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Review

Oxysterols in Metabolic Syndrome: From Bystander Molecules to Bioactive Lipids

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Oxysterols are cholesterol metabolites now considered *bona fide* bioactive lipids. Recent studies have identified new receptors for oxysterols involved in immune and inflammatory processes, hence reviving their appeal. Through multiple receptors, oxysterols are involved in numerous metabolic and inflammatory processes, thus emerging as key mediators in metabolic syndrome. This syndrome is characterized by complex interactions between inflammation and a dysregulated metabolism. Presently, the use of synthetic ligands and genetic models has facilitated a better understanding of the roles of oxysterols in metabolism, but also raised interesting questions. We discuss recent findings on the absolute levels of oxysterols in tissues, their newly identified targets, and the mechanistic studies emphasizing their importance in metabolic disease, as there is a pressing need to further comprehend these intriguing bioactive lipids.

Oxysterols as Key Players in Metabolic Syndrome?

Obesity and sedentary lifestyle are on the rise and pose serious threats to people's health and wellbeing. Obesity is often associated with other pathologies and is one of the criteria included in the definition of **metabolic syndrome** (**MetS**) (see Glossary). This syndrome regroups a cluster of metabolic disorders such as hyperglycemia, dyslipidemia, obesity, insulin resistance, or hypertension. MetS also increases the incidence of pathologies such as atherosclerosis, type 2 diabetes (T2D), **nonalcoholic fatty liver disease** (NAFLD), stroke, or cancer. Over the years, numerous endogenous mediators have been investigated to better understand and treat this syndrome. Among these, bioactive lipids such as bile acids, endocannabinoids, ceramides, and oxysterols are clearly of interest. Oxysterols were long considered as mere byproducts of cholesterol metabolism, generated as simple intermediates in the synthesis of the more-studied bile acids. The discovery of their affinity for specific receptors, such as liver X receptors (LXRs), and, more recently, EBI2 and CXCR2, favors their study as endogenous mediators. These molecules are now fully considered bioactive lipids that exert their pleiotropic effects through multiple receptors.

Following a brief outline of the metabolism and main targets of oxysterols, we discuss the evidence supporting the interplay among oxysterols, obesity, and MetS. We also discuss the main pharmacological and genetic tools that can be used to interrogate the roles of oxysterols in MetS.

Oxysterol Metabolism and Molecular Targets

Metabolic Network of Oxysterols

Oxysterols are oxygenated species derived from cholesterol. They are generated through enzymatic reactions mainly involving **cytochrome P450** (CYP) or through chemical reactions

Trends

Two G-protein-coupled receptors (GPCRs) have been recently found to bind oxysterols, CXCR2 and EBI2. These two receptors are involved in immune processes. The discovery has further widened the action spectrum of oxysterols, suggesting a role in immunity.

Recent studies in humans and mice have shown that the levels of these bioactive lipids are drastically altered during obesity and metabolic syndrome (MetS).

The recent use of synthetic ligands for retinoid-related orphan receptors (RORs), receptors activated by oxysterols, has facilitated the study of their potential implication in MetS. This has further extended our understanding of these receptors in the pathophysiology of MetS.

The study of human genetic polymorphisms and the use of multiple mouse genetic models targeting oxysterol receptors or oxysterol transport proteins in the context of MetS are increasing our understanding of the role played by the oxysterol system in MetS.

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involving reactive oxygen species (ROS). As shown in Figure 1, a single oxysterol can be metabolized by different enzymes [e.g., 24(*S*)-hydroxycholesterol (24(*S*)-OHC)] and a single enzyme can react with different oxysterols [e.g., sterol 27-hydroxylase (CYP27A1)], rendering the study of oxysterol metabolism very complex [1].

Although the liver is the most studied organ responsible for oxysterol generation (Figure 1), oxysterols are also produced in other organs due to the expression of CYP in numerous tissues. For instance, the hypothalamus, which plays a key role in obesity settings and MetS, constitutes, as the rest of the central nervous system (CNS), a distinct and separate compartment regarding cholesterol metabolism. This is due to the fact that the cholesterol synthetized in the CNS cannot cross the blood–brain barrier (BBB) and is removed from the CNS in the form of oxysterols, mainly 24(*S*)-OHC. In human brain, in addition to 24(*S*)-OHC, other oxysterols have been shown to cross the BBB towards the periphery [e.g., 7β-hydroxycholesterol (7β-OHC) and 7-ketocholesterol] while a net flux of 27-hydroxycholesterol (27-OHC) towards the CNS has been found [2]. In terms of metabolic enzymes, CYP expression levels in both mice and humans vary depending on the brain region [3]. Although difficult to study in humans, CYP is expected to influence the levels of oxysterols produced in a given CNS area. The adipose tissue is another example of a tissue involved in MetS for which information on oxysterol metabolism remains sparse. Indeed, although the human adipose tissue expresses some of the enzymes involved in oxysterol metabolism [e.g., Chol-25-OHase producing 25-hydroxycholesterol (25-OHC), CYP3A5 producing 4β-hydroxycholesterol (4β-OHC) and 25-OHC, and CYP27A1 producing, for instance, 27-OHC], little is known about the actual *in situ* generation of these lipids [4–6].

Oxysterols are further transformed into **bile acids** by either the classical (or neutral) or the alternative (or acidic) pathways. Of note, a sulfate moiety can be added to oxysterols at the 3β-hydroxy position by cytosolic sulfotransferases SULT2B1b, SULT2B1a, and SULT2A, which leads to the formation of sulfated oxysterols (e.g., 25-hydroxycholesterol-3-sulfate) [7]. (See Figure I in Box 1 for a description of the effects of 25-OHC-3S).

Molecular Targets Mediating Actions of Oxysterols

While the most-studied molecular targets of oxysterols remain the LXRs, numerous other targets, including 'non-receptor proteins' such as lipid transporters, have been recently identified (Figure 2). This variety of receptors (or proteins) activated (or antagonized) by oxysterols help explain the large array of effects that have been described for these bioactive lipids in cholesterol, lipid, and glucose metabolism, as well as in inflammation and immunity (see later). It is important to bear in mind that oxysterols are a large family of mediators that despite their structural similarities, do not always share the same molecular target(s) or pharmacological effects (Table 1).

Liver X Receptors

LXRs are **nuclear receptors** consisting of two isoforms; both bind oxysterols, although with different affinities, depending on the isoform and oxysterol. Although many oxysterols behave as LXR agonists [e.g., 24(*S*),25-epoxycholesterol, 25-OHC, and 24(*S*)-OHC], several other oxysterols [e.g., 22(*S*)-hydroxycholesterol (22(*S*)-OHC)] have been described as antagonists [8]. In both rodents and humans, LXRα (or NR1H3) is largely expressed in the liver, intestine, and adipose tissue, whereas LXRβ (or NR1H2) is ubiquitously expressed. LXRs mainly act as heterodimers with their partner retinoid X receptor (RXR); this heterodimer binds to the LXR response element (LXRE) to modulate gene expression [9].

In both rodents and humans, LXRs are involved in the control of important metabolic pathways such as cholesterol homeostasis, lipogenesis, and glucose homeostasis [9]. Their

Glossary

ApoE$^{-/-}$(apolipoprotein, apoE) knockout mice: model used to study atherosclerosis. These mice display a significant increase in plasma cholesterol and develop atherosclerotic lesions that evolve into more advanced stages with age.
Bile acid metabolism: bile acids are produced from oxysterol through enzymatic reactions regrouped into two main pathways: the classical (or neutral) pathway, where CYP7A1 catalyzes the rate-limiting step, and the alternative (or acidic) pathway, where the first step is catalyzed by CYP27A1.
CCL2: chemokine (C–C motif) ligand 2 or monocyte chemoattractant protein 1 (MCP-1) is produced by a wide variety of cells but mainly by monocytes and macrophages. This potent chemoattractant is secreted during inflammation or oxidative stress, recruiting monocytes to the lesion sites. In atherosclerosis, it is central for the extravasation process of monocytes to the intima of the vessel.
Chylomicrons: lipoproteins containing mainly triglycerides (around 90%) that are formed in enterocytes and transport dietary lipids through lymphatic vessels into the blood.
Cytochrome P450 (CYP): large superfamily of heme-containing enzymes responsible for the oxidative metabolism of endogenous and exogenous molecules. The catalytic action of CYP transforms lipophilic molecules into more polar compounds.
G-protein-coupled receptor (GPCR): membrane receptors located at the cell surface comprising seven membrane-spanning segments. Upon activation by a ligand, GPCRs interact with heterotrimeric guanine nucleotide binding proteins (G proteins) to modulate intracellular second messengers.
Kupffer cells: resident macrophages of the liver, mostly located in the sinusoids. They play a crucial role in initiating and maintaining the inflammatory response in the liver.
M1/M2 macrophage polarization: very simplistically, macrophages can be characterized according to their activation state, M1 'proinflammatory' (or classically activated) or M2 'anti-inflammatory' (or alternatively