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Adipocyte NCoR Knockout Decreases PPARγ Phosphorylation and Enhances PPARγ Activity and Insulin Sensitivity

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SUMMARY

Insulin resistance, tissue inflammation, and adipose tissue dysfunction are features of obesity and Type 2 diabetes. We generated adipocyte-specific Nuclear Receptor Corepressor (NCoR) knockout (AKO) mice to investigate the function of NCoR in adipocyte biology, glucose and insulin homeostasis. Despite increased obesity, glucose tolerance was improved in AKO mice, and clamp studies demonstrated enhanced insulin sensitivity in liver, muscle, and fat. Adipose tissue macrophage infiltration and inflammation were also decreased. PPARγ response genes were upregulated in adipose tissue from AKO mice and CDK5-mediated PPARγ ser-273 phosphorylation was reduced, creating a constitutively active PPARγ state. This identifies NCoR as an adaptor protein that enhances the ability of CDK5 to associate with and phosphorylate PPARγ. The dominant function of adipocyte NCoR is to transrepress PPARγ and promote PPARγ ser-273 phosphorylation, such that NCoR deletion leads to adipogenesis, reduced inflammation, and enhanced systemic insulin sensitivity, phenocopying the TZD-treated state.

INTRODUCTION

The adipocyte uses well regulated transcriptional programs to adapt to environmental inputs through storage of calories as triglycerides and secretion of adipokines and other factors (Rosen and Spiegelman, 2006). PPARγ is a key factor controlling the importance of adipose tissue in whole-body glucose metabolism (Evans et al., 2004; Lehrke and Lazar, 2005; Saltiel and Olefsky, 1996; Sugii et al., 2009; Tontonoz and Spiegelman, 2008). PPARγ is a member of the nuclear hormone receptor (NR) family and is highly enriched in adipose tissue, where it plays a critical role in adipocyte differentiation, insulin sensitivity, and adipokine/cytokine secretion (Evans et al., 2004; Imai et al., 2004; Rangwala and Lazar, 2004; Tontonoz and Spiegelman, 2008). Although its endogenous ligand is poorly understood, PPARγ is the molecular target for the thiazolidinedione (TZD) class of insulin-sensitizing drugs used to treat type 2 diabetes.

Transcriptional control by NRs, including PPARγ and others, depends on multiprotein coregulatory complexes (Feige and Auwerx, 2007; Fowler and Alarid, 2004; Hermanson et al., 2002). In general, corepressor complexes are recruited to NRs in the absence of ligand, whereas coactivator complexes are recruited to NRs in the presence of agonists (Lonard and O'Malley, 2005). Coactivators and corepressors modulate gene transcription by a variety of mechanisms including histone acetylation, chromatin remodeling, and direct interactions with basal transcription complexes (Collingwood et al., 1999). There are several coactivators, such as CBP, PGC1α, and CRTC2, that are known to play important roles in metabolic control (Handschin and Spiegelman, 2008; Revilla and Granja, 2009; Wang et al., 2010). However, the role and underlying mechanisms of corepressor function in metabolic tissues remains unclear. Two major NR corepressors are the silencing mediator of retinoid and thyroid hormone receptors (SMRT) and the nuclear receptor corepressor (NCoR) (Chen and Evans, 1995; Horlein et al., 1995). It has been shown that downregulation of SMRT and NCoR expression in 3T3-L1 cells leads to enhanced adipocyte differentiation, in part through increased PPARγ transcriptional activity (Yu et al., 2005). However, their role in adipogenesis, adipocyte function, and glucose metabolism in vivo remains uncertain. Since whole body NCoR deletion is embronically lethal (Jepsen et al., 2000), we generated adipocyte-specific NCoR knockout (AKO) mice to assess the role of this corepressor in glucose metabolism, insulin sensitivity, and adipogenesis. We show that AKO mice develop increased adiposity on HFD relative to WT controls. Despite this increase in obesity, the AKO animals exhibit enhanced systemic insulin sensitivity, improved glucose tolerance, and decreased adipose tissue inflammation. Taken together, these features phenocopy the effects of systemic TZD treatment.

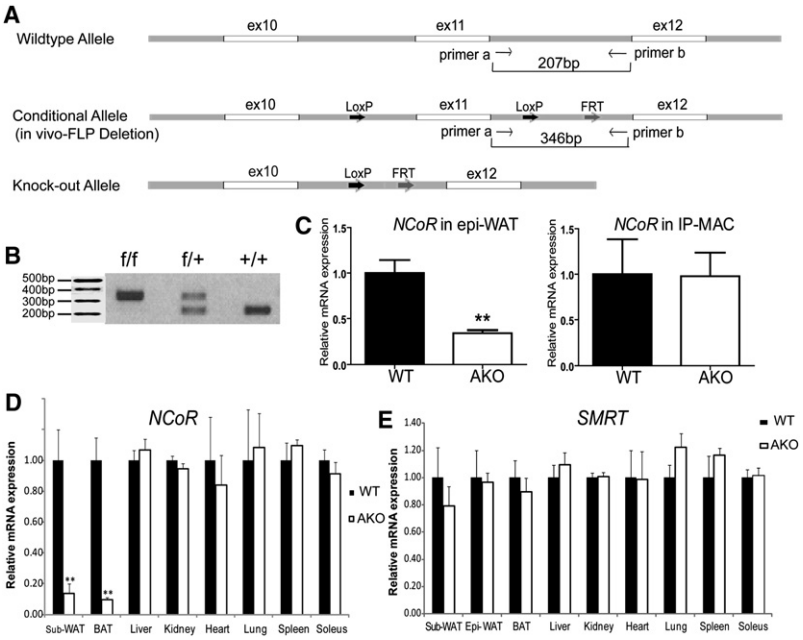

Figure 1. NCoR Targeting Strategy and Adipocyte-Specific Deletion

(A) Shown (top to bottom) are wild-type, floxed, and deleted NCoR gene loci. Primers used to distinguish WT and floxed alleles and sizes of the expected PCR products are indicated.

(B) Genotyping results of wild-type +/+, f/+, and f/f mice.

(C) Relative messenger RNA levels of NCoR in adipose tissue and macrophages.

(D and E) Relative NCoR (D) and SMRT (E) mRNA levels in various tissues.

Values are fold induction of gene expression normalized to the housekeeping gene *Gapdh* and expressed as mean ± SEM, n = 8–10 in (C)–(E), *p < 0.05, **p < 0.01 for AKO versus WT. See also Table S1.

RESULTS

NCoR Deletion in Adipocytes

To investigate the specific role of adipocyte NCoR on adipogenesis and on the development of insulin resistance in response to HFD feeding, we generated adipocyte-specific knockout mice (AKO) using the Cre-lox system (*NCoRfl/fl; aP2-Cre*$^{+/-}$) (Figures 1A and 1B). As controls, floxed *NCoR* mice that do not express Cre recombinase (Cre) were used (*NCoRfl/fl; ap2-Cre*$^{-/-}$), referred to, hereafter, as WT. As expected, *NCoR* expression was greatly diminished in the epididymal adipose tissue of AKO mice (Figure 1C). However, since aP2/Fabp4 can also be expressed in macrophages, we determined whether aP2 Cre-mediated deletion of NCoR could be detected in this cell type. There was no decrease in *NCoR* expression in intraperitoneal (IP)-macrophages from the AKO mice (Figure 1C), consistent with previous studies using this ap2-cre mouse line, showing adipocyte restricted expression(He et al., 2003; Qi et al., 2009; Sabio et al., 2008; Sugii et al., 2009). This explains the ~70% decrease in *NCoR* expression in whole epididymal adipose tissue, since *NCoR* is not deleted in macrophages or other nonadipocyte cell types present in this tissue. Interestingly, the magnitude of *NCoR* depletion in subcutaneous WAT and BAT was closer to 90%, most likely reflecting a lower amount of nonadipocytic cells, such as immune cells, in these depots. As also shown in Figure 1D, there was no *NCoR* deletion in any other tissue examined. *SMRT* is another corepressor, which in some contexts can function similarly to NCoR, but there were no changes in *SMRT* expression in the AKO mice in any tissues (Figure 1E).

AKO Mice Are More Obese Than WT after HFD Feeding

To investigate the functional significance of adipocyte-specific NCoR deletion, both WT and AKO mice were fed a 60% high-fat diet (HFD) for up to 17 weeks, starting at 8 weeks of age. As expected, WT mice became obese, but the AKO mice were even more obese as shown in Figure 2A. Thus, the body weight of the AKO mice was ~15% greater than WT (Figure 2B) and this was accompanied by a 10% increase in food intake (Figure 2C). To further assess body composition changes accompanying this increase in obesity, MRI analyses were performed. As shown in Figures 2D–2F, the AKO mice exhibited an increased volume of both subcutaneous and visceral fat. Accordingly, epididymal fat mass doubled in AKO mice compared to WT (Figure 2G), while there was no difference in lean body mass (data not shown). Given that PPARγ plays a central role in the promotion of adipogenesis, these results suggest constitutive activation of adipose tissue PPARγ. Consistent with this interpretation, Figure 2H shows increased expression of the adipogenic PPARγ response genes *FAS, ACC, SREBP1c, SCD1*, and *SCD2* in AKO adipose tissue (Djaouti et al., 2010; Lessard et al., 2007; Paton and Ntambi, 2009; Sugii et al., 2009).

Deletion of NCoR in Adipose Tissue Protects against HFD-Induced Systemic Insulin Resistance

Obesity leads to glucose intolerance as well as insulin-resistance in adipose tissue, liver, and muscle. Therefore, we assessed glucose homeostasis and insulin sensitivity in lean chow-fed and HFD-fed WT and AKO mice. No changes in glucose or insulin tolerance, BW, fat-pad weight, or fasting insulin level, were noted in the lean chow-fed mice between genotypes (Figure S1 available online). In contrast, in the context of HFD/obesity, marked phenotypic changes in glucose and insulin homeostasis emerged in the AKO mice. Basal insulin level was reduced (Figures 3A and 3B), and upon glucose and insulin tolerance test, the AKO mice showed an enhanced hypoglycemic response to the injected insulin (Figure 3C), as well as improved glucose tolerance compared to controls (Figure 3D). In aggregate, these results argue strongly for improved systemic insulin sensitivity as a result of adipocyte NCoR deletion.

To more accurately quantify in vivo insulin action and to assess tissue-specific sites of insulin sensitivity, hyperinsulinemic/euglycemic clamp studies were performed. The amount of