Resveratrol administration or SIRT1 overexpression does not increase LXR signaling and macrophage-to-feces reverse cholesterol transport *in vivo*

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The natural polyphenol resveratrol has cardiometabolic protective properties. Resveratrol has been reported to be an activator of NAD^+-dependent deacetylase sirtuin 1 (SIRT1), which may regulate liver X receptor (LXR) activity, thereby upregulating the expression of genes crucial in reverse cholesterol transport (RCT). In the present study, the effects of resveratrol and SIRT1 overexpression on RCT from macrophages-to-feces *in vivo* in C57BL/6 mice were determined. (^{3}H)cholesterol-labeled mouse macrophages were injected intraperitoneally into mice treated with intragastric doses of the well-known LXR agonist T0901317, resveratrol, or a vehicle solution, and radioactivity was determined in plasma, liver, and feces. T0901317-treated mice presented increased (^{3}H)cholesterol in plasma and HDL 48 h after the label injection. Treatment with T0901317 also increased liver ABCA1, G1, and G5 gene expression and reduced intestinal cholesterol absorption which were changes that were associated with a 2.8-fold increase in macrophage-derived (^{3}H)cholesterol in feces. In contrast, resveratrol treatment had no effect on liver LXR signaling or fecal (^{3}H)cholesterol excretion. A separate experiment was conducted in SIRT1 transgenic mice. Liver LXR-target gene expression and magnitude of macrophage-derived (^{3}H)cholesterol in plasma, liver, and feces of SIRT1 transgenic mice did not differ from those of wild-type mice. We conclude that neither resveratrol administration nor SIRT1 overexpression upregulate liver LXR-target genes and macrophage-to-feces RCT *in vivo*. (Translational Research 2013;161:110–117)

Abbreviations: ABC = adenosine triphosphate-binding cassette transporter; apo = apolipoprotein; CYP7A1 = cholesterol 7 alpha-hydroxylase; HDL = high-density lipoprotein; LXR = liver X receptor; SIRT1 = NAD^+-dependent deacetylase sirtuin 1; RCT = reverse cholesterol transport; SR-BI = scavenger receptor class-BI

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AT A GLANCE COMMENTARY

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Background

The natural polyphenol resveratrol has cardiometabolic protective properties. Resveratrol may activate SIRT1, which has been proposed to regulate liver X receptor (LXR) activity, thereby upregulating the expression of genes crucial in reverse cholesterol transport (RCT), 1 of the most important high-density lipoprotein antiatherogenic function. This work aimed to test the ability of resveratrol and SIRT1 expression to induce LXR-target genes and the macrophage-specific RCT pathway *in vivo* using a validated mouse assay in which radiolabeled cholesterol from macrophages is traced in plasma, liver, and feces.

Translational Significance

Our results demonstrate that neither resveratrol nor SIRT1 overexpression are activators of macrophage-specific RCT *in vivo*, thereby indicating that resveratrol- and SIRT1-mediated effects on atherosclerosis are not related to this major HDL antiatherogenic function.

Resveratrol is a naturally-occurring polyphenolic compound present in various dietary components such as peanuts, grapes, red wine, and berries. Studies conducted in mouse models of atherosclerosis indicated that resveratrol inhibited atherosclerosis progression.[1,2] Resveratrol has been shown to have several atheroprotective activities including hypolipidemic,[2,3] antioxidant, and anti-inflammatory properties.[4-8] The results of some studies, though not all, indicate that resveratrol increased high-density lipoprotein (HDL) cholesterol levels in experimental animal models (reviewed in Reference[9]). HDL promotes cholesterol efflux from lipid-laden macrophages located in the artery wall and delivers that cholesterol to the liver, where it will be partly eliminated through bile and feces. This process is termed macrophage-specific reverse cholesterol transport (RCT) and is crucial for antiatherogenic HDL function (reviewed in References[10-12]).

Resveratrol has also been identified as an activator of the NAD^+-dependent deacetylase sirtuin (SIRT) 1,[13] thereby producing beneficial effects on glucose homeostasis and insulin sensitivity in mice and humans.[14-16] However, it has not been proven that resveratrol activates SIRT1 directly.[17] Further, resveratrol may modulate liver X receptor (LXR) α target genes in human monocyte-derived macrophages *in vitro*[18] and enhances ATP-binding cassette transporter (ABC) A1-mediated cholesterol efflux (the first RCT step),[7] although the effects of resveratrol on SIRT1 or the entire RCT pathway were not addressed in those studies. The link between SIRT1 and macrophage RCT was initially studied in SIRT1-deficient mice.[19] Indeed, SIRT1-deficient mice showed lowered HDL cholesterol levels, decreased expression of LXR-target genes, and reduced macrophage cholesterol efflux *in vitro*.[19] However, SIRT1 overexpression did not affect HDL cholesterol levels.[20] Furthermore, splitomicin, a SIRT1 inhibitor, or SIRT1 overexpression, did not affect macrophage cholesterol efflux *in vitro*.[20,21] Nevertheless, the effects of SIRT1 on the entire macrophage-dependent RCT pathway are unknown.

As macrophage-specific cholesterol transport enhancement is currently considered a major HDL antiatherogenic action, the present study tested the ability of resveratrol and SIRT1 expression to induce LXR-target genes and the macrophage-specific RCT pathway *in vivo* using a validated mouse assay in which radiolabeled cholesterol from macrophages was traced in plasma, liver, and feces.[10,11,22]

METHODS

Mice and diet. All animal procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication no. 85–23, revised 1996), and all procedures were reviewed and approved by the Institutional Animal Care Committee of the Hospital de la Santa Creu i Sant Pau. Male and female wild-type C57BL/6 mice were obtained from Jackson Laboratories (Bar Harbor, ME; #000664). SIRT1 transgenic mice were kindly provided by Dr. Gu (Columbia University, New York, NY). SIRT1 transgenic mice on a C57BL76 background[23] were mated with wild-type mice to generate littermates of both genotypes. At 8 weeks of age, mice were randomized into 4 groups and fed for 4 weeks with a Western-type diet (TD88137; Harlan Teklad, Madison, WI, containing 21% of fat [saturated fat/total fat ratio = 0.64] and 0.2% cholesterol) and treated, respectively, with daily intragastric doses of a vehicle solution (1.0% v/v methyl sulfoxide and 1.0% w/v carboxymethylcellulose medium viscosity), LXR agonist (T0901317, 20 mg/kg body weight; Cayman Chemicals, Ann Arbor, MI), resveratrol (40 mg/kg; Sigma Diagnostics, St. Louis, MO), or resveratrol (400 mg/kg) for the last 7 days. SIRT1 and