Conjugated Linoleic Acid Negatively Regulates TR4 Activity in 3T3-L1 Adipocytes

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Abstract

Dietary conjugated linoleic acid (CLA) play key roles in lipid metabolism. Here, we investigated the effect of CLA on the transcriptional activity of TR4, an orphan nuclear receptor that plays an important role in lipid homeostasis. CLA increased TR4 gene mRNA level in 3T3-L1 adipocytes, but inhibited TR4 transcriptional activity in a dose-dependent manner. TR4 induced perilipin expression in 3T3-L1 adipocytes by activating perilipin promoter activity. In a gel shift assay, TR4 bound direct to the putative TR4 response element in the perilipin promoter. Interestingly, CLA reduced the interaction between TR4 and consensus DR1, a well-known TR4 binding site. Additionally, CLA inhibited TR4-induced perilipin promoter activity in a dose-dependent manner. Together, our results suggest that CLA may play a role in lipid homeostasis in adipocytes by functionally regulating TR4.

Key words: Conjugated linoleic acid, TR4, adipocytes, perilipin

Introduction

Adipose tissues are key reservoir for energy excess and properly control body lipid fluxes in response to nutrition status (Guilherme et al., 2008). Recently, adipose tissues are recognized not only as a fat storage site but also as an important endocrine organ secreting a variety of adipocytokines such as TNFα, leptin, adiponectin and resistin. These adipocytokines have been known to participate in various physiological processes including insulin sensitivity, cell proliferation, immune response and food intake (Kershaw and Flier, 2004; Rosen and Spiegelman, 2006). Dysregulated lipid metabolism by altered expression of genes involved in lipogenesis and lipid storage increases lipid accumulation in adipocytes and consequently leads to obesity. Obesity causes insulin resistance and thus has been considered as a major risk factor for the development of diabetes, hypertension, and coronary artery disease (Gregor and Hotamisligil, 2007; Kershaw and Flier, 2004; Shi and Burn, 2004).

Conjugated linoleic acid (CLA) is a group of positional and geometric isomers of linoleic acid. The prominent isomers known for biological action of CLA are cis-9, trans-11 and trans-10, cis-12 CLA. Human acquires these primary isomers from milk products and ruminant meats. Several studies have shown that CLA has potential anticarcinogenic, antiatherogenic, and antiobesity properties (Ha et al., 1990; Larsen et al., 2003; Lee et al., 1994). In vitro studies also showed that trans-10, cis-12 CLA inhibited adipocyte differentiation with prevention of triglyceride (TG) accumulation in adipocytes (Kang et al., 2003; Sakuma et al., 2010). Although CLA effect on lipid accumulation in adipocytes has been reported, molecular mechanism on CLA action in adiposity remains unclear. Several reports have shown that trans-10, cis-12 CLA competitively inhibits effect of PPARγ agonist on PPARγ activity while it rather plays a role as a partial agonist of PPARγ in the absence of PPARγ agonist (Alibin et al., 2008; Granlund et al., 2003; Kennedy et al., 2009; Miller et al., 2008). However, trans-10, cis-12 CLA in the absence of PPARγ agonist was still able to inhibit adipocyte differentiation of 3T3-L1 cells, suggesting that CLA may be involved in the transcriptional regulation of adipogenic genes required for lipid accumulation in a PPARγ-dependent and -independent manner (Miller et al., 2008).

TR4, a member of the nuclear receptor superfamily, regulates gene expression via binding to various TR4 response elements (TR4REs) in target gene promoters

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with highest affinity for DR1 element (Kim et al., 2003; Lee et al., 1995). TR4-deficient mice show reduced body fat mass with reduced TG accumulation in white adipose tissues (Kang et al., 2011; Kim et al., 2011).

Interestingly, various polyunsaturated fatty acids including γ-linoleic acid have been shown to stimulate TR4 transcriptional activity (Tsai et al., 2009). Moreover, Thiazolidinedione (TZD), a well-known PPARγ agonist, also promotes TR4 activity in the reporter gene assay (Xie et al., 2009). These previous reports strongly suggest that TR4 may function as a fatty acid sensor to modulate lipid homeostasis of our body (Xie et al., 2009). Thus, we hypothesized that CLA, a dietary component of milk products and ruminant meats, may participate in lipid homeostasis in adipocytes in part by modulation of TR4 activity. Here, we identified CLA is a functional modulator of TR4 and thus, suppresses TR4-induced perilipin promoter activity. Our findings suggest that TR4 is a key transcription factor which is negatively regulated by CLA during adipogenesis and this study will help to understand transcriptional network to control obesity-related diseases.

Materials and Methods

Materials
CLA and fatty acid free bovine serum albumin (BSA) was purchased from Sigma-aldrich. Rosiglitazone was purchased from Cayman. CLA was complexed to BSA to generate 32 mM CLA stock solution with 1% fatty acid free BSA.

Cell culture and adipocyte differentiation
NIH-3T3, 3T3-L1 cells were maintained in Dulbecco’s Modified Eagle’s Medium (DMEM) with 10% newborn calf serum and HEK293T cells were maintained in DMEM with 10% FBS. Adipocyte differentiation was described previously (Kim et al., 2010). Briefly, 2-day post confluent 3T3-L1 cells (designated day 0) were differentiated with the differentiation induction medium (MDI) containing 5 µg/mL insulin, 0.25 mM 3-isobutyl-1-methylxanthine (IBMX) and 0.25 µM dexamethasone in DMEM supplemented with 1% FBS.

Plasmids and Luciferase assays
Plasmids, pCMX-TR4, pCMX-4A4, and TR4RE-Luc (pGL-tk-(DR1)3-Luc) were described previously (Kim et al., 2003; Lee et al., 1995; Lee et al., 1997). The mouse perilipin promoter region consisting of -2000 to +75 bp was amplified by PCR from 3T3-L1 genomic DNA and cloned into pGL3-luciferase (Promega, USA) to generate pGL3-Plin-Luc. Three copies of synthesized DR1 element of the perilipin promoter and consensus DR1 (cDR1) were subcloned into pGL3-∆32TK-Luc to create pGL3-Plin-DR1-Luc and pGL3-cDR1-Luc, respectively. Transfections were performed using SuperFect (Qiagen, USA) according to the manufacturer’s instructions in HEK293T cells. After transfection, cells were incubated for 24 h in the absence of presence of CLA in DMEM supplement with 1% FBS and luciferase activities were measured in a Luciferase reporter assay system (Berthold). Relative luciferase activity (fold) was expressed based on the induction fold relative to transfection of an empty vector (set as 1-fold) and the results were expressed as means± SD of three separate experiments.

Reverse transcriptase PCR (RT-PCR) and gel shift assay
RNA isolation, cDNA synthesis and RT-PCR were performed as previously described (Kim et al., 2010). The sequences of TR4, aP2, and 36B4 primers used for RT-PCR were described previously (Cariou et al., 2006; Kim et al., 2003). Primer sequences for perilipin gene were: sense; 5’-CTCTGGGAAGCATCGAGAA-3’ and antisense; 5’-GATCCACATGGCCAGAGAT-3’. Gel shift assay was performed as described previously (Kim et al., 2003). Briefly, TR4 was in vitro synthesized using a T7-quick coupled TNT reticulocyte lysate system (Promega). The following oligomers were used for gel shift assays: cDR1 (5’-GATCTCTCTAGGTCAAAGGTCAATTTC-3’), Plin-DR1 (5’-GATCCTTGTCACCTTTCACCCACAT-3’) and mutant Plin-DR1 (5’-GATCCCCTTTTGCAAGGCAGATC-CCACATCC-3’).

Results
CLA increased TR4 expression in 3T3-L1 adipocytes
TR4-deficient mice have less fat mass with reduced TG accumulation. In agreement with this report, we recently found that TR4 regulates lipid accumulation in 3T3-L1 adipocytes via regulation of FATP1 expression. To investigate the possible involvement of TR4 in CLA effect on adipocyte biology, we added 20, 40 or 80 µM CLA together with standard adipogenic stimuli to 2-day post confluent 3T3-L1 cells (designated day 0) and CLA was continuously treated to the differentiating cells until day 6 of differentiation. Since CLA has been shown to inhibit