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Oleate rescues INS-1E β-cells from palmitate-induced apoptosis by preventing activation of the unfolded protein response

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ABSTRACT

Background: Saturated free fatty acids (FFAs), such as palmitate, cause β-cell apoptosis whereas unsaturated FFAs, e.g. oleate, are not harmful. The toxicity of palmitate could be mediated through endoplasmic reticulum (ER) stress which triggers the activation of a signal responding cascade also called unfolded protein response (UPR). We investigated whether or not palmitate induced β-cell apoptosis through UPR activation and whether or not oleate as a monounsaturated fatty acid could counteract these effects.
Methods: INS-1E β-cells were incubated with palmitate [0.5 mM], oleate [1 mM] or the combination [0.5/1 mM] for 1, 6 and 24 h. Viability and induction of apoptosis were measured by WST-1 assay and FITC-Annexin/PI-staining, respectively. Western blot analyses were performed for UPR specific proteins and mRNA expression of target molecules was determined by qPCR.
Results: Palmitate significantly decreased viability (29 ± 8.8%) of INS-1E β-cells compared to controls after 24 h. Stimulation with oleate showed no effect on viability but the combination of oleate and palmitate improved viability compared to palmitate treated cells (55 ± 9.3%) or controls (26 ± 5.3%). The number of apoptotic cells was increased 2-fold after 24 h incubation with palmitate compared to controls. Again, oleate showed no effect but in combination ameliorated the effect of palmitate to control level. Phosphorylation of eIF2α was increased after 6 and 24 h incubation with palmitate. In contrast, oleate had no effect and in combination prevented phosphorylation of eIF2α. Increased Xbp1 splicing was visible already 6 h after palmitate treatment and remained elevated at 24 h. The combination with oleate abolished Xbp1 splicing. Interestingly, mRNA expression of the chaperones Bip, Pdi, Calnexin and Grp94 was not altered by FFA treatment. Only the proapoptotic transcription factor Chop was significantly enhanced by palmitate incubation. In accordance with sustained cell survival the combination as well as oleate alone, did not result in increased Chop levels compared to controls. In summary, we showed that oleate protects INS-1E β-cells from palmitate-induced apoptosis by the suppression of ER stress which was independent of chaperone activation.

1. Introduction

The increasing prevalence of type 2 diabetes represents a serious worldwide health problem. Free fatty acids (FFAs) have emerged as an interesting target to explain different aspects in the development of type 2 diabetes as especially saturated fatty acids (SFA) promote insulin resistance and lead to β-cell apoptosis, a phenomenon also called lipotoxicity [1,2]. The plasma concentration of FFAs varies between 400 and 800 μmol/l [1]. Higher levels are associated with greater risk to develop diabetes [3–5]. Especially the amount of saturated or unsaturated fatty acids seems to influence glycaemic control. Whereas diets rich in SFAs were reported to result in impaired insulin sensitivity, high monounsaturated fatty acid (MUFA) intake did not negatively affect it or even improved insulin sensitivity [4,6–10]. The main component of

Abbreviations: ATF6, activating-transcription-factor 6; Bip/Grp78, immunoglobulin heavy-chain binding protein; Chop, C/EBP homologous protein; Grp94, glucose-regulated protein 94 kDa; eIF2α, eukaryotic initiation factor 2α; ER, endoplasmic reticulum; FFA, free fatty acid; IRE1, inositol-requiring-enzyme1; MUFA, monounsaturated fatty acid; Pdi, protein disulfide isomerase; PERK, protein-kinase-regulated-by-RNA-like-ER-associated-kinase; SCD1, stearoyl-CoA desaturase 1; SFA, saturated fatty acid; UPR, unfolded protein response; Xbp1, X-box-binding protein 1.

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MUFA in food is oleic acid. Its principal sources are olive oil, nuts and seeds. SFAs, e.g. palmitic acid, are found in animal and dairy fats [8]. The mechanism by which FFAs exercise their adverse effects is not fully understood. Increasing stress in the endoplasmic reticulum (ER) has been proposed to mediate the toxicity and can lead to apoptosis. The ER plays an important role in β-cells. It is responsible for calcium storage as well as protein folding and secretion [11,12]. When stress occurs in the ER the unfolded protein response (UPR), a stress responding cascade, is activated. The UPR is beneficial for the preservation of homeostasis but can as well induce apoptosis when stress is irresolvable [11,12]. The UPR consists of three main pathways. They are named ATF6, PERK and IRE1, which are transmembrane ER proteins. After dissociation of the chaperone Bip (immunoglobulin heavy-chain binding protein) from their luminal side these UPR pathways become activated. Bip dissociates when unfolded proteins accumulate in the lumen of the ER or other stressors perturb homeostasis [12–14]. An example for the importance of the UPR in humans is the Wolcott–Rallison syndrome (WRS). It is a rare autosomal recessive disease, characterized by early onset diabetes associated with skeletal dysplasia and growth retardation. A mutation in the gene encoding for PERK was found in patients with WRS [11–13]. Given the link between ER dysfunction and diabetes we asked whether or not saturated FFAs induced the activation of the UPR ultimately leading to β-cell apoptosis and lipotoxicity and whether or not oleate as an unsaturated fatty acid could counteract the toxic effects of palmitate by preventing the activation of the UPR.

2. Materials and methods

2.1. Cell culture

The rat insulinoma cell line INS-1E was a generous gift from Prof. Claes Wollheim, Geneva. Cells were cultured in RPMI 1640 medium containing 25 mM Hepes and 300 mg/l glutamine (PAA Laboratories, Pasching, Austria) additionally supplemented with 2 mM glutamine (PAA), 1 mM Pyruvate (Gibco, Carlsbad, California), 5% FCS (PAA) and 50 μM β-mercaptoethanol (Sigma, Munich, Germany) at 37 °C in an atmosphere of 5% CO_2. After seeding cells were maintained for 72 h in serum containing medium. 24 h before stimulation medium was changed to serum free medium (SFM) containing 0.2% BSA (Gibco).

2.2. FFA preparation

Oleate and palmitate (Sigma) were dissolved in 0.1 M NaOH to a concentration of 100 mM heated to 70 °C, and diluted in RPMI medium containing 10% fatty acid free BSA (Serva Heidelberg, Germany) to a stock solution of 5 mM. Working solution of 1 mM oleate, 0.5 mM palmitate and 1% fatty acid free BSA medium was always freshly prepared by dilution in RPMI medium (PAA Laboratories).

2.3. Cell viability and apoptosis

Cells were stimulated with 0.5 mM palmitate, 1 mM oleate or the combination of both, 1 μM thapsigargin (Sigma), camptothecin 2 μM (Sigma) and 85 μM etoposide (Calbiochem, Merck KGaA, Darmstadt, Germany) for 1, 6 or 24 h. Viability was measured by WST-1 assay (Roche, Mannheim, Germany) according to manufacturer's instruction. Apoptosis was assessed by a FITC-AnnexinV (An) and propidium iodide (PI) staining kit (BD, Heidelberg, Germany) followed by flow cytometric analysis. An-positive and double-stained An/PI positive cells were considered as apoptotic.

2.4. Protein extraction and Western blotting

For Western blot analyses cells were incubated as described above and lysed in RIPA-buffer containing 50 mM Tris–HCl, 1% NP-40, 0.25% Na-deoxycholate, 150 mM NaCl, 1 mM EDTA, 1 mM vandate, 1 mM NaF and Roche complete (Roche). Protein content was determined by BCA assay (Thermo Scientific, Waltham, Massachusetts). Equal amounts of protein were loaded on 10% SDS gels and semidry transferred to 0.45 μm nitrocellulose membranes (Biostep, Jahnsdorf, Germany). Primary antibodies P-eIF2α (#9721), eIF2α (#9722), Bip (#3183) were purchased from Sigma or Millipore (GAPDH (#MAB374)) and used as indicated. Secondary antibodies were purchased from Dako (Glostrup, Denmark). Specific bands were detected with the chemiluminescence substrate Classico (Millipore) or an ECL Advance Western Blotting Detection Kit (GE Healthcare). Images were analysed densitometrically using ImageJ software.

2.5. RNA extraction, quantitative real time PCR and TaqMan

After stimulation RNA was extracted using the RNeasy Mini kit (Qiagen, Hilden, Germany). RNA was reverse transcribed using M-MLV (Invitrogen, Carlsbad, California, USA). Taqman and SybrGreen analyses were performed using qPCR mastermix Low Rox (Eurogentec, Belgium) and SybrGreen mastermix absolute blue (Thermo Scientific). For standardization, expression of target gene was normalised to the expression of TATA-box-binding protein (Tbp) in each sample. For detection of the spliced form of *Xbp1* the PCR product was digested with 10–15 U PstI (Fermentas, Thermo Fisher Scientific) for 4 h and separated on a 2% agarose gel. PstI cuts only the unspliced fraction of *Xbp1* allowing a differentiation between the unspliced and spliced transcript. Primers are summarized in S1 (Supplementary Material).

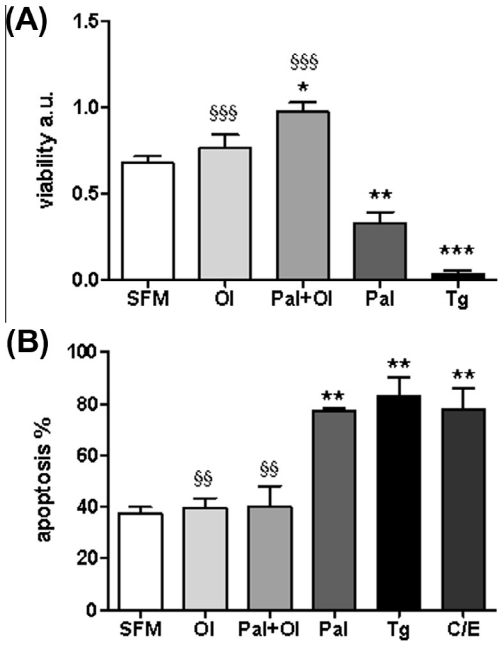

Fig. 1. Oleate rescues INS-1E cells form palmitate-induced apoptosis and restores viability. INS-1E β-cells were treated with serum free medium (SFM), palmitate (Pal) [0.5 mM], oleate (Ol) [1 mM] or the combination of both (Pal + Ol) [0.5/1 mM], thapsigargin (Tg) [1 μM] and camptothecin [2 μM] and etoposide [85 μM] (C/E) for 24 h. (A) Viability was determined by WST-1 assay and (B) apoptosis was determined as Annexin-positive and double-stained Annexin/Propidium Iodide positive cells by flow cytometric analysis. The results are shown in arbitrary units (a.u.). Data represent four to seven independent experiments performed sixfold (WST) or in duplicates (apoptosis) shown as means ± SEM $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ compared to control, $^{§§}p < 0.01$ $^{§§§}p < 0.001$ compared to palmitate.

2.6. Statistical analyses

Significances were determined using GraphPad Prism5 software and one-way or two-way ANOVA with Bonferronis Multiple Comparison Test as posthoc test. Significance was defined as $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ compared to control; $^{\S}p < 0.05$; $^{\S\S}p < 0.01$; $^{\S\S\S}p < 0.001$ compared to palmitate. Data represent means ± SEM of three or more independent experiments.

3. Results

3.1. Oleate rescues INS-1E β-cells from palmitate-induced apoptosis and restores viability

To analyse the different effects of saturated and unsaturated FFAs on β-cells, INS-1E β-cells were treated with palmitate [0.5 mM], oleate [1 mM] or the combination of both [0.5/1 mM]

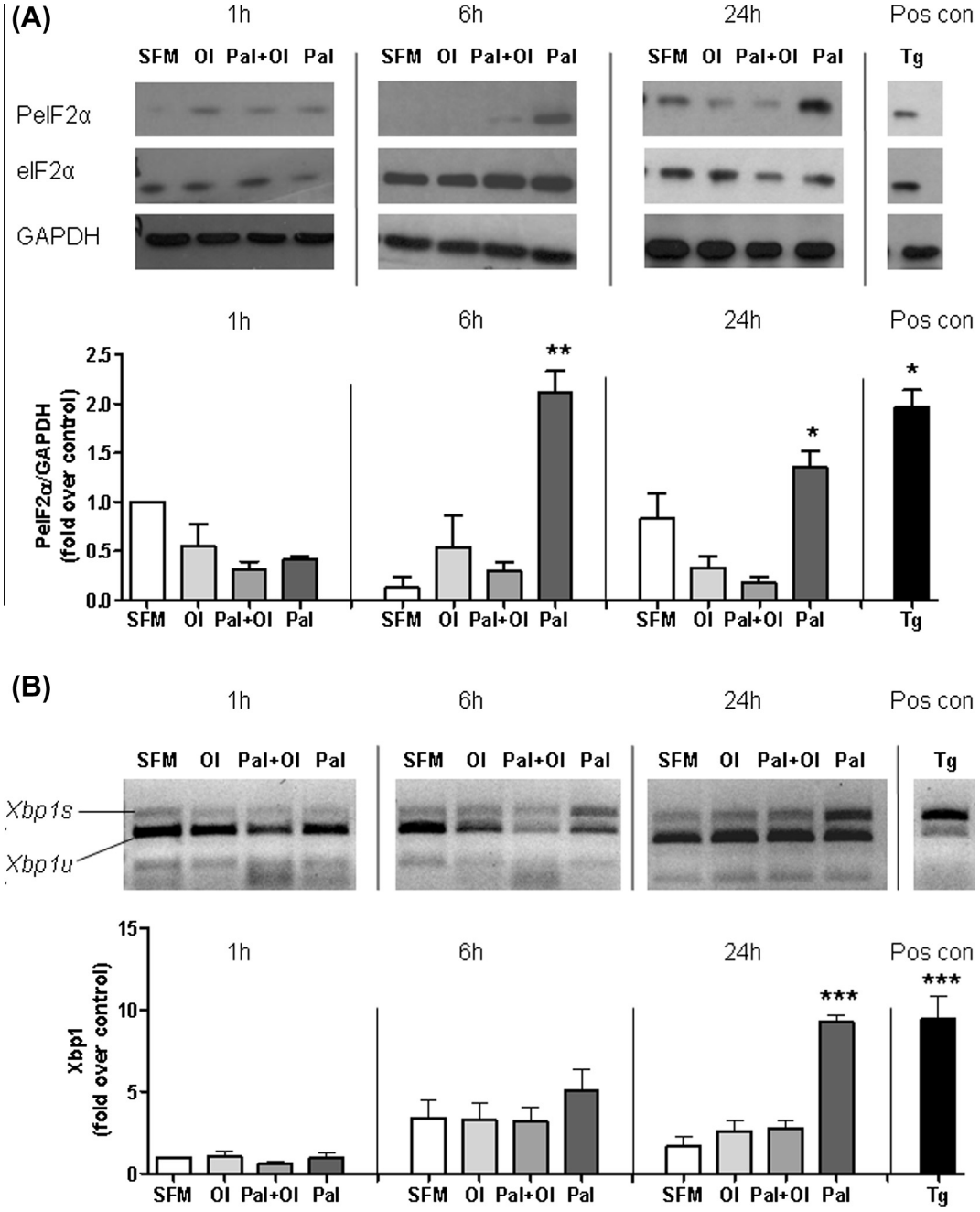

Fig. 2. (A) Palmitate-induced phosphorylation of eIF2α is counteracted by oleate. Western Blot analyses of eIF2α and phosphor-eIF2α were performed after 1 , 6 and 24 h incubation with serum free medium (SFM), palmitate (Pal) [0.5 mM], oleate (Ol) [1 mM] and the combination (Pal + Ol) [0.5/1 mM]. Thapsigargin (Tg) [1 μM] at 1 h represents the positive control for ER stress. One representative blot out of 4 independent experiments is shown. Blots were analysed densitometrically and normalised to GAPDH and the mean of SFM at 1 h which was set 1. Results are shown as means ± SEM $^{*}p < 0.05$; $^{**}p < 0.01$ compared to control. (B) Oleate prevents splicing of Xbp1. *Xbp1* mRNA was amplified after 1, 6 and 24 h treatment with serum free medium (SFM), palmitate (Pal) [0.5 mM], oleate (Ol) [1 mM], the combination (Pal + Ol) [0.5/1 mM] and thapsigargin (Tg) [1 μM]. The product was digested with PstI. The unspliced (non-stressed) variant of *Xbp1* results in two smaller fragments of 311 bp and 141 bp (*Xbp1u*) whereas the spliced (stressed) form is larger with 426 bp (*Xbp1s*). GAPDH was used as loading control. The picture is one representative result out of three independent experiments. The blots were analysed densitometrically and are presented as fold over control with SFM at 1 h set 1. Results are shown as means ± SEM $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ compared to control.

for 1, 6 and 24 h. Thapsigargin [1 μM], a well-established ER stress inducer, was employed as positive control. Palmitate significantly decreased viability (34.6 ± 7.2%) of INS-1E β-cells compared to cells treated with serum-free medium (SFM) after 24 h incubation (Fig. 1A). In contrast, viability was not changed after 6 h incubation with palmitate (data not shown). Stimulation with oleate showed no effect on viability but the combination of oleate and palmitate improved viability compared to palmitate treated cells (64.4 ± 8.3%) or controls (29.8 ± 6.9%) at 24 h (Fig. 1A). The number of apoptotic cells was increased 2-fold after 24 h incubation with palmitate compared to control cells (Fig. 1B). Again, oleate did not alter the level of apoptosis but in combination completely ameliorated the effect of palmitate. Apoptosis was not detected after 6 h incubation with palmitate (data not shown). In accordance with other publications [6,15–17] 24 h incubation with palmitate [0.5 mM] significantly reduced the ratio of glucose stimulated insulin secretion (GSIS) to basal secretion when INS-1 β-cells were challenged with 3 mM glucose or 20 mM glucose, respectively ($p < 0.05$). In contrast cells stimulated with oleate or the combination maintained a stimulatory index comparable to controls (S3).

3.2. Palmitate-induced phosphorylation of eIF2α is counteracted by oleate

To investigate whether the cytotoxic effects of palmitate are mediated by ER stress we determined activation levels of the main UPR pathways. PERK activates its main target eIF2α through phosphorylation [11,14]. In Western blot analyses we examined the phosphorylation state of eIF2α. Phosphorylation of eIF2α was significantly increased after 6 h (+112 ± 18%) and 24 h (+35 ± 17%) incubation with palmitate, but diminished to control levels after stimulation with the combination of palmitate and oleate. In contrast, oleate alone did not enhance eIF2α phosphorylation. Thapsigargin as ER stress inducer significantly increased phosphorylation of eIF2α to 2.0 ± 0.2-fold (Fig. 2A).

3.3. Oleate prevents splicing of Xbp1

After activation IRE1 cleaves Xbp1 mRNA producing an alternatively spliced variant [11,14]. To determine the splicing rate of Xbp1 we employed a technique recently described by Toda et al. [18]. Xbp1 mRNA was amplified and afterwards digested by Pst1. The Pst1 recognition site is retained only in the unspliced variant. Thus, the unspliced (non-stressed) variant of Xbp1 results in two smaller fragments of 311 bp and 141 bp whereas the spliced (stressed) form is larger with 426 bp. 6 h treatment with palmitate tended to increase splicing of Xbp1. After 24 h incubation splicing was elevated to 8.2 ± 0.4-fold whereas the combination of palmitate and oleate abolished the effects of palmitate. Again, oleate alone had no effect (Fig. 2B).

3.4. Incubation with palmitate and oleate does not influence Bip expression level

To measure the activation of the ATF6 pathway we performed analyses of mRNA and protein expression of Bip as one of the main targets of this pathway [11,19–21]. Interestingly, neither palmitate and oleate nor thapsigargin significantly altered Bip protein (Fig. 3A) or mRNA expression levels (Fig. 3B).

3.5. Oleate antagonizes palmitate-mediated upregulation of Chop but does not alter chaperone levels

We further wanted to investigate the regulation of downstream targets of the UPR pathways. Therefore we performed mRNA expression analyses of the main chaperones *Grp94*, *Pdi* and *Calnexin*. These chaperones represent targets of the IRE1 and ATF6 pathway [12,19]. Besides a slight but not persistent upregulation of *calnexin* after 1 h of palmitate treatment (Fig. 4C), chaperone levels were unaltered by FFA incubation (Fig. 4A and B). In contrast, palmitate significantly induced mRNA expression of the proapoptotic transcription factor *Chop* to 1.6 ± 0.5-fold after 6 h and to 2.8 ± 0.5-fold after 24 h. (Fig. 4D). *Chop* expression remained

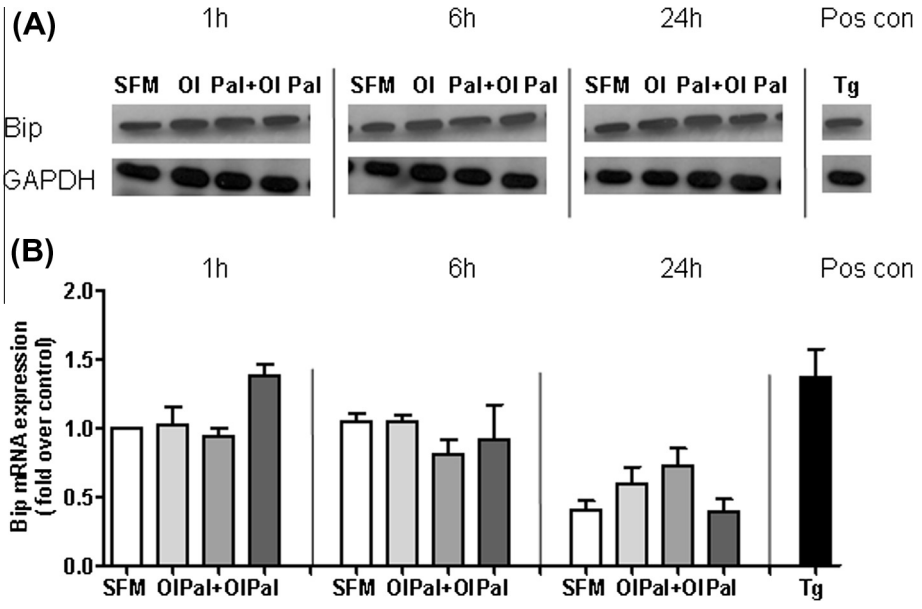

Fig. 3. Incubation with palmitate and oleate does not influence Bip expression level. (A) Western Blot analyses of Bip were performed three times after 1, 6 and 24 h incubation with serum free medium (SFM), palmitate (Pal) [0.5 mM], oleate (Ol) [1 mM], the combination (Pal + Ol) [0.5/1 mM] and thapsigargin (Tg) [1 μM]. One representative blot is shown. (B) *Bip* mRNA expression was quantified by qPCR and normalised to *Tbp*. The expression is presented as fold over control with SFM at 1 h set 1. The data represents means ± SEM of three independent experiments.

constant during oleate treatment compared to controls. The combination of oleate and palmitate was able to restore *Chop* mRNA to basal levels (Fig. 4D).

4. Discussion

ER stress has emerged as an interesting target contributing to the decline in β-cell mass during the development of diabetes. Elevated ER stress markers in islets of patients with type 2 diabetes compared to healthy subjects have been reported [22,23]. Accumulation of misfolded proteins, perturbation of ER calcium flow and production of reactive oxygen species (ROS) were all discussed to induce ER stress [13,14]. We wanted to analyse whether or not the effects of palmitate and oleate were also mediated by the UPR.

In accordance with several other studies we showed that the detrimental effects of the saturated fatty acid palmitate were completely reversed by addition of the unsaturated fatty acid oleate [17,24–28]. We further asked whether the differences observed were caused by a distinct activation of the UPR. We found that palmitate activated the PERK and IRE1 pathway of the UPR as shown by phosphorylation of eIF2α and splicing of *Xbp1*.

Apart from a slight but transient upregulation of *calnexin* mRNA after 1 h of palmitate treatment neither the harmful effects of

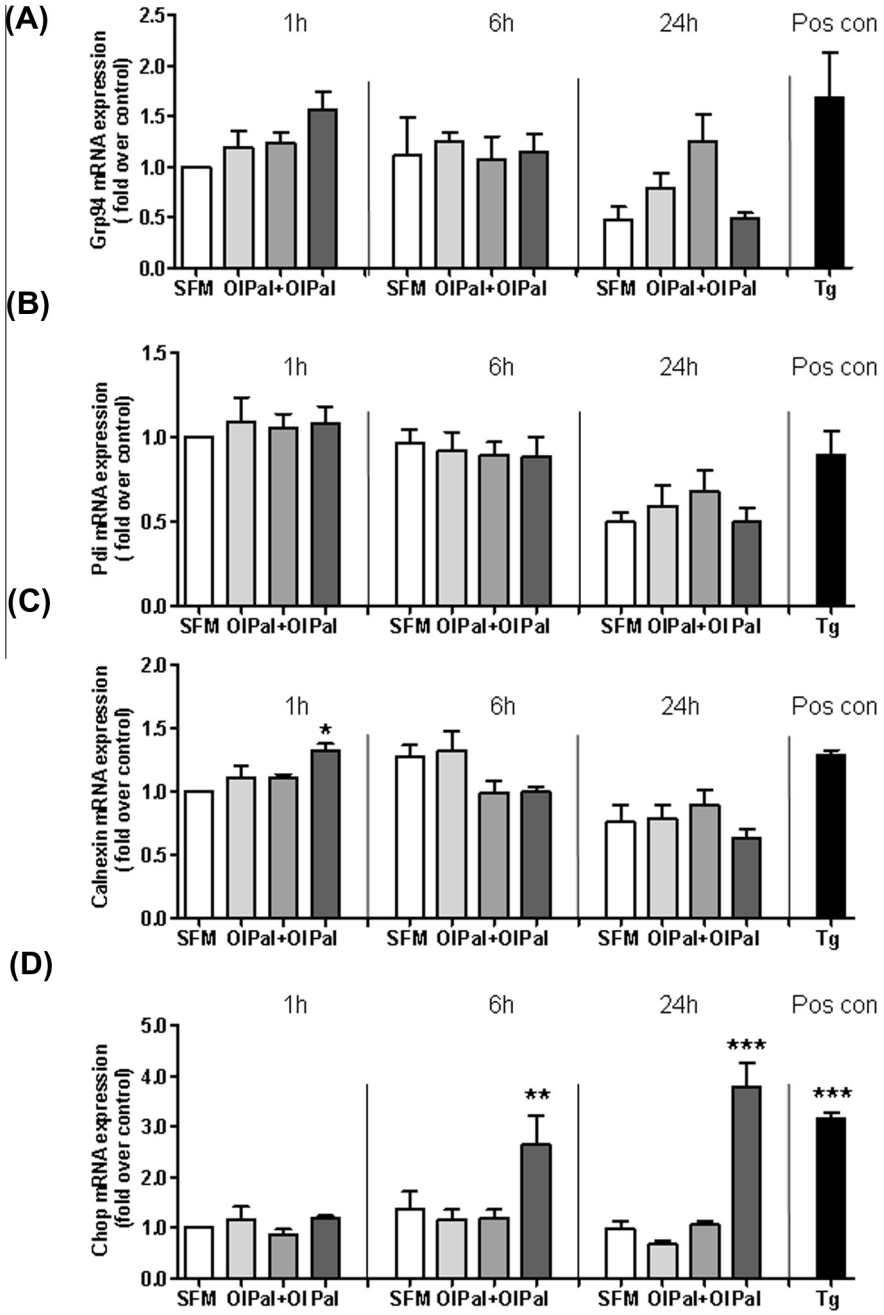

Fig. 4. Oleate antagonizes palmitate-mediated upregulation of *Chop* but does not alter chaperone levels. mRNA expression of (A) *Grp94*, (B) *Pdi*, (C) *Calnexin* and (D) *Chop* was analysed by qPCR. Ins-1E β-cells were incubated for 1, 6 and 24 h with serum free medium (SFM), palmitate (Pal) [0.5 mM], oleate (Ol) [1 mM], the combination (Pal + Ol) [0.5/1 mM] and thapsigargin (Tg) [1 μM]. *Tbp* was used as housekeeping gene and data were referred to SFM at 1 h set 1. Three to four independent experiments were performed and presented as means ± SEM $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ compared to control.

palmitate nor the protective effects of oleate were accompanied by changes in chaperone levels, although Bip, Grp94 and Pdi are reported to be downstream targets of the UPR pathways [14,21]. This finding is quite unexpected as chaperone upregulation during UPR activation has been postulated before [15,19,23,25,28–30]. However, Karsakov et al. [31,32] also observed unaltered levels of Grp94, Bip and Pdi after palmitate treatment in INS-1 β-cells. We therefore reasoned that palmitate activates the UPR in a manner to bypass its beneficial effects on protein folding. This assumption was supported by the finding of Lai et al. [33] who showed no protection of INS-1 β-cells from palmitate-induced apoptosis by Bip overexpression. In contrast, Laybutt et al. [23] reported attenuated cell death after Bip overexpression. However, they used MIN6 cells, so that differences in the cell type may account for the different results. Furthermore, other studies suggested either the existence of an additional but yet unknown transcription factor that regulates the promoter in chaperones or the presence of a different *cis*-acting element that is induced independently of ATF6 or Xbp1 during ER stress to activate the transcription of chaperones [34,35]. Shang and Lehrman could not detect a correlation between Xbp1 splicing, ATF6 cleavage and accumulation of Bip transcripts, but activation levels of the IRE1 and ATF6 pathway correlated with each other. They therefore assumed the existence of an alternative factor influencing chaperone transcription [20].

The proapoptotic activation of Chop that we detected after 6 h of palmitate incubation is under the control of the PERK pathway and probably represents one of the main mechanisms by which the UPR ultimately leads to apoptosis [2,25,36]. Chop decreases the expression of antiapoptotic Bcl-2 and initiates the translocation of Bax from the cytosol to the mitochondria [12]. Chop deletion delayed β-cell death and improved β-cell function in animal models of ER stress [37,38]. The combination of palmitate and oleate as well as oleate alone, in accordance with sustained cell survival, did not result in increased Chop levels compared to controls.

The question why saturated fatty acids activated the UPR whereas unsaturated can prevent it remains to be answered. One hypothesis states that palmitate incubation decreases the content of unsaturated phospholipids in the ER membrane and thereby alters its fluidity and structure [36,39]. Similar to cholesterol, palmitate accumulation in the ER membrane could compromise SERCA pump function which would lead to deficient calcium uptake in the ER and activation of the UPR [25,40,41]. We could also assume that oleate facilitates palmitate storage in neutral non-toxic lipids [24,26]. Palmitate in contrast is retained in the ER mostly in form of insoluble tripalmitin. This compromises the ER leading to enhanced membrane stiffness and subsequent activation of the UPR [27]. Nile red staining of oleate treated cells showed large lipid droplets in the cytoplasm (S2A) whereas cells incubated with palmitate (S2B) presented a diffuse pattern of staining. These lipid-filled structures were characterized to be dilated ERs [27,31,32,39]. Oleate and palmitate treatment resulted in more droplets and less vacuoles (S2C). This theory is supported by the finding that SCD1 knockdown cells showed enhanced apoptosis and UPR activation. SCD1 overexpression in contrast reduced palmitate-induced toxicity [36,42]. SCD1 is the key enzyme that catalyses the desaturation of C16 and C18 fatty acids [36].

In summary, we proved that oleate protects INS-1E β-cells from palmitate-induced apoptosis by the suppression of ER stress. Our results support the potential of MUFAs in the therapy of diabetes.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.10.130.

References

[1] G. Boden, Effects of free fatty acids (FFA) on glucose metabolism: significance for insulin resistance and type 2 diabetes, Exp. Clin. Endocrinol. Diab. 111 (2003) 121–124.
[2] L. Pineau, T. Ferreira, Lipid-induced ER stress in yeast and β cells: parallel trails to a common fate, FEMS Yeast Res. 10 (2010) 1035–1045.
[3] G.F. Lewis, Disordered fat storage and mobilization in the pathogenesis of insulin resistance and type 2 diabetes, Endocr. Rev. 23 (2002) 201–229.
[4] U. Riserus, W. Willett, F. Hu, Dietary fats and prevention of type 2 diabetes, Prog. Lipid Res. 48 (2009) 44–51.
[5] B. Salgin, K.K. Ong, A. Thankamony, et al., Higher fasting plasma free fatty acid levels are associated with lower insulin secretion in children and adults and a higher incidence of type 2 diabetes, J. Clin. Endocrinol. Metab. 97 (2012) 3302–3309.
[6] K. Maedler, J. Oberholzer, P. Bucher, et al., Monounsaturated fatty acids prevent the deleterious effects of palmitate and high glucose on human pancreatic – cell turnover and function, Diabetes 52 (2003) 726–733.
[7] K. Esposito, M.I. Maiorino, A. Ceriello, et al., Prevention and control of type 2 diabetes by Mediterranean diet: a systematic review, Diab. Res. Clin. Pract. 89 (2010) 97–102.
[8] Fats and fatty acids in human nutrition, Report of an Expert Consultation 10–14 November 2008, Food and Agriculture Organization of the United Nations, Rome, Geneva, 2010.
[9] G. Riccardi, R. Giacco, A.A. Rivellese, Dietary fat, insulin sensitivity and the metabolic syndrome, Clin. Nutr. 23 (2004) 447–456.
[10] B. Vessby, M. Uusitupa, K. Hermansen, et al., Substituting dietary saturated for monounsaturated fat impairs insulin sensitivity in healthy men and women: the KANWU sudy, Diabetologia 44 (2001) 312–319.
[11] S.H. Back, S.-W. Kang, J. Han, et al., Endoplasmic reticulum stress in the β-cell pathogenesis of type 2 diabetes, Exp. Diab. Res. 2012 (2012) 1–11.
[12] D.L. Eizirik, A.K. Cardozo, M. Cnop, The role for endoplasmic reticulum stress in diabetes mellitus, Endocr. Rev. 29 (2008) 42–61.
[13] S.G. Fonseca, J. Gromada, F. Urano, Endoplasmic reticulum stress and pancreatic β-cell death, Trends Endocrinol. Metab. 22 (2011) 266–274.
[14] D. Scheuner, R.J. Kaufman, The unfolded protein response: a pathway that links insulin demand with beta-cell failure and diabetes, Endocr. Rev. 29 (2008) 317–333.
[15] S.-E. Choi, Y.-J. Lee, H.-J. Jang, et al., A chemical chaperone 4-PBA ameliorates palmitate-induced inhibition of glucose-stimulated insulin secretion (GSIS), Arch. Biochem. Biophys. 475 (2008) 109–114.
[16] K. Pinnick, M. Neville, A. Clark, et al., Reversibility of metabolic and morphological changes associated with chronic exposure of pancreatic islet β-cells to fatty acids, J. Cell. Biochem. 109 (2010) 683–692.
[17] R. Spinnler, T. Gorski, K. Stolz, et al., The adipocytokine Nampt and its product NMN have no effect on beta-cell survival but potentiate glucose stimulated insulin secretion, PLoS ONE 8 (2013) E54106.
[18] H. Toda, G. Suzuki, M. Nibuya, et al., Behavioral stress and activated serotonergic neurotransmission induce XBP-1 splicing in the rat brain, Brain Res. 1112 (2006) 26–32.
[19] T. Okada, H. Yoshida, R. Akazawa, et al., , et al., Distinct roles of activating transcription factor 6 (ATF6) and double-stranded RNA-activated protein kinase-like endoplasmic reticulum kinase (PERK) in transcription during the mammalian unfolded protein response, Biochem. J. 366 (2002) 585.
[20] J. Shang, M.A. Lehrman, Discordance of UPR signaling by ATF6 and Ire1p-XBP1 with levels of target transcripts, Biochem. Biophys. Res. Commun. 317 (2004) 390–396.
[21] K. Yamamoto, T. Sato, T. Matsui, et al., Transcriptional induction of mammalian ER quality control proteins is mediated by single or combined action of ATF6α and XBP1, Develop. Cell 13 (2007) 365–376.
[22] C.-J. Huang, C.-Y. Lin, L. Haataja, et al., High expression rates of human islet amyloid polypeptide induce endoplasmic reticulum stress mediated beta-cell apoptosis, a characteristic of humans with type 2 but not type 1 diabetes, Diabetes 56 (2007) 2016–2027.
[23] D.R. Laybutt, A.M. Preston, M.C. Åkerfeldt, et al., Endoplasmic reticulum stress contributes to beta cell apoptosis in type 2 diabetes, Diabetologia 50 (2007) 752–763.
[24] M. Cnop, J.C. Hannaert, A. Hoorens, et al., Inverse relationship between cytotoxicity of free fatty acids in pancreatic islet cells and cellular triglyceride accumulation, Diabetes 50 (2001) 1771–1777.
[25] D.A. Cunha, P. Hekerman, L. Ladrière, et al., Initiation and execution of lipotoxic ER stress in pancreatic beta-cells, J. Cell. Sci. 121 (2008) 2308–2318.
[26] L.L. Listenberger, Triglyceride accumulation protects against fatty acid-induced lipotoxicity, Proc. Natl. Acad. Sci. 100 (2003) 3077–3082.

[27] J.H. Moffitt, B.A. Fielding, R. Evershed, et al., Adverse physicochemical properties of tripalmitin in beta cells lead to morphological changes and lipotoxicity in vitro, Diabetologia 48 (2005) 1819–1829.
[28] V. Němcová-Fürstová, R.F. James, J. Kovář, Inhibitory effect of unsaturated fatty acids on saturated fatty acid-induced apoptosis in human pancreatic β-cells: activation of caspases and ER stress induction, Cell Physiol. Biochem. 27 (2011) 525–538.
[29] P. Pirot, N. Naamane, F. Libert, et al., Global profiling of genes modified by endoplasmic reticulum stress in pancreatic beta cells reveals the early degradation of insulin mRNAs, Diabetologia 50 (2007) 1006–1014.
[30] L. Salvadó, T. Coll, A.M. Gómez-Foix, et al., Oleate prevents saturated-fatty-acid-induced ER stress, inflammation and insulin resistance in skeletal muscle cells through an AMPK-dependent mechanism, Diabetologia 56 (2013) 1372–1382.
[31] E. Karaskov, C. Scott, L. Zhang, et al., Chronic palmitate but not oleate exposure induces endoplasmic reticulum stress, which may contribute to INS-1 pancreatic beta-cell apoptosis, Endocrinology 147 (2006) 3398–3407.
[32] E. Diakogiannaki, H.J. Welters, N.G. Morgan, Differential regulation of the endoplasmic reticulum stress response in pancreatic beta-cells exposed to long-chain saturated and monounsaturated fatty acids, J. Endocrinol. 197 (2008) 553–563.
[33] E. Lai, G. Bikopoulos, M.B. Wheeler, et al., Differential activation of ER stress and apoptosis in response to chronically elevated free fatty acids in pancreatic -cells, Am. J. Physiol. Endocrinol. Metab. 294 (2008) E540.
[34] A.-H. Lee, N.N. Iwakoshi, L.H. Glimcher, XBP-1 Regulates a subset of endoplasmic reticulum resident chaperone genes in the unfolded protein response, Mol. Cell. Biol. 23 (2003) 7448–7459.
[35] Y. Ma, J.W. Brewer, J.A. Diehl, et al., Two distinct stress signaling pathways converge upon the CHOP promoter during the mammalian unfolded protein response, J. Mol. Biol. 318 (2002) 1351–1365.
[36] H. Ariyama, N. Kono, S. Matsuda, et al., Decrease in membrane phospholipid unsaturation induces unfolded protein response, J. Biol. Chem. 285 (2010) 22027–22035.
[37] B. Song, D. Scheuner, D. Ron, et al., Chop deletion reduces oxidative stress, improves β cell function, and promotes cell survival in multiple mouse models of diabetes, J. Clin. Invest. 118 (2008) 3378–3389.
[38] S. Oyadomari, A. Koizumi, K. Takeda, et al., Targeted disruption of the Chop gene delays endoplasmic reticulum stress-mediated diabetes, J. Clin. Invest. 109 (2002) 525–532.
[39] N.M. Borradaile, X. Han, J.D. Harp, et al., Disruption of endoplasmic reticulum structure and integrity in lipotoxic cell death, J. Lipid Res. 47 (2006) 2726–2737.
[40] B. Feng, P.M. Yao, Y. Li, et al., The endoplasmic reticulum is the site of cholesterol-induced cytotoxicity in macrophages, Nat. Cell Biol. 5 (2003) 781–792.
[41] Y. Li, M. Ge, L. Ciani, et al., Enrichment of endoplasmic reticulum with cholesterol inhibits sarcoplasmic-endoplasmic reticulum calcium ATPase-2b activity in parallel with increased order of membrane lipids: implications for depletion of endoplasmic reticulum calcium stores and apoptosis in cholesterol-loaded macrophages, J. Biol. Chem. 279 (2004) 37030–37039.
[42] A.K. Busch, E. Gurisik, D.V. Cordery, et al., Increased fatty acid desaturation and enhanced expression of stearoyl coenzyme A desaturase protects pancreatic – cells from lipoapoptosis, Diabetes 54 (2005) 2917–2924.