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RESEARCH ARTICLE

LIPOTOXICITY IN OBESITY: BENEFIT OF OLIVE OIL

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ABSTRACT

The clinical implication of Lipotoxicity in obesity derives primarily from its potential to progress to insulin resistance, endothelial dysfunction and atherosclerosis. Olive oil rich diet decrease accumulation of triglyceride in the liver, improved postprandial triglyceride levels, improve glucose and GLP-1 response in insulin resistant subjects, and up regulate GLUT-2 expression in the liver. The exact molecular mechanism is unknown but, decreasing NFkB activation, decreasing LDL oxidation and improving insulin resistance by less production of inflammatory cytokines (TNF-a, IL-6) and improvement of kinases JNK-mediated phosphorylation of IRS-1 are the principle mechanisms. The beneficial effect of the Mediterranean diet derived from monounsaturated fatty acids (MUFA), mainly from olive oil. In this review we document lipotoxicity in obesity and the benefit of olive oil.

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INTRODUCTION

Obesity reduce life expectancy of up to 7 years compared with normal weight individuals and predict the future development of metabolic syndrome, Diabetes (T2DM), and cardiovascular disease [(1)]. Excess adiposity and poor cardiorespiratory fitness drive the epidemic of T2DM and early cardio vascular disease [(2).] Cardiovascular complications are the main cause of morbidity and mortality in obese, insulin resistance and type 2 diabetes mellitus (T2DM). Complications can be divided in micro vascular (retinopathy, neuropathy, nephropathy) and macrovascular (atherosclerosis, CAD, PVD, and Stroke). Multiple mechanisms contribute to these clinical outcomes including hyperglycemia, hyperinsulinemia, insulin résistance, inflammation, change in circulating adipokines, and alteration in intracellular signaling pathway. Increased circulating concentration of lipids and altered tissue metabolism of lipids is consistent feature of this prevalent condition and contribute importantly to cardiovascular complications.

Lipotoxicity: Lipotoxicity is the diverse effect of fatty acid accumulation in non-adipose tissue, the liver, muscle, pancreatic beta-cell, cardiac and arteries are the main targets. The term was coined by Unger to describe the deleterious effects of tissue fat accumulation on glucose metabolism; however the term has assumed added significance [(3)].

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Mechanism of Toxicity

Spectroscopic studies by MRI have demonstrated that intramyocellular and intrahepatic fat accumulations are closely associated with organ specific insulin resistance by impairing insulin signaling and multiple intracellular steps of glucose metabolism [(4-7)]. The deleterious effect of increase intracellular fat on insulin sensitivity is supported by the work of Kim *et al*. who overexpressed lipoprotein lipase in muscle and or liver in mice[(8)]. Plasma NEFA elevation by lipid infusion also increased intramyocellular diacylglycerol, a potent activator of protein kinase C (PKC) isoforms, which inhibits insulin signaling through serine phosphorylation of IRS-1[(9)],were demonstrated in animals and humans[((10-11)]. Ceramide levels are also increased in muscle and plasma in obese and T2DM individuals, correlating with severity of insulin resistance [(12-14)] Adiposities have a great ability to adapt to overfeeding by means of hypertrophy and hyperplasia. Within this context, adipose tissue must be viewed primary as a protective tissue that store and prevent excessive exposure of other organs to

fatty acid[15-16].Protection from chronic energy supply and access triglyceride accumulation in tissue (liver, pancreatic beta-cell, muscle) require an extraordinary adaptation by adiposities that involves activation of several inflammatory pathway but at the cost of insulin resistance, the most relevant of this pathway in obesity are the inhibitor kB Kinase/ nuclear factor kB pathway in which FFA active Toll-like-receptor (TLR-4) in macrophage and adiposities. Pharmacologically decreasing plasma FFA levels restores hepatic insulin sensitivity [15-17]

Role of lipotoxicity in liver, muscle, beta-cells, cardiac and vessel disease

In Skeletal muscle: increase plasma FFA concentration impairs insulin signaling and cause skeletal muscle insulin resistance, and increase in intra myocellular lipids[18]. Intramyocellular diacylglycerols concentration have been reported to be markedly high, this metabolites impair insulin signaling and activate inflammatory pathway, including certain protein kinas C isoforms and IkB/nuclear factor kB. A decrease in plasma FFA improves rapidly insulin sensitivity[19].

In Pancreatic beta-cell: One-third of obese adults aged>60 have "pre-diabetics" in the USA. Sustained elevation of plasma FFA levels impairs insulin secretion in lean subjects, and in non-diabetic subjects genetically predisposed to develop T2DM[20]. FFA induced pancreatic bet-cell dysfunction and can be rapidly reversed in these subjects by decreasing the release of FFAs [18]

Liver: Two third of fatty acid delivery are supplies by the adipocytes. In lean subjects insulin resistance can be induced rapidly by a lipid infusion[21-22].The presence of diabetes is associated with advanced liver disease, cirrhosis, and hepatocellular carcinoma[23-28]. There is close relationship between obesity, T2DM and non alcoholic fatty liver disease (NAFLD) [29].The prevalence of steatohepatitis (NASH) among patients with NAFLD range from 15% to 40% and increases in the presence metabolic syndrome [25-31]. Disease progression from bland steatosis to NASH is associated with mitochondrial dysfunction, endoplasmic reticulum stress, reactive oxygen species formation, and active inflammatory pathway by toxic lipids metabolites such as diacylglycerols, ceramides and others [32-33]. Development of severe advanced fibrosis is believed to occur in 10%-20% of patients with NASH [34-36]. Recent metabolic studies in patients with NASH suggests that liver fibrosis correlates closely with sever adipose tissue insulin resistance[37], rendering further support to the link between obesity and fibrosis and making adipose tissue a potential target for the prevention of disease progression.

Vessel: Endothelial dysfunction is a hallmark in obesity and diabetes-related vascular dysfunction. Central aspect of endothelial dysfunction is reduced nitric oxide (NO) bioactivity. Multiple alterations such as hyperglycemia, oxidative stress, activation of rennin-angiotensin system and increased pro-inflammatory cytokines contribute independently and synergistically to endothelial dysfunction. Vascular insulin resistance correlates with endothelial dysfunction [38-39]. This relationship is driven by a pathway-selective inhibition of insulin mediated activation of NO synthase (eNOS) by PI3K and AKT, whereas MAPK signaling to endothelin 1 (ET-1) is intact or even augmented [40]. The resulting endothelial cell dysfunction renders the vascular wall more susceptible to atherosclerosis and less responsive to agonist- induced vasodilatation [41-42].

Lipotoxicity, Insulin resistance and Atherosclerosis (Figure 1)

Increase FFA impair endothelial cell insulin signaling and NO production through the activation of the inhibitor β/nuclear factor kB pathway, and experimentally induced plasma FFA elevation in humans alter endothelial function[43-44].In vivo studies provide that insulin promoteatherogenesis [45-47]. Non diabetic chickens fed high-cholesterol diet develops sever atherosclerosis, which regress when switched to low-cholesterol diet [47]. Insulin administration prevented regression of coronary atherosclerosis[46]. In other experiment; dogs receiving a low dose insulin infusion into hindlimb develop sever atherosclerosis, whereas all other arteries remained free of atherosclerosis plaque[45].Other effect of FFA is inducing inflammation. Palmitic, Oleic, and Linoleic acid comprise 70% of the total circulating FFA[48]. Palmitate signals via TLR4, a pattern recognition receptor that is essential for initiating inflammatory responses associated with innate immunity[49-50]. When palmitate signal via TLR4, allowing nuclear translocation of NF kβ-kinase (IKK), a transcriptional activator of the expression of many gens involved in inflammation[49].

Myocardial steatosis and cardiac function

Recently, proposed mechanisms involved in cardiac lipid utilization and the development of lipotoxicity in the heart that lead to contractile dysfunction. The mechanism includes altered AMPK signaling, ceramide accumulation, endoplasmic reticulum (ER)-stress, ROS, and mitochondrial dysfunction [51]. Studies via MRI [52-53]have increased the understanding of myocardial lipid accumulation and metabolic fat of circulating fatty acid. Analysis of cardiac tissue has provided impair cardiac function in subjects with obesity and T2DM, with or without heart failure[54-55].Change in cardiac lipids content can also be induced in otherwise healthy individual subjected to short- term hyperinsulinemia and hyperglycemia[56], temporally increasing circulating FFAs in healthy individuals exercising the fasted state. Reducing lipid accumulation in obese individual leading to improved cardiac function[57].Heart failure is associated with increased lipid accumulation in cardiac tissue[58], lipid accumulation in the failing heart may be reversed by mechanical unloading, which partially alleviate mitochondrial dysfunction and insulin resistance[59]. However reducing circulating FFAs during heart failure is not sufficient to restore cardiac function [60].This accumulation of FFAs reflect complex interaction between the metabolic and neurohumoral milieu and change in mechanisms governing cardiac lipid uptake and metabolism. However the functional implication of myocardial steatosis is context-dependent, where it may be maladaptive, or could represent adaptation that might be cardio protective. Where triglycerides per se are not likely to be toxic, but may be a biomarker for the accumulation of more toxic and reactive lipids. Paradoxically, increase dietary fat, may lead to cardio protection in heart that are already undergoing left ventricular remodeling[39].

Table 1. Mechanisms of action of olive oil on Lipotoxicity

Mechanism	Component involved
Anti-inflammatory & immuno modulatory effects	Oleic acid Phenolic compounds
Anti-oxidants: Decrease lipid peroxidation Decrease oxidative DNA damage	Oleic acid Phenolic compounds: hydroxytyrosol, oleuropein, caffeic acid, o-coumaric acid, vanillic acid, and dihydroxyphenylethanol
Modulation of transduction pathways: Decrease arachidonic acid Inhibit lipoxygenase Inhibit HMG-CoA reductase Decrease in RAS activation Regulation of gene expression in liver regeneration: (Oleic acid inhibit ð6-desaturase which decrease PGE2 and inhibit liver regeneration)	Oleic acid Phenolic compounds: protocatecuic acid Hydroxytyrosol Squalene Squalene Oleic acid Minor compounds
Change in membranes fluidity and membrane Peroxidation (estrogen modulator, regulate G protein)	Oleic acid Lignans

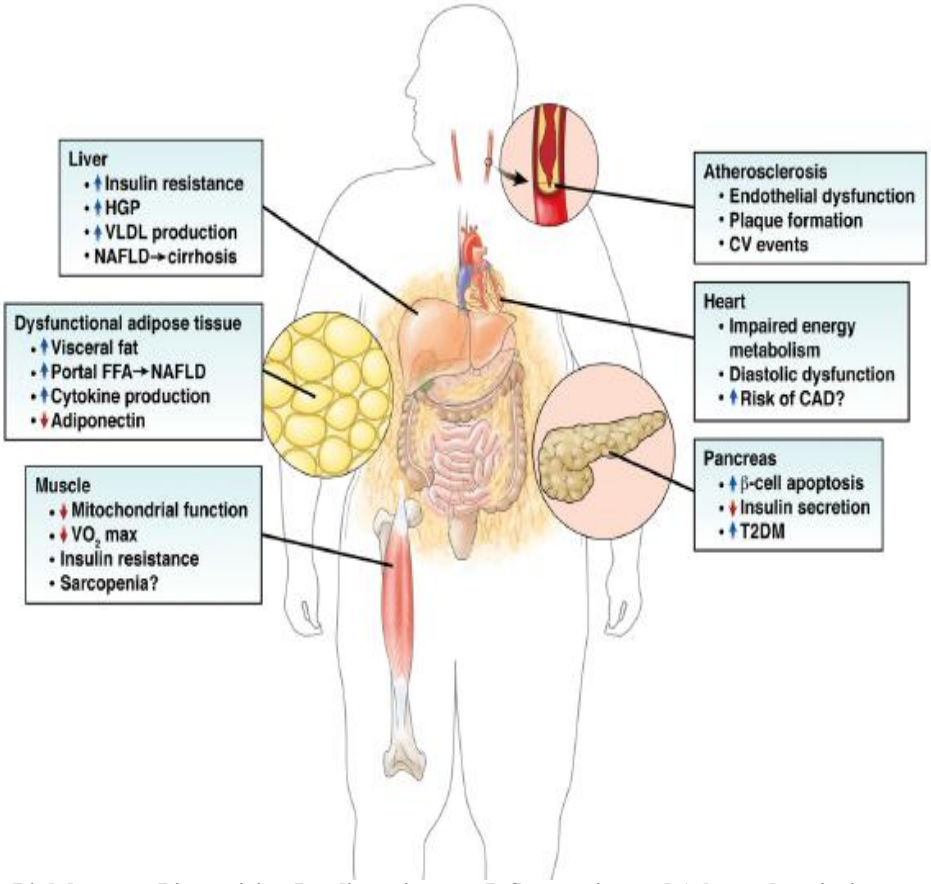

Figure 1. Link between Lipotoxicity, Insulin resistance, Inflammation and Atherosclerosis: increased plasma NEFA/intramyocellular levels of toxic lipids metabolites (long- chain fatty acyl CoAs, diacylglycerol, ceramides) play a role in the pathogenesis of muscle, liver and other tissue insulin resistance

Benefit of olive oil

Diet and nutrition, in particular the amount and type of fat intake, has been linked to insulin resistance, an increased risk of developing type 2 diabetes and impaired postprandial lipid metabolism[61-62]. "Mediterranean diet" has been associated with higher survival and for lower all-causemortality. The main characteristics of the Mediterranean diet include an abundance of plant food (fruits, vegetables, whole-grain cereals, nuts, and legumes); olive oil as the principal source of fat; fish and poultry consumed in low-to moderate amounts; relatively low consumption of red meat; and moderate consumption of wine[63-64].

Composition of olive oil

Each 100 g of olive oil contains the following fatty acids: MUFA 73.7 g (n-9 oleic acid 18:1); saturated fatty acids (SFA) 13.5 g (16:0 palmitic acid); polyunsaturated fatty acids (PUFA) 7.9 g (n-6 linoleic acid 18:2, and n-3 alpha linoleic acid 18:3)[14]. MUFAs include palmitic (C16:1), oleic (C18:1), elaidic (C18:1) and vacentic acids (C18:1). The most abundant MUFA in the diet is oleic acid (C18:1 n-9) [15]. In Mediterranean countries, the main source of MUFA is olive oil (74 g/100 g). Other oil sources of MUFAs are canola (59 g/100 g), peanut (46 g/100 g), sunflower (32 g/100 g), corn (29

g/100 g), soybean (24 g/100 g) and safflower oils (14 g/100 g) [16]. Additionally, new oil variants, rich in oleic acid have been developed including high-oleic acid sunflower oil (84 g/100 g) and high oleicacid safflower oil (74 g/100 g) [17]. In addition to a high MUFA content, virgin (unrefined) olive oil contains a significant amount of antioxidants and α-tocopherol and phytochemicals. However, when refined or heated, olive oil loses these natural compounds[65]. Olive oil is graded according to its acidity. Extravirgin olive oil, the first pressed oil, having maximum freeacidity, contains an abundance of squalene and phenolicantioxidants including simple phenols (hydroxytyrosol,tyrosol), aldehyde secoiridoids, flavonoids and lignans(acetoxypinoresnol, pinoresinol). Interestingly, itcontains significantly higher concentrations of phenolic antioxidants and squalene than refined virgin andseed oils. In addition, seed oils, which contain verylow amounts of squalene, have none of the phenolicantioxidants that are present in virgin and refined oliveoils[66]. The exact composition of olive oil depends notonly on the growth conditions in the year preceding theharvest, but also on the degree of ripeness of the fruitand the technical processing (cold pressing, refining)[65].

Oleic Acid: Olive oil is approximately 72-percent oleic acid, a monounsaturated fatty acid. Olive oil is unique with respect to the high oleic acid content because the majority of seed oils are composed primarily of polyunsaturated fatty acids, including the essential omega-6 fatty acid, linoleic acid. Compared to polyunsaturated fatty acids, oleic acid is monounsaturated, meaning it has one double bond, making it much less susceptible to oxidation and contributing to the antioxidant action, high stability, and long shelf life of olive oil.

Mechanism action of oleic acid and other minor compounds of olive oil: The main mechanism by which the components of olive oil influence endothelial activation involves inhibition and/or scavenging of ROS. Oleic acid and h-sitosterol may reduce intracellular ROS by creating a less-oxidant environment through inhibition of intracellular ROS production. H-sitosterol may also enhance SOD activity, hence decreasing O À 2 levels. This reduction has also observed for the terpenoid oleanolic acid, although the mechanism not known, tocopherols and phenolic compounds are potent antioxidant that may help lipid peroxidation and scavenge intracellular free ROS and free NO, reduction of OONO **À.** ROS can active the NF n B, which is then translocated into the nucleus, where it binds to recognition sequences in DNA to induce gene expression. This mobilization of NF n B is blocked by a-tocopherolsuccinate but not by a-tocopherol. In contest, phenolic compounds have been proposed to act blocking the formation of NF n B/DNA binding complexes. NF n B modulation the expression of cytokines. LOX and COX, thereby affecting the levels of adhesion molecules and eicosanoids. However, some of the minor compounds of olive oil may act directly on this enzymes and cytokines. LOX and COX activities are inhibited at different points by phenolic and triterpenoids whereas IL-1 expression is inhibited by phenolicand tocopherols, contributing to protect the endothelium against vasoconstriction, platelet aggregation and monocyte adhesion. Vasodilatation is also suggested to be enhanced by oleuropien and oleanolic acid through an increase in the production of NO[67].

The Effect of Olive Oil on Specific condition [68]

***Coronary Heart Disease*:** Oxidation of LDL cholesterol has been identified as one of the first steps in the development of atherosclerotic lesions by promoting injury to the arterial wall through several mechanisms, including growth factor and chemotactic protein expression, inflammation, and increased local macrophages. Macrophages bind to and engulf oxidized LDL – an innate immune response to tissue damage. This engulfment produces a fatty foam cell, which, when combined with other cells, produces a fatty streak in the blood vessel[69]. Oxidized LDL can also be taken up directly by endothelial and smooth muscles cells, leading to formation of fatty streaks, which is the first sign of atherosclerosis. The lesions forming atherosclerotic plaques are made up of lipids, endothelial and smooth muscle cells, and extracellular matrix. The plaque environment is proinflammatory. Inflammation occurring prior to the formation of fatty streaks and atherosclerotic lesions causes alterations to the endothelial cell wall, which increases the adhesion of leukocytes, LDL cholesterol, and platelets. This contributes to the development of atherosclerosis and cardiovascular disease[69]. The Giovanna study[70] have demonstrate for the first time that olive oil enhanced fat oxidation and regulated myocardial metabolic enzymes, optimizing cardiac energy metabolism in obesity conditions. Olive oil and its minor phenolic compounds, oleuropein and cafeic acid had myocardial antioxidant activity in standard-fed conditions.

***Hypertension*:** As with other aspects of cardiovascular diseases, there is a reduced incidence of hypertension in populations that consume the Mediterranean diet,[71-72] and adherence to the Mediterranean diet is inversely related to systolic and diastolic blood pressure. [73] Several studies have demonstrated the antihypertensive properties of olive oil[74-76]. Epidemiological data from studies in three Mediterranean countries- Italy, Greece, and Spain – as well as non-Mediterranean countries, suggest a protective effect for monounsaturated fatty acids or olive oil, while non Mediterranean countries show little or no positive effects[77]. Ferrara *et al* compared a diet rich in polyunsaturated fatty acids (from sunflower oil) with a diet high in monounsaturated fatty acids (from olive oil) in patients taking antihypertensive medications[78] and found individuals who consumed an olive oil-rich diet were able to reduce the dosage of antihypertensive medication. Either tan the mechanism of action for blood pressure reduction is unknown, although several theories have been proposed. Giuliani *et al* concluded that olive oil is a calcium channel antagonist; closely mimicking the effects of the calcium channel blocker drug verapamil.1 another suggested mechanism is via improved endothelial function[71-77-79]. Phenols and oleic acid may contribute to improved endothelial function by reducing ROS. Other potential mechanisms have been suggested, including decreasing vascular tone and changes to the fatty acid and phospholipid composition of the aorta[78].

Effect on low density lipoprotein (LDL): Animal studies have shown Squalene added to the diet of rats resulted in an 80-percent increase in serum squalene levels and inhibition of the hepatic enzyme HMG-CoA reductase the enzyme inhibition may be due to squalene or its metabolites. HMG-CoA reductase[80], the rate-limiting enzyme in the biosynthesis

of cholesterol, results in decreased production of cholesterol and the intermediates formed during its biosynthesis[80]. Following acute administration of squalene, the rate of cholesterol synthesis increased 9-24 hours post-administration .This apparent conflicting data may be a result of the single dose of squalene used[81]. These observed differences may be due to the dose ofsqualene. Short-term studies have shown increased dietary squalene, while increasing serum squalene levels, does not cause an increase in serum cholesterol or atherosclerosis[81-82].

Liver: Decreasing total fat consumption and shifting to MUFAs found in olive oil (20%-40% of total energy) or n-3 PUFAs found in fish oil (2 g/d) could lead to a decrease in postprandial lipidemia and steatosis. In one study [83]a modified Mediterranean diet (42% carbohydrates) was associated with lower alanine aminotransferase (ALT) levels at both 6 and 12 months compared with both the 2003 American Diabetes Association (ADA) diet and a low-glycemic-index diet, independent of weight changes. People allocated to a Mediterranean diet have lower circulating levels of triglyceridesand less abdominal obesity, as compared with control diets. [84-86].

List of Index words

Glut-2,glucose transporter; GLP-1, Glucagon-like peptide 1; NF-Kb, nuclear factor Kb, TNF-a, Tumor necrosis factor alpha, IL-6 interleukin- 6, *IRS-1*, insulin receptor substrate 1, CAD, coronary artery diseases, PVD, peripheral vascular disease, NEFA nonesterified fatty acids, TLR-4,Toll-like receptor 4, AMPK activated protein kinase, IKK-β, I-Kb kinas- β; TNFR, tumor necrosis factor receptor, FFA, free fatty acids; CETP, cholesterol ester transfer protein; PKC, protein kinase C; MCDD, methionine-choline deficient diet; MDA, SREBP, sterol regulatory element-binding protein,IRS-1 Insulin receptor substrate 1 , monounsaturated fatty acids (MUFA), Lipoxygenases (LOX) and Cyclooxygenases (COX) metabolizing enzymes,

Conclusion

Dietary fat content modified fat accumulation in obese subjects. Obese patients with NAFLD have a higher postprandial TG response and an increased production of VLDL suggesting that the metabolism of dietary fat is impaired in these individuals. Decreasing total fat consumption and shifting to monounsaturated fat found in olive oil could lead to a decrease in postprandial lipidemia and in Lipotoxicity. Further studies in humans are needed to ascertain whether the consumption of olive oil may be helpful in obese individuals with lipotoxicity and NAFLD

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