Suppressing the activity of ERRα in 3T3-L1 adipocytes reduces mitochondrial biogenesis but enhances glycolysis and basal glucose uptake

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Abstract

Estrogen-related receptor α (ERRα) is thought to primarily regulate lipid oxidation and control the transcription of genes in the oxidative phosphorylation pathway in skeletal and cardiac muscles. However, its role in white adipose tissue is not well studied. In this study, we aimed to establish a role for ERRα in adipocytes by down-regulating its activity through its inverse agonist XCT-790 in differentiated 3T3-L1 adipocytes. We found that XCT-790 differentially reduced the expression of ERRα target genes. Specifically, XCT-790 reduced the expressions of peroxisome proliferator-activated receptor γ co-activator-1β (PGC-1β), resulting in reductions of mitochondrial biogenesis, adiogenesis and lipogenesis. Through suppressing the expression of another ERRα target gene pyruvate dehydrogenase kinase 2 (PDK2), we found that XCT-790 not only enhanced the conversion of pyruvate to acetyl-CoA and hyper-activated the tricarboxylic acid (TCA) cycle, but also led to higher levels of mitochondrial membrane potential and reactive oxidant species (ROS) production. Additionally, XCT-790 treatment also resulted in enhanced rates of glycolysis and basal glucose uptake. Therefore, ERRα stands at the crossroad of glucose and fatty acid utilization and acts as a homeostatic switch to regulate the flux of TCA cycle, mitochondrial membrane potential and glycolysis to maintain a steady level of ATP production, particularly, when mitochondrial biogenesis is reduced.

Keywords: ERRα • PGC-1β • mitochondrial biogenesis • glycolysis • basal glucose uptake

Introduction

Estrogen-related receptors α, β and γ (ERRα, ERRβ and ERRγ) are considered to be orphan nuclear hormone receptors that display constitutively active transcriptional activities [1–4]. Like other nuclear hormone receptors, ERRα for example is comprised of a N-terminal ligand-independent transcriptional activation domain (AF-1), a centrally located DNA binding domain (DBD), a variable hinge region and a C-terminally located ligand binding domain (LBD) that harbors the ligand-dependent transcriptional activating function (AF-2) [4].

ERRα is primarily thought to regulate energy homeostasis through interacting with peroxisome proliferator-activated receptor γ co-activator-1α and -1β (PGC-1α, and -1β) and co-ordinately control the transcription of genes in the oxidative phosphorylation (OXPHOS) pathway [5–7]. ERRα has also been shown to modulate fatty acid and glucose utilization through directly regulating the expression of medium chain acyl dehydrogenase (MCAD)[8] and pyruvate dehydrogenase kinase 2 and 4 (PDK2 and 4) [9]. In skeletal muscle of human type 2 diabetic patients, the expression levels of mitochondrial OXPHOS genes are reduced, suggesting that the functions of ERRα and PGC-1α are altered [10]. This reduction of ERRα/PGC-1α activities may contribute to the development of insulin resistance.

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If a reduction of ERRα expression or activity would lead to a compromised OXPHOS capacity, then ERRα knock out mice would be expected to develop impaired glucose tolerance or even insulin resistance. Paradoxically, ERRα knock out mice are lean and resistant to high-fat-diet-induced obesity [11]. The size of adipocyte in ERRα knock out mice is smaller compared to wild-type mice and these adipocytes show reduced lipogenesis [11]. Since these ERRα knock out mice delete ERRα in all tissues including other insulin-sensitive tissues like liver and muscle in addition to adipose, the phenotypes seen in adipose tissue may be subjected to modification by an altered glucose homeostasis initiated in other tissues. We therefore use a specific inverse agonist of ERRα XCT-790 to investigate the role of ERRα in vitro in murine 3T3-L1, a well-established adipocyte model.

In the present study, we demonstrate that suppressing the activity of ERRα through its inverse agonist XCT-790 has a direct and immediate effect on energy homeostasis in 3T3-L1 adipocytes. We show that XCT-790 suppresses the expression of ERRα target genes including ERRα, PGC1β and PDK2. Surprisingly the expression of PDK4 is induced by XCT-790 while the expression of PGC-1α remains unchanged. The reduction of PGC1 β expression leads to a reduction of mitochondrial biogenesis. In addition, the co-activating function for PPARγ is also reduced, leading to reduced expressions of PPARγ target genes like PPARγ, fatty acid binding protein 4 (FABP4/aP2) and liver X receptor α (LXRα). Consequently, the expression levels of LXRα target gene sterol regulatory element binding protein-1c (SREBP-1c) and its lipogenic target gene fatty acid synthase (FAS) are lowered, resulting in a reduced level of adipogenesis and cellular triglyceride (TG) amount.

On the other hand, the differential regulations of PDK2 and PDK4 expressions result in releasing the negative regulation of PDKs on pyruvate dehydrogenase complex (PDC), increasing the amount of pyruvate being converted to acetyl-CoA and enhancing the rate of tricarboxylic acid (TCA) cycle, and finally resulting in a compensatory increase in mitochondrial membrane potential. Additionally, the rate of glycolysis is enhanced to replenish the pyruvate pool. The enhanced rate of glycolysis also leads to an enhanced rate of glucose uptake. Therefore, suppressing the activity of ERRα through its inverse agonist XCT-790 enhances glucose uptake and reduces TG level in adipocytes, which are reminiscent of the adipocyte phenotypes of the ERRα knock out mice, suggesting that ERRα plays an important role in adipocytes in addition to the more extensively studied muscle tissue.

Materials and methods

Cell culture and treatment

3T3-L1 pre-adipocytes were cultured in DMEM (Gibco, Grand Island, NY, USA) with 10% FBS (Hyclone, Logan, UT, USA) and 100 U/ml penicillin-streptomycin (Gibco) under humidified air containing 5% CO2 at 37°C. Two-days post-confluent cells were induced to differentiate with 5 μg/ml insulin (Sigma, Saint Louis, MO, USA), 0.5 mM 3-isobutyl-1-methylxanthine (Calbiochem, Darmstadt, Germany) and 1 μM dexamethasone (ICN, Costa Mesa, CA, USA) in DMEM supplemented with 10% FBS for 2 days. Cells were then maintained in 10% FBS/DMEM with 5 μg/ml insulin for another 2 days. Medium was subsequently changed into 10% FBS/DMEM every other day. Cells were fully differentiated to adipocytes at day 6–8. To suppress the activity of ERRα, adipocytes were treated with different concentrations of XCT-790 for 48 hrs with DMSO as vehicle control.

Succinate dehydrogenase (SDH) activity assay

SDH activity was determined by the ability of mitochondrial SDH to reduce methyl thiazolyl tetrazolium (MTT) (MBChem, Shanghai, China). Experiments were performed with the addition of 5 mg/ml MTT to drug-treated cells for 2 hrs. Absorbance of methyamethyst crystal product was measured at 570 nm.

ATP production

The amount of ATP in adipocytes was measured with ATPlite-glo, a luciferase-based luminescence assay kit (PerkinElmer, Boston, MA, USA). Briefly, treated cells were mixed to shake with detection regent for 5 min, luminescence was measured with VERITASSTM Microplate luminometer (Turner Biosystems, Sunnyvale, CA, USA).

Mitochondrial mass

Mitotract green was used to determine the mitochondrial mass in adipocytes. Briefly, cells were incubated in serum-free medium with 150 nm Mitotract Green (Invitrogen, Carlsbad, CA, USA) for 30 min, then spectral characteristics of harvested cells were analyzed with fluorescence-assisted cell sorter (FACS), (excitation at 490 nm, emission at 516 nm).

Mitochondrial membrane potential

Mitochondrial membrane potential was analyzed by a fluorescent dye, JC-1 (Beyotime, Jiangsu, China). Monomer JC-1 of green fluorescence forms dimer of red fluorescence when membrane potential is high. Assays were initialized by incubating adipocytes with JC-1 for 30 min and fluorescence of separate cells was detected with FACs.

Reactive oxidant species (ROS) assay

The determination of ROS was based on the oxidation of 2,7-dichlorodihydrofluorescein (DCHF) (Beyotime) by peroxide, as previously described [12]. In brief, adipocytes were washed and incubated with DCHF for 20 min. Cells were then washed several times and harvested in PBS. The fluorescence of 2,7-dichlorofluorescein (DCF) was detected with FACs (excitation at 488 nm, emission at 530 nm).
Glucose uptake

Glucose uptake assays were performed in adipocytes as previously described [13]. Briefly, differentiated cells were plated to 24-well plate, 300,000 cells/well. After drug treatment, cells were incubated in FCB (Kreb’s Ringer with 2 mM pyruvate and 2% bovine serum albumin [BSA]) for 20 min and another 20 min in FCB with or without 100 nM insulin at 37°C. Cells were then maintained in FCB with the 4.15 μCi/ml [3H]-2-deoxyglucose (Atom HighTech, Beijing, China) for 10 min. Reactions were terminated by washing with cold PBS for several times and cells were harvested in the scintillation liquid. Radioactivity was determined by liquid scintillation counting.

Glycolytic rate

Glycolytic rate was measured according to previous report [14]. In brief, adipocytes were cultured in 6-well plate, after treating with vehicle or XCT-790, cells were incubated in Kreb’s buffer for 30 min and another 1 hr with the addition of 5 μl 4-3H-glucose (Atom HighTech) at 37°C. A total of 50 μl of 10-2.5% trypsin was added to the reaction system for harvesting cells. Open tubes with 100 μl reaction system terminated with 50 μl 0.2 N HCl were transferred to 4 ml scintillation vials containing 1 ml H2O. After a 2-day incubation in the closed environment at room temperature, 3H2O generated from glycolysis by evaporating into H2O in the vials was counted, as well as the remaining radioactivity in the open tube. Glycolytic rate was determined by calculating the ratio of diffused 3H2O and undiffused 5-3H-glucose.

Quantitative real-time PCR

Total RNA from adipocytes was extracted by Trizol reagent (Invitrogen). The first-strand cDNA was generated with random primer by reverse transcription kits (Invitrogen). Real-time PCR reactions were performed with sybr green kit (Takara Biotechnology, Dalian, China) in MJ research PTC 200. The sequences of primers are available upon request. glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression was taken as internal control.

Western blot analysis

Protein was extracted by Trizol reagent according to the manufacturer’s protocol. The concentration was measured by the protein quantification kit (Shenergy Biocolor, Shanghai, China). Equal amounts of each protein sample were analyzed by SDS gel electrophoresis. Transferred proteins were incubated with ERRα antibody (Upstate) followed by an anti-rabbit immunoglobulin G–horseradish peroxidase antibody. β-actin was taken as internal control.

Measurement of TG content and Oil-Red-O staining

Adipocytes were lysed by sonication. TG content was measured by the triglycerides kit (Dongling Diagnostic products, Shanghai, China) and normalized with protein concentration. Oil-Red-O staining was performed as described previously [15].

Results

Suppressing ERRα activity in adipocytes down-regulates PGC-1β expression

While ERRα is well-established to be expressed and play an important role in both skeletal [10] and cardiac muscles [16, 17], ERRα is less well studied in adipocytes. We first examined the expression levels of ERRα in 3T3-L1 pre-adipocytes upon differentiation. 3T3-L1 pre-adipocytes were induced to differentiate into mature adipocytes by IBMX and insulin. We found that ERRα expression level did not change significantly during various time-points upon differentiation (Fig. 1A). Since ERRα acts together with PGC-1α or PGC-1β to regulate the expression of a cohort of genes involved in OXPHOS and the electron transport chain [10, 16], and that the expression levels of PGC-1α and PGC-1β themselves can be regulated by ERRα [18], we examined the mRNA levels of both PGC-1α and PGC-1β in 3T3-L1 adipocytes upon differentiation. We found that the expression level of PGC-1α increased by fourfolds at most (Fig. 1B), whereas, the expression level of PGC-1β increased by >500 folds during the later stages of adipocyte differentiation (Fig. 1C).

In order to address the role of ERRα in adipocytes, we used an ERRα–specific inverse agonist XCT-790 [19] as a tool to probe into the function of ERRα. Since the mRNA expression level of ERRα itself is regulated by ERRα and PGC-1α [20], we expected that XCT-790 would down-regulate the activity of ERRα and lead to a reduction of the endogenous ERRα mRNA expression level. We studied the status of ERRα in differentiated 3T3-L1 adipocytes under the treatment of XCT-790. RT-PCR and Western blotting were performed for mRNA and protein expression levels of ERRα, respectively. We found that ERRα mRNA expression was reduced by about 40% in the presence of 20 μM XCT-790 compared to DMSO as a control (Fig. 1D). Consistent with the mRNA level, protein levels of ERRα were also down-regulated in a dose-dependent manner (Fig. 1E). In addition, we also measured and found that the expression level of another ERRα target gene MCAD in adipocytes was down-regulated by XCT-790 (data not shown).

Intriguingly, of the two PGC-1 co-activators that are shown to be regulated by ERRα, only the expression level of PGC-1β was dose-dependently reduced by XCT-790 (Fig. 1F), suggesting that ERRα selectively regulates PGC-1β expression in 3T3-L1 adipocytes. We also detected a moderate increase in PGC-1α expression in XCT-790 treated cells (Fig. 1G). Since some of the functions of PGC-1 co-activators are overlapping [21], the
Fig. 1 Effects of XCT-790 on the expressions of ERR\(\alpha\), PGC-1\(\alpha\) and PGC-1\(\beta\). The expression levels of ERR\(\alpha\), PGC-1\(\alpha\) and PGC-1\(\beta\) during the differentiation of 3T3-L1 were analyzed by RT-PCR. Total RNA of differentiating 3T3-L1 cells was extracted at day 0, 2, 4 and 8. The expression levels of genes were normalized with GAPDH level. The relative expression levels of genes at day 0 are set as 1 (A, B and C). Differentiated 3T3-L1 adipocytes were treated with XCT-790 of different concentrations for 48 hrs. mRNA levels of ERR\(\alpha\) (D), PGC-1\(\alpha\) (G) and PGC-1\(\beta\) (F) were determined by RT-PCR. The relative expression levels of genes treated with DMSO control (0 \(\mu\)M XCT-790) are set as 1. Values are shown as mean ± S.D. of three independent experiments. Protein levels of ERR\(\alpha\) were determined by Western blot analysis. Total protein was extracted from treated cells and ERR\(\alpha\) antibody was used for the analysis with \(\beta\)-actin as an internal control. The results are representative of three independent experiments (E).
increase in PGC-1α expression may be derived from a compensatory response due to the reduction of PGC-1β expression.

XCT-790 reduces lipogenesis in 3T3-L1 adipocytes through down-regulating PGC-1β

Since PGC-1 co-activators are important for mitochondrial biogenesis and functions, we examined the consequences of PGC-1β down-regulation by ERRα inverse agonist XCT-790. One potential consequence of a lowered level of PGC-1β expression would be a reduction of mitochondrial biogenesis. We treated adipocytes with a mitochondrial dye Mitotracker Green that stains mitochondrion independent of mitochondrial membrane potential. The intensity of Mitotracker Green staining reflects cellular mitochondrial mass. We found that XCT-790 dose-dependently reduced mitochondrial mass (Fig. 2A).

In addition to regulating mitochondrial biogenesis, PGC-1 co-activators also function to enhance the transcriptional activities of a number of nuclear hormone receptors [22], including peroxisome proliferators-activated receptor γ (PPARγ), a key regulator of adipocyte differentiation and lipogenesis [23]. Since the expression level of PPARγ mRNA itself is regulated by PPARγ activity level, which is in part determined by the expression levels of PGC-1 co-activators, we therefore examined for the consequences of reduced PGC-1β expression level by checking the expression levels of PPARγ and its target gene aP2, a marker of adipocyte differentiation. We found that XCT-790 dose-dependently reduced the expression of PPARγ and aP2 (Fig. 2B). In addition, we also examined the expression level of another PPARγ target gene LXRα, which is involved in cholesterol homeostasis and lipogenesis through inducing SREBP-1c and in turn FAS. We found that XCT-790 also dose-dependently reduced the expression of LXRα, its target gene SREBP-1c and SREBP-1c lipogenic target gene FAS, respectively (Fig. 2C). To further confirm the influences on adipocyte differentiation and lipogenesis, we measured the TG level in differentiated 3T3-L1 adipocytes. We found that XCT-790 lowered the TG content (Fig. 2D). In addition, the sizes of oil droplets were smaller in XCT-790-treated cells revealed by Oil-Red-O staining (Fig. 2E). Therefore, down-regulating ERRα activity in adipocytes may directly result in reduced adipocyte differentiation and lipogenesis, mirroring the lean phenotype and reduced adipocyte size seen in ERRα knock out mice [11].

XCT-790 alters mitochondrial functions

Not only does ERRα regulate mitochondrial biogenesis with PGC-1 co-activators, ERRα also regulates the rate of glucose and fatty acid utilization through controlling the expressions of PDK2 and PDK4 [9]. PDK2 and PDK4 phosphorylate PDC and negatively regulate the activity of PDC, which is responsible for the conversion of pyruvate to acetyl-CoA for entry into the TCA cycle. Unlike in muscle cells in which ERRα positively regulates the expressions of both PDK2 and PDK4, we found that XCT-790 reduced the expression level of PDK2 but enhanced the expression of PDK4 in adipocytes (Fig. 3A).

Reductions in PDK2 and PDK4 activities would be expected to relieve the negative control of PDK2 and PDK4 on PDC, therefore, enhance the rate of acetyl-CoA conversion and the amount for entry into the TCA cycle. Since the expression levels of PDK2 and PDK4 mRNA are differentially regulated by ERRα in adipocytes, and that the summation of these regulations would affect PDC activity and the rate of acetyl-CoA entry into the TCA cycle, we instead examined the effect of down-regulating ERRα activity on the TCA cycle by measuring the activity of SDH, a key enzyme in the TCA cycle, upon XCT-790 treatment. A significant and dose-dependent increase in SDH activity was observed (Fig. 3B), indicating that the TCA cycle was hyper-activated and suggesting that the total amount of PDK activity was reduced.

The hyper-activated TCA cycle is then expected to generate more NADH and FADH2 to enter into the electron transport chain and elevate the mitochondrial membrane potential (Δψm). We then used a fluorescent dye JC-1, which gives a red fluorescence when Δψm is high and green fluorescence when Δψm is low to determine the overall electron transport chain activity. In adipocytes treated with protonophore m-chlorophenylhydrazine (CCCP), a known compound that reduces Δψm, the amount of red fluorescence decreased from 9.37% of control to 2.71% in CCCP-treated cells. As expected, the percentage of cells with red fluorescence increased from 9.37% of control to 41.17% in cells treated with 20 μM XCT-790 (Fig. 3C), indicating that Δψm was elevated in cells in which the activity of ERRα was suppressed. This elevated Δψm would be expected to generate more ATP per mitochondrion in order to compensate for the lost of ATP production capacity due to an overall lower amount of mitochondrial resulting from the reduction of ERRα and PGC-1β activities. Indeed, the total cellular ATP levels did not show any significant changes even when in the presence of reduced mitochondrial mass levels (Fig. 3D).

However, a sustained elevation in Δψm is also known to elevate the production of reactive oxygen species (ROS). By using a flow cytometry method to quantify the amount of fluorescence, we next measured the amount of ROS by a fluorescent probe H2DCFDA that detects intracellular ROS. Compared to a positive control (Rose Up) that elevates ROS, we found that XCT-790 also dose-dependently increased ROS level in adipocytes (Fig. 3E).

XCT-790 enhances glycolysis and glucose uptake

As more pyruvate is converted to acetyl-CoA to feed into the hyper-activated TCA cycle, the pool of pyruvate is expected to be reduced. A potential consequence would be an increased rate of glycolysis in order to replenish the pyruvate pool. Therefore, we examined the glycolytic rate in 3T3-L1 adipocytes with or without suppressing ERRα activity. 5-3H-glucose was incubated with cells after treatment with XCT-790 and the glycolytic rate was
Fig. 2 Effects of XCT-790 on mitochondrial biogenesis and lipogenesis in 3T3-L1 adipocytes. Mitochondrial mass was determined with Mitotracker Green. Relative mass was presented as the fold of DMSO-treated cells (A). Expression of lipogenesis marker genes PPARγ, aP2, LXRα, SREBP-1c, FAS was determined by RT-PCR. Relative expression level was normalized with GADPH and presented as the fold of DMSO control (B, C). XCT-790-treated adipocytes were disrupted by sonication. Triglyceride (TG) content was measured by a TG kit and normalized by total protein concentration. TG concentration was presented as the percentage of DMSO-treated control (D). Values are shown as mean ± S.D. of three independent experiments. Intracellular lipid was stained with Oil-Red-O. Oil droplets were examined with a microscope and photographed (E).
Fig. 3 Effects of XCT-790 on mitochondrial functions in 3T3-L1 adipocytes. Expression levels of PDK2 and PDK4 were determined by RT-PCR. Relative expression levels were normalized with GADPH and presented as the fold of DMSO (A). SDH activity was determined by MTT method. Absorbance was read at 570 nm. The relative activity of SDH was presented as the fold of control, values were shown as mean ± S.D. of triplicate (B). Mitochondrial membrane potential was analyzed by calculating the ratio of cells with red and green fluorescence generated by JC-1. Cells with more red fluorescence mean higher membrane potential. Fluorescence of harvested adipocytes was detected by FACs. CCCP, which uncouples the membrane potential serves as the positive control. PE: red fluorescence FITC: green fluorescence (C). Total cellular ATP levels were measured by ATPlite-glo, a luciferase-based luminescence assay kit (PerkinElmer) (D); ROS levels were determined by measuring the fluorescence of DCF with FACs (excitation at 488 nm, emission at 530 nm). The relative levels are presented as the fold of DMSO with Rose up as a positive control. Values were shown as mean ± S.D. of triplicate (E).
determined by counting $^3$H$_2$O generated from glycolysis. We found that the glycolytic rate was increased by about fourfold in the presence of 20 μM XCT-790 compared to vehicle control (Fig. 4A).

The enhanced rate of glycolysis may lead to an increase in glucose uptake in order to feed the glycolytic pathway forward. Thus, we investigated the rate of glucose uptake in 3T3-L1 adipocytes by treating cells with $^3$H-2-deoxyglucose. Compared to insulin as a control that increases glucose uptake, we observed a more than twofold enhancement on basal glucose uptake after treatment with 20 μM XCT-790 (Fig. 4B). On the other hand, the amount of glucose uptake induced by insulin was lowered compared to DMSO control (Fig. 4C).

We then checked to see if this increase in basal glucose uptake was primarily due to alterations of glucose transport-1 (Glut-1) and glucose transport-4 (Glut-4) expressions. Glut-4 in cardiomyocytes has recently been shown to be positively regulated by ERRα [10, 16]. Consistent with Glut-4 being an ERRα target, we found that adipocyte Glut-4 mRNA expression level was reduced by XCT-790 (Fig. 4D). On the other hand, Glut-1 expression level was induced and likely responsible for mediating the increase in basal glucose uptake (Fig. 4D).

**Discussion**

ERRα has been reported to regulate energy metabolism with PGC-1α by inducing fatty acid oxidation and OXPHOS in high-energy expenditure tissues like muscle, heart and brown fat [1–4]. However, the function of ERRα in energy storage tissue, namely white fat, has not been clearly defined. Paradoxically, ERRα knock out mice are lean and resistant to high-fat-diet-induced obesity [11]. It has been reported that these mice were slightly defective in energy absorption in the gut, therefore, reducing the amount of...
calories intake and leading to a lean phenotype. This phenotype may also be partly explained by a compensatory increase in ERR\textsubscript{\gamma} expression, which may substitute for some of the function of ERR\textsubscript{\alpha} in promoting fatty acid oxidation.

In our present study, we tried to establish a direct role for ERR\textsubscript{\alpha} in adipocyte by mimicking an ERR\textsubscript{\alpha} loss of function through using its inverse agonist XCT-790 as a probe. While XCT-790 does not affect the activities of two closely related members ERR\textsubscript{\beta} and ERR\textsubscript{\gamma}, if functions as a specific inverse agonist of ERR\textsubscript{\alpha} [19]. The effect of XCT-790 on ERR\textsubscript{\alpha} goes beyond just altering its interaction with co-activators but also extends to promoting its degradation by the proteasome [24]. We not only observed that ERR\textsubscript{\alpha} protein level was lowered by treating with XCT-790 (Fig. 1E), we also found that its mRNA level was reduced in 3T3-L1 adipocytes (Fig. 1D), which is consistent with the idea that the expression of ERR\textsubscript{\alpha} mRNA is self-regulated by ERR\textsubscript{\alpha} protein [20, 25]. On the other hand, only the ERR\textsubscript{\alpha} protein level was lowered in breast cancer cells treated with XCT-790 [24], perhaps reflecting the dominant influence of estrogen receptor in governing ERR\textsubscript{\alpha} gene expression in estrogen-responsive tissues [26]. Having established that XCT-790 effectively suppressed ERR\textsubscript{\alpha} expression, we investigated into the consequences of inhibiting ERR\textsubscript{\alpha} activity in differentiated 3T3-L1 adipocytes, a well-established in vitro model of white fat. In this system, ERR\textsubscript{\gamma} expression was not altered by XCT-790 treatment (data not shown), suggesting that the effects observed were primarily the results of suppressing ERR\textsubscript{\alpha} activity. We found that down-regulating ERR\textsubscript{\alpha} activity would reduce the storage of TG and size of oil droplets in adipocytes, partly recapitulating the lean phenotype and smaller adipocyte size seen in ERR\textsubscript{\alpha} knock out mice. PGC-1s are notable for their major roles in metabolic regulation as PPAR\textsubscript{\gamma} and ERR\textsubscript{\alpha} co-activators [22]. These nuclear hormone receptors physically interact with PGC-1s using distinctive LXXLL interacting motifs and regulate largely non-overlapping sets of genes. Nonetheless, we demonstrated that down-regulating ERR\textsubscript{\alpha} activity would still have a major consequence on PPAR\textsubscript{\gamma} activity. During differentiation of 3T3-L1 adipocytes, the expression of PGC-1\textalpha\textsubscript{\gamma} gradually increases but PGC-1\textbeta\textsubscript{\gamma} is markedly induced accompanying the increase in PPAR\textsubscript{\gamma} activity (Fig. 1B and 1C). We found that down-regulating ERR\textsubscript{\alpha} activity would selectively reduce the expression level of PGC-1\textbeta\textsubscript{\gamma} but not PGC-1\textalpha\textsubscript{\gamma}. Down-regulating the activity of ERR\textsubscript{\alpha} by siRNA at early stages of adipocyte differentiation reduces PGC-1\textalpha\textsubscript{\gamma} expression [27]. However, using ERR\textsubscript{\alpha} inverse agonist XCT-790 to suppress ERR\textsubscript{\alpha} activity at later stages of adipocyte differentiation only represses PGC-1\textbeta\textsubscript{\gamma} but not PGC-1\textalpha\textsubscript{\gamma} expression (Fig. 1F and 1G), perhaps suggesting that once PGC-1\textalpha\textsubscript{\gamma} expression is established, the regulation by ERR\textsubscript{\alpha} becomes non-essential while the expression of PGC-1\textbeta\textsubscript{\gamma} requires the full activity of ERR\textsubscript{\alpha}. With the reduction of PGC-1\textbeta\textsubscript{\gamma} expression, the transcriptional activity of PPAR\textsubscript{\gamma} is reduced, leading to lowered levels of adipogenesis and lipogenesis. The reduction in lipogenesis was likely due to the reductions of LXRE\textsubscript{\alpha}, SREBP-1c and FAS expression (Fig. 2C). Noticeably, the expression levels of SREBP-1c and FAS were down-regulated in the white adipose tissue of ERR\textsubscript{\alpha} knock out mice [11]. Our data collectively implies that in adipocytes PGC-1\textbeta\textsubscript{\gamma} exerts a more important role in lipogenesis than PGC-1\textalpha\textsubscript{\gamma}. In addition, through regulating the transcriptional level of a co-activator, ERR\textsubscript{\alpha} can also cross-regulate the activities of other nuclear hormone receptors like PPAR\textsubscript{\gamma}.

In addition to regulating lipid metabolism, ERR\textsubscript{\alpha} is also a key modulator of glucose metabolism through regulating PDK2 and PDK4 expressions. PDK2 and PDK4 control the flux of acetyl-CoA entering into the TCA cycle through suppressing the activity of PDC. Intriguingly, the expression levels of PDK2 and PDK4 are differentially regulated by ERR\textsubscript{\alpha} in adipocytes in contrast to their unison regulation by ERR\textsubscript{\alpha} in skeletal and cardiac muscle tissues [9]. In fact, in the ERR\textsubscript{\alpha} knock out mice, the expression of PDK4 in white adipose tissue was indeed elevated compared to the wild-type control [11]. In addition, we observed that the expression of PDK4 was indeed down-regulated in rat myotubes treated with XCT-790 (data not shown). It is conceivable that cell-type-specific coregulators and other signaling pathways may contribute to this differential effect. Nonetheless, we found that down-regulating ERR\textsubscript{\alpha} activity would not only enhanced the conversion of pyruvate to acetyl-CoA and hyper-activated the TCA cycle, but also led to higher levels of mitochondrial membrane potential and ROS production. Additionally, down-regulating ERR\textsubscript{\alpha} activity also results in enhanced rates of glycolysis and glucose uptake in order to replenish the pool of pyruvate being converted into acetyl-CoA for feeding into the TCA cycle. Therefore, ERR\textsubscript{\alpha} stands at the crossroad of glucose and fatty acid utilization and acts as a homeostatic switch to regulate the flux of TCA cycle, mitochondrial membrane potential and glycolysis to maintain a steady level of ATP production, particularly, when mitochondrial biogenesis is reduced.

Our unexpected observations that reducing the activity of ERR\textsubscript{\alpha} enhanced the rate of basal glucose uptake, lowered the level of TG, and reduced the sizes of adipocytes, which are associated with higher insulin sensitivity raise an intriguing question whether ERR\textsubscript{\alpha} inverse agonists would have therapeutic potential for treating diabetes. However, the reduced level of mitochondrial biogenesis and increased level of ROS production would be expected to contribute to insulin resistance if left unchecked. In fact, the expression of Glut4 was lowered and the insulin-induced glucose uptake in adipocytes was reduced by XCT-790 treatment. Therefore, the jury on ERR\textsubscript{\alpha} inverse agonists as therapeutic compounds for metabolic diseases is still out.

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