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# Adipose tissue regulates hepatic cholesterol metabolism via adiponectin

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ABSTRACT

*Aims:* Lipid metabolic disorder involves multiple tissues and organs. Hepatic cholesterol metabolism is an important physiological process, which is tightly related to obesity and lipid metabolic disorders. In this study, we examined the direct effects of adipocytes on hepatic cholesterol metabolic factors and investigated the role of potential adipocytokines in it.
*Main methods:* Male SD rats were induced by a high-fat diet (HFD) and hepatic cholesterol metabolic factors, 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMGR) and ATP-binding cassette transporter A1 (ABCA1) were measured by immunoblotting. Then the effects of adipocytes on the expressions of hepatic cholesterol metabolism proteins were examined in the co-culture system. Finally, the concentrations of several adipocytokines were detected by ELISA and the effect of adiponectin (APN) on hepatic cholesterol metabolism was confirmed by short interference RNA (siRNA) in vitro.
*Key findings:* Our results showed that adipocytes significantly increased ABCA1 and decreased HMGR in hepatocytes after co-culture. Lipopolysaccharide (LPS) treatment in this co-culture system reversed cholesterol metabolism compared with the untreated group. APN, which also decreased in obese rats, had a significant positive correlation with ABCA1 and inversed correlation with HMGR in vitro. Co-culturing with APN-silenced adipocytes partially restored ABCA1 and HMGR levels.
*Significance:* The present study demonstrates that adipocytes regulate hepatic cholesterol metabolism partly via APN.



## Introduction

Recently, nonalcoholic fatty liver disease, hyperlipidemia and other lipid metabolic disorders are on the rise among the general population. It has been demonstrated that lipid metabolic diseases are systemic disorders affecting many organs such as the liver, muscle, and adipose tissue. Cholesterol homeostasis is presumed to be primarily responsible for the development of many lipid metabolic disorders. The liver plays an important role in cholesterol homeostasis, such as biosynthesis, via 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMGR), reverse cholesterol transport through ATP-binding cassette transporter A1 (ABCA1), (Weber et al., 2004). The major precursor of cholesterol synthesis is acetyl-CoA which gives rise to hydroxyl methylglutaryl-CoA (HMG-CoA). The rate limiting step in the cholesterol biosynthetic pathway is the conversion of HMG-CoA to mevalonic acid (MVA) by HMGR, and ABCA1 plays a crucial role in the efflux of cellular cholesterol (Espenshade and Hughes, 2007; Segatto et al., 2011). ABCA1 is a critical factor in the maintenance of plasma HDL-C levels, and is important for the transfer of cellular cholesterol, phospholipids, and other molecules to lipid-poor apolipoproteins (Ye et al., 2011).

According to recent surveys, obesity is a major risk factor for metabolic syndrome and strong relationship exists between obesity and cholesterol metabolic disorder (Fabbrini et al., 2010; Oda, 2012). One characteristic of obesity is the enlargement of adipose tissue, and scores of studies have reported that adipose tissue can be regarded as a major secretory and endocrine organ. Further, numerous proteins secreted by adipocytes potentially play physiological roles in metabolism (Galic et al., 2010). Leptin, adiponectin (APN), resistin and several other adipocytokines have been thought to be involved in the regulation of lipid metabolism (Lago et al., 2009). Studies have clearly demonstrated that APN signaling have beneficial effects on lipid and glucose metabolism. Current research has indicated that APN dramatically promoted reverse cholesterol transport in the liver by increasing high density lipoprotein assembly (Matsuura et al., 2007). APN exerts its effects by two receptors, AdipoR1 which involved in AMP-activated kinase (AMPK) pathway, and AdipoR2 which is involved in PPARα pathway (Yamauchi et al., 2014). Many clinical studies pointed out the association between APN and serum lipoprotein (Chan et al., 2009; Park et al., 2010; Tsuchiya et al., 2009), and patients with high cholesterol level revealed significantly elevated levels of pro-inflammatory cytokines such as interleukin 6(IL-6) and tumor necrosis factor α(TNF-α)

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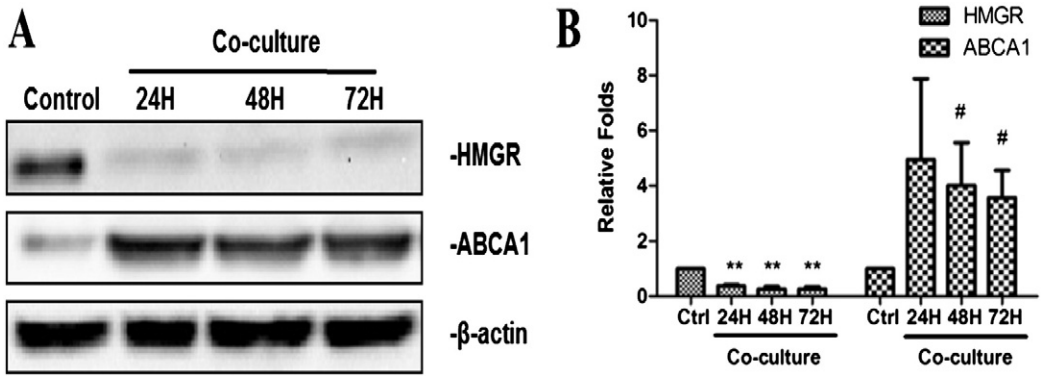


**Fig. 1.** The effects of adipocytes on the expressions of ABCA1 and HMGR in hepatocytes. (A): Hepatocytes were treated in the presence or absence of adipocytes for 24 h, 48 h, and 72 h. (B): Densitometric scanning of band intensities obtained from three independent experiments were sent to quantify the change of protein expressions (control value taken as one-fold in each case) are showed. Bars indicate the means ± S.D., *P < 0.05, **P < 0.01 compared with the Ctrl group (HMGR), #P < 0.05, ##P < 0.01 compared with the Ctrl group (ABCA1).

(Kumar et al., 2012). Although direct actions of some adipocyte-secreted factors on cholesterol metabolism have been reported (eg. IL-1β and TNF-α up-regulated cholesterol influx and down-regulated cholesterol efflux in vivo and in vitro), the effects of adipocytes on hepatic cholesterol metabolism are unclear.

In this study, the association between adipocytes and hepatic cholesterol metabolism was verified by a co-culture system in vitro. Then we detected the adipocytokines in the co-culture system and obese rats. Furthermore, we tried to find out which adipocytokine plays a role between adipocytes and hepatic cholesterol metabolism. The results demonstrate the direct involvement of adipocytes in hepatic cholesterol metabolism partly via APN.

## Material and methods

### *Culture of 3T3-L1 cells*

Mouse embryonic fibroblast-adipose like cell line 3T3-L1 (ATCC) were cultured in high glucose Dulbecco's modified Eagle's medium (DMEM) supplemented with 100 U/ml penicillin, streptomycin and 10% newborn bovine serum. Cells were incubated at 5% $CO_2$ and 37 °C and differentiated into adipocytes according to the previously described methods (Kim et al., 2002). Briefly, cells were propagated and allowed to reach 100% of confluence. After 2 days, the medium was changed to IDX I (DMEM containing 10% fetal bovine serum and 150 nmol/L insulin, 250 nmol/L dexamethasone, and 0.5 mmol/l 3-isobutyl-1-methylxanthine). Two days later, the medium was switched to IDX II (DMEM containing 10% fetal bovine serum and 150 nmol/L insulin). After another 2 days, the cells were switched back to DMEM supplemented with 10% fetal bovine serum and cultured for an additional 4 days. Cells in two differentiation periods (0 and 8 days after the differentiation, respectively) were fixed with p-formaldehyde and stained with 0.5% Red-Oil O (Sigma), in 60% isopropanol (Miki et al., 2001).

### *Isolation and culture of primary hepatocytes (Wang et al., 2006)*

Primary hepatocytes were isolated from adult male Sprague–Dawley rats (250 to 300 g). In brief, the liver was initially perfused with $Ca^{2+}$-free Hank's buffer and then dissociated with collagenase type IV (GIBCO) in Hank's buffer plus 5 mmol/L $CaCl_2$. Isolated hepatocytes were resuspended in Hank's buffer containing 1.0 mmol/L $CaCl_2$ and 0.6 mmol/L $MgSO_4$. Cells were then filtered through a 90 μm nylon mesh, counted, and tested for viability using a trypan blue (GIBCO) exclusion. Isolated cell pellets were resuspended in DMEM supplemented with 10% fetal bovine serum.

### *Co-culture (Suganami et al., 2007; Yamashita et al., 2007)*

Co-culture of adipocytes and hepatocytes was conducted using a transwell system (Millipore) and transwell insert has a 0.4 μm PET membrane to separate upper and lower chambers. Briefly, 3T3-L1 cells ($2 \times 10^5$ cells/well) were seeded in six-well plates (lower chamber) and differentiated into mature adipocytes as described above. Then the hepatocytes ($2 \times 10^5$ cells/insert) were plated onto the transwell insert (upper chamber) and transferred to plate wells and incubated with mature adipocytes. The medium in the transwell system was changed to DMEM without serum; this resulted in an assembly of the two cells types sharing the same culture medium but being separated by the membrane of the insert.

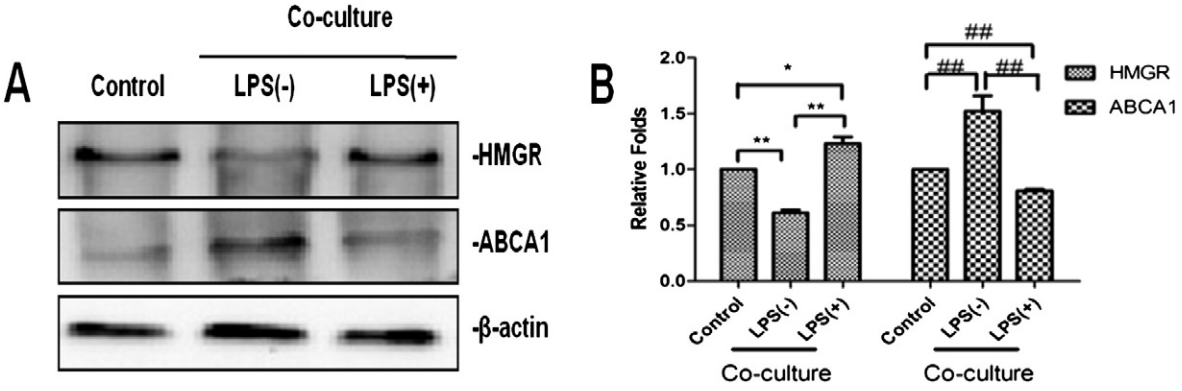


**Fig. 2.** The effects of LPS-induced adipocytes on the expressions of ABCA1 and HMGR in hepatocytes. (A): Hepatocytes were treated in the presence or absence of LPS-induced adipocytes for 48 h. (B): Densitometric scanning of band intensities obtained from three independent experiments were sent to quantify the change of proteins expressions (control value taken as one-fold in each case) are showed. Bars indicate means ± S.D., *P < 0.05, **P < 0.01 compared with the control group, #P < 0.05, ##P < 0.01 compared with the control group.