2.1. High Fat Diet

Excess fat intake is stored as fat, whereas excess carbohydrate is mostly oxidized in the short term but can lead to substantial gain in fat stores because of reduced fat oxidation and considerable de novo lipogenesis in the long term. Different types of fat in our diet affect our health differently. Some effects are beneficial, but others can lead to serious health risks. Fat, regardless of the type, is high in calories and should be a substitute for another source of energy, such as simple carbohydrates, in one's diet. By enriching our diets with fruits, vegetables, nuts, fish, lean meats, olive oil, whole grains, and low-fat dairy, we provide our bodies with the healthy fats they require. The UK Food Standards Agency (FSA) convened an expert group to agree upon definitions for high, medium and low in fat, saturated fat, total sugars and salt, which can be used in the context of front-of-pack ‘signposting’ of these nutrients. The FSA recommends using of this signposting indicating the level of these nutrients in food. Another definitions have been also developed by Coronary Prevention Group and food product retailers. CPG has setted up a definition of high fat for all food and drink as 49.5 kcal from fat/100 kcal of food, while FSA has setted up ≥20 g of fat/100 g of solid food and ≥21 g of fat/serving, if serving size ≥250 g, respectively. Those agencies use patterns to generate more rational definitions of high, medium and low in fat.9-11
2.1.1. Complication of high fat diet

Atherosclerosis and cardiovascular disease are the major complications of high fat diet and these diseases are the main cause of death in developed countries. Endothelial dysfunction, characterized by reduced bioavailability of nitric oxide, is considered the first pathological symptom of atherosclerosis. Recent evidence suggests that oxidative stress plays a role in the process of endothelial dysfunction. Several observational and epidemiological studies have associated the Mediterranean diet with a lower incidence of coronary artery disease. It has been postulated that the high content of certain antioxidant compounds on the diet may slow the atherogenic process by inhibiting oxidative damage and restoring endothelial function. Virgin olive oil is a major constituent of the Mediterranean diet. Olive oil, in addition to oleic acid, contains a range of micronutrients, such as phenolic compounds, which have been shown to possess antioxidant, anti-inflammatory action. Cardiovascular disease generally refers to conditions that involve narrowed or blocked blood vessels that can lead to a heart attack, chest pain (angina) or stroke. Other heart conditions, such as infections and conditions that affect heart's muscle, valves or beating rhythm, also are considered forms of heart disease.12,13

2.1.2. Risks caused by high fat diet

Diet is a very important factor in determining body health, the types of food we eat are having a substantial impact on our body to the point where they may either protect or increase the risk of developing body diseases.
A high fat diet is dangerous because of having too much fat in the diet can increase the risk of obesity, heart disease, cancer and diabetes. A high fat diet also can be dangerous because of the amount or the types of fat eaten. Too much of any macronutrient, carbohydrate, protein, or fat can lead to weight gain. Fat provides more than double the calories that carbohydrate or protein provide per gram, too much dietary fat sometimes leads to excess weight gain. Eating high amounts of certain types of fat can also be dangerous because of their impact on heart health. A diet high in saturated fat causes cholesterol to build up in the arteries (blood vessels). Cholesterol is a soft, waxy substance that can cause clogged or blocked arteries. This causes risk for arteriosclerosis, heart attack, stroke, and other major health problems. Another form of fats called trans fatty acids are also unhealthy fats that form when vegetable oil hardens in a process called hydrogenation. They are often used to keep food fresh for a long time, and for cooking in fast food restaurants.\textsuperscript{14,15}

There are also some other risks caused by high fat diet as mentioned below:

\subsection*{2.1.2.1. Diabetes mellitus}

Diabetes mellitus, or simply diabetes, is a group of metabolic diseases in which a person has high blood sugar, either because the pancreas does not produce enough insulin, or because cells do not respond to the insulin that is produced. This high blood sugar produces the classical symptoms of frequent urination, increased thirst, and increased hunger. Type 2 DM results from insulin
resistance, a condition in which cells fail to use insulin properly, sometimes combined with an absolute insulin deficiency. High-fat intake leading to obesity contributes to the development of non-insulin-dependent diabetes mellitus (NIDDM type 2).16

2.1.2.2. Obesity

The main reason for obesity is consumption of energy-dense food high in saturated fats and sugars, and reduced physical activity. And high fat diet is one of the main environmental factors that contributes to this disease. The studies have shown that increased fat intake is associated with body weight gain which can lead to obesity and other related metabolic diseases. Obesity has been cited as a contributing factor to approximately 100,000–400,000 deaths in the United States per year. The prevalence of obesity has been increasing all over the world. This increase is frequently attributed to the change in the life style of western societies, particularly important among them the consumption of high-fat diets. Fat is the dietary nutrient with the greatest energy density since it provides 9 kcal per gram, while carbohydrate and protein provide only 4 kcal. Thus, and increased fat intake can promote a high energy consumption and for this reason dietary fat is considered to be one of the environmental factors that most contribute to the current epidemic of obesity.16-18
2.1.3. Lipids Metabolism of high fat diet

2.1.3.1. Roles of Lipids

Lipids have a wide variety of roles in biological systems. These roles are a consequence of their chemical and physical properties. Fatty acids and their derivatives can act as highly concentrated energy storage molecules. The high energy density (i.e. the relatively large amount of energy released per unit of mass) of fat stores is due to three main factors. 1) The completely reduced carbons of fatty acids have higher energy content than the partially oxidized carbons of carbohydrates and proteins. 2) The fortuitous fact that the reduced carbons have covalent bonds to light atoms (hydrogen rather than to the heavier oxygen) means that the fully reduced hydrocarbon compounds are lighter than the partially oxidized carbohydrates. 3) Lipids are hydrophobic molecules and therefore fat stores contain little water, which would add to the weight of the molecules without adding to the energy content. Because layers of lipids are good insulators, and because adipose tissue has limited metabolic activity, fat stores can reduce the exchange of heat between an organism and its environment.\textsuperscript{19,20}

2.1.3.2. Digestion of lipids

The majority of lipids in a normal diet are presented in the form of triacylglycerols. Digestion of these compounds begins in the stomach, which contains acid-stable lipases that release some free fatty acids from dietary triacylglycerols. The stomach is not capable of efficiently cleaving triacylglycerols, because these hydrophobic molecules tend to aggregate, and the
Lipases are only capable of hydrolyzing the triacylglycerols at the surface of the aggregates. The small intestine has mechanisms for emulsifying lipids. The process begins by dispersing the lipid aggregates mechanically as a result of the muscles of the small intestine forcing the partially digested material through the relatively small spaces of the intestinal lumen. In addition, the intestine contains bile acids and bile salts, detergents that break up the lipid aggregates into smaller micelles. Finally, the small intestine also contains a variety of digestive enzymes produced in the pancreas. These enzymes include pancreatic cholesteryl ester hydrolase, which releases free cholesterol from cholesteryl esters, pancreatic lipase, which releases free fatty acids from the 1- and 3-positions of triacylglycerols, and several phospholipases, which release free fatty acids from phospholipids. The monoacylglycerols, partially hydrolyzed phospholipids, and free fatty acids act as additional detergents and assist in further disrupting the larger lipid aggregates. Short chain fatty acids enter the circulation directly but most of the fatty acids are reesterified with glycerol in the intestines to form triglycerides that enter into the blood as lipoprotein particles called chylomicrons. Lipoprotein lipase acts on these chylomicrons to form fatty acids. These stored as fat in adipose tissue.\textsuperscript{19,21,22,23}

2.1.3.3. Lipoproteins

Lipoproteins are synthesized in the liver, in the intestines, arise from metabolic changes of precursor lipoproteins. Lipoproteins consist of a mixture of protein, phospholipid, cholesterol, and triacylglycerol. The proportions of each vary depending on the specific type of particle. Lipid is less dense than protein or
water. Initial studies on lipid transport separated the different transport forms on the basis of density, with the density differential being largely the result of differing protein content.\textsuperscript{24,25}

2.1.3.4. Types of Dietary Fat

Fat is a major source of energy that helps human’s body absorb vitamins, important for growth, and to keep body healthy. A completely fat-free diet would not be healthy, but it is important that fat can be consumed in moderation. Since human’s body only needs a certain amount of fat each day, any extra fat that is consumed is stored in fat tissue. Fat also affects human heart, but the effect depends on which kind of fat is consumed. Different types of fats in our diet affect our health differently. Some effects are beneficial, but others can lead to serious health risks. Fat are divided into some types as follows:

2.1.3.4.1. Saturated fat

Saturated fat is fat that consists of triglycerides containing only saturated fatty acids. Saturated fatty acids have no double bonds between the individual carbon atoms of the fatty acid chain. That is, the chain of carbon atoms is fully saturated with hydrogen atoms. Saturated fats tend to raise both LDL-cholesterol and HDL-cholesterol. In excess, they also tend to promote insulin resistance. Therefore, saturated fat should be consumed only in limited quantities. Saturated fat occurs naturally in all dietary sources of fat derived from both plant and animal, but it is highest in fats from animals, especially dairy fats. In the human diet, the most common fatty acids of this type are palmitic acid and stearic acid.
We cannot avoid saturated fats, but we can reduce the proportion of them in our diets, limiting our intake.\textsuperscript{26,27}

2.1.3.4.2. Trans fat

Trans fat promotes inflammation, aggravates insulin resistance, and increases the risk of developing diabetes or even sudden cardiac death. Furthermore, it increases LDL-cholesterol and decreases HDL-cholesterol concentrations in the blood, while also increasing concentrations of VLDL-cholesterol and triglycerides. These effects can contribute to a proatherogenic lipoprotein profile. Food sources of trans fats include margarines, shortening, baked goods, and deep-fried foods such as French fries or chicken. Trans fats are recommended to be kept below 1\% of total energy intake.\textsuperscript{27,28}

2.1.3.4.3 Monounsaturated fatty acids

Monounsaturated fatty acids (MUFAs) increasing the dietary proportion of these fatty acids increases HDL-cholesterol and reduces LDL-cholesterol, thus improving blood cholesterol profiles. Increasing the proportion of MUFAs also reduces overall factors for cardiovascular risk, as has been demonstrated by dietary approaches that use olive oil as a staple along with other healthy constituents, such as the Mediterranean diet approach. Olive oil contains a high proportion of oleic acid (71\%), which is the most common MUFA in our diets. Olive oil also has anti-inflammatory properties, although it is not clear whether these properties come from oleic acid alone or are a synergistic effect coming from other components of olive oil.\textsuperscript{27-29}
2.1.3.4.4. Polyunsaturated fatty acids

Polyunsaturated fatty acids (PUFAs) or omega fatty acids. PUFAs are designated as omega fatty acids. Two of the omega-3 fatty acids that come from fish, eicosapentaenoic (EPA) and docosahexaenoic (DHA). The omega-3 fatty acids in fish oil reduce the risk of sudden cardiac death, lower blood triglycerides, slightly raise HDL-cholesterol concentrations, and reduce the proinflammatory.

2.2. Extra virgin olive oil

Extra virgin olive oil is produced from olive tree. The olive tree, Olea europaea, is native from the Mediterranean basin and parts of Asia Minor. The fruit and compression extracted oil have a wide range of therapeutic and culinary applications. EVOO also constitutes a major component of the Mediterranean diet. Olive oil is the main source of unsaturated fatty acids in the Mediterranean region, a nutritional regimen gaining ever-increasing renown for its beneficial effects on inflammation, cardiovascular disease, and cancer. EVOO is the oil obtained solely from the fruit of the olive tree (Olea europaea L), to the exclusion of oil obtained using solvents or re-esterification processes and of any mixture with other kinds of oil. EVOO is the fresh juice from the olive and is considered to be the highest grade olive oil. The main determinants are low acidity and the absence of flavour defects. Like other food products, extra virgin olive oil doesn’t stay fresh forever, the beneficial nutrients and fresh flavours will decrease and change as the oil ages. There are international and local voluntary industry standards that provide guidance for producers and consumers as to what chemical
and flavour properties extra virgin olive oil should have. The oil that comes from the first "pressing" of the olive solely by mechanical or other physical means and is extracted without using heat or chemicals. The oil must not be altered in any way. It can only be treated by washing, decanting, centrifuging and filtering. Extra Virgin Olive Oil contains no more than 0.8% acidity (0.8 grams per 100 grams, expressed as oleic acid), and is judged to have a superior taste. Extra virgin olive oils are best because they have the highest phenol content. Blood vessel response and function was improved for after the high-phenol olive oil meal. This improvement was associated with increased of dilating blood vessels, and reduced levels of oxidative stress - a process whereby the metabolic balance of a cell is disrupted by exposure to substances that result in the accumulation of free-radicals, which can damage the cell.\textsuperscript{4,30,31,32}

Decreasing fat consuming, particularly saturated fats which is known as low fat will be better for health. Thus choosing unsaturated fats or oils, especially monounsaturated fat (eg olive oil) as these types of fat are better for heart. Since fat is the greatest source of calories, consuming less fat will help you to lose weight.\textsuperscript{15}

2.2.1. Active compound of extra virgin olive oil

The chief active components of olive oil include oleic acid with percentages ranging from 56\% to 84\%. And also of other fatty acids such as palmitic, palmitoleic, stearic, linoleic, and a-linolenic acids and squalene. In addition to fatty acids, olive oil also contains phenolic compounds. Studies (human, animal, in vivo and in vitro) have demonstrated that olive oil phenolic
compounds have positive effects on certain physiological parameters, such as plasma lipoproteins, oxidative damage, inflammatory markers, platelet and cellular function, antimicrobial activity and bone health. The biological activities of olive oil are considered linked with its phenolics constituents, and linked to the direct or indirect antioxidant activity of olive oil phenolics and their metabolites, which are exerted more efficiently in the gastrointestinal (GI) tract, where dietary phenolics are more concentrated when compared to other organs.\textsuperscript{4,33,34}

2.2.2. The benefits of extra virgin olive oil

Several components of olive oil show beneficial health effects on the atherosclerotic and thrombotic formation processes, that include lipid oxidation, hemostasis, platelet aggregation, coagulation, and fibrinolysis. Oleic acid, a major component, and the polyphenols tocopherol, hydroxytyrosol, and oleuropein-of olive oil exhibit the most substantial antiatherosclerotic effects. The majority of olive oil fatty acid chains contain 16 or 18 carbon atoms. The carbon chains of all fatty acids have a carboxyl group (COOH) at one end. Olive oil composition includes a large proportion of unsaturated fatty acids (oleic, linoleic and linolenic acids), micronutrients, represented mainly by vitamins (A, E and b-carotene), and microconstituents (e.g. phenolic compounds or chemicals present in the unsaponifiable fraction). The total phenol content in virgin olive oil has been reported to vary from 100 mg/kg to 1 g/kg.\textsuperscript{31,35}
2.3. Endothelial cells

The endothelium is one of the largest systems in human body spread throughout the capillaries and arterioles in all tissues, forming a selectively permeable barrier between the outer vascular wall and the bloodstream. It also the tissue producing nitric oxide responsible for vasorelaxation, platelet aggregation, leukocyte endothelium adhesion and vascular smooth muscle cell migration and proliferation. Nitric oxide is synthesized from L-arginine in a reaction catalyzed by the endothelial eNOS. Thus, any factors decreasing eNOS activity and/or increasing NO degradation i.e. affecting the eNOS/NO system have been recognized as a potential source of disturbed endothelium function. Three isoforms of nitric oxide synthase have been identified: an endothelial type (eNOS), a neuronal type (nNOS), and an inducible type (iNOS). eNOS and nNOS are two forms of constitutive Ca++- and calmodulin-dependent NO synthases, i.e. requiring calcium ions and calmodulin for their activation. The eNOS is expressed constitutively in endothelial cells and synthesizes the NO needed for regulation of blood pressure. Nitric oxide produced by endothelial nitric oxide synthase, is recognised as a central anti-inflammatory and anti-atherogenic principle in the vasculature. Decreased availability of NO in the vasculature promotes the progression of cardiovascular diseases.6,36

2.3.1. Endothelial nitric oxide synthase (eNOS)

Endothelial NOS is a nitric oxide synthase that generates NO in blood vessels and is involved with regulating vascular tone by inhibiting smooth muscle
contraction and platelet aggregation. A constitutive Ca2+ dependent NOS provides a basal release of NO. eNOS is associated with plasma membranes surrounding cells and the membranes of Golgi bodies within cells. Nitric oxide that is generated by endothelium, is a key signaling molecule in vascular homeostasis, is a potent vasodilator and acts as an important key factor in the anti-atherosclerotic properties of the endothelium. It is a small molecule having a molecular weight of 30 Daltons, which is synthesized, via L-arginine oxidation, by a family of nitric oxide synthase (NOS) enzymes. eNOS is a potent cell-signaling molecule that plays important and diverse roles in biological processes such as neurotransmission, inflammatory response, and vascular homeostasis because of its importance biological effects. Cardiovascular diseases are the leading cause of death in the developed world and are emerging as a cause of death in developing countries. Endothelial dysfunction, characterised by a reduced capacity of endothelial cells to suppress processes of inflammation, thrombosis and oxidative stress is a central pathophysiologic process during the initiation and progression of atherosclerotic lesions. A key mechanism underlying endothelial dysfunction and increased expression of adhesion molecules and chemoattractants is the loss of endothelial nitric oxide bioavailability. Loss of NO bioavailability due to reduced synthesis and increased scavenging by reactive oxygen species is a cardinal feature of endothelial dysfunction in vascular disease states. Reduction in the production of nitric oxide is the first step for the occurrence of many diseases.\textsuperscript{37-39}
2.3.2. Nitric oxide

Nitric oxide is synthesized by a family of enzymes called NOS. Furthermore nitric oxide is a soluble gas with a half-life of 6–30s, continuously synthesized from the amino acid l-arginine in endothelial cells by the constitutive calcium-calmodulin-dependent enzyme nitric oxide synthase. This heme-containing oxygenase catalyzes a five-electron oxidation from one of the basic guanidino nitrogen atoms of l-arginine in the presence of multiple cofactors and oxygen. The substance responsible for the acetylcholine-stimulated relaxation was initially called endothelium-derived relaxant factor, and subsequently found to include nitric oxide. It is now known that a variety of agonists (e.g., acetylcholine, histamine, thrombin, serotonin, ADP, bradykinin, norepinephrine, substance P, and isoproterenol) can increase the synthesis and release of nitric oxide from the endothelium, although many of these same agonists constrict vascular smooth muscle in the absence of endothelium. High fat diet impairs nitric oxide bioavailability. The link between NO availability and the metabolic adaptation to high fat diet is not well characterized. Nitric oxide has a wide range of biological properties that maintain vascular homeostasis, including modulation of vascular dilator tone, regulation of local cell growth, and protection of the vessel from injurious consequences of platelets and cells circulating in blood. A growing list of conditions, including those commonly associated as risk factors for atherosclerosis such as hypertension and hypercholesterolemia, are associated with diminished release of nitric oxide into the arterial wall either because of impaired synthesis or excessive oxidative degradation. The endothelium is a
metabolically active organ system that maintains vascular homeostasis by (1) modulating vascular tone, (2) regulating solute transport into cell components of the vessel wall, local cellular growth, and extracellular matrix deposition, (3) protecting the vessel from the potentially injurious consequences of substances and cells circulating in blood, and (4) regulating the hemostatic, inflammatory, and reparative responses to local injury. Growing list of conditions, including hypercholesterolemia, systemic hypertension, smoking, diabetes, congestive heart failure, pulmonary hypertension, estrogen deficiency, hyperhomocysteinemia, and the aging process itself, have been associated with impaired functions of the endothelium. As a result, the vessel wall in these conditions may promote inflammation, oxidation of lipoproteins, smooth muscle proliferation, extracellular matrix deposition or lysis, accumulation of lipid-rich material, platelet activation, and thrombus formation. All of these consequences of endothelial dysfunction may contribute to development and clinical expression of atherosclerosis. Endothelial NOS activity plays a critical role in angiogenesis and endothelial function. Survival, proliferation and migration of endothelial cells, and their secretion of angiogenic factors, are dependent upon NO. Nitric oxide synthase (NOS) metabolizes L-arginine to L-citrulline and nitric oxide (NO), a key regulator of vascular and metabolic homeostasis. In the vasculature, the endothelial isoform (eNOS) exerts significant control over vessel tone, structure and interaction with circulating blood elements.\textsuperscript{5,6,37,40}
Figure 1. Summary of vasoprotective effects of nitric oxide in atherosclerosis.

(Adopted from: Yasa M, Turkseven S. 2005)\textsuperscript{5}

2.4. Intercellular adhesion molecule-1 (ICAM-1)

Intercellular adhesion molecule-1 (ICAM-1, CD54) is a member of the immunoglobulin superfamily. The main function of ICAM-1 is to participate in recognition and adhesion between cells. (ICAM-1, CD54) a transmembrane glycoprotein of 505 amino acids, has a molecular mass ranging from 80 to 114 kDa depending on the degree of glycosylation. The receptors of ICAM-1 are lymphocyte function associated antigen-1 (LFA-1) and macrophage surface antigen-1 (Mac-1), and the type of combine is heterogeneous affinity. ICAM-1 is the essential molecule in the interaction between LFA-1-dependent T-cells and fibroblasts, as well as the agglutination between lymphocytes or lymphocyte and endothelial cell under the stimulation by phorbol ester. ICAM-1 is a large class of glycoprotein molecules which located on the surface of cells. They mainly distribute on lymphocytes in the bone marrow, whereas, there is almost none on the peripheral blood cells. In normal tissues, ICAM-1 is expressed mostly in the spleen. Because the normal lymphocytes express low levels of ICAM-1,
lymphocytes provide a good environment for ICAM-1. In addition, in vascular endotheliocytes, mucous epitheliums of the tonsils, the thymus epitheliums, hepatic sinusoids lining cells, as well as some fibroblasts in some tissues have low levels of ICAM-1’s expression. SICAM-1 has been detected in various body fluids including serum, cerebrospinal fluid, synovial fluid, sputum, urine and bronchoalveolar fluid. SICAM-1 is present in normal human serum at concentrations between 100-450 ng/ml, whilst elevated sICAM-1 has been found in serum from patients with cardiovascular disease, cancer, autoimmune disease and correlated serum levels of sICAM-1 with severity of disease.\textsuperscript{41-43}

2.4.1. Role ICAM-1

ICAM-1 plays a critical role in the homing of leukocytes to sites of atherosclerotic lesions. ICAM-1 is to increase the association of leukocytes with the luminal surface of endothelial cells in the vicinity of an inflammatory stimulus. Bound leukocytes are activated and participate in the induction of interendothelial gaps that increase vascular leak and enable cellular transmigration into the extravascular tissue.\textsuperscript{7,41,43}
2.5. Theoretical Framework

**Figure 2.** Theoretical framework
2.6. Conceptual Framework

![Diagram](image)

**Figure 3.** Conceptual framework of study

2.7. Hypothesis

Administration of EVOO has effect in reducing ICAM-1 and increasing eNOS in high fat diet wistar rats.