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PPAR γ Activation Mitigates Glucocorticoid Receptor-Induced Excessive Lipolysis in Adipocytes *via* Homeostatic Crosstalk[†]

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Abstract

Proper balance between lipolysis and lipogenesis in adipocytes determines the release of free fatty acids (FFA) and glycerol, which is crucial for whole body lipid homeostasis. Although, dysregulation of lipid homeostasis contributes to various metabolic complications such as insulin resistance, the regulatory mechanism remains elusive. This study clarified the individual and combined roles for glucocorticoid receptor (GCR) and peroxisome proliferator-activated receptor (PPAR) γ pathways in lipid metabolism of adipocytes. In mature 3T3-L1 adipocytes, GCR activation using dexamethasone upregulated adipose triglyceride lipase (ATGL) and downregulated phosphoenolpyruvate carboxykinase (PEPCK), resulting in enhanced glycerol release into the medium. In contrast, PPAR γ ligand pioglitazone modestly upregulated ATGL and hormone sensitive lipase (HSL), but markedly enhanced PEPCK and glycerol kinase (GK), thereby suppressed glycerol release. Dexamethasone showed permissive like effect on PPAR γ target genes including perilipin A and aP2, therefore co-administration of dexamethasone and pioglitazone demonstrated synergistic upregulation of these enzymes excepting PEPCK, of which downregulation by dexamethasone was abolished by pioglitazone to the level above control. Thus, the excessive glycerol release was prevented as the net outcome of the co-administration. Consistently, the bodipy stain demonstrated that dexamethasone reduced the amount of cytosolic lipid, which was preserved in co-treated adipocytes. Moreover, silencing of PPAR γ suppressed the

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synergistic effects of co-treatment on the lipolytic and lipogenic genes, and therefore the GCR pathway indeed involves PPAR γ . In conclusion, crosstalk between CGR and PPAR γ is largely synergistic but counter-regulatory in lipogenic genes, of which enhancement prevents excessive glycerol and possibly FFA release by glucocorticoids into the circulation.

Keywords

3T3-L1 ADIPOCYTE; HYPERCHOLESTEROLEMIA; LIPOGENESIS; PERMISSIVE EFFECT; TRIACYLGLYCEROL; TYPE 2 DIABETES MELLITUS

Adipocytes play pivotal roles in whole body lipid homeostasis. In general, the process mostly depends upon the equilibrium between lipolysis (triacylglycerol hydrolysis) and lipogenesis (fatty acid synthesis and esterification) [Zimmermann et al., 2009]. It is conceivable that as a master regulator of adipocyte biology, peroxisome proliferator-activated receptor (PPAR) γ should take active parts in the dynamics of lipid metabolism [Evans et al., 2004; Lefterova et al., 2008; Lehrke and Lazar, 2005]. In particular, regarding the lipolysis, the major lipases such as hormone sensitive lipase (HSL) and adipose triglyceride lipase (AGTL) are the direct transcriptional targets of PPAR γ [Kim et al., 2006; Lowe et al., 2011; Yajima et al., 2007]. These lipases breakdown the triacylglycerol (TG) stored in adipocytes to release free fatty acid (FFA) and glycerol [Peckett et al., 2011; Walker, 2006; Wang, 2005].

On the other hand, during lipogenesis, FFAs are esterified with glycerol-3-phosphate to form TGs to be deposited in lipid droplets [Macfarlane et al., 2008; Zimmermann et al., 2009]. In this context of (*de novo*-)lipogenesis, insulin and PPAR γ promotes formation of TGs by enhancing glucose uptake through lipoprotein lipase (LPL) and converting them to glycerol-3-phosphate *via* activation of fatty acid synthetase N (FASN) [Macfarlane et al., 2008; Nye et al., 2008]. Recent studies demonstrated that other than glucose, glycerol-3-phosphate are mostly produced either from pyruvates by glyceroneogenesis *via* phosphoenolpyruvate carboxykinase (PEPCK) [Tordjman et al., 2003], or from glycerol by stimulating its recycle *via* glycerol kinase (GK) [Guan et al., 2002]. Both of these key enzymes (PEPCK and GK) needed for (re-)esterification are the direct transcriptional targets of PPAR γ [Guan et al., 2002; Tordjman et al., 2003].

However, in obesity, due to their saturated capacity to store lipids, adipocytes typically fail to accommodate or esterify excess FFA, and thus, circulating FFA levels are elevated [Wueest et al., 2009]. This disequilibrium between lipolysis and lipogenesis promotes obesity-induced insulin-resistance and type 2 diabetes mellitus [Lehrke and Lazar, 2005]. Therefore, besides fatty acid synthesis, which actually contributes to only a minor proportion of lipogenesis [Macfarlane et al., 2008], PPAR γ activation enhances proliferation of preadipocytes [Hasan et al., 2011] and additionally participates in FFA re-esterification to TG and facilitates the ability of adipose tissue to store excess lipids [Evans et al., 2004]. Thus, in spite of its lipolytic effects, PPAR γ activation enhances a ‘futile’ cycle of TG hydrolysis and re-synthesis; and thereby rather reduces release of FFA and glycerol [Guan et al., 2002; Tordjman et al., 2003].

A recent review explored that about 1 in 500 patients with metabolic syndrome has Cushing's syndrome [Loriaux, 2017]. Moreover, in obesity, 11 β -hydroxysteroid dehydrogenase type 1 enzyme is overexpressed, which enhances corticosterone concentration in the adipose tissue [Walker, 2006; Wang, 2005]. From this perspective, activation of glucocorticoid receptor (GCR) by the glucocorticoids (GCs) could increase transcription of HSL and AGTL at least in acute conditions [Campbell et al., 2011; Xu et al., 2009]. However, excessive GCs in patients with type 2 diabetes mellitus or Cushing syndrome and in obese rodents are known to show adipocyte hypertrophy [Loriaux, 2017], suggestive of a lipogenic effect of GCs, through a currently not well defined mechanism [Peckett et al., 2011].

Considering the similarities between GCR and PPAR γ on lipid metabolism, we hypothesized that a crosstalk exists between GCR and PPAR γ pathways playing a role in lipid homeostasis in adipocytes. Thus, we studied, first, the effect of GCR activation on lipid metabolism; second, the existence of any crosstalk between GCR and PPAR γ ; and third, the outcome of the crosstalk on lipid metabolism.

MATERIALS AND METHODS

CELL CULTURE AND INDUCTION OF DIFFERENTIATION

3T3-L1 preadipocytes were cultured and induced to differentiate to adipocytes following our previous report with slight modifications [Hasan et al., 2011]. Briefly, two days post confluent cells grown in DMEM (Sigma-Aldrich, St Louis, MO, USA) supplemented with 10% fetal bovine serum (Nichirei Bioscience Inc. Tokyo, Japan) and 1% penicillin-streptomycin-glutamine (Invitrogen, Grand Island, NY, USA), were induced to differentiation using the same medium additionally containing 0.5 mM 3-isobutyl-1-methylxanthine, 1 μ M dexamethasone and 170 nM insulin (all from Sigma-Aldrich) for 2 days. For the next four days cells were treated with medium containing only 170 nM insulin. During this 6 days intervention, most of the preadipocytes show lipid droplets in their cytoplasm, an indicator of mature adipocytes. The day of induction was indicated as day 0, and unless otherwise mentioned adipocytes at 8th post induction day were used for different types of interventions for 6 days. The adipocytes were treated with dexamethasone alone, with pioglitazone (LKT laboratories Inc., St Paul, MN, USA) alone, or their combination at indicated doses, while controls received dimethyl sulfoxide (DMSO, Wako Pure Chemical Industries, Osaka, Japan) as the vehicle.

RNA ISOLATION AND qRT PCR

Total RNA extraction, cDNA preparation and qRT-PCR was performed following our previous report [Hasan et al., 2017]. Primers are as reported previously for ATGL [Nishino et al., 2008], fatty acid binding protein 4 (aP2) [Kosteli et al., 2010], GK [Patsouris et al., 2004], and PEPCK [Buler et al., 2012] used with Fast SYBR Green Master Mix (Life Technologies, Japan); and using inventoried primers and probes from TaqMan Gene Expression Assays for FASN, HSL, and LPL with Premix ExTaq ROX plus (Takara Bio Inc. Japan). Results were normalized to Cyclophilin B [Kosteli et al., 2010], and expressed as fold change over control.

WESTERN BLOTTING

Western blotting was done following conventional method as we have described previously [Hasan et al., 2011; Ishihara et al., 2010]. Other than for detecting PPAR γ , for which 50 μ g was used, 20 μ g of protein was subjected to SDS-PAGE and then transferred to Immobilon-PSQ membrane (Millipore). Membranes were blocked with 5% skimmed milk, and incubated with anti-PPAR γ (1: 1000, Cell Signaling Technology, Danvers, MA, USA), anti-HSL (Santa Cruz Biotechnology, Dallas, TX, USA), anti-perilipin A (Santa Cruz Biotechnology), anti-aP2 (1:1000, Cell Signaling Technology). Anti- β -actin (1:3000, Santa Cruz Biotechnology) was used as loading control. Protein levels were expressed as fold change over control.

CO-IMMUNOPRECIPITATION ASSAY

About 20 μ g of anti-HSL (Santa Cruz Biotechnology) antibody were added to 1.5 mg cell lysate, and were incubated with overnight at 4°C. After an additional gentle rocking for 3 h with protein A/G beads (Santa Cruz Biotechnology), bound antibodies were precipitated by centrifugation for 3 minutes at 8500 rpm. The precipitates were washed and then subjected to SDS-PAGE and western blotted with anti-PPAR γ or anti-aP2 (1:1000, Cell Signaling Technology) antibodies as mentioned above.

GLYCEROL ASSAY

Culture medium was collected from the 12-well plates where the adipocytes had been grown and was preserved in -80°C until further procedure. Glycerol content was determined enzymatically by a colorimetric assay kit (Free Glycerol Reagent, Sigma-Aldrich) from the absorption of 540 nm and served as an index of lipolysis.

BODIPY STAINING TO ASSESS LIPID CONTENTING AREA

Cellular neutral lipid droplets of adipocytes grown in 96-well plates were stained with Bodipy® 493/503 (1:1000, Thermo Fisher Scientific, Waltham, MA, USA) following manufacturer's instruction. DAPI (Millipore, County Cork, Ireland) was used to stain the nucleus. At least four randomly chosen areas were captured using a BioRevo BZ9000 (Keyence, Osaka, Japan) fluorescent microscope.

siRNA TRANSFECTION

Silencing of PPAR γ gene was done using siRNA reported previously [Liao et al., 2007]. Scrambled universal negative control (Sigma Aldrich) was used as control siRNA. Eight days after induction of differentiation, 3T3-L1 cells were electroporated with about 2 nmol of siRNAs or scrambles at 250 V and 950 μ farad following our previous study [Hasan et al., 2017]; and plated on 12-well collagen coated plates. From the next day the cells were incubated in the fresh medium (DMEM-10%FBS) with DMSO, or dexamethasone, or pioglitazone as indicated in the figure legends for six days until further maneuver.

STATISTICAL ANALYSES

All experiments were performed as triplicates. In comparison between two groups two-tailed Student's *t*-test was performed. Difference was considered significant if $p < 0.05$. Error bars in graphs denote the standard error of mean.

RESULTS

PPAR γ AUGMENTS GCR MEDIATED LIPOLYTIC PROCESS IN ADIPOCYTES

Eight days after initiation of differentiation in over-confluent 3T3-L1 preadipocytes, most of the cells showed lipid droplets in their cytoplasm, which confirmed their successful differentiation. To delineate the impacts of GCR and/or PPAR γ stimulation, the mature adipocytes were treated with DMSO (as control), or different doses of dexamethasone, and/or 1 μ M pioglitazone for 6 days.

Firstly, we examined the effects on lipolytic enzymes. Dexamethasone slightly but significantly upregulated basal ATGL expression, and the effect was more than doubled in the presence of pioglitazone (Fig. 1A). Although dexamethasone showed no effects on basal mRNA expression of HSL, cells co-treated with pioglitazone showed significantly increased expression of HSL (Fig. 1B). The magnitude of upregulation of both AGTL and HSL expressions by dexamethasone in the presence of pioglitazone was dose-dependent with the optimal effects at 20 nM (Fig. 1A and B). Therefore, all the subsequent experiments were performed using this dose. The mRNA expression of aP2, a well-known HSL binding protein [Smith et al., 2007] showed the similar trend in dexamethasone and/or pioglitazone treatment as AGTL and HSL did (Fig 1C). Thus, PPAR γ stimulation by pioglitazone seems to enhance upregulation of lipolytic enzymes by dexamethasone at transcriptional level in adipocytes.

Western blot analysis revealed that co-treatment with pioglitazone augmented the effects of dexamethasone on HSL expression also at protein level. In addition, expressions of perilipin A, an anti-lipolytic protein, and aP2 were also enhanced by both dexamethasone and pioglitazone and to somewhat greater extent by their combination (Fig. 1D). Moreover, during co-immunoprecipitation assay, both PPAR γ and aP2 were detected in anti-HSL antibody precipitated lysates in pioglitazone treated adipocytes (Fig. 1E). Although dexamethasone alone did not allow binding of PPAR γ or aP2 to HSL, these bindings were most augmented by the combination of the two (Fig. 1E). These data suggest that in the presence of GCs, pioglitazone activates binding of both HSL and aP2 with PPAR γ , and thus could additionally augment GC mediated lipolytic actions.

PPAR γ ACTIVATION COUNTERACTS GCR MEDIATED ANTI-LIPOGENIC ACTIONS IN ADIPOCYTES

Secondly, to determine the effects of CG and/ or PPAR γ stimulation on new lipid synthesis, mRNA expression of lipogenic enzymes, GK and PEPCK, was assessed. Although dexamethasone alone showed no effect on mRNA expression of GK, pioglitazone alone significantly enhanced it. By co-treatment with dexamethasone and pioglitazone, GK expression was substantially (more than 3 times of control or dexamethasone alone)

upregulated (Fig. 2A). On the other hand, dexamethasone slightly but significantly downregulated the expression of PEPCK, while pioglitazone alone markedly enhanced the PEPCK expression. This lipogenic effect of pioglitazone completely counteracted the dexamethasone-induced down regulation of PEPCK expression on co-administration, which was more than 4 times of control (Fig. 2B). In addition, mRNA expressions of LPL and FASN, both of which take part in lipogenesis, were unaffected by dexamethasone alone, but significantly upregulated by the co-treatment (Supplementary Fig. S1).

Thirdly, glycerol release into the culture medium was assessed as an indicator of the net lipolysis-lipogenesis balance. Dexamethasone treatment enhanced, while pioglitazone reduced glycerol release (Fig. 2C). Co-treatment with pioglitazone significantly reduced the enhanced glycerol release by dexamethasone (Fig. 2C). Subsequently, we assessed lipid-containing area in the adipocytes using bodipy staining. Due to clustering of many adipocytes we could not reliably quantify the fluorescent intensities of each groups. However, consistent with enhanced lipolysis, bodipy staining demonstrated that most of dexamethasone-treated cells contained scanty and very minute lipid droplets, and that the cells treated with pioglitazone alone or in combination with dexamethasone contained similarly larger lipid droplets in the cytoplasm (Fig. 2D and Supplementary Fig. S2). The above findings (Figs. 1 and 2) imply the lipolytic role of GCs, and further suggest that by the greater augmentation of lipogenesis than lipolysis, net effect of PPAR γ activation rather abolishes the lipolytic effect of GCR.

PPAR γ PLAYS BI-DIRECTIONAL ROLES IN ACTIONS OF DEXAMETHASONE

Because both GCs and PPAR γ can independently control lipid metabolism, it is possible that the alteration observed in the combined dexamethasone and pioglitazone treatment could be independent processes. To confirm that PPAR γ activation indeed reverses dexamethasone induced lipolysis, endogenous PPAR γ of adipocytes were depleted by RNA interference, which reduced both PPAR γ protein and mRNA levels ranging within 20% and 42% (Figs. 3A, B and E); validates the efficiency of the maneuver.

The silencing of PPAR γ also lowered both protein (Fig. 3A, C) and mRNA levels of HSL (Fig. 3G) and aP2 (Fig. 3A, D and I); and mRNA expressions of ATGL (Fig. 3F) and GK (Fig. 3H). For all these consequences of silencing of PPAR γ significantly abrogated the pioglitazone-induced effects on actions of dexamethasone (Fig. 3A–I). Consistently, we found that glycerol release was significantly reduced in all four PPAR γ -silenced groups compared to scrambled siRNA treated groups (Fig. 3J). Taken together the findings suggest existence of homeostatic crosstalk between GCR and PPAR γ , where the net effects of PPAR γ activation can reverse the deleterious lipolytic effects of GCR.

DISCUSSION

Dysregulation of lipid metabolism in adipocytes is a major predisposing factor for developing obesity induced metabolic disorders [Ishihara et al., 2010; Kadowaki et al., 2003; Lehrke and Lazar, 2005]. More specifically, a failure of adipocytes to efficiently hydrolyze TGs to release FFA and glycerol, and to reuptake or re-esterify them as TGs increases the basal FFA release. Thereby deteriorates insulin signaling and glucose metabolism pathways

[Evans et al., 2004; Lehrke and Lazar, 2005]. Due to involvement of multifactorial process the exact mechanism of lipid metabolism still remains elusive. Therefore, to get a more integrated view of the lipid metabolism we studied the molecular connections and respective contributions of GCR and PPAR γ pathways. In the current study we demonstrated first, that GCR activation enhances lipolysis; second, that a crosstalk exists between GCR and PPAR γ , which is largely synergistic in lipid metabolism but counter-regulatory in some lipogenic genes; and third, that PPAR γ activation rather mitigate the lipolytic effect of GCR through enhancing lipogenesis. Interestingly, we additionally found a permissive like effect of GCR that enables pioglitazone to mitigate dexamethasone-induced lipogenesis via PPAR γ (Supplementary Fig. S3).

In respect to lipid metabolism, several studies showed that GCs have pronounced effects on lipolysis [Macfarlane et al., 2008; Xu et al., 2009]. To explore the effects of GCR activation on adipocytes, these studies exposed the cells to different types of GCs for relatively shorter durations, up to 48 hours [Macfarlane et al., 2008; Xu et al., 2009]. In contrast, their roles to lipogenesis are somewhat not straightened [Campbell et al., 2011; Macfarlane et al., 2008]. One report showed that GCs can at least enhance adipogenesis by acting on preadipocytes [Campbell et al., 2011]; while other showed that in chronic exposure GCs may also increase lipogenesis in mature adipocytes [Patsouris et al., 2009; Peckett et al., 2011; Walker, 2006].

Some of the above mentioned studies have provided compelling evidences that adipocyte-lipolysis as a rapid response of GCs. Considering the chronic nature of obesity induced metabolic disorders we intervene the 3T3-L1 adipocytes for a longer period of 6 days. Our study clearly showed that chronic exposure to dexamethasone increased lipolysis mostly through upregulating expression of ATGL, as well as additionally reducing lipogenesis through downregulating PEPCK. The net effect is the enhanced glycerol release and reduced lipid content in the adipocytes (Figs. 1 and 2). Taking into account the activated GCR in obesity [Loriaux, 2017], our findings are consistent with the human studies those showing that in obesity adipose tissue releases more FFA and glycerol, and thus their plasma levels are elevated [Stinkens et al., 2015].

We found that dexamethasone enhanced expression of only ATGL, but not HSL (Fig. 1A–C). It is noteworthy that when expressed at endogenous level, ATGL functions mainly in the hydrolysis of TG to diacylglycerol, and HSL mainly diacylglycerol to monoacylglycerol and to glycerol [Lampidonis et al., 2011; Macfarlane et al., 2008; Peckett et al., 2011]. However, at high level ATGL alone can completely hydrolyze all tri-, di- and mono-acylglycerols to release glycerol [Yang et al., 2011]. The finding of increased ATGL by dexamethasone was in agreement to the complete hydrolysis of TGs that led to increase release of glycerols in the culture medium (Fig. 2C), leaving scanty lipid droplets within the cells (Fig. 2). Due to low concentration in the medium, we were not able to report the condition of FFAs in this study. However, the above mentioned findings reveal that dexamethasone indeed enhanced basal lipolysis, and that excessive TGs have been depleted from the cells during this long term intervention implemented in our study.

Moreover, we observed that dexamethasone ~50% reduced PEPCK expression below the basal level (Fig. 2B). Dexamethasone induced downregulation of PEPCK was also reported

in 3T3-F442a adipocytes, coincides with our findings [Franckhauser et al., 1995]. Notably, adipose tissue utilizes both glucose as well as non-glucose sources such as lactate and pyruvate to produce glycerol-3-phosphate to generate TGs through PEPCK activity [Nye et al., 2008]. Therefore, it is conceivable that as stress hormones, GCs should reduce PEPCK to make glucose and other sources of energy available to tissues such as muscles [Franckhauser et al., 1995].

From the perspective of GCR and PPAR γ interaction, it is noteworthy that in obesity, due to enhanced expression of 11 β -hydroxysteroid dehydrogenase type 1, corticosterone concentration into the adipose tissue is increased [Walker, 2006; Wang, 2005]. In the current study, we exposed the adipocytes in the presence of pioglitazone with addition of gradually increasing concentrations of dexamethasone ranging from 10 nM to 100 nM (Fig. 1A and B). Given the 30 times potency of dexamethasone over corticosterone [Pavlaki et al., 2011], the dose is equivalent to 120 to 1200 ng/ml, within the reported corticosterone level of 334.8 and 676.7 ng/ml in epididymal adipose tissue of normal fed and high fat fed mice, respectively [Patsouris et al., 2009]. We observed that optimal synergistic effect for ATGL and HSL mRNA expression was achieved from 20 nM, which almost corresponds to the normal corticosterone levels in mice-epididymal adipose tissue (240 ng/ml equivalent). Our results demonstrate that to accomplish lipogenesis, even the physiological concentrations of GCs in adipocytes are sufficient for activating PPAR γ mediated lipogenic effects (Fig. 2).

We attempted to delineate the process by which the synergistic effects of GCR and PPAR γ activation occurs. One possible mechanism could be the permissive effect of GCs [Gebhardt and Mecke, 1979; Macfarlane et al., 2008]. Until now, permissive effects of GCs has been reported for glucagon and catecholamines; where GCs exert the calorogenic effect of glucagon and lipolytic effects of catecholamines [Barrett et al., 2010]. Indeed, we observed that despite dexamethasone caused no significant effects on basal mRNA expression of HSL and GK; it significantly enhanced pioglitazone mediated expressions of these genes (Fig. 1B and 2A, respectively). This observation is suggestive of a novel evidence of permissive effect of activated GCR on PPAR γ activation.

In this regard, we also found that in the presence of dexamethasone, pioglitazone-induced binding of HSL to PPAR γ has been facilitated (Fig. 1E). This constitutes a permissive effect like activity of GCRs *via* PPAR γ that most plausibly modulates transcription of ligand-activated PPAR γ to regulate lipolysis and lipogenesis. Nevertheless, silencing of PPAR γ significantly attenuated the synergistic effect of dexamethasone and pioglitazone (Fig. 3). These findings suggest that GCR-induced upregulation of these genes is indeed PPAR γ dependent.

Interestingly, the combined dexamethasone and pioglitazone vastly increased GK expression (Fig. 2C). As mentioned above, this co-treatment also significantly enhanced the activity of lipases (ATGL and HSL), which increased release of glycerol (Figs. 2A–C). It can be assumed that due to abundance of the products of lipolysis including glycerol, pioglitazone treatment tremendously recycled glycerol to incorporate into TGs by GK. Thereby, as the net effect the combined treatment reduced glycerol in the medium, indicating enhanced

lipogenesis. This condition more mimics the fed state, where GK is activated by the PPAR γ agonists and glycerol is a main source of energy [Guan et al., 2005; Guan et al., 2002].

Moreover, in fed state LPL increases hydrolysis of FFAs and allows them to enter in to the adipocytes [Macfarlane et al., 2008]. Indeed, we observed significant upregulation of LPL by the combined dexamethasone and pioglitazone treatment (Supplementary Fig. S1A). This finding implies an increase of FFA re-entry to the adipocytes. A parallel upregulation was observed for FASN mRNA expression (Supplementary Fig. S1B). Protein synthesized from this gene is responsible for synthesizing fatty acid by *de novo* lipogenesis, utilizing acetyl CoA formed from pyruvate [Ferré and Foufelle, 2007; Macfarlane et al., 2008]. Thereby recycling FFAs back to TGs in adipocytes, PPAR γ counter-balances the GCR-mediated release of FFA and glycerol into the circulation to attenuate lipotoxicity.

Thus, our findings suggest that in long term exposure of GCs, TGs stored in adipocytes are subjected to ATGL-mediated lipolysis leading to enhanced release of glycerol. We also found that activated GCR augments PPAR γ -target genes through a permissive like effect. Utilizing this effect of GCR, PPAR γ ligands enhance re-entry and re-esterification of glycerol along with FFA in the adipocytes by upregulating, respectively LPL and GK expression through the PPAR γ activity. Thus in spite of the strong lipolytic stimulation of GCR in obese adipocytes, PPAR γ could counter-balance the release of excess FFA and glycerol into the circulation. The net effect is preserved accumulation of lipids in the adipocytes through lipogenesis. This crosstalk between GCR and PPAR γ may exert beneficial effects in lipid homeostasis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1. PPAR γ augments dexamethasone mediated lipolytic effects in adipocytes

3T3-L1 adipocytes after 8 days of adipogenic induction were treated with DMSO (as a control), or 20 nM dexamethasone (Dexa), or 1 μ M pioglitazone (Pio) alone, or different doses (10, 20 and 100 nM) of dexamethasone in combination with 1 μ M pioglitazone for 6 days. Medium was changed every alternative day. mRNA expressions of (A) ATGL and (B) HSL were determined by qRT-PCR after normalizing with that of cyclophilin B. The data are expressed as the mean $\pm$ SEM ($n = 3$). * $P < 0.05$ by two-tailed unpaired Student's t -test. Similarly, 3T3-L1 adipocytes were treated with DMSO (as a control), or 20 nM dexamethasone (Dexa), or 1 μ M pioglitazone (Pio) alone or with 20 nM dexamethasone for

6 days. (C) mRNA expression of aP2 was also assessed as mentioned above. The data are expressed as the mean $\pm$ SEM ($n = 3$). * $P < 0.05$ by two-tailed unpaired Student's t -test. (D) HSL, perilipin-A, aP2 and the internal control β -actin protein levels in whole cell lysate were estimated by western blot. (E) The adipocytes were also immunoprecipitated using anti-HSL antibody, then immunoblotted (western blot) with anti-PPAR γ and anti-aP2 antibodies.

Fig. 2. PPAR γ activation counteracts dexamethasone induced anti-lipogenic action in adipocytes 3T3-L1 adipocytes after 8 days of adipogenic induction were treated with DMSO (as a control), or 20 nM dexamethasone (Dexa), or 1 μM pioglitazone (Pio) alone or with 20 nM dexamethasone for 6 days. mRNA expressions of (A) GK and (B) PEPCK were determined by qRT-PCR after normalizing with that of cyclophilin B. The data are expressed as the mean $\pm$ SEM ($n = 3$). * $P < 0.05$ by two-tailed unpaired Student's t -test. (C) Lipolysis was assessed as the total glycerol released in the culture medium during last 2 days by ELISA. The data are expressed as the mean $\pm$ SEM ($n = 3$). (D) Cellular lipid content was assessed

by the bodipy staining. Nucleuses were stained with DAPI. Corresponding full images are presented in Supplementary Fig. S2.

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Fig. 3. PPAR γ is involved in the bi-directional roles of dexamethasone induced lipid metabolism 3T3-L1 adipocytes after 8 days of adipogenic induction were electroporated with siRNA against PPAR γ (si-Py) or scrambled siRNA (Scr) and allowed to recover for 48 hours. Then the cells were treated with DMSO (as a control), or 20 nM dexamethasone (Dexa), or 1 μ M pioglitazone (Pio) alone or with 20 nM dexamethasone for 6 days. Protein levels of (A) PPAR γ , HSL, aP2 along with the loading control β -actin were assessed by western blot. Densitometric values of (B) PPAR γ , (C) HSL and (D) aP2 after normalizing with that of β -actin are presented. mRNA expressions of (E) PPAR γ , (F) ATGL, (G) HSL, (H) GK and (H) aP2 were determined by qRT-PCR after normalizing with that of cyclophilin B. The data are expressed as the mean $\pm$ SEM ($n = 3$). * $P < 0.05$ by two-tailed unpaired Student's t -test. (C)

Total glycerol released in the culture medium during last 2 days was estimated by ELISA. The data are expressed as the mean $\pm$ SEM ($n = 3$).