyme responsible for transferring a phosphate group for phosphocreatine to Adenosine Triphosphate (ATP). Elevated levels of this enzyme in the blood can be used to diagnose muscle damage. The pain may be expanded and turn to myopathy, and in rare cases to rhabdomyolysis, a state in which muscle tissue is dissociated, leading to an excess in circulating myoglobin, a state which can cause serious glomerular injury and ultimately, kidney failure. Parker *et al* reported that there is a correlation between elevated physical exercise and statin induced myopathy. She describes that because during exercise muscle glycogen is reduced along with Adenosine Triphosphate (ATP), permeability of the muscle's cellular membrane increases, a state that leads to muscular fiber dispersion and damage. In addition, Sakamoto *et al* suggested that statin induced myopathy is also related to GGPP depletion. We have already described the beneficial impact of GGPP depletion through HMG-CoA reductase inhibition in the development of atherosclerosis. However, other unwanted effects take place because of this depletion. GGPP is responsible for protein prenylation, and that seems to be involved in a complex pathway resulting in muscle cell apoptosis. Vulnerable groups include the elderly (especially women) in which muscle tissue is already in a smaller amount than healthy young people have. (46,47,48)

Glucose intolerance is one of the major health problems in developed countries. FDA (Food and Drug Administration) issued a warning in patients receiving statins in 2012, concerning their effect on glucose tolerance. According to that warning, patients receiving statin treatment are more susceptible to develop type 2 diabetes and at the same time, patients already diagnosed with diabetes have an increased risk of glucose intolerance. The JUPITER study stated that there was an increase in incidence

of Diabetes Mellitus type II due to rosuvastatin usage. Sampson *et al* suggests that the JUPITER and WOSCOP (West of Scotland Coronary Prevention Study) trials provided us with insight on the risk of developing Diabetes Mellitus after statin usage. This insight suggests that the risk of developing DM after statin intervention increases slightly. However, the overall risk of developing CVD is greatly reduced with statins, because of their mechanism of action and pleiotropic effects, and according to Sampson *et al* the benefits of statin usage far outweigh the risk of DM development. (33,49)

Myopathy is an adverse effect that usually assists statin usage. Some other agents seem to potentiate and induce that effect by inhibiting the cytochrome isoform responsible for statin metabolism. They are thus contraindicated or used with caution with some agents (antifungal azole derivatives, macrolides, Cardio-depressive Calcium channel blockers, grapefruit juice). These effects are of high clinical significance especially with statins who are potent substrates for the CYP-450 (3A4) metabolizing pathway. One example of such a statin is simvastatin, which can exert increased muscular toxicity if co-administered with one or more of the agents mentioned above. (50,51)

Statins are also reported to cause mitochondrial defects. Because of the muscular tissue histophysiology, muscle fibers tend to be less dependent on mitochondrial defects when compared to other organs like the liver and kidneys. Non-muscular pathological conditions associated with statins, according to Golomb *et al*, may arise because of this effect statins in the mitochondrion. According to the tissue affected, adverse effects on a patient receiving statin therapy can be manifested as glucose elevation, insomnia and other psychological disturbances, encephalopathy (cognitive impairment), as well as Gastrointestinal and neurological abnormalities. These effects can be very risky especially in patients with co-morbidities. In severe cases, hepatotoxicity and nephrotoxicity can be evident, and thus, statins are generally just used with caution in patients with chronic hepatic or renal insufficiency. (51)

20 DEFINITION OF FIBRATES

Fibrates are agents derived from fibric acid and are widely used to normalize the lipid profile in both the human tissue and circulation. As Goldenberg *et al* pointed out, fibrates activate a receptor known as Peroxisome Proliferator Activated Receptor alpha (PPAR-α), which in turn binds to the Retinoid X Receptor alpha (RXR-α). A cascade pathway is responsible for specific gene transcription time alteration. Target genes are either increased or decreased, which ultimately increases or decreases their activity respectively. The major and most affected gene is called Lipoprotein Lipase (LPL), which is responsible for TAG hydrolysis. Other genes affected by this mechanism affect HDL and LDL synthesis. Barter *et al* stated that these agents decrease TAG levels by up to 50% and increase HDL by up to 20%. However, their effect on LDL varies and can be both positively and negatively influenced. Examples of fibrates include Fenofibrate, Bezafirate and Gemfibrozil. (52,53)

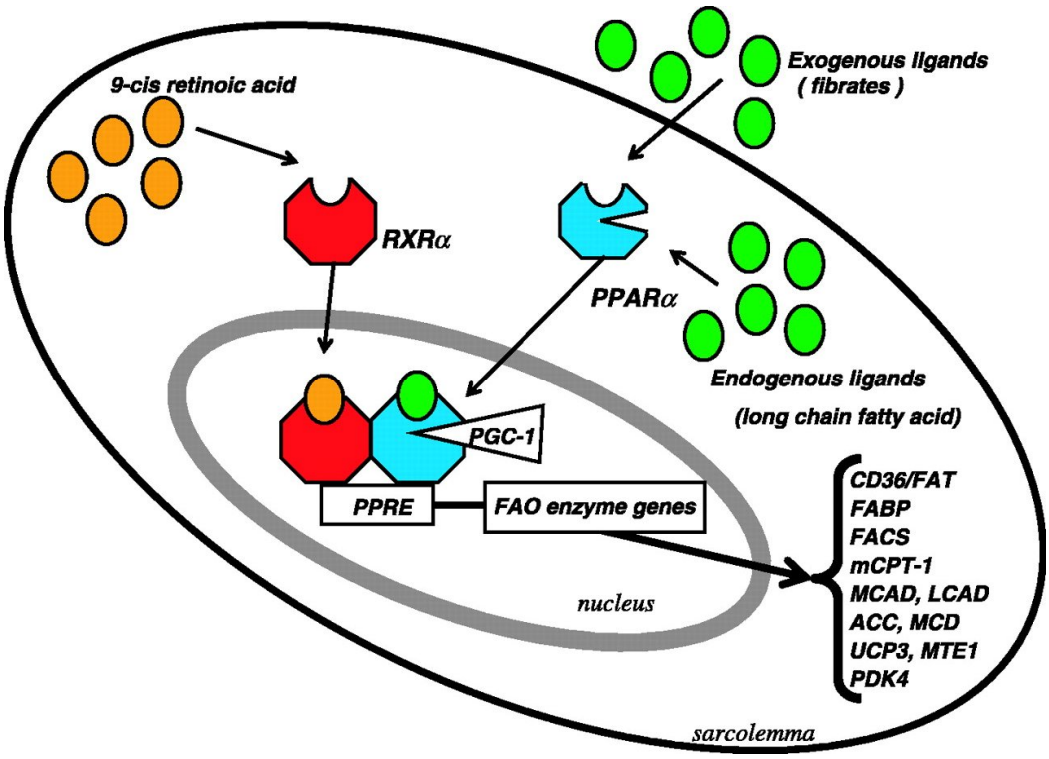

Figure 4: The mechanism of action of fibrates, their binding to PPARα and its corresponding binding to RXR, actions that lead to gene transcription time modification, ultimately affecting many different pathways.

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21 FIBRATE USAGE IN THE PREVENTION AND TREATMENT OF ATHEROSCLEROTIC ALTERATIONS

Fibrates are widely used to normalize altered lipid profiles since they affect many lipid producing pathways through their mechanism of action. Hypertriglyceridemia is a major risk factor for atherosclerosis development. The mechanism of action of fibrates, although complex, results in a modest decrease in TAG levels in the circulation. Through gene transcription time alteration, fibrates also diminish lipid production in muscular and hepatic tissue. The result is diminished VLDL (Very Low Density Lipoprotein) production, a precursor of LDL, which is well known as a major risk factor for atherosclerosis development. Their effects, however, on lipid profile are somewhat different from statins because of their different and indirect course of action. LDL and HDL levels are affected mostly positively, but they are not as evident as with statin treatment. As Tenenbaum *et al* states, fibrates are evident to decrease the risk of developing CVD complications due to atherogenic dyslipidemia, a state in which circulating TAG levels are elevated while HDL is decreased. (21)

As atherosclerotic pathophysiology is regarded, a lowering in TAG levels reduces the risk of complication development by diminishing the LDL available to be beta-oxidized and create foam cells. (21)

It is clear nowadays that atherosclerosis is related to inflammatory response. Fibrates, and more specifically Fenofibrate, demonstrate pleiotropic effects like statins. The pathway by which they do so, however, is somewhat different from that of statins. Through their complex mechanism of action, as Moutzouri *et al* suggests ,fibrates not only benefit lipid profile, but they are also evident to decrease many inflammatory response markers such as hs-CRP and Interleukin-6 (IL-6), both of which are associated atherosclerosis development. Other pleiotropic effects of fibrates include fibrinogen, platelet aggregation and uric acid modulation, actions that are all significant in the correction of endothelial dysfunction (21,54)

22 FIBRATES ADVERSE EFFECTS AND CONTRAINDICATIONS

Fibrates include various adverse drug reactions, most of which are mild. Nausea, Diarrhea, muscle pain and mild CK elevation are the most common among them. Other effects may include headaches, vertigo, insomnia and libido loss. However, fibrate treatment may result in elevated homocysteine, urea and creatinine levels, as well as a moderate decrease in Glomerular Filtration Rate (GFR). (54)

Usage with caution or contraindication when administering fibrates is usually with the following agents. Coumarin derivatives may be displaced and increase the risk of bleeding when used at the same time with Fenofibrate, since it is highly protein bound. Nephrotoxicity risk is multiplied when administering Fenofibrate with other nephrotoxic agents such as Cyclosporine and caution is advised when they are co-administered. When hepatic or renal insufficiency is present, Fenofibrate is contraindicated. The most controversial issue so far, regarding contraindications, is co-administration of fibrates with statins. Fibrates seem to potentiate the muscular adverse effects of statins, especially with Gemfibrozil, since it is metabolized by the same enzyme metabolizing statins. (54)

23 POSSIBILITIES OF STATIN AND FIBRATE COMBINATION-BENEFITS AND RISKS INCLUDED

Physicians have the possibility to prescribe more than one cholesterol decreasing agent according to the nature of the dyslipidemia. A patient with evident hypertriglyceridemia, decreased HDL and elevated LDL levels would normally not achieve maximum effect if treated with either statins or fibrates. However, when a statin is combined to a fibrate, they each have complementary effects on the lipid profile (see Figure 5). Fibrates are generally combined with statins when atherogenic dyslipidemia is evident, a state usual in patients suffering from DM. Since PPAR-alpha activation involves more pathways than just regulating lipid profile, we can safely assume that they have pleiotropic effects like statins. Bezafibrate has a somewhat distinct mechanism of action in comparison with the other agents of this group. Letting aside its lipid normalizing potential, bezafibrate is also able to modulate and normalize glucose levels, since it acts as an agonist in all three PPAR

isoforms, namely alpha, gamma and delta. It is known that PPAR-gamma agonists are used to modulate glucose intolerance in patients with Type II DM. Since PPAR-gamma is responsible for glucose regulation, the pleiotropic effect of bezafibrate and its concomitant PPAR-gamma agonistic effect is evident and beneficial in these patients. (21)

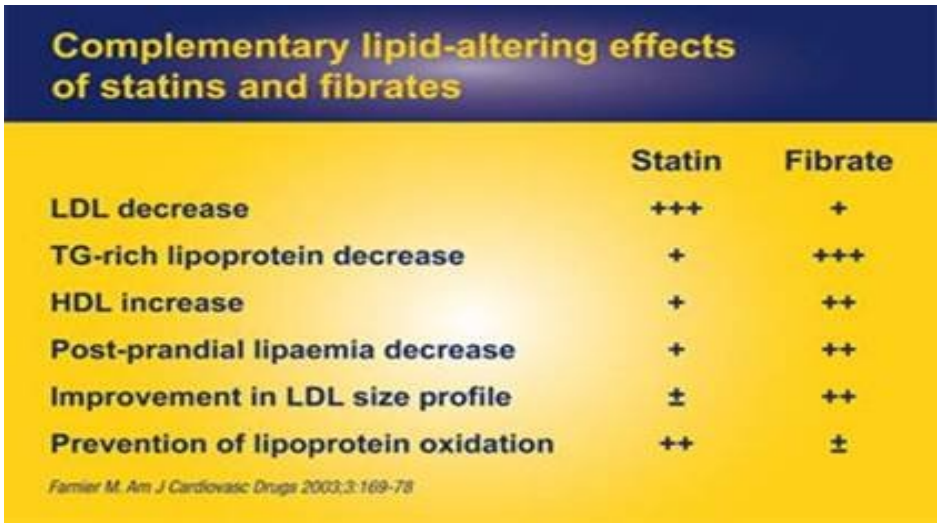

Figure 5: Comparison between statins and fibrates effects in different lipoprotein physiologies.

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Fenofibric acid, a fibrate marketed as Trilipix, was found to be beneficial when combined with statins in decreasing CVD events up to 35%, especially in secondary prevention of atherosclerotic complications. Opposing the general rule, the combination of fenofibric acid and statins was not found to have adverse muscular, renal and hepatic effects. (18)

As Agouridis *et al* clearly mentions, many studies including combination of different statins with fenofibrate were conducted in patients with DM suffering atherogenic mixed dyslipidemia, and the result was a decrease in LDL and TAG with the concomitant HDL increase. These resulting effects showed that these patients had a decrease in the risk of developing CVD. Thus, a combination of these agents is beneficial when it comes to cases like this. The risk, however, of developing some

adverse effect is also increased, with the most clinically important one being myositis leading to rhabdomyolysis. (21,55)

Opposing this statin and fibrate combination-induced muscular adverse effect, however, studies such as FIELD (Fenofibrate Intervention and Event Lowering in Diabetes), ACCORD (Action to Control Cardiovascular Risk in Diabetes), as well as other sub-studies containing statin/fibrate combination, showed that there was a significant decrease in the risk of developing Type II DM induced morbidities. The results showed a decrease in the risk of developing microvascular retinopathy and necessity for laser intervention, nephropathy and albuminuria as well as DM induced limb amputation. (55)

There are other benefits included in the usage of statin/fibrate combination. PPARα activation is shown to activate many pleiotropic effects of statins. Balakumar *et al* explained that when statins are followed by PPAR activation, they have beneficial effect on arterial hypertension, inflammatory response, chemotherapy (carboplatin) induced nephrotoxicity, cyclooxygenase (COX) mediated aggregation, cardiac hypertrophy and fibrosis as well as urinary tract protecting effects against inflammation and fibrosis. Many of these effects, however, are still under preclinical studies and they are yet to be confirmed in humans. (56)

24 CONCLUSIONS

In our modern society, many bad habits such as sedentary lifestyle and lack of physical exercise, smoking, poor quality food consumption and alcohol abuse tend to lead to a gradual and silent building of risk factors of developing atherosclerosis over the years. CVD is becoming clearly the most threatening cause of morbidity and mortality in many countries, while in others it is already the leading cause of death. Due to pharmaceutical and medical breakthroughs accompanying the last 100 years, population aging is becoming more and more relevant to this CVD development. Until, however, an intervention slowing this process is developed, our focus remains on countering the risk factors of developing atherosclerosis and CVD, increasing the patient's quality of life and reducing the risk of developing complications.

Atherosclerosis includes a wide range of risk factors and complications, and that is the main reason why more target-specific medicine becomes more and more

important. Many agents exert beneficial abilities on prevention and treatment of atherosclerosis. However, their impact is usually in one of the risk factors that may lead to it (Arterial Hypertension, Diabetes Mellitus, Dyslipidemia).

Statins and Fibrates are agents suggesting an undeniably giant step in preventing and treating one of the major causes of atherosclerosis. With their potency on diminishing the levels of LDL cholesterol, statins provide a great resort for countering elevated LDL levels, which tend to oxidize when endothelial dysfunction is evident, leading to the formation of foam cells, and hence, development of atherosclerotic plaque. As most of the pharmaceutical agents, statins also have a number of adverse effects. In the recent years, interventions have been made to develop a statin capable of producing the same, if not more, diminishing effects on LDL, with a minimized expression of adverse drug reactions. Pitavastatin has shown a benefit on both those parameters, even when administered in a much lesser strength compared to other statins. Statins also exert pleiotropic effects, and this is their most significant difference among other pharmaceutical interventions against dyslipidemias. These beneficial effects are evident on inflammatory and aggregation response, on LDL beta-oxidation and on the vasculature (NO synthesis and expression).

Fibrates is another class of lipid lowering interventions, acting through a different mechanism of action than that of statins. Atherogenic dyslipidemia is a common sight in patients with Type II DM, and it is characterized by diminished HDL levels, while free LDL and TAG levels in the circulation are elevated. Fibrates are effective on diminishing TAG through a gene expression inhibitory mechanism, while moderately increasing HDL.

These effects are complementary to statins, and this is why combination of these 2 classes is advised in cases like this. The risk of developing adverse effects due to monotherapy or combination of these agents must be always considered, especially in vulnerable patients suffering hepatic or renal insufficiency.

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