

PPAR δ is a fatty acid sensor that enhances mitochondrial oxidation in insulin-secreting cells and protects against fatty acid-induced dysfunction

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Abstract The peroxisome proliferator-activated receptor δ (PPAR δ) is implicated in regulation of mitochondrial processes in a number of tissues, and PPAR δ activation is associated with decreased susceptibility to ectopic lipid deposition and metabolic disease. Here, we show that PPAR δ is the PPAR subtype expressed at the highest level in insulinoma cells and rat pancreatic islets. Furthermore, PPAR δ displays high transcriptional activity and acts in pronounced synergy with retinoid-X-receptor (RXR). Interestingly, unsaturated fatty acids mimic the effects of synthetic PPAR δ agonists. Using short hairpin RNA-mediated knockdown, we demonstrate that the ability of unsaturated fatty acids to stimulate fatty acid metabolism is dependent on PPAR δ . Activation of PPAR δ increases the fatty acid oxidation capacity in INS-1E β -cells, enhances glucose-stimulated insulin secretion (GSIS) from islets, and protects GSIS against adverse effects of prolonged fatty acid exposure. The presented results indicate that the nuclear receptor PPAR δ is a fatty acid sensor that adapts β -cell mitochondrial function to long-term changes in unsaturated fatty acid levels. ■ As maintenance of mitochondrial metabolism is essential to preserve β -cell function, these data indicate that dietary or pharmacological activation of PPAR δ and RXR may be beneficial in the prevention of β -cell dysfunction.—Ravnskjaer, K., F. Frigerio, M. Boergesen, T. Nielsen, P. Maechler, and S. Mandrup. PPAR δ is a fatty acid sensor that enhances mitochondrial oxidation in insulin-secreting cells and protects against fatty acid-induced dysfunction. *J. Lipid Res.* 2010. 51: 1370–1379.

Supplementary key words peroxisome proliferator-activated receptor δ • β -cells • β -oxidation • fatty acids • glucose-stimulated insulin secretion

The peroxisome proliferator-activated receptors (PPARs) are a family of transcription factors belonging to the nuclear receptor superfamily. The PPAR family consists of three subtypes, PPAR α , PPAR γ , and PPAR β/δ , all of which are regulated by fatty acids and their derivatives. The PPARs bind to peroxisome proliferator response elements (PPREs) as heterodimers with the retinoid-X-receptor (RXR). Upon ligand binding, the PPAR-RXR heterodimers stimulate expression of each their subset of genes involved in glucose and fatty acid metabolism, insulin signaling, cell differentiation, or cell proliferation (reviewed in Ref. 1). The PPAR subtypes have distinct transactivation characteristics and display preference for distinct subsets of target genes (2–4).

All three PPAR subtypes are expressed in pancreatic β -cells (5, 6). We previously reported that PPAR α governs β -cell fatty acid catabolism, while activation of PPAR γ primarily directs fatty acids toward esterification and accumulation as triglycerides (7). These specific roles are reflected in the metabolic control of PPAR α and PPAR γ expression. Under hypoglycemic fasting conditions, where there is increased demand for energy production from nonglucose substrates, PPAR α is most abundant, whereas PPAR γ is upregulated in β -cells under hyperglycemic and hyperlipidemic conditions (6, 8, 9).

PPAR δ is widely expressed in mammalian tissues and cell types; however, until recently, the function of PPAR δ in metabolic regulation has been elusive. Evidence now accumulates in favor of a pivotal role for PPAR δ in basal lipid oxidation. The importance of PPAR δ in lipid catabolism has been described for metabolically important tis-

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Abbreviations: CPT-1b, carnitine palmitoyltransferase 1b; GSIS, glucose-stimulated insulin secretion; PPAR, peroxisome proliferator-activated receptor; PPRE, peroxisome proliferator response element; RXR, retinoid-X-receptor; UCP2, uncoupling protein 2.

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sues, such as skeletal muscle (10), adipocytes (11), as well as cardiomyocytes (12), pointing at PPAR δ as a potential drug target in treatment of metabolic disease. Interestingly, hypolipidemic drugs, unsaturated fatty acids, and derivatives hereof directly activate PPAR δ as ligands (13, 14).

In the endocrine pancreas, a key regulator of whole-body metabolism, the role of PPAR δ remains unexplored. It is recognized that the PPAR δ gene is expressed in pancreatic β -cells (5, 9, 15), but the function of PPAR δ in β -cell physiology has not been studied. PPARs, in particular PPAR α and PPAR δ , have been shown to mediate effects of unsaturated fatty acids on gene expression in some cell types (13, 14), but it is unknown whether this is the case in pancreatic β -cells.

Fatty acids have profound effects on β -cell function and viability. While they acutely stimulate insulin release (16, 17), long-term exposure of β -cells to elevated levels of fatty acids leads to lipid accumulation, β -cell dysfunction, and eventually apoptosis (18–20). These long-term effects are associated with changes in β -cell gene expression. Moreover, the effects of fatty acids on β -cell function depend on their chain length and degree of saturation (17, 21, 22). Saturated fatty acids, such as palmitic acid, negatively affect insulin production and β -cell viability (23, 24), whereas unsaturated fatty acids raise the basal insulin secretion, thereby desensitizing the β -cell to glucose without causing apoptosis (20, 25). Notably, the adverse effects of fatty acids on β -cell function are generally inversely related to the rate of fatty acid oxidation (19, 22).

In this study, we show that PPAR δ is abundant in pancreatic β -cells. We show that PPAR δ activity is highly inducible and that PPAR δ displays a pronounced synergy with agonists of RXR α in the activation of endogenous target genes in isolated rat islets and clonal β -cells. Interestingly, using short hairpin RNA (shRNA)-mediated knockdown, we find that the ability of unsaturated fatty acids to stimulate expression of genes involved in fatty acid metabolism is dependent on PPAR δ and is potentiated by RXR agonists. The transcriptional regulation by PPAR δ increases β -cell fatty acid oxidation capacity, potentiates glucose-stimulated insulin secretion (GSIS), and counteracts fatty acid-induced alterations in insulin secretion. Our results show that the nuclear receptor PPAR δ is both a main transcriptional effector of unsaturated fatty acids and a central regulator of fatty acid metabolism in pancreatic β -cells. The modulation of PPAR δ activity using selective agonists hence provides new perspectives for protection of β -cell function and prevention of diabetes.

MATERIALS AND METHODS

Cell culture, islet isolation, and agonist stimulation

The cell line INS-1E was cultured as previously described (26). The cells were at passage numbers between 50 and 78 and were used for experiments at 70–80% confluence. Rat islets were isolated from adult male Wistar rats by collagenase perfusion and cultured for 24 h in RPMI with 11 mM glucose (27). Medium and supplements were from Invitrogen-GIBCO and serum from

HyClone. L165041, a selective PPAR δ agonist, was kindly provided by Merck Research Laboratories, PPAR γ and RXR α agonists BRL49653 and LG100268 were kindly provided by Novo Nordisk, and WY14.643, a PPAR α -specific agonist, was purchased from Calbiochem. All fatty acids were purchased from Sigma. Agonists were used in the following concentrations [EC₅₀ values for the relevant PPAR subtype in parentheses (28)]; L165041, 1 μ M (3.8 μ M); WY14.643, 30 μ M (0.63 μ M); BRL49653, 1 μ M (0.076 μ M); LG100268, 0.2 μ M; and oleic acid, 100 μ M. A table of EC₅₀ values can be found in supplementary Table I. Fatty acids were added directly to the medium containing 10% FBS. Unless otherwise indicated, cells were stimulated with agonists for 24 h before harvest or further experiments. Rat handling was carried out in a certified animal facility according to procedures that were approved by the animal care and experimentation authorities of the Canton of Geneva.

Quantitative PCR

RNA isolation and cDNA synthesis were performed as described previously (7). Quantitative three-step PCR was performed on the ABI-7700 Prism quantitative PCR instrument using 2xSyBRgreen master mix (Sigma) and Sigma passive reference according to instructions from the manufacturer. PCR reactions were made in duplicates. Primers for quantitative PCR were designed using Primer Express 2.0 (Applied Biosystems) and specificity and efficacy validated before use. For each primer set, a standard curve was generated from cDNA stepwise diluted 10-fold over a 10,000-fold range. The slope of the standard curve for each primer set was used to determine the efficacy, which in turn was used to calculate expression levels from C_t values. Only primer sets with efficacies 0.95 < E < 1.05 were used for analysis. All quantifications were performed with TFIIB as internal standard and presented as fold over control. TFIIB expression remained unaffected under the described experimental conditions. Primer sequences and gene information are also found in the supplementary data.

Transient transfection and luciferase reporter assay

Cells were seeded in 24-well plates and grown until 70% confluence. Medium was changed the day before transfection. Transfection with the 3xACO-PPRE luciferase reporter construct (described in Ref. 29) was performed in triplicate using Lipofectamine2000 from Invitrogen for 4 h, and cells were then incubated for 24 h in new media containing DMSO or indicated agonists. Transfected cells were harvested in lysis buffer from Tropix, and cell lysates were assayed for luciferase activity. All measurements were performed as duplicates in 96-well microtiter plates in a LUMistar luminometer from BMG-Labtech. It was verified that the observed reporter stimulation by the agonists was mediated through the PPRE elements with no stimulation of the parent vector activity (thymidine kinase promoter-driven luciferase reporter) or plasmid uptake (data not shown). All transfections were repeated a minimum of three times.

Adenovirus generation and transduction

pSuper-shPPAR α and pSuper-shPPAR δ were constructed with specific oligos directed against sequences 5'-GAGGCAGAGGTC-CGATTCT-3' and 5'-GAGGAGAAAGAGGAAGTGG-3', respectively, as described (30). The H1 promoter cassette was then excised using *Sma*I and *Hind*III and ligated into pShuttle (EcoRV and *Hind*III). Recombinant adenoviruses containing shRNA against PPAR α or δ were generated using the AdEasy cloning system from Stratagene. The linearized plasmids were transfected into 293-HEK cells, and the viruses were amplified and purified. The viruses were initially titrated by a plaque assay-based approach. Subsequently, relative titers of

functional viruses were equalized based on quantification of the adenoviral transcript AdE4 by quantitative PCR. Twenty-four hours after transduction with AdshEmpty, AdshPPAR α , or AdshPPAR δ , agonists (L165041, LG100268, or oleic acid) were added to the medium, and cells were incubated further 24 h before harvest for RNA and protein extraction.

Protein analysis by Western blotting and ECL detection

For total protein extraction, INS-1E cells were harvested in hypotonic lysis buffer containing 2.5% SDS. Nuclear extracts were prepared essentially as described in Ref. 31. Protein extracts were separated by SDS-PAGE and proteins blotted onto polyvinylidene difluoride membranes (Millipore) and probed with the indicated antibodies. Primary antibodies anti-PPAR γ (SC-7273) and anti-TFIIB (SC-225) were obtained from Santa Cruz and secondary horseradish peroxidase-coupled anti-Fc γ antibodies, anti-mouse (P0447) and anti-rabbit (P0399), were obtained from DAKO Cytomation. SC-7273 (E-8) is raised against the PPAR γ C terminus, which is highly conserved between the PPAR subtypes. Therefore, SC-7273 detects all three PPAR subtypes with a slight preference for PPAR α over PPAR δ and PPAR γ , as determined by comparing ECL signals of HA-tagged versions of the three subtypes (data not shown).

[1-¹⁴C]Oleic acid oxidation

INS-1E cells were treated with agonists or vehicle for 24 h. The fatty acid oxidation capacity was subsequently determined by incubating the cells with [1-¹⁴C]oleic acid complexed to BSA in serum free medium containing 5 mM glucose for 4 h and collecting ¹⁴CO₂ essentially as described by Berge et al. (32). Data were normalized to total cellular protein. Dishes with medium but without cells were treated in parallel and used for background subtraction. Experiments were performed in triplicate.

GSIS

INS-1E cells and primary rat islets were treated with fatty acids or ligands as indicated for 24 h before insulin secretion. After an incubation for 30 min in KRBS with basal glucose (2.5 mM for INS-1E cells and 2.8 mM for rat islets) or stimulatory glucose (15 mM for INS-1E cells and 11.2 mM for rat islets), secreted insulin was measured by radioimmunoassay (Linco Research) using rat insulin as standard as described (26) and normalized to total insulin content or to the number of islets. Experiments were performed in triplicate.

Statistical analysis

Statistical evaluation of the data was performed using two-way ANOVA with Bonferroni corrections where applicable. Data relative to control samples are presented as means \pm SD.

RESULTS

PPAR δ is expressed as the most abundant PPAR subtype in pancreatic β -cells

PPAR δ has recently been implicated in the regulation of fatty acid metabolism in metabolically important tissues, such as skeletal muscle, white adipose tissue, and heart. In pancreatic β -cells, PPAR δ is reported to be highly expressed at the mRNA level (5). **Figure 1** shows that PPAR δ is abundantly expressed at the RNA level in INS-1E insulinoma cells and in isolated rat islets. PPAR α is also expressed at significant levels, whereas PPAR γ is expressed at very low levels (Fig. 1A, B). The primers used for quantitative PCR

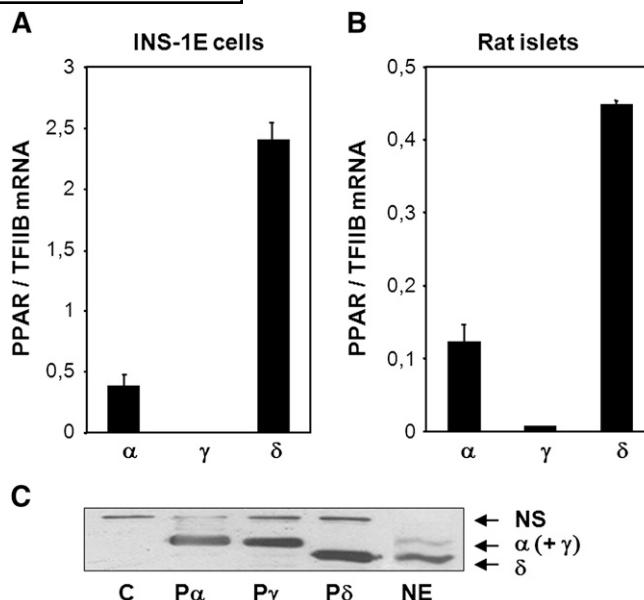


Fig. 1. PPAR δ is the most abundant PPAR subtype in pancreatic β -cells. Total RNA was extracted from INS-1E cells (A) and Wistar rat islets (B) cultured in RPMI with 11 mM glucose and supplemented as described. Expression of the PPAR subtypes α , γ , and δ was quantified by quantitative PCR and normalized to TFIIB expression. RNA was harvested in duplicates and range is indicated. The presented results are representative of at least three independent experiments. C: Nuclear proteins were extracted from INS-1E cells (NE) and separated by SDS-PAGE, and PPAR levels were visualized by immunoblotting. Protein extracts from NIH-3T3 cells ectopically expressing murine PPAR α (P α), PPAR γ (P γ), PPAR δ (P δ), or control adenoviral vector (E) were run in parallel as control for protein migration. Arrows indicate the PPAR subtypes (NS, nonspecific band).

were subtype specific and tested for equal efficacy of amplification. Previous reports have indicated that PPAR δ may be subject to translational control (33). Thus, to investigate if the PPAR protein levels reflected the PPAR mRNA abundances, nuclear protein extracts were prepared from INS-1E cells, and proteins were analyzed by Western blotting. As control for protein migration, protein extracts from NIH-3T3 cells ectopically expressing mPPAR α , mPPAR γ , or mPPAR δ were run in parallel. An antibody recognizing all three PPAR subtypes with a slight preference for PPAR α over PPAR γ and PPAR δ (data not shown) was used for ECL detection. In keeping with the relative mRNA abundances, the lower band corresponding to PPAR δ had the highest intensity (Fig. 1C). The less intense upper band corresponds to nonseparated PPAR α and PPAR γ proteins. Hence, PPAR δ is the predominant PPAR subtype in INS-1E insulin-secreting cells under normal culture conditions.

PPAR δ is transcriptionally highly active and displays a high degree of synergy with RXR in insulin-secreting cells

The finding that PPAR δ is the most abundant PPAR subtype in β -cells prompted us to investigate if this is reflected at the functional level, i.e., whether endogenous PPAR δ is also transcriptionally highly active in insulin secreting cells. We therefore transiently transfected INS-1E cells with a luciferase reporter construct containing a

multimerized PPRE and incubated 24 h with DMSO or specific agonists for PPAR α (WY14.643), PPAR γ (BRL49653), or PPAR δ (L165041) in the presence or absence of the RXR α agonist LG100268. PPAR α and PPAR γ agonists were administered at concentrations that give maximal but specific activation of the respective receptors, whereas the PPAR δ agonist was kept at a suboptimal concentration to ensure specific activation of PPAR δ (28). Whereas neither the PPAR α nor the PPAR γ agonist had any significant effect alone, activation of either RXR α or PPAR δ increased the activity of the reporter construct (Fig. 2A). Simultaneous activation of both PPAR δ and RXR α resulted in a pronounced (8.5-fold) synergistic activation. The combined activation of PPAR α and RXR α resulted in a much weaker but still significant activation of the reporter, while activation of PPAR γ and RXR α was without effect compared to RXR α activation alone.

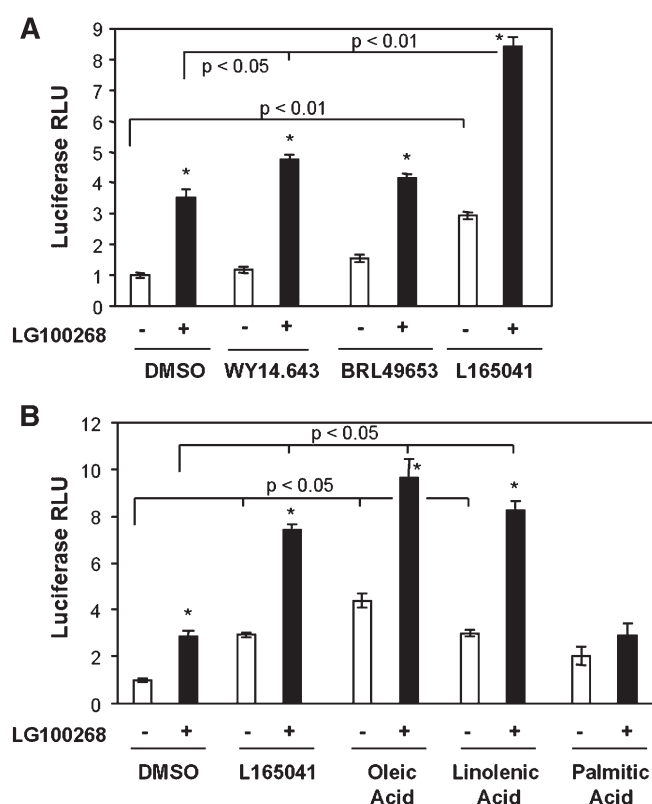


Fig. 2. A PPAR δ -specific agonist and unsaturated fatty acids activate a PPAR-responsive reporter in synergy with RXR α in INS-1E cells. INS-1E cells were transfected with the 3xACO-PPRE luciferase reporter construct and incubated in RPMI (11 mM glucose) for 24 h with DMSO, PPAR subtype-specific agonists, or fatty acids in the presence or absence of the RXR α agonist LG100268 (0.2 μ M). A: The specific PPAR agonists used were for PPAR α , WY14.643 (30 μ M); for PPAR γ , BRL49653 (1 μ M); and for PPAR δ , L165041 (1 μ M). B: The fatty acids used were oleic acid (100 μ M), γ -linolenic acid (100 μ M), or palmitic acid (100 μ M). Luciferase activities were measured and presented relative to DMSO control. Transfections were performed in triplicate, and standard deviations are indicated. Results are representative of three independent experiments, and *P* values were calculated using two-way ANOVA. Addition of LG100268 differs from corresponding DMSO control (**P* < 0.05).

Unsaturated fatty acids have previously been described as potential endogenous ligands of the PPARs (34). Interestingly, oleic acid (C18:1), γ -linolenic acid (C18:3), and arachidonic acid (C20:4; data not shown) all activated the PPRE reporter construct alone and synergistically with LG100268 to a similar degree as the specific PPAR δ agonist L165041 (Fig. 2B). By contrast, the saturated fatty acid palmitic acid (C16:0) did not have any significant effect on reporter activity. In keeping with unsaturated fatty acids acting through PPAR δ , there was no additive effect of combining L165041 and fatty acids neither in the absence nor in the presence of LG100268 (data not shown). These results indicate that PPAR δ is a transcriptionally highly responsive PPAR subtype in INS-1E cells and that the effect of unsaturated fatty acids on the expression of PPAR target genes in β -cells may be mediated by PPAR δ in synergy with RXR α .

The expression of endogenous PPAR target genes is synergistically activated by selective agonists of PPAR δ and RXR α as well as by unsaturated fatty acids

PPAR δ potentially activated transcription from an artificial PPRE in synergy with RXR α . To investigate the impact of PPAR δ activation on the expression of endogenous genes, we treated INS-1E cells with DMSO or the indicated agonists for 24 h. Based on previous results (7), expression of PPAR-regulated genes involved in β -cell fatty acid metabolism was investigated using quantitative PCR and normalized to TFIIB expression. CD36, muscle carnitine palmitoyltransferase 1b (CPT-1b), and uncoupling protein 2 (UCP2) were all significantly induced by the specific PPAR δ agonist L165041, but only minimally, or not at all, by specific activators of PPAR α and PPAR γ (Fig. 3A). The RXR-specific agonist LG100268 alone resulted in a minor activation of all three genes; however, the combination of PPAR δ and RXR specific agonists resulted in a significant and synergistic activation of target genes. Simultaneous activation of PPAR α and RXR α also resulted in significant, although less pronounced, synergistic activation of the genes, as we have shown previously (7). In contrast, no synergy was observed between the specific PPAR γ and RXR agonists.

Notably, as observed in the transient transfections, unsaturated fatty acids, such as oleic acid and γ -linolenic acid, mimicked selective activation of PPAR δ and activated all the PPAR target genes investigated (Fig. 3B).

In keeping with high expression of PPAR δ in primary cells (see Fig. 1B), PPAR δ and RXR α agonists significantly activated CPT-1b and CD36 expression in isolated rat islets (Fig. 3C). These results indicate that the response to PPAR δ activation is comparable between primary β -cells and INS-1E cells.

Taken together, these results show that PPAR δ regulates genes involved in fatty acid metabolism in both rat islets and INS-1E β -cells and is a probable mediator of transcriptional effects of unsaturated fatty acids.

Oleic acid induces fatty acid handling genes through activation of PPAR δ

The above results suggest that PPAR δ , and possibly also PPAR α , are mediators of the transcriptional effects of un-

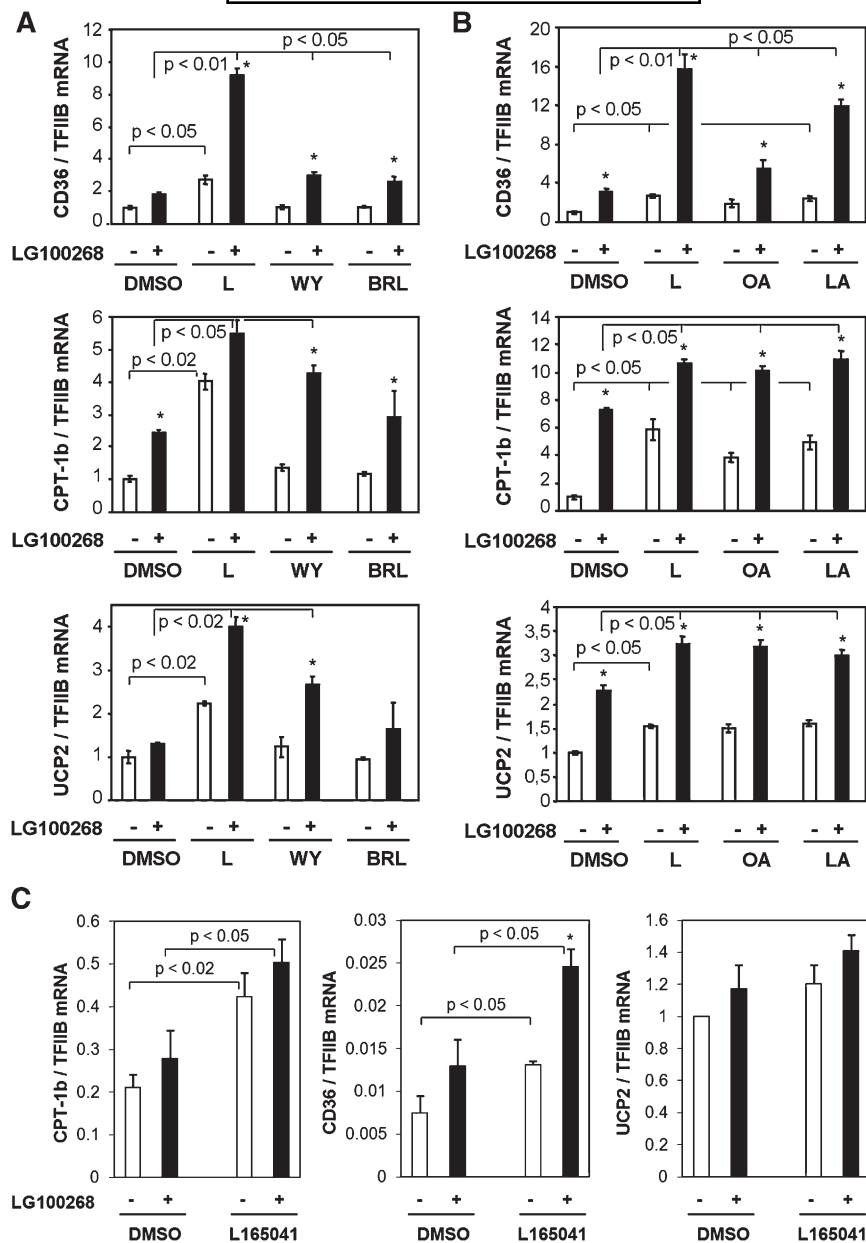


Fig. 3. PPAR δ activation and fatty acids stimulate expression of fatty acid handling genes in synergy with RXR α in INS-1E cells and rat islets. INS-1E cells were incubated for 24 h in RPMI (11 mM glucose) with DMSO, PPAR subtype-specific agonists, or fatty acids in the presence or absence of the RXR α agonist LG100268 (0.2 μ M). A: The specific PPAR agonists used were for PPAR α , WY14,643 (WY, 30 μ M); for PPAR γ , BRL49653 (BRL, 1 μ M); and for PPAR δ , L165041 (L, 1 μ M). B: The fatty acids used were oleic acid (OA, 100 μ M) or γ -linolenic acid (LA, 100 μ M). Total RNA was extracted, and expression levels of CD36, muscle CPT-1, and UCP2 were quantified using quantitative PCR and normalized to TFIIB expression. Expression levels are presented relative to DMSO control and are representative of three independent experiments. For each experiment, RNA was harvested in triplicates. C: Rat islets were isolated and incubated for 24 h in RPMI (11 mM glucose) with the indicated ligands. Results are means of three independent experiments, and *P* values are calculated using two-way ANOVA. Addition of LG100268 differs from corresponding DMSO control (**P* < 0.05).

saturated fatty acids. To determine the relative importance of PPAR α and PPAR δ in mediating these effects, we constructed adenoviral vectors for acute knockdown of each of these receptors in INS-1E cells. Adenoviral transfer of shRNA targeting specifically PPAR α or PPAR δ resulted in knockdown of mRNA expression levels by 70% and 60%, respectively, compared with control-infected cells (Fig.

4A). A nearly complete depletion of either of the two PPAR subtypes was confirmed at the protein level (Fig. 4B). Notably, the slower migrating protein band corresponding to PPAR α and PPAR γ was totally absent in the PPAR α knockdown cells, confirming that PPAR γ is virtually absent in INS-1E cells under the described culture conditions.

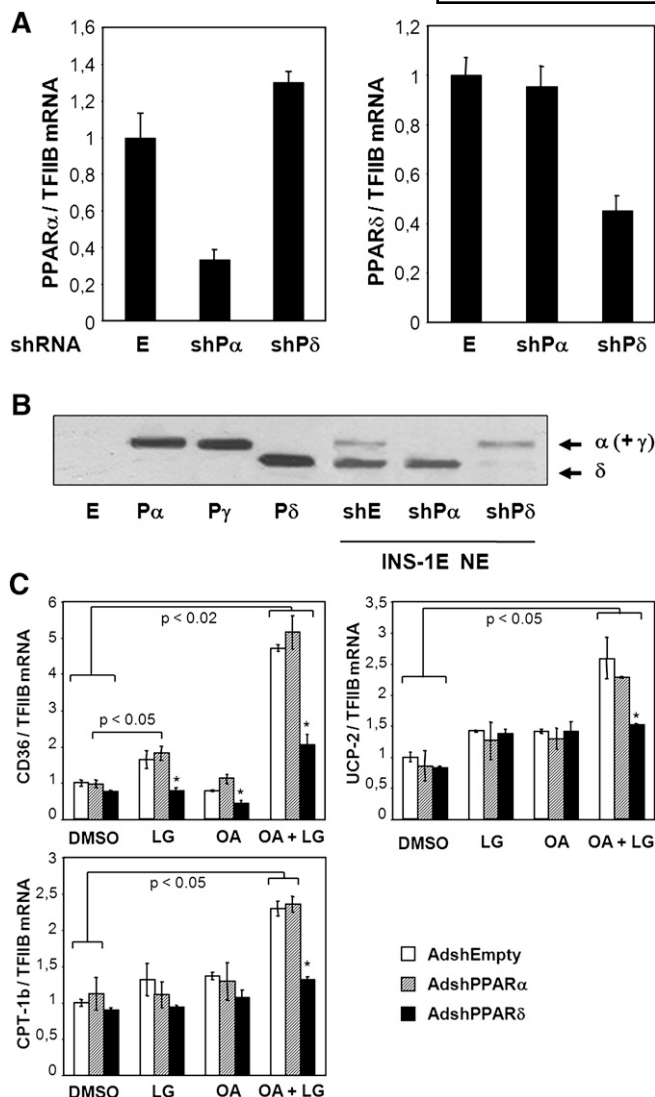


Fig. 4. Oleic acid activation of fatty acid handling genes is dependent on PPARδ in insulin-secreting cells. INS-1E cells were transduced with adenoviral vectors (~20 pfu/cell) expressing shRNA targeting PPARα [AdshPPARα (shPα)] or PPARδ [AdshPPARδ (shPδ)] or with empty control virus [AdshEmpty (E)] and cultured for 48 h in RPMI (11 mM glucose) before harvest of total RNA and nuclear protein extracts. **A:** Expression levels of PPARα and PPARδ were quantified using quantitative PCR, normalized to TFIIB expression, and presented relative to AdshEmpty control transduced cells. **B:** Nuclear proteins (NE) were separated by SDS-PAGE, and PPAR levels were visualized by immunoblotting. Protein extracts from NIH-3T3 cells ectopically expressing murine PPARα (Pα), PPARγ2 (Pγ), PPARδ (Pδ), or control adenoviral vector (E) were run in parallel as control for protein migration. Arrows indicate migration of the subtypes. **C:** INS-1E cells were transduced with the indicated adenoviral vectors and cultured for 24 h in RPMI (11 mM glucose) before incubation with DMSO or the fatty acid oleic acid (OA, 100 μM) in the presence or absence of the RXRα agonist LG100268 (0.2 μM) for additional 24 h. Total RNA was extracted, and expression levels of CD36, muscle CPT-1, and UCP2 were quantified using quantitative PCR and normalized to TFIIB expression. Expression levels are presented relative to DMSO control. Results are means of three independent experiments, and *P* values were calculated by two-way ANOVA. Addition of LG100268 differs from corresponding DMSO control (**P* < 0.05).

The basal expression levels of the investigated PPAR target genes were unaffected by PPARδ knockdown as well as by PPARα knockdown. Importantly, however, PPARδ knockdown abolished induction of these genes in response to oleic acid and the RXRα agonist LG100268 (Fig. 4C) and in response to γ-linolenic acid and LG100268 (data not shown). By contrast, PPARα knockdown had no effect on the induction of these PPAR target genes by unsaturated fatty acids or the RXR agonist under the experimental conditions described. These results show that in insulin-secreting cells; PPARδ is an endogenous mediator of the induction of genes involved in fatty acid oxidation by unsaturated fatty acids.

Activation of PPARδ increases fatty acid oxidation capacity in insulin-secreting cells

Others and we have previously shown that activation of PPARα in pancreatic β-cells is associated with an increased potential to oxidize fatty acids (7). Activation of the endogenous PPARδ in INS-1E cells by the specific agonist L165041 or unsaturated fatty acids induced genes involved in fatty acid uptake and oxidation. To investigate whether the PPARδ-induced changes in gene expression affected INS-1E cell fatty acid oxidation capacity, we incubated cells for 24 h with DMSO, the PPARα agonist WY14.643, the PPARδ agonist L165041, or oleic acid in the presence or absence of the RXRα agonist LG100268, respectively, and subsequently determined [¹⁴C]oleic acid oxidation. In keeping with previous observations (7), activation of PPARα with or without concomitant RXRα activation increased oleic acid oxidation (Fig. 5A), whereas agonists for either PPARδ or RXRα alone only modestly affected fatty acid oxidation. However, simultaneous activation of RXRα and PPARδ by L165041 or oleic acid synergistically increased fatty acid oxidation to the same extent as PPARα/RXRα activation. The effect of incubation with oleic acid and LG100268 was blunted when the PPARδ level was reduced by shRNA-mediated knockdown (Fig. 5B). These results establish PPARδ as a lipostat required for oleic acid-induced fatty acid oxidation in INS-1E cells, and the pronounced synergy between PPARδ and RXRα reflects our observations at the transcriptional level.

PPARδ protects β-cells against fatty acid-induced changes in insulin secretion

Prolonged exposure of β-cells to fatty acids is known to have profound effect on both basal insulin secretion and GSIS (35). The notion that excess fatty acids ablate mitochondrial function and that these effects are inversely related to fatty acid oxidation prompted us to investigate the effects of PPARδ activity on insulin secretion. INS-1E cells were preincubated for 24 h with the PPARδ agonist L165041 or DMSO. Cells were then exposed to basal 2.5 mM or stimulatory 15 mM glucose for 30 min and supernatant was collected. The secreted insulin was quantified and calculated as percentage of total cellular insulin content (Fig. 6A). The pretreatment with the PPARδ agonist increased GSIS 1.5-fold (*P* < 0.02) without affecting basal insulin secretion. To extend this finding to primary β-cells, we isolated rat pancreatic islets and pretreated these for 24 h

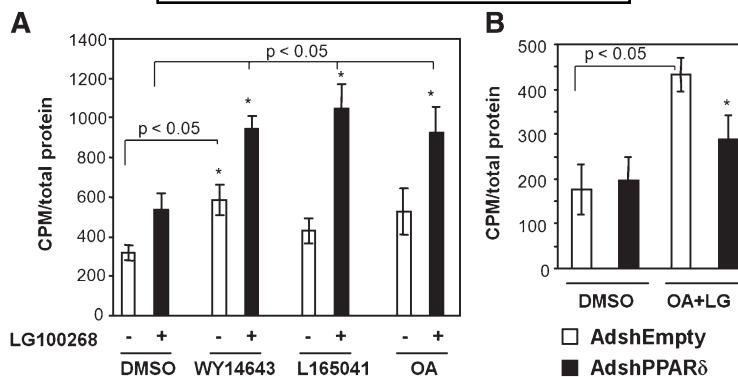


Fig. 5. Oleic acid activation of PPAR δ increases fatty acid oxidation capacity in synergy with RXR α in insulin-secreting cells. **A:** INS-1E cells were incubated for 24 h in RPMI (11 mM glucose) with DMSO, the specific PPAR α agonist WY14.643 (30 μ M), or the specific PPAR δ agonist L165041 (1 μ M) in the presence or absence of the RXR α agonist LG100268 (0.2 μ M). Cells were incubated with [1-14C]oleic acid for 4 h in serum-free RPMI1640 containing 5 mM glucose, and oxidation was measured in triplicate, background was subtracted, and values were normalized to total cellular protein. **B:** INS-1E cells were transduced with adenoviral vectors (\sim 20 pfu/cell) expressing shRNA against PPAR δ (AdshPPAR δ) or a control shRNA vector (AdshEmpty). After 24 h in RPMI (11 mM glucose), DMSO or oleic acid (OA; 100 μ M) together with LG100268 (LG; 0.2 μ M) was added to the media. Cells were incubated for another 24 h, and fatty acid oxidation was measured as described above. The experiments were performed in triplicate, and standard deviations are indicated. Results are representative of three independent experiments, and *P* values were calculated using two-way ANOVA. Addition of LG100268 differs from corresponding DMSO control (**P* < 0.05).

with DMSO or the PPAR δ agonist L165041. The rat islets were then subjected to basal 2.8 mM or stimulatory 11.2 mM glucose for 1 h and supernatant was collected. Similar to INS-1E cells, the PPAR δ agonist had no effect on basal

insulin secretion but increased GSIS by 35% (*P* < 0.05) (Fig. 6B).

Knowing that a PPAR δ agonist enhances GSIS, we wanted to see if PPAR δ activity was important for preserva-

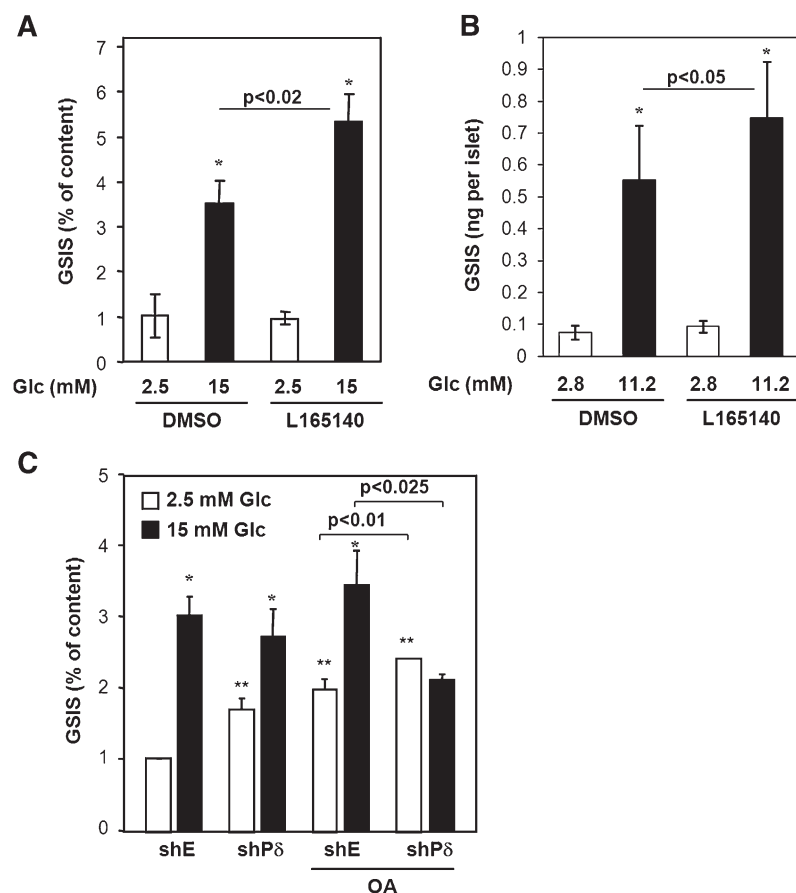


Fig. 6. PPAR δ activity potentiates GSIS and protects against fatty acid-induced insulin secretion defects. **A:** INS-1E cells were incubated for 24 h in RPMI (11 mM glucose) with DMSO or the specific PPAR δ agonist L165041 (1 μ M). Insulin secretion over 30 min at 2.5 and 15 mM glucose was determined and normalized to total insulin content. GSIS is significantly different from basal insulin secretion (**P* < 0.01). GSIS in cells preincubated with L165041 is elevated compared with DMSO-treated cells (*P* < 0.02). **B:** Rat islets were incubated for 24 h in RPMI (11 mM glucose) with DMSO or the specific PPAR δ agonist L165041 (1 μ M). Insulin secretion over 30 min at 2.8 and 11.2 mM glucose was determined and normalized to islet number. GSIS is significantly different from basal insulin secretion (**P* < 0.01). GSIS in cells preincubated with L165041 is elevated compared with DMSO-treated cells (*P* < 0.05). **C:** INS-1E cells were transduced with adenoviral vectors (\sim 20 pfu/cell) expressing shRNA against PPAR δ (shP δ) or control shRNA (shE). After 24 h of incubation in RPMI (11 mM glucose) with DMSO or oleic acid (OA; 400 μ M), insulin secretion over 30 min at 2.5 and 15 mM glucose was determined and normalized to total insulin content. Where indicated, GSIS is significantly different from basal insulin secretion (**P* < 0.05) as assessed by two-way ANOVA. ** Significantly different from control transduced, DMSO-treated cells at 2.5 mM glucose (*P* < 0.001). Insulin secretion was significantly reduced at 15 mM glucose (*P* < 0.025) and increased at 2.5 mM glucose (*P* < 0.01) for shP δ cells compared with wild-type cells treated with oleic acid. The results are means of three independent experiments.

tion of insulin secretion when β -cells were exposed to fatty acids. INS-1E cells were transduced with adenovirus expressing shPPAR δ or control virus. Cells were then cultured in medium with or without 0.4 mM oleic acid for 24 h, and insulin secretion at 2.5 or 15 mM glucose was determined. Treatment of control cells for 24 h with oleic acid increased basal insulin secretion ($P < 0.001$) without affecting insulin secretion stimulated by 15 mM glucose, thereby lowering the fold increase normally induced by glucose (Fig. 6C). This phenotype was resembled by knockdown of PPAR δ in the absence of oleic acid, as these cells still responded to 15 mM glucose but the overall response was blunted. Importantly, oleic acid completely abolished the insulin response to glucose from cells lacking PPAR δ . The basal insulin secretion was even further elevated compared with oleic acid-treated control cells ($P < 0.01$), and the glucose stimulation was completely ablated. Insulin content was unaffected under all of the described experimental conditions. These data show that PPAR δ expression is important for the β -cells to cope with the increased fatty acid load possibly by adjusting the fatty acid turnover.

DISCUSSION

The presence of abundant levels of PPAR δ , mRNA in pancreatic islet is well established (9, 15), but until now, no function has been ascribed to this nuclear receptor in pancreatic β -cells. In this work, we show that PPAR δ is the most abundant PPAR subtype in pancreatic β -cells at both the mRNA and protein level. Consistently, selective activation of PPAR δ by the use of a specific agonist leads to efficient induction of genes involved in lipid metabolism. By contrast, little activation of target genes was observed by activation of PPAR α and PPAR γ . Gene activation by PPAR δ is synergistically potentiated by co-stimulation of RXR α . Also, this synergism is more pronounced than the synergy observed by agonist activation of the other PPAR subtypes and RXR but is similar to the synergy between ectopically expressed PPAR α and RXR α in INS-1E cells (7). Notably, mono- and polyunsaturated fatty acids behave highly similar to the specific PPAR δ agonist in both luciferase reporter assays and in the induction of endogenous genes in INS-1E cells. The sensing of unsaturated fatty acids at physiological levels and their influence on gene expression is synergistically potentiated by co-stimulation of RXR α and abolished by specific knockdown of PPAR δ . These data show that PPAR δ is an important mediator of the transcriptional effects by unsaturated fatty acids in INS-1E insulinoma cells. In accordance with these findings in insulin secreting cells, PPAR δ was previously described as a fatty acid sensor also in macrophages (36). In contrast, the saturated fatty acid palmitic acid had minimal effect on PPAR δ activity, although saturated and unsaturated fatty acids reportedly are bound by the PPAR δ ligand-binding domain with comparable affinities (37, 38). Possibly, PPAR δ binding of different fatty acids could give rise to different structural conformations and recruit-

ment of different cofactor complexes as it has been described for PPAR γ (39). Moreover, the fatty acids used in our study could be derivatized prior to PPAR δ ligand-binding domain binding, and little can be concluded about the bona fide PPAR δ agonist.

In some tissues, PPAR δ has been implicated in regulation of growth and differentiation (40, 41). Recently, however, evidence has accumulated that PPAR δ is also involved in the maintenance of mitochondrial oxidative capacity, in particular fatty acid oxidation. This has been described in various tissues, including white adipose tissue (11), skeletal muscle (10), and cardiomyocytes (12). Importantly, stimulation of PPAR δ activity protects against diet-induced obesity and insulin resistance, whereas loss of PPAR δ causes ectopic lipid accumulation, obesity, and cardiac lipotoxicity. These findings are in agreement with our observations suggesting a similar role for PPAR δ in pancreatic β -cells. PPAR δ is a central regulator of genes involved in mitochondrial handling of fatty acids and, hence, important for lipid partitioning in pancreatic β -cells. Indeed, activation of PPAR δ with a specific agonist or the monounsaturated oleic acid increases the potential for fatty acid oxidation in INS-1E cells. The induction of fatty acid oxidation by PPAR δ and PPAR α is of similar magnitude. As PPAR δ agonists induced PPAR target genes involved in fatty acid metabolism more potently than the PPAR α agonist but produced a comparable increase in fatty acid oxidation capacity, other parameters must be limiting for PPAR δ -induced fatty acid oxidation under the experimental conditions described. Notably, the effects of PPAR δ /RXR activation appear to be confined to enhancement of mitochondrial substrate utilization with no effect on mitochondrial density (data not shown).

As PPAR δ activation by unsaturated fatty acids or synthetic ligands in INS-1E cells increased mitochondrial capacity to oxidize fatty acids, we wanted to investigate if PPAR δ activation would also affect GSIS, which is tightly coupled to mitochondrial metabolism. Indeed, activation of the transcriptional program regulated by PPAR δ enhanced GSIS in both primary rat islets and INS-1E cells without affecting basal insulin secretion. Most likely, this effect corresponds to increased mitochondrial activity and possibly increased anaplerotic glucose flow. As previously reported for PPAR α (7), activation of PPAR δ by either natural or synthetic agonists increased PDK4 mRNA expression in both rat islets and INS-1E cells (supplementary Figure II). By inhibiting the pyruvate dehydrogenase complex, PDK4 is believed to increase the anaplerotic carbon flow through pyruvate carboxylase and sensitize the β -cells to glucose (42, 43).

It is recognized that the adverse chronic effects of fatty acids on β -cell function is counteracted by conditions increasing mitochondrial fatty acid oxidation. Stimulation of β -cells with leptin, AICAR, and troglitazone increases fatty acid oxidation and protects against the lipotoxic effects of fatty acids (44–46). Likewise, glucose is known to direct fatty acids from mitochondrial oxidation to lipid accumulation and thereby aggravate the toxicity of fatty acids (23, 47). Moreover, unmetabolized fatty acids, such as 2-bromopalmitate, are generally more lipotoxic than

their metabolizable analogs (48, 49). We therefore wanted to investigate whether PPAR δ activity would counteract alterations of the insulin response to glucose by fatty acids. Exposure of the cells to 0.4 mM oleic acid for 24 h increased basal insulin secretion ($P < 0.001$) and blunted the glucose response. Similar effects of oleic acid have recently been described in other studies (25, 35). In the presence of oleic acid, PPAR δ is presumably already active, and addition of synthetic ligands would not further increase PPAR δ activity. We therefore employed shRNA-mediated knock-down to evaluate if PPAR δ preserves insulin secretion during exposure to elevated fatty acid levels. Importantly, the effect of oleic acid on insulin secretion was much more pronounced in PPAR δ -depleted cells than in control infected cells. Basal insulin secretion was further increased, and GSIS was totally ablated. In the absence of PPAR δ , fatty acids and their derivatives would accumulate to higher levels and override the tight coupling between glucose stimulation and fatty acid turnover known to be important for the normal glucose response (50). Activated PPAR δ may hence protect against the adverse effects of fatty acids on glucose responsiveness by adjusting the mitochondrial capacity to the increased load of fatty acid. A β -cell protective role is in keeping with previous studies showing that loss of PPAR δ in cardiomyocytes caused lipid accumulation and lipotoxic cardiomyopathy (12). A recent study showed that feeding mice a high-fat diet rich in oleic acid increased islet mitochondrial metabolism (51). The observed increase in mitochondrial capacity is reminiscent of PPAR δ activation in other tissues (10, 11), but whether this adaptation of the islet cells in vivo to increased fatty acid loads is dependent on PPAR δ remains to be determined.

In conclusion, we show that PPAR δ is abundant in pancreatic β -cells and plays a central role as effector of unsaturated fatty acids in the regulation of fatty acid metabolism and β -cell function. The transcriptional activity of PPAR δ is synergistically potentiated by simultaneous activation of RXR by agonists, and co-stimulation of PPAR δ and RXR leads to a pronounced increase of the oxidative potential of the insulin secreting cells. Our results indicate that this increase in oxidative potential may counteract the harmful actions of fatty acids and therefore may represent an adaptive mechanism for protection of β -cell function and a promising target for pharmacological prevention of lipid induced β -cell dysfunction.

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