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REVIEWS: CURRENT TOPICS

The impact of dietary fatty acids on macrophage cholesterol homeostasis☆

Milessa da Silva Afonso[a], Gabriela Castilho[a], Maria Silvia Ferrari Lavrador[a], Marisa Passarelli[a], Edna Regina Nakandakare[a], Simão Augusto Lottenberg[b], Ana Maria Lottenberg[a,*]

[a]Lipids Laboratory (LIM10), Faculty of Medical Sciences of the University of Sao Paulo, Sao Paulo, Brazil
[b]Endocrinology Service from of the Clinical Hospital of the University of São Paulo, Sao Paulo, Brazil

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Abstract

The impact of dietary fatty acids in atherosclerosis development may be partially attributed to their effect on macrophage cholesterol homeostasis. This process is the result of interplay between cholesterol uptake and efflux, which are permeated by inflammation and oxidative stress. Although saturated fatty acids (SAFAs) do not influence cholesterol efflux, they trigger endoplasmic reticulum stress, which culminates in increased lectin-like oxidized LDL (oxLDL) receptor (LOX1) expression and, consequently, oxLDL uptake, leading to apoptosis. Unsaturated fatty acids prevent most SAFAs-mediated deleterious effects and are generally associated with reduced cholesterol efflux, although α-linolenic acid increases cholesterol export. Trans fatty acids increase macrophage cholesterol content by reducing ABCA-1 expression, leading to strong atherosclerotic plaque formation. As isomers of conjugated linoleic acid (CLAs) are strong PPAR gamma ligands, they induce cluster of differentiation (CD36) expression, increasing intracellular cholesterol content. Considering the multiple effects of fatty acids on intracellular signaling pathways, the purpose of this review is to address the role of dietary fat in several mechanisms that control macrophage lipid content, which can determine the fate of atherosclerotic lesions.

Keywords: Macrophage; Fatty acids; Cholesterol uptake; Cholesterol efflux

1. Introduction

In recent decades, cardiovascular diseases have become the leading cause of morbidity and mortality in developed countries and are increasingly prevalente in developing nations [1]. In particular, atherosclerosis is characterized as a chronic inflammatory disease that is initiated by the subendothelial retention of low-density lipoprotein (LDL), followed by physicochemical modifications such as oxidation. Macrophages located in the arterial intima layer present several scavenger receptors that uptake modified LDL. This contributes to intracellular cholesterol accumulation and inflammation and leads to monocyte recruitment [2,3].

Atherosclerotic lesions are characterized by progressive macrophage lipid accumulation, which leads to foam cell formation. All of the steps in these processes are mediated, in part, by dietary fatty acids. Lipid-laden macrophages release many chemoattractants and inflammatory mediators, leading to lesion progression. Later stages of atherosclerosis are marked by rupture-susceptible lesions that are prone to events such as arterial occlusion and atherothrombotic processes that culminate in apoptosis and the formation of a necrotic core [4]. Macrophage lipid homeostasis is determined by the balance between cholesterol uptake and the efflux of excess cholesterol to extracellular acceptors such as high density lipoprotein (HDL) and apolipoprotein A-I (apo A-I) [5]. Cholesterol is delivered to these cells mainly from modified lipoproteins as well as in a minor amount from native LDL. The accumulation of lipids within macrophagic cells is detrimental to arterial wall thickening because these cells engulf cholesterol depending on the amount of modified lipoproteins available and the expression of scavenger receptors. Lipid-laden macrophages ultimately generate foam cells, a hallmark of the early stages of atherosclerosis.

In this scenario, the ATP-binding transporters A-1 (ABCA-1) and G-1 (ABCG-1) play a central role in maintaining the macrophage lipid content by driving reverse cholesterol transport (RCT), an anti-atherogenic system that promotes the trafficking of excess cholesterol from arterial wall macrophages to the liver for excretion in the bile and feces [6].

According to clinical and epidemiological studies, dietary fat plays an important role in the plasma cholesterol concentration and also modulates several steps in RCT [7–9]. However, the specific role of each fatty acid in the signaling pathways that modulate macrophage cholesterol homeostasis remains elusive. In addition, depending on the experimental protocol, fatty acids exert multiple effects that can be dose-dependent and/or tissue specific and also related to the

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* Corresponding author. Endocrinology Service from of the Clinical Hospital of the University of São Paulo, CEP: 01246–000 Sao Paulo, Brazil. Tel./fax: +55 11 30621255.

E-mail address: amlottenberg@uol.com.br (A.M. Lottenberg).

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animal model utilized. Furthermore, the same fatty acid may exhibit different actions in similar metabolic contexts. Therefore, this review explores the role of different fatty acids in the cellular and molecular mechanisms involved in macrophage cholesterol balance, which is an important factor in the initiation, progression and outcome of atherosclerotic lesions.

1.1. Macrophage cholesterol homeostasis

Intracellular lipid content is tightly regulated to maintain cellular function and mitosis and to avoid lipotoxicity. The cellular cholesterol content is modulated by sterol regulatory element binding protein (SREBP)-dependent gene regulation, including cholesterol biosynthesis and expression of the LDL receptor. In addition, the intracellular lipid concentration is determined by the HDL-mediated cholesterol and oxysterol efflux. 3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase is the key regulatory enzyme in endogenous cholesterol biosynthesis and converts acetate to mevalonate [10]. The transcriptional regulation of HMG-CoA reductase and the modulation of the native-LDL receptor (B-E) are influenced by the intracellular cholesterol and oxysterol concentrations. These molecules inhibit the proteolytic cleavage of SREBP-2, blocking the transport of its active transcription factor. During sterol depletion, a sensitive domain of the complex that keeps SREBP anchored to the endoplasmic reticulum (ER) releases the transcription factor for nuclear binding, where it induces HMG-CoA reductase and B-E receptor gene expression [11]. The ER is also a resident site for other proteins involved in cellular lipid metabolism. When the load of intracellular free cholesterol rises above the physiological concentration, the ER resident enzyme acyl-coenzyme A: cholesterol acyltransferase (ACAT) esterifies the excess cholesterol, creating a pool of lipids that is maintained in an inert form within lipid droplets in macrophages. However, neutral cholesterol ester hydrolase (nCEH) can reconvert esterified cholesterol into free cholesterol upon sterol demand [10]. Many cells rely on the transcriptional and translational modulation of the B-E receptor by SREBP-2 and proprotein convertase subtilisin/kexin type 9, which increases B-E receptor degradation. By contrast, macrophages have few B-E receptors on their surface, even though these cells present many scavenger receptors, such as the lectin-like oxLDL (LOX-1), the cluster of differentiation 36 (CD36), and the class A and class B scavenger receptors [SRA and scavenger receptor class B type 1 (SR-BI), respectively] [2].

These receptors are quite promiscuous and bind a wide range of molecules, such as advanced glycation end products, oxidized lipids, inflammatory mediators and chemically modified peptides. RCT is particularly relevant for adjusting the intracellular lipid content in arterial wall macrophages. In this regard, RCT acts to prevent atherosclerosis and, disturbances in one or many steps of this pathway are usually related to the premature development of atherosclerosis in humans and animal models [6].

Cholesterol efflux from arterial macrophages occurs by active mechanisms involving ABC transporters. ABCA-1 is a cell membrane protein that requires energy released by ATP hydrolysis to export cholesterol to the outer leaflet of the cell membrane and then to lipid-poor apoA-I and pre-beta HDL. ABCA-1 is up-regulated by oxysterols such as 22-hydroxycholesterol and 24,25-hydroxycholesterol, which activate liver X receptor (LXR). The latter protein dimerizes with retinoic X receptor (RXR) and interacts with a DR-4 sequence in the ABCA-1 gene promoter to induce gene transactivation [12].

ABCA-1 protein content is mostly regulated by post-transcriptional mechanisms that regulate its half-life, including degradation by the ubiquitin–proteasome system, lysosomal degradation through the endosomal sorting complex required for transport pathway and surface proteases [13].

The half-life of ABCA-1 is extended in the plasma membrane by its interaction with apo A-I, which promotes receptor dephosphorylation of a PEST (proline, glutamic acid, serine, threonine) sequence, impairing ABCA-1 degradation by proteases such as calpains [14]. Studies have revealed a synergistic role for ABCA-1 and ABCG-1 in cholesterol export. ABCG-1 can also remove cholesterol by interacting with large mature HDL particles. In addition, ABCG-1 exports oxysterols, thus minimizing their deleterious effects in macrophages, for instance, by reducing inflammation and apoptosis, which are related to plaque instability and rupture. Likewise, ABCA-1 and ABCG-1 are up-regulated by LXR/RXR [15].

In addition to the active process, intracellular cholesterol can be exported to HDL by a passive mechanism involving scavenger receptor class B type 1 (SR-BI). This receptor is located in cholesterol- and sphingomyelin-rich domains in the plasma membrane called caveolae and forms a hydrophobic channel through which cholesterol crosses the membrane. This transport depends on the HDL composition of phospholipids and cholesterol and on a membrane composition that favors a concentration gradient that propels cholesterol across the membrane [10]. The exact contribution of SR-BI to macrophage RCT is not fully recognized, and studies using animal models have revealed that this receptor does not seem to contribute to RCT in vivo.

However, several findings have suggested that SR-BI has a role in the uptake of esterified cholesterol from HDL in the liver during the final step of RCT. In mice, the overexpression of SR-BI is related to the decreased development of atherosclerosis despite elevated plasma HDL cholesterol levels, and SR-BI knockout mice present the opposite effect, displaying increased atherosclerosis despite the presence of high levels of HDL cholesterol [15]. In cholesterol-overloaded macrophages, ABCA-1 contributes to most of the cholesterol export, with a minor contribution ascribed to ABCG-1 and even less to SR-BI [16]. The main mechanisms involved in macrophage cholesterol homeostasis are presented in Fig. 1.

1.2. Fatty acids and macrophage cholesterol uptake

The degree of saturation and the amount of fatty acids in the diet can modulate not only the plasma lipoprotein concentration but also cellular cholesterol uptake [7,8,17].

1.2.1. Lectin-like oxLDL receptor (LOX1)

It is well established that palmitic acid is positively associated with atherosclerotic plaque development [18]. A recent work conducted in RAW 264.7 and THP-1-derived macrophages demonstrated that different concentrations of palmitic acid were able to increase LOX-1 expression, an effect that was not obtained with other scavenger receptors (CD36, SR-A, SR-BI and CD68) [18]. In the same study, the authors showed that palmitic acid induced LOX-1 expression is mediated by the oxidative stress/mitogen activated protein kinase (p38 MAPK) pathway. In fact, when these cells were treated with the antioxidant n-acetylcysteine and a specific MAPK inhibitor, the deleterious effect of palmitic acid was reversed. Subsequent data from the same group revealed that palmitic acid-mediated LOX-1 expression is also associated with ER stress, which is characterized by an accumulation of misfolded peptides [19]. The overload of misfolded proteins leads to an adaptive response known as the unfolded protein response, which counts with metabolic sensors, such as protein kinase-like ER kinase (PERK), inositol requiring kinase/endonuclease-1 (IRE-1) and activating transcription factor-6, or ultimately leads to pro-apoptotic signaling [20].

1.2.2. Endoplasmic reticulum stress and apoptosis

Prolonged ER stress is detrimental in macrophages and is present in many steps of atherogenesis, including foam cell formation. Moreover, in advanced lesions, ER-stressed macrophages are prone to apoptosis, leading to less stable plaques, which correlate with