Susceptibility of Pancreatic Beta Cells to Fatty Acids Is Regulated by LXR/PPARα-Dependent Stearoyl-Coenzyme A Desaturase

Karine H. Hellemans1, Jean-Claude Hanaert1, Bart Denys1, Knut R. Steffensen2, Cindy Raemdonck1, Geert A. Martens1, Paul P. Van Veldhoven3, Jan-Åke Gustafsson2,4, Daniel Pipeleers1

Abstract

Chronically elevated levels of fatty acids—FA can cause beta cell death in vitro. Beta cells vary in their individual susceptibility to FA-toxicity. Rat beta cells were previously shown to better resist FA-toxicity in conditions that increased triglyceride formation or mitochondrial and peroxisomal FA-oxidation, possibly reducing cytoplasmic levels of toxic FA-moieties. We now show that stearoyl-CoA desaturase—SCD is involved in this cytoprotective mechanism through its ability to transfer saturated FA into monounsaturated FA that are incorporated in lipids. In purified beta cells, SCD expression was induced by LXR- and PPARα-agonists, which were found to protect rat, mouse and human beta cells against palmitate toxicity. When their SCD was inhibited or silenced, the agonist-induced protection was also suppressed. A correlation between beta cell-SCD expression and susceptibility to palmitate was also found in beta cell preparations isolated from different rodent models. In mice with LXR-deletion (LXRα-/- and LXRβ-/-), beta cells presented a reduced SCD-expression as well as an increased susceptibility to palmitate-toxicity, which could not be counteracted by LXR or PPARα agonists. In Zucker fatty rats and in rats treated with the LXR-agonist TO1317, beta cells show an increased SCD-expression and lower palmitate-toxicity. In the normal rat beta cell population, the subpopulation with lower metabolic responsiveness to glucose exhibits a lower SCD1 expression and a higher susceptibility to palmitate toxicity. These data demonstrate that the beta cell susceptibility to saturated fatty acids can be reduced by stearoyl-CoA desaturase, which upon stimulation by LXR and PPARα agonists favors their desaturation and subsequent incorporation in neutral lipids.

Citation: Hellemans KH, Hanaert J-C, Denys B, Steffensen KR, Raemdonck C, et al. (2009) Susceptibility of Pancreatic Beta Cells to Fatty Acids Is Regulated by LXR/PPARα-Dependent Stearoyl-Coenzyme A Desaturase. PLoS ONE 4(9): e7266. doi:10.1371/journal.pone.0007266

Editor: Kathrin Maedler, University of Bremen, Germany

Received May 3, 2009; Accepted September 2, 2009; Published September 29, 2009

Copyright: © 2009 Hellemans et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This work was supported by grants from the Research Foundation Flanders to KH (FWO-1.5.195.05, FWO-1.5.193.08), the Interuniversity Attraction Poles/CP227 and the Interuniversity Attraction Poles/C223, from the Dutch Research Council (NWO), from the 6th EU-framework (IAP P6/40 - DP coordinator) from the geconcentreerde onderzoeksacties van de Vlaamse Gemeenschap (GOA 2004/08 to PPVV), the Norwegian Research Council (KRS), the Swedish Research Council (JAG) and a general nuclear receptor research grant from karobio Ab. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: ÅG is shareholder, research grant receiver and consultant of Karobio AB. The funders did not play a role in this particular study. The Diabetes Research Center is a partner of the Juvenile Diabetes Research Center for Beta Cell Therapy in Diabetes. The other authors have no financial conflict of interest to disclose.

* E-mail: karine.hellemans@vub.ac.be

Introduction

Chronically elevated levels of saturated fatty acids may cause a reduction in beta cell mass during the pathogenesis of type 2 diabetes. Supporting evidence comes primarily from animal models and in vitro studies in which high fatty acid concentrations induce beta cell dysfunction and death [1-4]. Depending on the laboratory model, the lipotoxic process is seen (in)dependently of glucotoxic influences [5]. In cultures of purified rat beta cells, we found that palmitate was cytotoxic irrespective of the glucose concentration but that the cells varied in their susceptibility, some of them surviving while others rapidly or progressively proceeded to necrosis or apoptosis [6,7]. This observation is another illustration of the functional heterogeneity in the beta cell population whereby cells differ in their individual sensitivities and/or defense mechanisms [8-10]. Fatty acid (FA) toxicity was found inversely related to the cellular ability to incorporate them as neutral lipids in the cytoplasm [6]. The capacity to form cytoplasmatic lipid droplets may thus serve as a cytoprotective mechanism by preventing accumulation of toxic non-esterified FA [6,11]. Un saturated FA such as oleate can channel palmitate into triglyceride pools away from pathways leading to cellular apoptosis [12-14]. A second protective mechanism consists in increasing breakdown of fatty acids in the beta cells [15,16]. We recently demonstrated that the cytoprotective effect of PPARα agonists against long-chain fatty acid toxicity is associated with increased rates of β-oxidation and peroxisomal activity [7]. During the latter study we noticed that PPARα agonists also induced a higher expression of stearoyl CoA Desaturase (SCD), which is known to generate monounsaturated FA from palmitate and thus facilitates their incorporation into lipid reserves [17,18], instead of their accumulation as non-esterified fatty acids or via an increased
incorporation in ceramides [19]. Elevated SCD-levels have been shown to protect against saturated fatty acids in a number of cell types, including MIN6 cells [19-21]. The present study was undertaken to investigate the potential contribution of SCD in the detoxifying action of PPARγ agonists.

Since the SCD1 and 2 enzymes are direct downstream targets of Liver X Receptors (LXR) [22], we started by examining whether LXR agonists can protect beta cells against palmitate through an SCD-dependent mechanism. LXRα and LXRβ are nuclear oxysterol receptors with established roles in cholesterol, lipid, and carbohydrate metabolism [23,24], and have recently been shown to play an important role in maintaining beta cell function [25,26].

Results

Activation of LXR protects beta cells against palmitate toxicity

Rat beta cells were cultured with palmitate (C16:0) in presence or absence of LXR agonists. Culture with 500 μM palmitate-2 days (500 μM-P) or 250 μM-8 days (250 μM-P) resulted in a cytotoxicity of 29 and 38% respectively (Figure 1a). The LXR agonists TO1317 and GW3965 protected against both conditions keeping cell death below 10%. On the other hand, no protection was seen with the FXR and PXR agonists GW4064 and pregnenolone carbonitrile (PCN), which supports LXR specific protection. TO1317 was also found to protect human beta cells (Figure 1a). The protective effect of TO1317 was maintained over 16 days: 10±2% dead cells at 250 μM-P with the agonist versus 55±5% without (Figure 1b). Protection was also observed when TO1317 was added 24 h after starting cultures with 250 μM-P: 16±2% dead cells after 8 days versus 40±5% in its absence (P<0.01) (data not shown). None of the tested agonists influenced beta cell survival in absence of palmitate.

Pre-treatment of beta cells with TO1317 also protected against a subsequent exposure to 250 μM-P (Table 1). During a first period of 8 days, beta cells were cultured with or without the agonist; 250 μM-P was added on day 8 and culture continued without agonist. Protection was sustained up to 8 days in cells exposed to palmitate after removal of the protective agonist:

Figure 1. Agonists for LXR protect primary beta cells against palmitate toxicity. a) Rat and human beta cells were exposed to 500 μM-P, or 250 μM-P in the absence or presence of agonists for LXR (10 μM TO1317 or 1 μM GW3965), FXR (5 μM GW4064), or PXR (30 μM PCN). Data shown as mean±sd, n = 4-10, * p<0.05, ** p<0.01, *** p<0.001, versus palmitate b) Time course over 16 days for the toxicity of 250 μM-P±TO1317 (1 μM) in rat beta cells. Data shown as mean±sd, n = 7-10.

doi:10.1371/journal.pone.0007266.g001
23 ± 3% dead cells versus 38 ± 3% (P < 0.05). Beta cells cultured under control conditions for 8 days showed a comparable cytotoxic response as freshly isolated cells when subsequently exposed to palmitate for 2 to 8 days, indicating that prolonged culture did not alter their susceptibility for palmitate.

In contrast to PPARα agonists that induce protection to saturated and mono-unsaturated FA [7], TO1317 did not protect against palmitoleate (C16:1), vaccenate (C18:1 n-7) or oleate (C18:1 n-9), while it protected against stearate (C18:0) (Table S1).

### Table 1. Effect of TO1317 pretreatment on palmitate toxicity.

| Pre-culture | Final condition | 2 days | 8 days |
|-------------|-----------------|--------|--------|
| Control C16:0 | 14 ± 2 | 38 ± 3 |
| TO1317 C16:0+TO1317 | 1 ± 2 $^5$ | 12 ± 3 $^5$ |
| TO1317 C16:0 | 5 ± 3 $^5$ | 23 ± 3 $^5$ |
| C16:0+TO1317 | 4 ± 2 $^5$ | 32 ± 6 |

Beta cells were pre-cultured for 8 days in absence or presence of 1 μM TO1317 or 250 μM-P; on day 8 these conditions were replaced by the final conditions, as indicated, and the resulting cytotoxicities were measured after 2 or 8 days. Mean ± SD, n = 4.

$^5$p < 0.001, versus control.

DOI:10.1371/journal.pone.0007266.t001

Figure 2. Effect of LXR agonist on intracellular lipid accumulation and palmitate metabolism. a) Representative figure showing electron microscopy and vital nile red staining on control cells, and beta cells exposed to 250 μM-P for 2 days + TO1317 (10 μM). Magnification: EM 2550x, Nile red (green) + nuclear Hoechst (red) staining: 60x. b) Effect of 250 μM-P + TO1317 on 14C-palmitate metabolism. Beta-cells were cultured for 24 h in the absence (white bars) or presence (gray bars) of 250 μM-P + TO1317, and subsequently incubated for 2 hrs with 14C-palmitic acid. Metabolism was measured as recovery of 14C-label in CO2, in lipids and lipid intermediates (pmol/2 h/103 cells) and expressed as percentages of the control condition. Data shown as mean ± se (n = 4), Student’s t-test, $^*$ P < 0.01 and $^{**}$ P < 0.001, versus control; * P < 0.05 and ** P < 0.01 versus palmitate.

DOI:10.1371/journal.pone.0007266.g002
We assessed the effect of TO1317 on the cellular ability to transfer palmitate into cellular lipids, by measuring the incorporation of 14C-palmitate-derived carbon over a period of 2 hrs in cellular lipids, or as 14CO2-production, in control cells and in cells pre-exposed for 24hrs to 250 μM-P. Presence of 1 μM TO1317 stimulated the 14C-flux into lipids by 50±16% as compared to control (p<0.01) (Figure 2b). In contrast to the recent study by Green et al. [29], TO1317 was found to suppress 14C-palmitate oxidation in both the palmitate and the control condition. The apparent negative effect of 250 μM-P pre-exposure on 14CO2-formation is explained by a dilution effect on the 14C-label due to the increased intracellular palmitate levels, as shown before [7]. These observations support the view that channeling FA into stored neutral lipids correlates with protection against the cytotoxicity of cytoplasmic free acyl moieties and indicates the involvement of LXR in this process.

Figure 3. Enzymes involved in conversion of palmitate in response TO1317. a) Overview of enzymes involved in conversion of palmitate (C16:0) to palmitoylate (C16:1), vaccinate (C18:1 n-7), stearate (C18:0) and oleate (C18:1 n-9): stearoyl CoA desaturase (SCD1, SCD2), elongases (ELOvl5, ELOvl6), diacylglycerol transferase (DGAT1, DGAT2), sterol-O-acyl transferase-1(SOAT1), and CE, cholesteryl esters. b) qPCR result examining the mRNA levels of the above enzymes in freshly isolated rat beta cells, alpha-cells and liver, white adipose and brain tissue. Gene expression levels were normalized to actin, n = 3. c) qPCR analysis for SCD1 and 2, showing the effect of 8 days 250 μM-P±TO1317. Data shown as mean±sd, n=4, *** p<0.001, compared to control; $ p<0.05, $$$ p<0.001, versus TO1317. d) Representative immunoblot showing SCD protein levels in beta cells cultured for 8 days with or without 250 μM-P±TO1317 (10 μM). Equal amounts of total protein were added on a 10% polyacrylamide gel. Representative for 4 independent experiments.

doi:10.1371/journal.pone.0007266.g003

Effect of LXR agonist on mRNA expression of desaturation enzymes in beta cells

We examined the effect of TO1317 on mRNA expression of enzymes involved in fatty acid elongation (ELOvl5 and 6), desaturation (SCD1 and 2) and esterification (DGAT1 and 2, SOAT 1 and 2) (Figure 3a). Since PPARγ agonists have been previously found to induce beta cell protection through effects on enzymes that regulate palmitate metabolism [7], we compared the effects of the LXR agonist with those of the PPARγ agonist combination clofibrate plus 9cis RA (Table 2).

When beta cells are compared with liver, brain, white adipose tissue (WAT) and endocrine alpha cells, they exhibit a high expression of SCD2, FA elongase-5 (ELOvl5) and sterol-O-acyl transferase-1(SOAT1), and a low expression of SCD1, ELOvl6, and the diacyl glycerol acyltransferase-1 and 2 (DGAT1 and DGAT2)
Inhibition of stearoyl-coenzyme A desaturase reverses the cytoprotective effects of LXR and PPARα agonists in beta cells

In view of their effects on expression of SCD1 and SCD2 we examined whether the cytoprotective effects of the LXR and PPARα agonists were influenced by 11,12-conjugated linoleic acid, a known inhibitor of SCD, or by its analog 9,11-CLA, which has no effect on SCD activity [20]. Rat beta cells were cultured with 250 µM-P plus TO1317 for 8 days in the presence or absence of one of these compounds. The SCD-active CLA (10,12-CLA - 40 µM) not only prevented the protective effect of TO1317 but also augmented palmitate cytotoxicity, while no effect was seen with 9,11-CLA (Figure 4a). This was also the case for the clofibrate-retinoic acid combination. None of the CLA-compounds were toxic by themselves, while they showed a mild protective effect on palmitate toxicity in the absence of TO1317 in rat beta cells. At this moment we can only speculate why both the active and the inactive CLA-isomers provided protection to palmitate in rat beta cells. 10,12-CLA did not show this effect on human cells where it was - as expected - found to increase 250 µM-P toxicity in the presence, as well as in the absence of TO1317 (Figure 4a). Up to 5 different SCDs have been described in mammals with their own specific FA-preferences, whereas the effects of the CLAs have only been described for SCD1. It can thus not be excluded that other SCDs could be influenced by CLA-protection. In contrast to PPARα agonists, the LXR agonist did not stimulate expression of enzymes involved in mitochondrial and peroxisomal oxidation. On the contrary, inhibitions up to 40% were measured (Table 2), which is compatible with the observed suppression of 14C-palmitate oxidation (Figure 2b).

Table 2. Effect of palmitate and LXR or PPARα agonists on gene mRNA expression levels calculated as ΔΔCt values compared to the indicated control conditions.

| Genes | C16:0 | TO1317 | C16:0-TO1317 | C16:0-clof/9-cis RA |
|-------|-------|--------|-------------|----------------------|
|        | Compared to control | Compared to control | Compared to C16:0 | Compared to C16:0 |
| LXRα  | 1.4±0.3 | 2.5±1.3 * | 1.3±0.7 | 1.7±0.6 ** |
| LXRβ  | 0.8±0.3 | 1.4±0.5 | 1.0±0.3 | 1.0±0.4 |
| PPARα | 0.7±0.2 * | 1.1±0.6 | 1.3±0.4 | 1.9±0.4 *** |
| FXR   | 0.7±0.1 * | 1.0±0.1 | 1.1±0.4 | 1.6±0.5 ** |
| SREBP1c | 0.8±0.2 | 3.3±0.7 *** | 3.5±1.2 *** | 2.8±0.5 ** |
| SCD1  | 0.5±0.2 *** | 2.2±0.3 *** | 2.5±0.6 *** | 5.8±2.7 *** |
| SCD2  | 0.7±0.1 *** | 1.8±0.3 ** | 2.4±0.3 *** | 2.6±0.6 *** |
| ELOV5 | 0.8±0.3 | 1.8±0.5 | 1.3±0.3 | 2.3±0.7 * |
| ELOV6 | 0.8±0.1 | 1.9±0.6 ** | 1.0±0.3 | 2.4±0.8 * |
| GPAT  | 1.1±0.3 | 1.2±0.5 | 1.2±0.1 | 1.5±0.2 ** |
| DGAT1 | 1.0±0.1 | 1.6±0.4 | 0.7±0.3 | 0.9±0.2 |
| DGAT2 | 1.7±0.6 | 1.9±0.7 ** | 1.0±0.3 | 1.1±0.3 |
| SOAT1 | 1.7±1.0 | 0.9±0.2 | 1.0±0.1 | 1.2±0.5 |
| CPT1  | 1.9±0.6 ** | 1.2±0.1 | 0.6±0.2 * | 1.9±0.2 *** |
| Acad l | 1.4±0.7 | 0.8±0.1 | 0.7±0.2 * | 1.5±0.2 ** |
| Acad m | 0.8±0.2 | 1.1±0.2 | 1.0±0.1 | 1.6±0.3 ** |
| Aca a2 | 1.3±0.4 | 1.5±0.3 | 0.6±0.3 * | 1.6±0.1 *** |
| Aca a1 | 0.8±0.2 | 1.9±0.5 * | 0.8±0.4 | 1.6±0.1 *** |
| Acox1 | 1.0±0.3 | 1.2±0.2 | 0.7±0.3 | 1.6±0.4 ** |
| Acox3 | 1.4±0.9 | 1.4±0.4 | 0.8±0.3 | 1.5±0.2 *** |

Beta cells were exposed for 2 days to 250 µM-P±1 µM TO1317, or 250 µM clofibrate/2 µM 9-cis RA. qPCR values were normalized to actin and compared relative to the respective control conditions. Unpaired student t-test, two tailed, mean±5D, n=4 – 6, * p<0.05, ** p<0.01, *** p<0.001.

doi:10.1371/journal.pone.0007266.t002
levels of C18:1 n-9 (oleic acid) and C18:1 n-7 (vaccenic acid) were lower, and those of C16:1 (palmitoleic acid) very low (Figure S1a). Culture with 250 μM-P led to a marked increase of the C16:1 fraction, and lowered C18:0 and C18:1 (n-7 and n-9) levels (Figure 4b). Consistent with a stimulatory effect on SCD, addition of TO1317 was found to normalize the C18:1 n-7 levels, but did not prevent the changes in C18:0, C18:1 n-9 and C16:1. The SCD-active CLA counteracted the effect of TO1317 on C18:1 n-7, and blocked formation of C16:1 and C18:1 n-9. No change was seen for the levels of C20:4 (arachidonic acid), a fatty acid derived from C18:2 (linoleic acid) through Δ5- and Δ6-desaturases, further indicating specific involvement of the Δ9 desaturase pathway. We noticed a higher C16:0 peak area after palmitate exposure, but it is impossible to discriminate whether this reflects uptake or adherence of the fatty acid. The effects of TO1317 and t10,c12 CLA on the ratios of saturated over monounsaturated fatty acids are shown in Figure S1b.

The role of SCD in beta cell protection against palmitate was further examined by RNA interference with this protein. Exposure of beta cells for 48 h to 50 nM siRNA-cationic lipid complexes targeted to both SCD1 and SCD2 resulted in a significant decrease in SCD-RNA levels (by 78±3% for SCD1, and 51±12% for SCD2 respectively), without affecting SREBP1c, LXR and PPARγ mRNA levels (not shown). Jetti-ENDO control and SiGLO risk free treated cells were used as control. Cell viability was not affected by the transfection protocol, and siRNA knockdown of SCD did not affect cell viability when compared to controls. Under these conditions, cells were taken 24 h after...
transfection and cultured for 72h in presence or absence of 250 μM-P with or without TO1317 (10 μM) (Figure 4c). SCD1/SCD2 siRNA-treated beta cells showed an increased palmitate toxicity (47±9%) as compared to control-transfected cells (Jetsi-ENDO control and SiGLO risk-free treated cells, p<0.05, n = 4) (Figure 4c). Moreover, protection by TO1317 was lost in SCD1/SCD2 siRNA-treated beta cells. Comparable results were obtained when cells were transfected with siRNAs directed to either SCD1 or SCD2 (data not shown). Cell viability was not affected by the transfection protocol, and siRNA knock-down of SCD did not affect cell viability when compared to controls.

Interaction between desaturation pathway and palmitate induced ER stress

A causal relation between palmitate toxicity, ER stress and the accumulation of spindle-shaped structures in the ER has been suggested [27,28,30]. In our experiments these structures accumulated under protective conditions (See Figure 2). We therefore studied the expression of ER-stress markers in response to palmitate with, or without TO1317. Using this strategy we could, however, not establish a clear correlation between ER-stress, palmitate induced toxicity and protection against it (Table 3). When rat beta cells were exposed to 500 μM-P for 2 days – a condition with acute cell death- the expression levels of DNA-damage inducible transcript 3 (Ddit3/Chop) were found in-condition with acute cell death- the expression levels of DNA-damage inducible transcript 3 (Ddit3/Chop) were found increased, in the absence, as well as in the presence of TO1317, while those of DnaJ homologue C3 (DnaJc3/P58) and heat shock 70 kDa protein 5 (HSPA5) remained unaffected (Table 3). No changes in expression for ER stress markers were found when cells were exposed to 250 μM-P with or without TO1317. We therefore further addressed this question by looking upstream from SCD. A9-desaturation requires electrons supplied via Ncb5or (NAD(P)H-cytochrome b5 oxidoreductase) [31]. Ncb5or was recently found to protect cells against palmitate induced ER-stress and lipotoxicity. Cells lacking Ncb5or expression showed more signs of ER stress and a higher cytotoxicity in response to palmitate [32]. We found the expression levels of Ncb5or induced by TO1317 in presence of 250 μM and 500 μM-P, but not influenced by palmitate on its own (Table 3). These observations are consistent with a role of SCD to channel palmitate into less harmful pathways such as accumulation into neutral lipids, and -in our view- dissociate the accumulation of these lipids in the ER from apoptosis, since more of these spindle-shaped structures were formed under protective conditions.

LXR isotype specificity in modulation of palmitate toxicity through SCD and SREBP1

The cytoprotective effects of LXR and PPARα agonists were measured in beta cell preparations isolated from LXRα-/-, LXRβ-/-, LXRαβ-/- and wild type mice. Beta cell survival in control conditions was similar for the 4 genotypes and was not influenced by the presence of TO1317 or clofibrate/9-cis RA. Culture with 250 μM-P was cytotoxic for the four preparations (Figure 5a). Beta cells from LXRβ-/- and LXRβ-/- mice showed a much higher susceptibility to palmitate-toxicity (almost 100% cell death) than wild type cells, whereas those from LXRα-/- mice were less susceptible. No differences in oleate toxicity were seen (Figure 5a). The same observations were made when cells were exposed to 100 μM-P, leading to almost 70% cell death in absence of LXRβ (results not shown). Beta cells from wild-type mice were strongly protected by the LXR-agonist TO1317, as well as by clofibrate/9-cis RA. This was not the case for LXRα-/-, LXRβ-/-, and LXRαβ-/- (Figure 5a). These observations indicate that the protective action of PPARα agonists on palmitate toxicity depend on the presence of both LXRα and β and downstream signaling pathways.

We also conducted the above-described mRNA analysis on islets isolated from LXRα-/-, LXRβ-/-, LXRαβ-/-, and wild type mice (Table S2). The islets did not present obvious differences in cellular composition with more than 65% percent beta cells in each preparation (results not shown). No significant differences were noticed between islets from wild-type mice and from LXRαβ-/- mice, while absence of LXRβ was associated with a markedly reduced expression of SCD1 and 2, and of SREBP1c (Figure 5b), and higher expression of DGAT1, SOAT1, and ELOVL5 (Table S2). This observation is compatible with a key role of LXRα in the expression of SCD and SREBP1c, and their influence on the cellular susceptibility to palmitate toxicity and further supports a role of SREBP1c downstream of LXR < PPARα [33].

Correlation between SCD expression in isolated beta cells and their susceptibility to palmitate

The view that SCD-expression in beta cells may vary with their environmental conditions in vivo, led us to explore three conditions in which rat beta cells could be expected to express higher SCD-levels after isolation. These preparations were then analyzed for their susceptibility to palmitate toxicity.

The first condition is represented by beta cells isolated from Zucker fatty rats lacking leptin receptor (fa/fa). Since leptin suppresses SCD [34] these animals show increased Δ9-desaturation activities and decreased saturated/mono-unsaturated fatty acid ratios in serum and tissues, including pancreas [35]. Compare to their lean controls (fa/−), beta cells from fa/fa animals showed 2-fold higher mRNA levels for SCD1, SCD2; their LXRα expression was also elevated, whereas PPARα expression was suppressed (Figure 6a), Table S3. Beta cells isolated from fa/fa animals showed a significantly lower susceptibility to palmitate-induced cell death than those isolated from lean controls (fa/−); after 8 days the cytotoxicity of 250 μM-P and 500 μM-P was, respectively, 6±2%

| Table 3. Effect of palmitate and LXR agonists on genes related to ER-stress. |

| Genes | TO1317 | 250 μM-P | 250 μM-P+TO1317 | 500 μM-P | 500 μM-P+TO1317 |
|-------|--------|----------|----------------|--------|----------------|
| Ddit3 | 1.2±0.2 | 1.1±0.1 | 1.3±0.2 | 1.8±0.3 | 1.8±0.2 |
| DnaJc3 | 0.7±0.1 | 1.0±0.2 | 0.7±0.02 | 0.8±0.2 | 0.8±0.1 |
| Hspa5 | 0.8±0.1 | 1.0±0.2 | 0.8±0.2 | 1.5±0.3 | 1.1±0.1 |
| Ncb5or | 1.5±0.3 | 1.3±0.1 | 1.5±0.1 | 1.8±0.1 |

qPCR values normalized against actin and expressed relative to the control condition (vehicle treated). Cells were exposed for 2 days to 250 μM-P or 500 μM-P with or without 10 μM TO1317. Student t-test, two tailed, mean±sd, N = 3 – 6, * p<0.05, ** p<0.01.

doi:10.1371/journal.pone.0007266.t003
and 13±1% for in fa/fa versus 28±4% and 48±5% in the fa/- controls (Figure 6b). In contrast, the cytotoxicity of oleate was low and similar in both preparations. When the LXR-agonist TO1317 was added to the palmitate conditions, it gave a mild reduction of palmitate toxicity in fa/fa beta cells, whereas fa/- cells were efficiently protected. Protection was counteracted by the SCD inhibitor t10,c12 CLA which even amplified the palmitate toxicity in both cell preparations. Beta cell vitalities of cells isolated from lean and Fatty Zucker rats were similar in control conditions. These data indicate that differences in SCD expression and activity in beta cells have consequences for their susceptibility to saturated fatty acids.

The second condition was experimentally induced by treatment of rats with the LXR agonist TO1317 (40 mg/kg body weight by oral gavage for 5 days) or with a control vehicle. The pancreases were then removed for purification of beta cells and alpha cell enriched fractions by FACS-sorting. Beta cells from TO1317-treated rats showed elevated SCD1, SCD2 and SREBP1c mRNA levels, and similar levels of LXRα, LXRβ and PPARα (Figure 6c). Their palmitate susceptibility was lower after 500 μM-P -2 days but could not be further reduced by addition of LXR or PPARα agonists, while these compounds induced protection in the vehicle-derived beta cells. Cells from TO1317-treated and vehicle-treated animals showed the same low oleate cytotoxicity (Figure 6f). Comparable data were obtained after culture at 250 μM-P for 8 days (cytotoxicity of 10±3% in TO1317-cells versus 30±1% in vehicle-cells). These observations indicate that in vivo treatment with the LXR-agonist has induced a beta cell phenotype with a sustained protection against palmitate. In prior work we showed that clof/9-cis RA did not influence palmitate toxicity in alpha-cell enriched preparations [7]. In the present study alpha-cell enriched preparations from TO1317- or vehicle-treated rats exhibited the same palmitate toxicity; this was also the case when TO1317 was added to the culture medium. No differences were seen in the viability of the beta cell or alpha cell-enriched preparations isolated from TO1317- or vehicle-treated rats and cultured in the control condition.

In prior work we have shown that primary beta cells can be less susceptible or even resistant to palmitate toxicity, and that this property is heterogeneously expressed [7]. To explore this observation, as a third condition, we have separated freshly isolated rat beta cells into two subpopulations according to their cellular metabolic responsiveness to an acute 7.5 mM glucose stimulation, as described in prior studies by our group [8,36]. The high-responsive beta cells were found to express higher levels of SCD1 and LXRα than the low-responsive beta cells; their higher expression of glucokinase was confirmed and served as an index for their higher metabolic responsiveness (Figure 6e) [37]. They also presented a lower cytotoxicity after 8 days culture with 250 μM-P (Figure 6f), or 2 days with 500 μM-P (data not shown). Addition of the LXR- or PPARα-agonists exerted a much more pronounced protective effect in the low-responsive subpopulation, which is in part related to their higher palmitate toxicity but which also indicates that they can generate cytoprotective mechanisms when receiving extracellular signals. As with the previously described conditions, the two tested cell populations underwent a similar low oleate cytotoxicity.

Discussion

The mechanisms regulating the balance between functional adaptation, lipid accumulation, dysfunction, or beta cell death in response to chronic elevated FA levels are poorly understood. Clarifying these mechanisms could help to develop new strategies to prevent beta cell deterioration in type 2 diabetes. Several studies have reported the potential of PPAR ligands to preserve functional
Figure 6. SCD levels regulate beta cell susceptibility for palmitate in vivo. Beta cells and alpha-enriched cell preparations were obtained from: a-b) Zucker fatty (fa/fa) and lean control rats (fa/-) and exposed for 8 days to 250 μM and 500 μM palmitate or oleate in the presence or absence of 10 μM TO1317 or 10,12 CLA. Percentages of cytotoxicity are shown as mean ± sd, n = 3, * p < 0.001 fa/fa versus lean control, $ p < 0.001 versus palmitate; or isolated from c-d) Wistar rats treated by oral gavages for 5 days with 40mg/kg BW TO1317 or vehicle (DMSO/PBS, 1/3), and exposed for 2 days to 500 μM palmitate or oleate in the presence or absence of 1 μM TO1317 or 250 μM clofibrate +2 μM 9-cis RA. Results are shown as mean ± sd, n = 3, * p < 0.001 TO1317-cells versus vehicle, $ p < 0.001 versus control; or e-f) Beta cells were FACS sorted based on their glucose-induced increase in [NAD(P)H]-autofluorescence into two subpopulations characterized by a high or low glucose-responsive phenotype [8], and exposed for 8 days to 250 μM palmitate or oleate in the presence or absence of 1 μM TO1317 or 250 μM clofibrate plus 2 μM 9-cis RA. Percentages of cytotoxicity are shown as mean ± sd, n = 3, * p < 0.01 low versus high NAD(P)H-cells; $ p < 0.01 agonist treatment versus control. a,c,e) Q-PCR analysis showing the differences in expression levels of glucokinase, SCD1, SCD2, LXRα, LXRβ, PPARα and SREBP1c. Results are shown as mean ± sd, n = 3-7, * p < 0.05, ** p < 0.01 versus the respective control preparations.

doi:10.1371/journal.pone.0007266.g006
beta cell mass in obesity models and in clinical type 2 diabetes [30,39], but only a limited number of reports has characterized the role of LXRαs in this context [25,26]. LXR activation has been shown to stimulate insulin secretion in vitro via de novo lipid synthesis [26,29,40], while in vivo LXR agonists are shown to reduce serum glucose levels and to improve glucose tolerance and insulin resistance in obesity models [41,42].

We have used primary beta cell preparations to examine ways to induce protection against beta cell death during prolonged exposure (up to 16 days) to elevated concentrations of the saturated fatty acid palmitate. Protection was seen with PPARα agonists and correlated with an increased oxidation rate and an increased expression of genes involved in β-oxidation [7], as well as in Δ9-desaturation and lipogenesis. In view of the prior reported protective action of SCD [19-21], which is mainly a LXR target gene, we now demonstrate that both PPARα and LXR-agonists provide long-term protection in rodent and human beta cells through a pathway involving Δ9-desaturation. We propose the following model (schematic overview in Figure 7): Δ9-desaturase expression in beta cells is regulated by LXR. Direct activation of LXR, or induction of LXR downstream of PPARα regulates SCD-transcription, possibly in a SREBP1c-dependent way. As a result exogenous palmitate is desaturated, elongated and more efficiently channeled to neutral storage pools associated with detoxification. This view is supported by the observation that TO1317 confers protection to saturated, but not to monounsaturated FA; and by the increased toxicity of palmitate upon SCD transcription, possibly in a SREBP1c-dependent way. As a result exogenous palmitate is desaturated, elongated and more efficiently channeled to neutral storage pools associated with detoxification. This view is supported by the observation that TO1317 confers protection to saturated, but not to monounsaturated FA; and by the increased toxicity of palmitate upon SCD inhibition, or by its silencing. SREBP1c, known to regulate unsaturated FA; and by the increased toxicity of palmitate upon SCD transcription from the SCD-promoter via SREBP1c. These results imply that both LXRα and LXRβ are required to gain protection by LXR or PPAR ligands, whereas LXRβ seems to control basal transcription from the SCD-promoter via SREBP1c. These results point to a complex regulation of the SCD promoter in response to LXRs, PPARs and SREBP1c. Comparable observations, where both LXR subtypes are required to control transcription in concert with PPAR and/or SREBP1c, have been reported for Elovl5, Δ6-desaturase and SREBP1c [33,49].

In the proposed model SREBP1c seems to play a central role downstream from PPARα and LXR. SREBP1c activation has already been implicated in the adaptive changes that underlie beta cell hypersecretion in response to elevated glucose [26,29,50]. In contrast however, hyper-activation of SREBP1c by its over expression or its activation by glucose and/or palmitate, has been

Figure 7. Hypothetical lipoprotection model using LXR or PPAR ligands. Long chain saturated FA, such as palmitate, induce cell death through reactive intermediates, ceramide generation or via ER stress. Activation of LXR downstream of PPARα activates SREBP1c and induces transcription of genes encoding SCD1 and SCD2. This results in an increased formation of monounsaturated FA and directs the flow of palmitate towards intracellular storage as neutral lipids. Next to inducing SCD levels, activation of PPARα results in an increased turn-over of FA by stimulation of β-oxidation, with detoxifying effects on both saturated and monounsaturated FA.

doi:10.1371/journal.pone.0007266.g007
linked to glucolipotoxicity, suppression of glucose-stimulated insulin secretion (GSIS), and was proposed as a mechanism to explain islet failure in Zucker Diabetic fatty rats (ZDF) [51-56]. Inactivation of SREBP1c in these islets, however, failed to normalize GSIS, thereby excluding SREBP1 and triglyceride-accumulation as the main cause behind defective secretion in this model [57]. Consistent with a role for SREBP1c in controlling insulin secretion, TO1317 has been shown to stimulate GSIS and insulin biosynthesis in beta cell lines and mouse islets via a stimulation of glucose and lipid metabolism [26,29]. Negative effects of TO1317 on cellular vitality and/or beta cell proliferation were however also reported and need attention when LXR agonists would be considered to treat type 2 diabetes [45,58,59].

We therefore examined TO1317±palmitate for a period up to 16 days; no adverse effects on cell viability and cell numbers were observed, and palmitate toxicity was suppressed by an average of 88% during the whole period. In this context it also needs to be mentioned that TO1317 was recently found, in parallel to SCD1, to induce CPT1 mRNA levels and to stimulate β-oxidation in INS1-cells [29], and in hepatocytes [60], whereas TO1317 showed the opposite effects in our experiments using primary rat beta cells.

These divergent results could relate to differences in experimental protocols such as FFA concentrations, FFA/BSA ratio’s, use of serum, or relate to differences in levels and duration of LXR-activation, but also point to differences between beta cell lines and primary cells. Lai et al. [61] recently postulated that the lower sensitivity of human beta cells and Min6 cells to palmitate toxicity as compared to INS-I cells, could be related to higher SCD1 levels.

In our experiments however, comparable cytotoxic responses to palmitate (250 and 500 μM) were found for FACS-purified rat, mouse and human beta cells. Comparison of the expression profiles of primary cells and cell lines in response to FA and agonists might reveal differences explaining these divergent observations. Cross talk between PPAR- and LXR-levels and competition to bind RXRs or with other nuclear receptors could play a role [62].

Busch et al. reported that palmitate-resistant Min6 cells were mainly characterized by an increase of SCD1 [20]. Our study cannot discriminate between the respective contribution of SCD1 or SCD2. Whereas SCD2 was more prevalent in freshly isolated beta cells, SCD1 was induced more potently by LXR and PPARα. SCD1 contains SREBP1c and LXR-responsive elements in its promoter region, whereas its expression is actively repressed by leptin [63,64]. Activation of SCD in animals lacking normal leptin signaling can thus be predicted to lower the cellular susceptibility to toxic palmitate concentrations. This hypothesis is supported by our observations on beta cells isolated from TO1317-treated animals and from Zucker fatty rats (lacking a functional leptin receptor), which were protected from palmitate but not oleate toxicity, and by a rise of palmitate toxicity in the presence of an SCD-inhibitor. The importance of SCD in beta cells is also documented by the loss of SCD1 in leptin−/− mice, leading to an accelerated progression to overt diabetes [65]. Absence of SCD in this model was accompanied by appearance of a morphologically distinct class of dysfunctional islets showing a suppression of PPARα and its target genes, increased triglycerides, FFA, and higher levels of saturated FA than a second class of islets displaying normal features [65]. These observations are also interesting in view of the differences in palmitate susceptibility and SCD1 expression levels observed in the high and low glucose-responsive beta cells, and in view of our prior work where it was shown that primary beta cells show differences in susceptibility or can even be resistant to palmitate toxicity [7]. Recent observations in leptin receptor-overexpressing obese db/db mice showed suppressed SREBP1c and adipogenic gene-expression levels, severe diabetes and beta cell loss 6 weeks before the db/db controls [66]. These findings support the idea that the inherent adipogenic phenotype of individual beta cells via chain elongation and desaturation provides the cells with a protective mechanism to cope with elevated FFA. Our observations contain an important message since SCD1-inhibitors are evaluated to treat obesity and the related metabolic syndrome [67,68]. Although suppression of SCD1 might show beneficial effects on adiposity, these benefits may come at the expense of pancreatic beta cells. Clinical studies already indicated an increased risk to develop diabetes in predisposed obese patients using a mixture of c9,t11 and t10,c12 CLA for weight management [69,70].

In conclusion, activation by PPAR and LXR agonists of a pathway controlled by LXR involving A9-desaturation, and chain elongation followed by esterification leads to detoxification of saturated FA and prevents beta cell death.

Methods

Materials

Chemicals and FA were purchased from Sigma-Aldrich (Bornem, Belgium). Stock solutions of FA were prepared in 90% ethanol. Stock solutions of clofibrate, TO1317, PCN and 9-cis RA were dissolved in absolute ethanol or DMSO. [U-13C]-palmitic acid was obtained from Perkin Elmer Life Sciences (Zaventem, Belgium).

Ethics statement

Animal experiments were approved by the local Ethical Committee for Animal Experimentation of the Vrije Universiteit Brussel. All manipulations were carried out in accordance with the European Community Council Directive (86/609/EEC). Human endocrine cells were obtained from the Brussels-Beta Cell Bank. Human pancreata from donors at European hospitals affiliated with Eurotransplant Foundation (Leiden, The Netherlands) are processed by the Beta-Cell Bank of the Juvenile Diabetes Research Foundation Center for Beta Cell Therapy in Brussels with the purpose of preparing islet cell grafts for clinical trials. Isolated fractions that do not fulfill the quality criteria for transplantation, can be made available to research projects approved by the Medical Ethics Committee of the University Hospital (UZ Brussel–Vrije Universiteit Brussel). Approval to use beta cells for this project was obtained from the Medical Ethics Committee of the UZ Brussels – Vrije Universiteit Brussel.

Isolation and culture of beta cell preparations

Pancreatic islets were isolated from 10 wks old male Wistar and Zucker rats (lean and fa/fa) and male LXR knock-out mice (8 to 12 wks), LXRα−/−, LXRβ−/−, and LXRαβ−/− mice were back-crossed from a 129/Sv to a C57BL/6 background for at least ten generations [71]. Rat and mouse islets were dissociated into single cells and purified by FACS into beta cells (mean purity 90% insulin-positive cells) and alpha enriched-cells (75% glucagon-positive cells, 25% insulin-positive cells) using cellular light-scatter and FAD autofluorescence as discriminating parameters. The methods for rodent islet isolation, dissociation and FACS purification of islet beta and alpha cells have been described previously [72]. In one set of experiments, rat beta cell subpopulations with, respectively, high and low glucose-responsiveness were FACS sorted using glucose-induced increases in cellular level of NAD(P)H-autofluorescence as discriminator, as described in our previous studies reporting on the identity and function of these cells [8,10,37]. Human endocrine cells were obtained from the Brussels-Beta Cell Bank and prepared as
described by Ling and Pipeleers [73]. These endocrine fractions were enriched by FACS to a mean purity of 60% beta cells and further enriched under culture conditions favoring beta cell survival.

For cell viability experiments, beta cells were cultured in polylysine-coated microtiter plates with Ham's F10 containing 10 mM glucose, 1% charcoal-extracted BSA (Fraction V, radioimmunoassay grade, Boehringer-Mannheim), 2 mM L-glutamine, 50 mM 3-isobutyl-1-methyl-xanthine, 0.075 mg/ml penicillin and 0.1 mg/ml streptomycin. Test reagents were added, with controls receiving similar dilutions of solvent. The unbound FFA concentrations in the presence of 1% albumin were calculated to correspond to 9 and 27 nM after addition of 250 and 500 μM palmitate using a multiple stepwise equilibrium model using the association constants for binding of palmitate to the first six binding sites of albumin, as described by Richieri et al. [74].

Media were refreshed every 2 to 3 days. The percent living and dead cells was determined as previously described and toxicity indices calculated [6]. For metabolic and gene expression studies, freshly isolated cells were re-aggregated and cultured in suspension [72].

Samples for electron microscopy were fixed in cacodylate-buffered glutaraldehyde (4.5%, pH 7.3), postfixed in osmium tetroxide (1%) and embedded in Spurr's resin. Ultrathin sections were stained with uranylacetate and lead citrate and examined on a Zeiss EM 109 electron microscope. Nile red (Molecular probes, Eugene, OR) were stained with uranylacetate and lead citrate and examined on 1% buffered glutaraldehyde (4.5%, pH 7.3), postfixed in osmium tetroxide (1%) and embedded in Spurr's resin. Ultrathin sections were stained with uranylacetate and lead citrate and examined on a Zeiss EM 109 electron microscope. Nile red (Molecular probes, Eugene, OR) was used to detect lipid accumulation in living cells (axioplan, Zeiss).

Palmitate oxidation was measured in KRH medium containing 0.2% BSA, 2 mM CaCl₂, 10 mM Hepes and 0.5 μCi [U-14C]-palmitic acid, and 250 μM unlabeled palmitic acid, as described prior [7]. The rate of [U-14C]-palmitic acid oxidation was assessed through formation of 14CO₂ [75].

LXR Agonist Treatment

Male Wistar rats (approx. 250 g), were treated with 40 mg/kg body weight/day of LXR agonist TO1317 (Sigma-Aldrich) dissolved in DMSO/PBS (1:3), given daily at the onset of the dark cycle by oral gavage for 5 days. Control rats were given only vehicle. After 5 days beta cells and alpha-enriched cells were FACS-purified and used for viability assays as described.

siRNA Transfection

Transfection was conducted in freshly isolated rat beta cells. Cells were aggregated in presence of 50 nM siRNA-lipid complexes using Jetsi-ENDO (Polyplus, France) as transfection agent [76]. siRNAs for rat SCD1 and SCD2 were siGENOME SMART pool reagents (Dharmacon), pools of four different siRNAs. Control cells were treated with Jetsi-ENDO only or transfected with non-targeting siGLO RISC-free (D-001600-01). Transfection did not result in cytotoxicity. After 24 h, aggregates were dissociated to single cells. Half of the cells were used in the toxicity assay with an additional 72 h culture; the other half was reaggregated and RNA extracted 24 h later. Over 95% of reaggregated siGLO-transfected beta cells showed cytoplasmic siRNA-associated fluorescence.

Gene, protein and lipid analysis of beta cell aggregates

RNA was extracted using RNeasy columns (Qiagen). RNA quality was verified by Agilent Bioanalyzer. Following removal of genomic DNA (TURBO DNA-free, Ambion, Austin, Texas, U.S.A.) and reverse-transcription (High-Capacity cDNA Archive Kit, Applied Biosystems, Foster City, U.S.A.), targets were amplified on ABI Prism 7700 using TaqMan Universal PCR Master Mix and specific TaqMan MGB probes (Applied Biosystems, assays’ IDs available on request). Expression levels of target genes were normalized to β-actin or 18S (ΔΔCt) and expressed versus a chosen calibrator (comparative ΔΔCt method).

Beta cell aggregates were lysed in RIPA buffer containing protease inhibitors (Sigma). Proteins were resolved by SDS-PAGE on 10% (w/v) acrylamide: bisacrylamide gels and transferred to nitrocellulose membranes using iblot (Invitrogen, Belgium). Immunodetection of SCAD was performed using mouse anti-SCD1/2 antibodies (Abcam). Hsp70 served as housekeeping control (Santa Cruz Biotechnology, CA, USA). Horseradish peroxidase-linked secondary antibodies (Santa Cruz Biotechnology) were used. Immunoreactive proteins were visualized by enhanced chemiluminescence (ECL, Pierce).

Lipid extracts were prepared from beta cell aggregates, fortified with 10 nmol tricosanoic acid (IS), dried and treated with 0.5 ml 0.5 N HCl in 90% acetonitrile at 100°C for 1 hr [77]. After adding water, released FA were extracted in 2x1 ml hexane, and an aliquot of the aqueous phase was analyzed for phosphate to estimate phospholipid content. The hexane extract was dried, silylated with N-trimethylsilyl-N-methyl trifluoroacetamide with 1% tert butyldimethyl chlorosilane (Pierce/pyridine (1:1, v/v) and analyzed by GC-MS (Trace GC-MS, Thermo Finnigan), equipped with an automatic cold-on-column injector and high oven temperature device connected to a RXP70 column (30 m x 0.25 mm; 0.25 μm). Fatty acids were identified by comparing elution times to standards and by their mass spectrum. Total ion current signals, obtained in EI+ mode at 35 eV, were related to the IS signal and converted to nmol fatty acid using relative response factors by analyzing a reference fatty acid mixture containing tricosanoic acid (GLC-538, free acids; Nu-Chek Prep), derivatized like the extracts.

Data analysis

Data are presented as means±SD of n independent experiments. Single comparisons were performed by the Student’s paired t-test. For multiple comparisons, data were analyzed by ANOVA, and group comparisons using Student’s paired or unpaired t test, with correction of the P values for multiple comparisons by the Bonferroni method.

Supplementary Information

Figure S1 Effect of inhibition of SCD on GC-MS fatty acid profiles. Beta cells were exposed for 8 days to 250 μM-P±1 μM TO1317±10,c12 CLA (40 μM). Lípid extracts were prepared and analyzed by GC-MS as described. a) Representative chromatograms, covering the elution of C14:0 till C22:6. Figure representative for 6 independent analyses. b) The ratios between the saturated and monounsaturated FA were calculated. Mean±SE, n = 5 - 6, * P<0.05, ** P<0.01 versus control, $ P>0.05, $<P 0.01 versus palmitate

Found at: doi:10.1371/journal.pone.0007266.s001 (1.47 MB TIF)

Table S1 Effect of TO1317 on cytotoxicity of FA with different chain length and saturation. Beta cells were exposed for 2 or 8 days to the indicated FA±1 μM TO1317. Mean±SD (n>4), # P<0.001 toxicity of different FA compared to C16:0; * P<0.05, *** P<0.001, the TO1317 condition compared to the respective control FA condition.

Found at: doi:10.1371/journal.pone.0007266.s002 (0.04 MB DOC)

Table S2 mRNA expression levels in islets from LXR KO mice relative to wild type. qPCR values normalized against 18S and compared relative to the expression levels in wild type islets.
Table S3 mRNA expression levels in beta cells from Zucker obese rats relative to lean controls. qPCR values normalized against β-actin and expressed relative to the expression levels in beta cells from Zucker lean controls. Unpaired student t-test, two tailed, mean±SD, n = 3, Differences were considered significant with p<0.01, ** p<0.001 and *** p<0.0001, compared to wild type control. ND = not detected

Acknowledgments

The authors thank the cell preparation team at the Diabetes Research Center and at the Beta Cell Bank for providing the preparations used in this study. They acknowledge the expert technical assistance of K. Suennens, L. Heylen and G. Stangé. Electron microscopy was performed by M. Berghmans at the Unit of Experimental pathology at the Vrije Universiteit Brussel.

Author Contributions

Conceived and designed the experiments: KHH KRS. Performed the experiments: KHH JCH KRS CR EDV PVV. Analyzed the data: KHH BD KRS GM PVV. Contributed reagents/materials/analysis tools: KHH KRS PVV JG DP. Wrote the paper: KHH. Contributed to writing the paper: KRS GM JAG DP.

References

1. Donath MY, Elhes JA, Maedler K, Schumann DM, Ellingsgaard H, et al. (2005) Mechanisms of beta-cell death in type 2 diabetes. Diabetes 54 Suppl 2: S108–S113.
2. Kaiser N, Leibowitz G (2009) Failure of beta-cell adaptation in type 2 diabetes: Lessons from animal models. Front Biosci 14: 1099–1115.
3. Pouto V, Robertson RP (2008) Glucolipotoxicity: fuel excess and beta-cell dysfunction. Endocr Rev 29: 351–366.
4. Cnop M (2008) Fatty acids and glucolipotoxicity in the pathogenesis of Type 2 diabetes. Biochem Soc Trans 36: 348–352.
5. Prenkli M, Joly E, El Assaad W, Rochuit R (2002) Malonyl-CoA signaling, lipid partitioning, and glucolipotoxicity: role in beta-cell adaptation and failure in the etiology of diabetes. Diabetes 51 Suppl 3: S405–S413.
6. Cnop M, Hannae JCT, Hoorians A, Ezizki D, Pippeeres D (2001) Inverse relationship between cytotoxicity of free fatty acids in pancreatic islet cells and cellular triglyceride accumulation. Diabetes 50: 1771–1777.
7. Hellemans K, Kredkovs H, Hannae JCT, Martens G, Van Veldhoven P, et al. (2007) Peroxisome proliferator-activated receptor alpha-retinoid X receptor agonists induce beta-cell protection against palmitate toxicity. FEBS J 274: 605–615.
8. Martens GA, Cai Y, Hinke S, Stange G, Van De Casteele M, et al. (2005) Glucose suppresses superoxide generation in metabolically responsive pancreatic beta-cells. J Biol Chem 280: 20389–20396.
9. Hoorens A, Van De Casteele M, Kloppel G, Pipeleers D (1996) Glucose promotes survival of rat pancreatic beta cells by activating synthesis of proteins which suppress a constitutive apoptotic program. J Clin Invest 98: 1568–1574.
10. Kirkens R, Ine Veld P, Mahler T, Schuit F, van de Winkel M, et al. (1992) Differences in glucose recognition by individual rat pancreatic B cells are associated with intercellular differences in glucose-induced biosynthetic activity. J Clin Invest 89: 117–125.
11. Borgen J, Klimn C, Wierup N, Larsen S, et al. (2009) Perilipin is present in islets of Langerhans and protects against lipotoxicity when overexpressed in the beta-cell line INS-1. Endocrinology 150: 3049–3057.
12. Maedler K, Oberholzer J, Buher P, Spinna GA, Donath MY (2003) Monounsaturated fatty acids prevent the deleterious effects of palmitate and high glucose on human pancreatic beta-cell turnover and function. Diabetes 52: 726–733.
13. Eitel K, Staiger H, Bredenel MD, Brandhorst D, Breetzel RG, et al. (2002) Different role of saturated and unsaturated fatty acids in beta-cell apoptosis. Biochim Biophys Acta Res Commun 299: 835–840.
14. Diakonogianaki E, Morgan NG (2008) Differential regulation of the ER stress response by long-chain fatty acids in the pancreatic beta-cell. Biochem Soc Trans 36: 959–962.
15. Sol EM, Sargsyan E, Akusjarvi G, Bergsten P (2008) Glucolipotoxicity in the beta cells from Zucker lean controls. Unpaired student t-test, two tailed, mean±SD, n = 3, Differences were considered significant with ** p<0.001 and *** p<0.0001, compared to wild type control. ND = not detected

Found at: doi:10.1371/journal.pone.0007266.s003 (0.05 MB DOC)
41. Lafitte RA, Chao LC, Li J, Walczak R, Hummasti S, et al. (2003) Activation of liver X receptor improves glucose tolerance through coordinate regulation of glucose metabolism in liver and adipose tissue. Proc Natl Acad Sci U S A 100: 5419–5424.

42. Cao G, Liang Y, Broderick CL, Oldham BA, Beyrer TP, et al. (2007) Antidiabetic action of a liver X receptor agonist mediated by inhibition of hepatic gluconeogenesis. J Biol Chem 278: 1131–1136.

43. Kumadaki S, Matsuzaka T, Kato T, Yahagi N, Yamamoto T, et al. (2008) Mouse Elovl6 promoter is an SREBP target. Biochem Biophys Res Commun 368: 261–266.

44. Shimanou H (2009) SREBP: Physiology and pathophysiology of the SREBP family. FEBS J 276: 610–621.

45. Choe SS, Choi AH, Lee JW, Kim KH, Chung JJ, et al. (2007) Chronic activation of liver X receptor induces beta-cell apoptosis through hyperactivation of lipopgenic liver X receptor-mediated lipotoxicity in pancreatic beta-cells. Diabetes 56: 1534–1541.

46. Morgan NG, Dhaval S, Diakogiannaki E, Welters HJ (2008) The cytoprotective actions of long-chain mono-unsaturated fatty acids in pancreatic beta-cells. Biochem Soc Trans 36: 905–908.

47. Francis GA, Fayard E, Picard F, Anwer XJ (2003) Nuclear receptors and the control of metabolism. Annu Rev Physiol 65: 261–311.

48. Lalloyer F, Vandewalle B, Percevault F, Torpier G, Kerr-Conte J, et al. (2006) Peroxisome proliferator-activated receptor alpha improves pancreatic adaptation to insulin resistance in obese mice and reduces lipotoxicity in human islets. Diabetes 55: 1603–1613.

49. Qin Y, Dallen KT, Gustafsson JA, Nebb HI (2009) Regulation of hepatic fatty acid elongase 3 by LXRalpha-SREBP-1c. Biochim Biophys Acta 1791: 140–147.

50. Diraison F, Ravier MA, Richards SK, Smith RM, Shimano H, et al. (2008) SREBP1 is required for the induction of glucose by pancreatic beta-cell genes involved in glucose sensing. J Lipid Res 49: 814–822.

51. Diraison F, Parent L, Ferre P, Fournie F, Briscos CP, et al. (2004) Overexpression of steroid-regulatory-element-binding protein-1c (SREBP1c) in rat pancreatic islets induces lipogenesis and decreases glucose-stimulated insulin release: modulation by 5-aminooximolizole-4-carboxamide ribonucleoside (AlCA). Biochem J 378: 769–778.

52. Wang H, Kouri G, Wollheim CB (2005) ER stress and SREBP-1 activation are implicated in beta-cell glucolipotoxicity. J Biol Chem 280: 21134–21144.

53. Shimano H, Amemiya-Kudo M, Takahashi A, Kato T, Ishikawa M, et al. (2007) Activation of liver X receptor induces beta-cell apoptosis through hyperactivation of lipopgenic liver X receptor-mediated lipotoxicity in pancreatic beta-cells. Cell Metab 4: 143–154.

54. Morgan NG, Dhaval S, Diakogiannaki E, Welters HJ (2008) The cytoprotective actions of long-chain mono-unsaturated fatty acids in pancreatic beta-cells. Biochem Soc Trans 36: 905–908.

55. Takahashi A, Motomura K, Kato T, Yoshikawa T, Nakagawa Y, et al. (2005) Transgenic mice overexpressing nuclear SREBP-1c in pancreatic beta-cells. Diabetes 54: 492–499.

56. Kato T, Shimano H, Yamamoto T, Ishikawa M, Kumaishi S, et al. (2008) Palmitate impairs and eicosapentaenoate restores insulin secretion through regulation of SREBP-1c in pancreatic islets. Diabetes 57: 2392–2392.

57. Parent LE, McMillen PJ, Shen Y, Docherty E, Sharpe E, et al. (2006) Limited role for SREBP-1c in defective glucose-induced insulin secretion from Zucker diabetic fatty rat islets: a functional and gene profiling analysis. Am J Endocrinol Metab 291: E540–E550.

58. Werny W, Bremer MB, Zitter H, Gromada J, Efimov AM (2007) Activation of liver X receptors and retinoid X receptors induces growth arrest and apoptosis in insulin-secreting cells. Endocrinology 148: 1843–1849.

59. Meng ZX, Ne J, Jing JF, Sun JX, Zha YX, et al. (2009) Activation of liver X receptors inhibits pancreatic islet beta cell proliferation through cell cycle arrest. Diabetologia 52: 125–135.

60. Hu T, Foxworthy P, Sieszky A, Ficorilli JV, Gao H, et al. (2005) Hepatic peroxisomal fatty acid beta-oxidation is regulated by liver X receptor alpha. Endocrinology 146: 5380–5387.

61. Lai E, Bikoopoulos G, Wheeler MB, Rozakio-Adcock M, Volchuk A (2006) Differential activation of ER stress and apoptosis in response to chronically elevated free fatty acids in pancreatic beta-cells. Am J Physiol Endocrinol Metab 294: E540–E550.

62. Ide T, Shimano H, Yoshikawa T, Yahagi N, Amemiy-i-Kudo M, et al. (2003) Cross-talk between peroxisome proliferator-activated receptor (PPAR) alpha and liver X receptor (LXR) in nutritional regulation of fatty acid metabolism. II. LXRs suppress lipid degradation gene promoters through inhibition of PPAR signaling. Mol Endocrinol 17: 1253–1267.

63. Chu K, Miyazaki M, Man WC, Ntambi JM (2006) Srearo1-coenzyme A desaturase-1 deficiency protects against hypertriglyceridemia and increases plasma high-density lipoprotein cholesterol induced by liver X receptor activation. Mol Cell Biol 26: 6786–6796.

64. Kakuma T, Lee Y, Unger RH (2002) Effects of lepin, trolgizazone, and dietary fat on srearo1 CoA desaturase. Biochim Biophys Res Commun 297: 1259–1263.

65. Flowers RB, Rahaglia ME, Schueler KL, Flowers MT, Lan H, et al. (2007) Loss of srearo1-Coa desaturase-1 improves insulin sensitivity in lean mice but worsens diabetes in leptin-deficient obese mice. Diabetes 56: 1228–1239.

66. Wang MY, Grayburn P, Chen S, Ravazza M, Orci L, et al. (2008) Adipogenic capacity and the susceptibility to type 2 diabetes and metabolic syndrome. Proc Natl Acad Sci U S A 105: 6139–6144.

67. Gutierrez-Juarez R, Pocai C, Malas C, Ono H, Bhanot S, et al. (2006) Critical role of srearo1-Coa desaturase-1 (SCD1) in the onset of diet-induced hepatic insulin resistance. J Clin Invest 116: 1686–1695.

68. Jiang G, Li Z, Liu F, Ellsworth K, Dallas-Yang Q, et al. (2005) Prevention of obesity in mice by antifein enoluroleide inhibitors of srearo1-Coa desaturase-1. J Clin Invest 115: 1030–1038.

69. Moloney F, Ycwo TP, Mullen A, Nolan JJ, Roche HM (2004) Conjugated linoleic acid supplementation, insulin sensitivity, and lipidrotein metabolism in patients with type 2 diabetes mellitus. Am J Clin Nutr 80: 887–895.

70. Riener U, Amilof I, Berglund I (2007) Long-term predictors of insulin resistance: role of lifestyle and metabolic factors in middle-aged men. Diabetes Care 30: 2920–2923.

71. Steffenen KR, Nilsson M, Schuster GU, Stuhling TM, Dahlan-Wright K, et al. (2003) Gene expression profiling in adipose tissue indicates different transcriptional mechanisms of liver X receptors alpha and beta, respectively. Biochim Biophys Acta 1310: 509–593.

72. Pipellees DG, Ii Ycvell PA, van de Winkel M, Maes E, Schuit FC, et al. (1985) A new in vitro model for the study of pancreatic A and B cells. Endocrinology 117: 806–816.

73. Lrnt Z, Pipellees DG (1996) Prolonged exposure of human beta cells to elevated glucose levels results in sustained cellular activation leading to a loss of glucose regulation. J Clin Invest 98: 2005–2012.

74. Richier GV, Anel A, Kleinfeld AM (1993) Interactions of long-chain fatty acids and albumin: determination of free fatty acid levels using the fluorescent probe ADIFAB. Biochemistry 32: 7574–7580.

75. Schuit F, De Vos A, Fabari S, Morsen K, Pipellees D, et al. (1997) Metabolic fate of glucose in purified islet cells. Glucose-regulated anaplerosis in beta cells. J Biol Chem 272: 10572–10579.

76. Martens GA, Vervoot A, Van De Castelee M, Stange G, Hellemans K, et al. (2007) Specificity in beta cell expression of L-3-hydroxylacyl-CoA dehydrogenase, shortchain (HADHSC) and potential role in down-regulating insulin secretion. J Biol Chem 282: 21314–2144.

77. Van Veldhoven PP, Bell RM (1988) Effect of harvesting methods, growth conditions and growth phase on dodecylglycerol levels in cultured human adherent cells. Biochim Biophys Acta 939: 185–196.