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Second-hand smoke stimulates lipid accumulation in the liver by modulating AMPK and SREBP-1☆

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***Background/Aims*: The underlying mechanisms of steatosis, the first stage of non-alcoholic fatty liver disease (NAFLD) that is characterized by the accumulation of lipids in hepatocytes, remain unclear. Our study aimed to investigate the hypothesis that cigarette smoke is known to change circulating lipid profiles and thus may also contribute to the accumulation of lipids in the liver.**

***Methods*: Mice and cultured hepatocytes were exposed to sidestream whole smoke (SSW), a major component of "second-hand" smoke and a variety of cellular and molecular approaches were used to study the effects of cigarette smoke on lipid metabolism.**

***Results*: SSW increases lipid accumulation in hepatocytes by modulating the activity of 5′-AMP-activated protein kinase (AMPK) and sterol response element binding protein-1 (SREBP-1), two critical molecules involved in lipid synthesis. SSW causes dephosphorylation/inactivation of AMPK, which contributes to increased activation of SREBP-1. These changes of activity lead to accumulation of triglycerides in hepatocytes.**

***Conclusion*: These novel findings are important because they point to another risk factor of smoking, i.e., that of contributing to NAFLD. In addition, our results showing that both AMPK and SREBP are critically involved in these effects of smoke point to the potential use of these molecules as targets for treatment of cigarette smoke-induced metabolic diseases.**

***Keywords*: Kinases; Fatty liver; Non-alcoholic fatty liver diseases; Transcription factors; Sidestream whole smoke**

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Abbreviations: ACC, acetyl-CoA carboxylase; ADRP, adipose differentiation-related protein; AICAR, 5-aminoimidazole-4-carboxamide ribonucleoside; AMPK, AMP-activated protein kinase; ApoB, apolipoprotein B; FAS, fatty acid synthase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HMGCR, 3-hydroxy-3-methylglutaryl CoA reductase; MSW, mainstream whole; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole; SREBPs, sterol-regulated element-binding proteins; SSW, sidestream whole; LDLR, low density lipopotein receptor; NAFLD, non-alcoholic fatty liver diseases; TPM, total particulate matter; TG, triglyceride; TC, total cholesterol.

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1. Introduction

Cigarette smoke contains more than 47,000 toxic substances which significantly harm almost every organ of the body, leading to a variety of diseases and syndromes [1]. Cigarette smoke is composed of MSW (mainstream whole, "first-hand") and SSW (side stream whole, major component of "second-hand" smoke) smokes. Smoking has been identified as one of the major risk factors for the development of atherosclerosis, the major component of cardiovascular disease [2–4] that manifests itself, among other things, by high lipid levels in the blood [5,6]. Furthermore, increasing evidence suggests that risk for cardiovascular disease incidence is associated with non-alcoholic fatty liver diseases (NAFLD) independently of the classical risk factors and features of this metabolic syndrome [7–10]. In the various stages of NAFLD, hepatic steatosis (the accumulation of lipid in the liver tissue) has become a significant public health concern because it tends to develop into more harmful hepatitis and cirrhosis. Because lipids in steatosis are stored as triglycerides in hepatocytes, understanding what causes this accumulation and how it occurs may contribute to elucidation of NAFLD [11].

Sterol regulatory element-binding proteins (SREBPs) are a family of transcription factors that control the expression of genes required for the biosynthesis of cholesterol, fatty acids, triglycerides, and phospholipids. The three isoforms of SREBP precursors located on the endoplasmic reticulum membrane, designated SREBP-1a, SREBP-1c, and SREBP-2 [12], have different functions and abundance in various animal tissues. The SREBP precursors are activated by a two-step cleavage process that releases the active form that then translocates to the nucleus of the cell to stimulate gene expression [13]. SREBP-1c preferentially controls the expression of genes involved in triglyceride synthesis and accumulation, such as fatty acid synthase (FAS) and acetyl coenzyme-A carboxylase (ACC), whereas SREBP-2 activity has been more closely linked to regulation of genes involved in cholesterol synthesis and uptake, such as low-density lipoprotein receptor (LDLR) and 3-hydroxy-3-methylglutaryl CoA reductase (HMGCR) [14–18]. In the liver tissue, the predominant form of SREBPs is SREBP-1c [19].

Another important modulator of lipid metabolism is 5′-AMP-activated protein kinase (AMPK). AMPK was first identified as a kinase that phosphorylates and inactivates ACC, the rate-limiting enzyme in fatty acid biosynthesis [20]. AMP binds and activates AMPK primarily by causing conformational changes that allows Thr172 phosphorylation to occur by upstream kinases. Activation of AMPK in the liver, skeletal muscle, and adipose tissue improves the status of type 2 diabetes by preventing ATP depletion, increasing fatty acid oxidation, decreasing blood glucose, etc. It has also been found that AMPK activity is inhibited in alcohol-induced fatty liver disease [21].

Although both AMPK and SREBP are related to the metabolism of the cell, the relationship between the two is not clear. We hypothesize that components of tobacco smoke cause lipid accumulation in the liver tissue of mice exposed to "second-hand" smoke by modulating the activities of AMPK and that this enzyme is important in the activation of SREBP-1, the central modulator for triglyceride synthesis. The elucidation of the mechanisms of lipid accumulation in hepatocytes caused by cigarette smoke may help understand processes involved in atherogenesis and in initiation of NAFLD, and suggest possible ways of treating both metabolic diseases.

2. Materials and methods

2.1. Smoke solution preparation

Sidestream whole (SSW) smoke solution was prepared from 1R3F research grade cigarettes (University of Kentucky, Louisville, KY). SSW smoke was bubbled into 10 mL serum free media for the duration of 30 puffs as previously described [22] using a puffer box built by the University of Kentucky. SSW smoke was collected from the burning end of the cigarette. The pH of the smoke solutions was adjusted to 7.4. The solution is aliquoted and kept at −20 °C (stable for up to 6 weeks).

2.2. Exposure of the animals to smoke

Six- to eight-week-old male apoB100 transgenic mice on 57BL/6SJL background [23] were fed a high-fat diet and were exposed to smoke for 19 weeks as described previously [24]. All animal experiments were conducted in accordance with US Public Health Service/US Department of Agriculture guidelines. Experimental protocols were approved by the University of California Riverside Institutional Animal Care and Use Committee.

2.3. Lipid content analysis

Lipid extraction and analysis were performed as described previously [24].

2.4. Oil Red O staining

Cells or cryo-sections of liver tissue were washed with cold PBS, fixed with 4% paraformaldehyde in PBS for 15 min, and stained for 20 min in freshly diluted Oil Red O solution (0.3% Oil Red O in isopropanol: $H_2O = 3:2$), and washed twice with water.

2.5. Immunoblotting

Immunoblot analysis was performed as previously described by us [25].

2.6. Infection of the hepatocytes to detect AMPK activity

AML12 cells or HEP3B cells were infected with null virus (Ad-null), an adenovirus expressing the constitutively active form of AMPK (Ad-AMPK-CA) [26], or the dominant negative form of AMPK (Ad-AMPK-DN), at 50 multiplicities of infection. AMPK phosphorylation was analyzed by immunoblotting using a phosphor-specific antibody, anti-phospho-AMPK Thr-172.