Available online at www.sciencedirect.com

ScienceDirect

Journal of Nutritional Biochemistry 25 (2014) 1207 – 1217

Journal of Nutritional Biochemistry

Alpha-lipoic acid improves high-fat diet-induced hepatic steatosis by modulating the transcription factors SREBP-1, FoxO1 and Nrf2 via the SIRT1/LKB1/AMPK pathway☆

Yi Yang[a,b,*], Wang Li[b], Yang Liu[b], Yuning Sun[b], Yan Li[b], Qing Yao[b], Jianning Li[b], Qian Zhang[b], Yujing Gao[b], Ling Gao[c], Jiajun Zhao[a,d,**]

[a]Department of Endocrinology, Provincial Hospital Affiliated to Shandong University, Jinan 250021, China
[b]Key Laboratory of Fertility Preservation and Maintenance of Ministry of Education, Ningxia Medical University, Yinchuan 750004, China
[c]Central Lab. Provincial Hospital Affiliated to Shandong University, Jinan 250021, China
[d]Institute of Endocrinology, Shandong Academy of Clinical Medicine, Jinan 250021, China

Received 12 December 2013; received in revised form 27 April 2014; accepted 1 June 2014

Abstract

Understanding the mechanism by which alpha-lipoic acid supplementation has a protective effect upon nonalcoholic fatty liver disease *in vivo* and *in vitro* may lead to targets for preventing hepatic steatosis. Male C57BL/6J mice were fed a normal diet, high-fat diet or high-fat diet supplemented with alpha-lipoic acid for 24 weeks. HepG2 cells were incubated with normal medium, palmitate or alpha-lipoic acid. The lipid-lowering effects were measured. The protein expression and distribution were analyzed by Western blot, immunoprecipitation and immunofluorescence, respectively. We found that alpha-lipoic acid enhanced sirtuin 1 deacetylase activity through liver kinase B1 and stimulated AMP-activated protein kinase. By activating the sirtuin 1/liver kinase B1/AMP-activated protein kinase pathway, the translocation of sterol regulatory element-binding protein-1 into the nucleus and forkhead box O1 into the cytoplasm was prevented. Alpha-lipoic acid increased adipose triacylglycerol lipase expression and decreased fatty acid synthase abundance. In *in vivo* and *in vitro* studies, alpha-lipoic acid also increased nuclear NF-E2-related factor 2 levels and downstream target amounts via the sirtuin 1 pathway. Alpha-lipoic acid eventually reduced intrahepatic and serum triglyceride content. The protective effects of alpha-lipoic acid on hepatic steatosis appear to be associated with the transcription factors sterol regulatory element-binding protein-1, forkhead box O1 and NF-E2-related factor 2.

Keywords: Alpha-lipoic acid; FoxO1; Nonalcoholic fatty liver disease; Nrf2; SIRT1; SREBP-1

1. Introduction

Nonalcoholic fatty liver disease (NAFLD), characterized by excessive triglyceride (TG) accumulation in the liver, is becoming increasingly prevalent in Asian-Pacific regions due to Westernization [1]. The central role of lipid accumulation in the pathogenesis of NAFLD has been confirmed in many clinical correlation studies and animal models [2]. Therapeutic options targeting hepatic lipid metabolism are therefore crucial to the management of NAFLD.

Sirtuin 1 (SIRT1) and AMP-activated protein kinase (AMPK) are known fuel-sensing molecules that modulate lipid metabolism [3]. AMPK serves as a cellular energy sensor and is activated by increased AMP/ATP ratio or by the upstream kinases liver kinase B1 (LKB1), Ca^{2+}/calmodulin-dependent protein kinase kinase (CaMKK) and transforming growth

Abbreviations: ACC, acetyl-CoA carboxylase; ALA, alpha-lipoic acid; ALT, alanine aminotransferase; AICAR, 5-aminoimidazole-4-carboxamide riboside; AMPK, AMP-activated protein kinase; AST, aspartate aminostransferase; ATGL, adipose triacylglycerol lipase; CaMKK, Ca^{2+}/calmodulin-dependent protein kinase kinase; CAT, catalase; CC, compound CC; DAPI, 4′,6-diamidino-2-phenylindole; DBC1, deleted in breast cancer-1; DHE, dihydroethidium; FAS, fatty acid synthase; FBS, fetal bovine serum; FITC, fluorescein isothiocyanate; FoxO1, forkhead box O1; HDL, high-density lipoprotein; H&E, hematoxylin and eosin; HFD, high-fat diet; HMG-CoA synthase, 3-hydroxy-3-methylglutaryl-coenzyme A synthase; HO-1, hemeoxygenase-1; IP, immunoprecipitation; LDL, low-density lipoprotein; LKB1, liver kinase B1; NA, nicotinamide; NAFLD, nonalcoholic fatty liver disease; ND, normal diet; NEFA, nonesterified fatty acids; NF-κB, nuclear factor-κB; Nrf2, NF-E2-related factor 2; PGC-α, peroxisome proliferator-activated receptorγcoactivator1α; PVDF, polyvinylidene difluoride; ROS, reactive oxygen species; SIRT1, sirtuin 1; SOD, superoxide dismutase; SRE-1, sterol regulatory element 1; SREBP-1, sterol regulatory element-binding protein-1; TAK1, transforming growth factor-β-activated kinase-1; TC, total cholesterol; TG, triglyceride.

☆ Grants support: This work was supported by the National Natural Science Foundation of China (81160103), the National Basic Research Program (2012CB524900) funded by the China government, and Science Research and International Cooperation Project of Ningxia Hui Autonomous Region (NXIC2011010).

* Correspondence to: Y. Yang, Key Laboratory of Fertility Preservation and Maintenance of Ministry of Education, Ningxia Medical University, Yinchuan, 750004, PR China. Tel.: +86 951 6980108.

** Correspondence to: J.J. Zhao, Department of Endocrinology, Provincial Hospital Affiliated to Shandong University, Jinan 250021, PR China. Tel.: +86 531 87921889.

E-mail addresses: yangyi73422@163.com (Y. Yang), jjzhao@medmail.com.cn (J. Zhao).

http://dx.doi.org/10.1016/j.jnutbio.2014.06.001

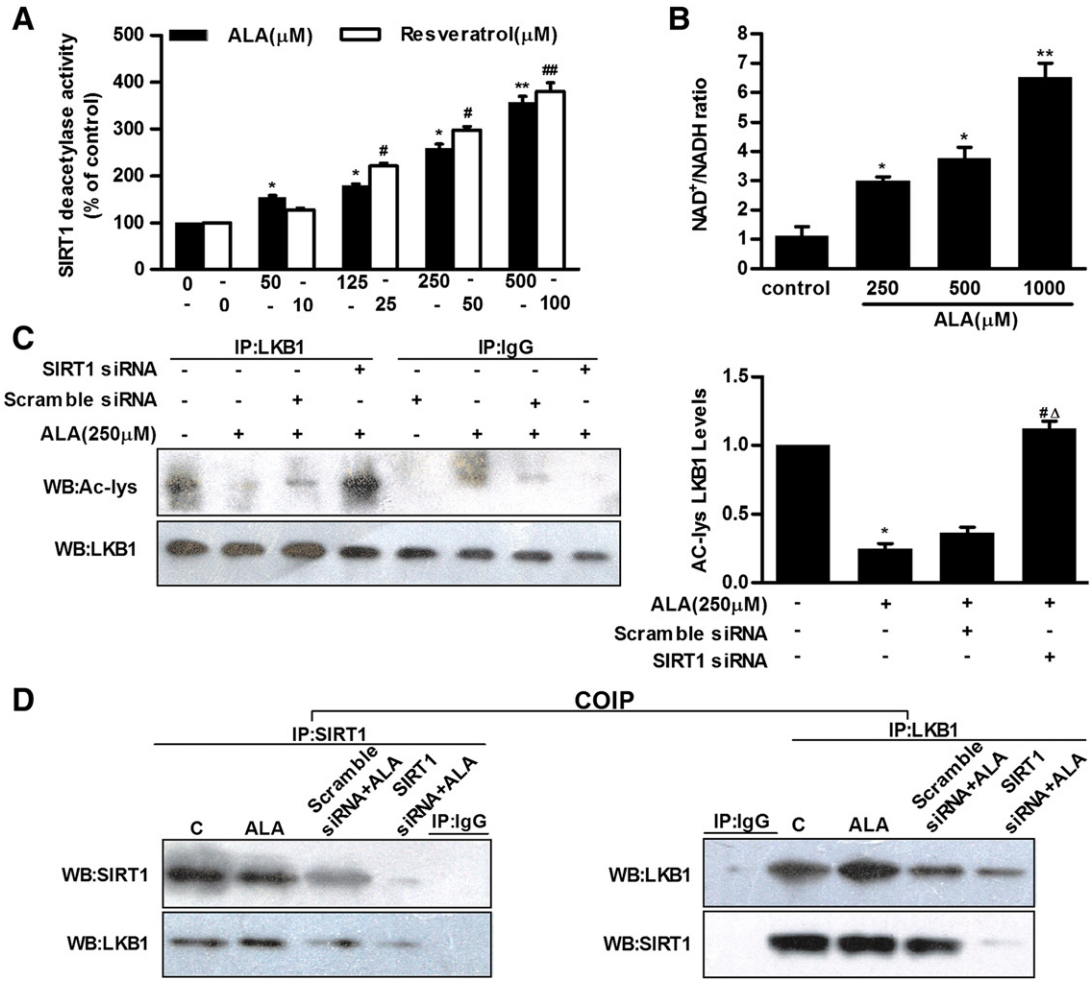

Fig. 1. ALA activates SIRT1/LKB1 pathway in HepG2 cells. (A) The dose–response effect of ALA on SIRT1 deacetylase activity. HepG2 cells were treated with 0, 50, 125, 250 and 500 μM ALA, or 0, 10, 25, 50 and 100 μM resveratrol used as a positive control for 24 h. Data are presented as mean±S.E.M. (n=5). *P<.05, **P<.01 vs. control (0 μM ALA); #P<.05, ##P<.01 vs. control (0 μM resveratrol). (B) Intracellular NAD^+/NADH ratio. HepG2 cells were treated with 0 (control), 250, 500 and 1000 μM ALA for 24 h. Data are presented as mean±S.E.M. (n=6). *P<.05, **P<.01 vs. control (0 μmol ALA). HepG2 cells were transfected with SIRT1siRNA or scramble siRNA for 24 h after incubation with ALA (250 μM, 6 h). (C) IP of acetylated liver kinase B1 (LKB1). *P<.05 vs. control (untreated HepG2 cells); #P<.05 vs. ALA group; ΔP<.05 vs. ALA+scramble siRNA group. (D) COIP of SIRT1 and LKB1. Nonspecific IgG was used as control.

factor-β-activated kinase-1 (TAK1) [4]. SIRT1 activation by polyphenols represents an upstream regulator in the LKB1/AMPK signaling axis [3]. Activation of SIRT1/AMPK signaling plays a central role in regulating hepatic fatty acid metabolism [5]. It abrogates ectopic fat accumulation by facilitating fatty acid oxidation and curbing the *de novo* fatty acid synthesis largely through deacetylation and phosphorylation, respectively, of transcription factors or coactivators, such as p53, forkhead box O (FoxO), nuclear factor-κB (NF-κB), peroxisome proliferator-activated receptorγcoactivator1α (PGC-α) and sterol regulatory element-binding protein 1 (SREBP-1) [6,7].

The transcription factor FoxO1 is regulated by dephosphorylation or deacetylation that causes its nuclear translocation to induce transcription of the rate-limiting enzymes of lipolysis, such as adipose triglyceride lipase (ATGL) [8]. In lipid metabolism, SREBP-1 up-regulates the expression of *de novo* lipogenesis via fatty acid synthase (FAS) [9]. SIRT1/AMPK signaling is intimately associated with the activation or inactivation of FoxO1 and SREBP-1 and therefore represents attractive targets in the development of therapies to repress hepatic steatosis.

Several lines of evidence suggest that chronic oxidative stress plays an important role in the progression of fatty liver due to a close link between dysregulated lipid homeostasis and oxidative stress [10]. The transcription factor NF-E2-related factors 2 (Nrf2) plays a central role in the defense against oxidative stress. Upon oxidative insults, Nrf2 translocates to the nucleus where it interacts with antioxidant response element (ARE) to mediate the transcription of its target genes, such as hemeoxygenase-1 (HO-1), superoxide dismutase (SOD), glutathione peroxidase and catalase [11]. The transcription of these genes enhances cellular resistance against oxidative stress. Previous studies have shown that Nrf2 plays an important role in the protection of hepatocytes from NAFLD [12]. SIRT1 is also known to markedly protect cells from oxidative

Fig. 2. ALA suppresses intracellular accumulation of lipids through the SIRT1/LKB1/AMPK signaling pathway in HepG2 cells. (A) HepG2 cells were treated with 0 (control, C), 50, 125, 250, 500 and 1000 μM ALA for 6 h. Protein expression of AMPK, p-AMPK, ACC, p-ACC, FAS and ATGL was determined by Western blot. (B) Measurement of intracellular triacylglycerol contents. HepG2 cells were treated with 125 μM PA and different concentrations of ALA for 12 h. (C) Effect of ALA (250 μM, 6 h) on protein expression levels in the presence or absence of AMPK inhibitor (CC, 20 μM, 0.5 h), SIRT1 inhibitor (NA, 10 mM, 12 h) and AMPK activator (AICAR, 2 mM, 1 h), respectively. (D) Measurement of intracellular triacylglycerol contents in the presence or absence of different activators and inhibitors. (E) HepG2 cells were transfected with SIRT1siRNA or scramble siRNA for 24 h after incubation with ALA (250 μM, 6 h). (F) Measurement of intracellular triacylglycerol in the presence or absence of SIRT1 siRNA. All data are presented as mean±S.E.M. (n=6). *P<.05 vs. control (untreated cells); #P<.05, ##P<.01 vs. PA group; ΔP<.05 vs. PA+ALA group.