

# The Perfect Storm: Non-Alcoholic Fatty Liver Disease (NAFLD) and Atheromatosis in Morbidly Obese Patients with or Without Diabetes. Effect of Bariatric Surgery



Júlia Carmona<sup>1</sup>, Eva Pardina<sup>1</sup>, Joana Rossell<sup>1</sup>, David Ricart Jané<sup>1</sup>, Juan A Baena Fustegueras<sup>2</sup> and Julia Peinado Onsurbe<sup>1\*</sup>

<sup>1</sup>Departament de Bioquímica i Biomedicina Molecular, Facultat de Biologia, Universitat de Barcelona, Spain

<sup>2</sup>Unitat de Cirurgia, Hospital Universitari Arnau de Vilanova (Universitat de Lleida), Spain

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**\*Corresponding author:** Julia Peinado Onsurbe, Department de Bioquímica i Biomedicina Molecular, Facultat de Biologia, Universitat de Barcelona, Diagonal 643, 08028 Barcelona, Spain, Tel: (34)-(93)-4021524/48; Fax: (34)-(93)-4021559; Email: [jpeinado@ub.edu](mailto:jpeinado@ub.edu)

## Abstract

In recent years, the prevalence of non-alcoholic fatty liver disease (NAFLD) has increased rapidly, paralleling the epidemic of type 2 diabetes mellitus (T2DM) and obesity leading to cardiovascular disease (CVD). It has been demonstrated that NAFLD is strongly associated with atherosclerosis [1]. With recently gained knowledge, it now appears that NAFLD might induce insulin resistance, dyslipidaemia, oxidative stress, inflammation, and fluctuation of the adipokines associated with atherosclerosis [1]. The association between NAFLD and atheromatosis in obese patients with or without T2DM has been controversial. It could be that liver disease participates in the origin of atheromatous plaques.

Excess liver fat is extremely common, and the prevalence of NAFLD has been increasing mainly because of the increased prevalence of obesity. The prevalence increases to 57% in obese subjects, 70% in diabetic subjects and 90% in morbidly obese people. Patients with NAFLD are at increased risk for cardio-metabolic complications, such as CVD and T2DM. Bariatric surgery corrects improves the steatosis, T2DM [2,3] and cardiovascular risk factors, and it reduces long-term cardiovascular events.

It is possible that a higher degree of liver disease indicates further progression of atheroma, but that finding could occur given that, due to a certain degree of liver injury, bariatric surgery is notable to improve or reverse vascular lesions [4].

**Keywords:** Steatosis; Atherosclerosis; Liver; Obesity; Diabetes; Cardiovascular disease

**Abbreviations:** NAFLD: Non-Alcoholic Fatty Liver Disease; T2DM: Type 2 Diabetes Mellitus; FFA: Free Fatty Acids; ROS: Reactive Oxygen Species; PUFA: Polyunsaturated Fatty Acids; VV: *Vasa Vasorum*

## Introduction

### Non-alcoholic fatty liver disease (NAFLD)

Excess liver fat is extremely common, and the prevalence of NAFLD has been increasing rapidly, paralleling the epidemic of type 2 diabetes mellitus (T2DM) and obesity leading to cardiovascular disease (CVD). It has been demonstrated that NAFLD is strongly associated with atherosclerosis [1]. Simple hepatic steatosis has been shown to be associated with marked silent carotid atherosclerosis [5]. The prevalence increases to 57% in obese subjects, 70% in diabetic subjects and 90% in morbidly obese people [6,7].

Increased hepatocyte triglyceride formation could play a protective role to prevent hepatocytes from FFA-induced damage. These toxic effects induced by FFA and other derived metabolites is known as lipotoxicity [8]. One important mediator of lipotoxicity is the over-production of reactive oxygen species (ROS). When ROS production exceeds the antioxidant capacity, it leads to oxidative stress. Numerous studies have demonstrated that oxidative stress is elevated in NAFLD patients [9]. Despite the powerful anti-oxidant capacity of the liver, excessive FFA oxidation in the steatotic hepatocytes could cause substantial oxidative stress [10].

Oxidative stress can cause mitochondrial injury by causing the reaction of ROS with polyunsaturated fatty acids (PUFAs) at the mitochondrial membrane. Insulin resistance (IR) plays a central role in these processes by allowing for the excessive flow of fatty acids from adipose tissue and also by impairing peripheral glucose disposal. Peroxisome proliferator-activated receptor  $\alpha$  (PPAR- $\alpha$ ) is a key transcription factor regulating the expression of genes involved in mitochondrial, peroxisomal and microsomal FFA oxidation [11]. Hepatocyte injury, a characteristic of NAFLD, which manifests as ballooning, is produced by the abnormal distribution of intermediate filaments induced by oxidative stress. This reaction is mediated by the Wnt/ $\beta$ -catenin pathway. These ROS attack and react with PUFAs (polyunsaturated fatty acids) present at the mitochondrial membranes, so ROS damage mitochondrial DNA and cause mitochondrial dysfunction. Apoptosis can be initiated by dysfunction of mitochondria via the Wnt/ $\beta$ -catenin pathway, and it is also the main mechanism of death in NASH, promoting the progression from simple steatosis to NASH.

Kupffer cells play a key role in liver inflammation [12], regulated by the balance of pro-inflammatory M1 Kupffer cells and anti-inflammatory M2 Kupffer cells [13]. Imbalanced M1/M2 phenotypic Kupffer cells have emerged as a central mechanism underlying steatohepatitis. Kupffer cells are exposed to various substances and they function to sense and remove pathogens and dangerous molecules via pattern recognition receptors (PRRs). The PRRs comprise at least two families of sensing proteins: the Toll-like receptors (TLRs) and the NOD-like receptors (NLRs). Both NLRs and TLRs detect danger signals, including pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs). PAMPs are pathogens originating from gut-derived microorganisms, while DAMPs include molecules endogenously released from stressed or injured hepatocytes. TLRs recognize bacterial products derived from gut microbiota, such as lipopolysaccharide (LPS, also known as endotoxin) and peptidoglycan. Kupffer cells are the primary sensors of PAMPs and DAMPs as well, and TLR and NLR receptors have emerged as important mediators of Kupffer cell activation [14].

### Atheromatous processes

The *vasa vasorum* (VV) are blood vessels that extend from the adventitia of large vessels and irrigate their walls. Its primary mission is to provide nutrients and oxygen to the layers that cannot penetrate the blood vessel lumen. One of the earliest changes that can be observed in the atheromatous process is the proliferation of VV in the adventitia [15]. The hypoxia is the main stimulus of intimal neovascularization by perpendicular adventitial vessels [16]. Hypoxia of the vessel wall, either by injury or by increasing demand, is the main factor inducing neoangiogenesis [17]. A correlation was shown between the density of the VV and the progression of atherosclerotic plaque [18]. Neovascularization and endothelial dysfunction are the

initial phenomena of atheromatosis, while increased IMT and appearance of the plaque occur later.

### Endothelial injury

The endothelial injury process begins with the molecular responses of transcription factors induced by hypoxia (HIF, hypoxia inducing factor) [19], where in the increase in low density lipoprotein [20] and reduced nitric oxide (NO, main vasodilator) initiate a cascade of endothelial activation, recruitment of inflammatory cells, production of reactive oxygen species (ROS) and monocyte infiltration, the direct migration of which is mediated by monocyte chemoattractant protein (MCP-1) [21]. The LDL are subjected to oxidative modification, resulting in a highly oxidized and aggregated lipoprotein called oxLDL, which is among the more atherogenic forms of LDL [22]. OxLDL stimulates the inflammatory signalling by endothelial cells, releasing chemotactic proteins, such as MCP1, and growth factors, such as monocyte colony-stimulating factor (M-CSF), which facilitate the recruitment of monocytes by the arterial wall, a process mediated by the selectins [23]. oxLDL also promotes monocyte differentiation into the macrophages that convert oxLDL into lipid-laden foam cells, which are cells with the hallmark of atherosclerosis [22]. oxLDL is recognized by the scavenger receptor (landfill) macrophages SR-A and SRB-1 or CD36 (scavenger receptor-A and B, respectively). In nondiabetic subjects, sCD36 (soluble CD36) was significantly associated with IR indices, carotid atherosclerosis and fatty liver. However, prospective studies are needed to further evaluate the role of sCD36 in the inter-relationship among atherosclerosis, fatty liver and insulin resistance [24].

Activated macrophages express cytokines, such as TNF- $\alpha$ , and IL-1  $\beta$  (Interleukin-1  $\beta$ ), which stimulate endothelial cells to express adhesion proteins, such as VCAM-1 and ICAM-1 (vascular-1 molecules and adhesion intercellular-1, respectively). ICAM-1 plays an important role in the recruitment of immune cells during the progression of plaque, and it was positively correlated with HOMA-IR (homeostasis model of resistance insulin), BMI (body mass index), leptin, and adiponectin and negatively correlated with high density lipoprotein (HDL) cholesterol [25]. Moreover, ICAM-1 is induced as a result of the binding of angiotensin-2 to its receptor, promoting the release of ROS and endothelin-1 by endothelial cells [26]. Other molecules involved in pathophysiology of the plaque are VEGF [26], PAI-1 and adiponectin [27].

It is well established that IR is the primary factor underlying hepatic steatosis. IR is present in almost all NAFLD patients [28]. Fat accumulation in the liver is associated with oxidative stress and lipid peroxidation. Furthermore, NAFLD subjects have increased secretion of inflammatory marker and plasma glucose and decreased HDL concentrations. The consequence of this physiological dysfunction is an increased risk for the development of diabetes and atherosclerosis and an increased risk of coronary artery disease [29].

Both diabetes and IR cause a combination of endothelial dysfunctions, which can diminish the anti-atherogenic role of the vascular endothelium [30]. Therefore, in patients with diabetes or insulin resistance, endothelial dysfunction might be a critical early target for preventing atherosclerosis and cardiovascular disease. The biochemical or cellular links between elevated blood glucose levels and vascular lesions remain incompletely understood.

### Molecular mechanisms

MicroRNAs (miRNAs) have emerged as a new class of gene regulators, recent studies of which have emphasized that they play a crucial role in atherosclerosis [15]. miRNAs have also been associated with oxidative stress, inflammation, insulin signalling, apoptosis and angiogenesis related to obesity. All of these processes contribute to the development of T2DM and atherosclerosis and therefore are associated with cardiovascular disorders [31]. Recent studies have reported that the miRNAs released by cells have endothelial anti-atherogenic properties, similar to those that increase vascular areas when the laminar flow is high or that decrease when the flow is low or abnormal, such as miRNA-10a [32], the miRNA-19 [33] or miRNA-143/-145 [34]. Other studies have reported pro-atherogenic roles for miRNA-712 [35] and miRNA-92a [36].

Studies of human NAFLD have identified approximately 44 miRNAs dysregulated in the NAFLD liver [37]. Several miRNAs have been identified as playing key roles in the development of steatosis and its progression to steatohepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma [38]. Overexpression of miR-185 resulted in increased insulin receptor substrate-2 (IRS-2) expression, improved insulin sensitivity and reduced steatosis [39]. The predicted targets of miR-122 include genes regulating cholesterol and lipid metabolism, proteasomal protein degradation, cell adhesion and extracellular matrix biology [40]. In addition to miR-122, several miRNAs have been associated with the pathogenesis of NAFLD. Cheung and colleagues found that miR-21 was heavily upregulated in the livers of patients with steatohepatitis [41].

Atheromatosis, NAFLD and endothelial changes may be reversible Bigornia et al. [42] demonstrated that reversing endothelial dysfunction at 12 months of weight loss was a more important metabolic change than the degree of weight loss. Mavri et al. [43] observed similar results in obese subjects submitted to a diet and found an improvement in endothelial dysfunction at one week after initiating the diet. Recently, decreased IMT was found after gastric bypass only in obese patients with T2DM but not in patients with impaired glucose tolerance [44]. Currently, we have several non-invasive techniques that allow us to assess endothelial dysfunction and adventitious arterial blood, more specifically VV, including the study of arterial tone in peripheral beds with EndoPAT and examining the density of the VV in the adventitia with carotid ultrasound echography after contrast

administration (microbubbles of hexafluoride sulphur). The use of micro bubbles allows us to study the vascular structures that these compounds leave in the bloodstream and thus allows for the visualization of VV [45].

All this finding showed that relatively early atherosclerotic changes might be reversible if it is explored with more sensitive measuring methods than IMT, presumably by measuring more development and obtaining early results.

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