Differential regulation of the ER stress response by long-chain fatty acids in the pancreatic β-cell

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Abstract
Recent evidence indicates that treatment of pancreatic β-cells with long chain fatty acids can lead to the development of an ER (endoplasmic reticulum) stress response. This is manifest as the activation of some components of the PERK [RNA-dependent protein kinase-like ER eIF2α (eukaryotic initiation factor 2α) kinase]-dependent arm of ER stress and is seen most dramatically when cells are treated with long-chain saturated fatty acids (e.g. palmitate). By contrast, the equivalent mono-unsaturates (e.g. palmitoleate) are much less effective and they can even attenuate the ER stress response to palmitate. This may be due to the regulation of eIF2α phosphorylation in cells exposed to mono-unsaturates. The present review discusses the differential effects of saturated and mono-unsaturated fatty acids on ER stress in β-cells and considers the extent to which regulation of this pathway may be involved in mediating their effects on viability.

Fatty acids and β-cell viability
The rising tide of obesity within the developed world constitutes one of the primary risk factors for Type 2 diabetes among these populations and is usually associated with hyperlipidaemia. This leads to alterations in the profile of circulating lipids and, more specifically, to elevations in the plasma levels of non-esterified fatty acids [1–4]. This, in turn, contributes to the progression of insulin resistance [5] and to impaired insulin secretion [6]. Sustained elevations of plasma non-esterified fatty acids also exert detrimental effects on β-cell viability and, as a result, they may contribute to the long-term decline in β-cell mass that is increasingly recognized as a feature of Type 2 diabetes [7]. This process has been termed lipotoxicity and it may be subject to potentiation by a concomitant rise in ambient glucose concentrations in vivo, such that the overall β-cell loss is driven by 'glucolipotoxicity' [8].

It is possible to study many of the intracellular events that culminate in (gluco)lipotoxicity by incubation of β-cells in vitro, and such work has revealed that not all fatty acids are equally toxic to these cells during chronic exposure. Rather, the cells respond differentially according to the degree of saturation and the length of the carbon chain. These differences are explored in more detail in an accompanying paper [9] but, in summary, it appears that long-chain saturated fatty acids [such as palmitate (C\textsubscript{16:0}) or stearate (C\textsubscript{18:0})] cause loss of viability, whereas shorter chain saturates (C\textsubscript{14} or less) or long-chain mono-unsaturated fatty acids [such as palmitoleate (C\textsubscript{16:1\,\textomega\textsubscript{9}}) or oleate (C\textsubscript{18:1\,\textomega\textsubscript{9}})] are well tolerated [10–16]. A particularly remarkable finding is that the presence of long-chain mono-unsaturated fatty acids abolishes the cytotoxicity caused by long-chain saturated species under in vitro conditions [10–12,14]. Assuming that these observations have a bearing on the response of β-cells to circulating fatty acids in vivo, then it seems reasonable to conclude that the precise ratio of fatty acid species impinging on the β-cells may be an important determinant of the final outcome and that either an elevation of saturated species or a net decrease in unsaturated molecules might be equally detrimental over time.

Involvement of the ER (endoplasmic reticulum) in fatty-acid-mediated toxicity
The molecular mechanisms involved in mediating the pro-apoptotic and/or the protective responses of β-cells to long-chain fatty acids remain unknown. However, increasing evidence suggests that perturbations in the ER may be associated with lipotoxicity and that, if sustained, could even be causative in mediating loss of cell viability. This is because the ER plays a central role in the determination of cell fate under conditions of stress. The ER acts as a conduit for many secretory and cellular proteins and, accordingly, it plays an important role in folding of these molecules during their transit through the organelle [17]. Any stimulus that leads to a disruption of this functionality (such as altered protein glycosylation, impaired trafficking, protein aggregation, virus infection, hypoxia or ischaemia) can disrupt ER homoeostasis and trigger a counter-regulatory response termed ‘ER stress’. The primary intention is to restore normal homoeostasis by promoting a temporary inhibition of general protein synthesis. However, if the stimulus is prolonged and persistent, ER stress is exacerbated and apoptosis may then be induced [18,19].

Key words: apoptosis, eukaryotic initiation factor 2α (eIF2α), integrated stress response, palmitate, palmitoleate, pancreatic β-cell.

Abbreviations used: ATF4, activating transcription factor 4; C/EBP, CCAAT/enhancer-binding protein; CHOP10, C/EBP-homologous protein 10; eIF2α, eukaryotic initiation factor 2α; ER, endoplasmic reticulum; GRP78, glucose-regulated protein 78; IRE1, inositol-requiring enzyme 1; ISR, integrated stress response; PERK, RNA-dependent protein kinase-like ER eIF2α kinase; XBP1, X-box-binding protein 1.

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Recent studies suggest that obesity and elevated levels of long-chain saturated fatty acids can cause ER stress in hepatocytes [20–22], although the precise mechanisms involved—remain unclear. Similarly, in the β-cell, long-chain saturated fatty acids also elicit an apparent ER stress response [23–26] as indicated by the increased expression of markers associated with this pathway such as the transcription factor CHOP10 [C/EBP (CCAAT/enhancer-binding protein)-homologous protein 10] [23,24,27,28]. This molecule is a well-established mediator of apoptosis during severe ER stress [29] and, therefore, the concept has emerged that the pro-apoptotic effects of saturated fatty acids in β-cells may be mediated, at least in part, by induction of ER stress.

ER-stress-dependent pathways activated by fatty acids

During ER stress in β-cells, the pathway primarily responsible for the induction of CHOP10 expression involves a protein kinase, PERK [RNA-dependent protein kinase-like ER eIF2α (eukaryotic initiation factor 2α) kinase], and one of its substrates, the eIF2α [30]. PERK activation may initially occur as a means to reduce ER stress since genetic deletion of the kinase increases the susceptibility to ER stress and leads to the development of diabetes in animals [31] and in humans [32]. Conversely, prolonged activation of PERK (e.g. in response to fatty acids) is also associated with β-cell loss [33]. Thus the balance between the acute and chronic regulation of the ER stress pathway may be critical to the overall control of cell viability.

Activation of the PERK-dependent arm of ER stress by saturated fatty acids has been reported in various pancreatic β-cell lines, including BRIN-BD11 [28], INS-1 [24] and MIN6 cells [34]. Although most of the studies concur that the PERK pathway is activated, a consensus on the effects of saturated fatty acids on the other two signal transduction cascades associated with ER stress [IRE1 (inositol-requiring enzyme 1) and ATF6 (activating transcription factor 6)] has been more difficult to reach. More specifically, neither XBP-1 (X-box-binding protein-1) splicing nor activation of JNK (c-Jun N-terminal kinase) (both of which are normally associated with activation of the IRE1 cascade [35]) was detected in palmitate-treated human islets or INS-1 cells by some workers [27,36], whereas XBP-1 splicing has been reported by others [25]. The involvement of ATF6 is also ambiguous, although both saturated and mono-unsaturated fatty acids have been shown to activate an ATF6 reporter construct in β-cells [23]. By contrast, it has been observed that the level of the major ER chaperone GRP78 (glucose-regulated protein 78) remains unaltered after fatty acid treatment [24,28] and, given that ATF6 activation drives the transcription of GRP78 [37,38], this implies that ATF6 had remained silent during treatment with saturated fatty acids in these studies. However, again the situation is not entirely straightforward, since GRP78 mRNA levels are increased in response to fatty acids [25].

Taken as a whole, the consensus implies that saturated fatty acids activate the PERK-dependent pathway of ER stress in β-cells. However, the extent to which they also control the IRE1 cascade and the pathway regulated by ATF6 remains to be clarified.

Fatty acids may not induce a classical ER stress response in β-cells

In drawing the conclusion that saturated fatty acids may activate the PERK-dependent arm of ER stress, it is important to re-emphasize that there is little evidence that they up-regulate the major ER chaperone, GRP78, at the protein level (although, as indicated above, changes in mRNA may occur). Nevertheless, several other components of the PERK pathway are activated, as shown by the detection of increased phospho-eIF2α in fatty acid-treated cells and by a rise in the levels of ATF4 and CHOP10 [24,27,28]. Thus it seems that full activation of the PERK-dependent ER stress response may not take place when β-cells are exposed to saturated fatty acids but that certain components of the pathway are induced. Based on these observations, it can be surmised that saturated fatty acids may not trigger a full ER stress response in β-cells. Rather, they appear to induce those components that are required for apoptosis, while they do not promote the more ‘beneficial’ responses, such as up-regulation of molecular chaperones. From this, it seems unlikely that fatty acids induce ER stress by causing the accumulation of unfolded (or aggregated) proteins within the ER, although this has not been formally proved. The finding that depletion of ER Ca2+ stores does not occur in fatty-acid-treated β-cells [24] is consistent with an incomplete activation of the ER stress response under these conditions. Moreover, it has also been shown that enforced overexpression of GRP78 in INS-1 β-cells failed to protect the cells from palmitate-induced apoptosis, thereby indicating that GRP78 does not directly regulate lipotoxicity in β-cells exposed to palmitate [27].

Differential activation of the ER stress response by saturated and mono-unsaturated fatty acids

As discussed above, it is clear that a partial ER stress response ensues in β-cells after exposure to saturated fatty acids. However, a recent work has shown that this response is strikingly attenuated (or even absent) during treatment with long-chain mono-unsaturated fatty acids [28]. Thus β-cells respond to these species in quite a different way: saturated molecules trigger defined components of the PERK arm of ER stress very strongly, whereas mono-unsaturates are much less effective. Moreover, when mono-unsaturated and saturated fatty acids are co-administered to the cells, the activation of ER stress is suppressed [28]. This is manifest as a reduction in the expression of markers such as phospho-eIF2α, ATF4 and CHOP10.
Thus mono-unsaturates antagonize the activation of the PERK arm of the ER stress pathway, and as a consequence, they are able to rescue the cells from the detrimental effects normally associated with sustained activation of this pathway. This mechanism may therefore contribute to the cytoprotective actions of mono-unsaturated fatty acids in β-cells.

The precise mechanism by which mono-unsaturates antagonize the activation of PERK-dependent processes has not been revealed. However, we have recently demonstrated that the protective effects are not accompanied by any alteration in the levels of GRP78 under conditions when expression of this molecule is enhanced, suggesting that the mono-unsaturates must intervene in the pathway at a point that lies distal to GRP78 induction [28]. Alterations in PERK phosphorylation are also unlikely to account for the effects. By contrast, the phosphorylation status of eIF2α is regulated in cells exposed to mono-unsaturated fatty acids, suggesting that these fatty acid species may influence the phosphorylation of this molecule. In support of this, it was observed that the induction of all markers of the PERK pathway that lie downstream of eIF2α are also suppressed in cells exposed to mono-unsaturates. This supports the conclusion that cytoprotection arises from the regulation of eIF2α phosphorylation.

These considerations raise a further important issue, namely the mechanism by which eIF2α phosphorylation might be regulated by mono-unsaturated fatty acids. In this context, it is important to note that eIF2α serves as a substrate for several kinases and that its regulation is not simply restricted to the ER stress pathway. On the contrary, a range of additional kinases, including the double-stranded RNA-dependent PKR (protein kinase R), HRI (haem-regulated eIF2α kinase) and GCN2 (general control non-derepressible 2), are all known to catalyse the phosphorylation of eIF2α [39]. Thus activation of one or more of these alternative kinases could elicit the downstream responses associated with ER stress, even under conditions when ER stress itself is not initiated. Hence, it might be more correct to suggest that eIF2α phosphorylation is a marker of a generalized ISR (integrated stress response) [40] and it is this that becomes activated during exposure of β-cells to fatty acids. Therefore we would propose that it is the ISR that is antagonized by mono-unsaturates and that they achieve this by acting at the level of eIF2α phosphorylation. Mono-unsaturates could bring about a change in eIF2α phosphorylation either by regulating an upstream kinase or, alternatively, by controlling an appropriate phosphatase. It remains to be proved which of these mechanisms is more likely, but we note that the ability of the eIF2α phosphatase inhibitor, salubrinal, to maintain eIF2α in a phosphorylated state is antagonized by palmitoleate [28]. This might imply that a kinase (rather than the phosphatase) is the primary target for the mono-unsaturate.

**Conclusion**

In conclusion, it is noteworthy that control of eIF2α phosphorylation was recognized as an important mechanism for β-cell survival and thus prevention of Type 2 diabetes several years ago [41]. There is increasing evidence suggesting that that one mechanism by which saturated fatty acids may promote β-cell toxicity is by enhancing the phosphorylation of eIF2α and thereby driving a sustained ISR in the cells. By contrast, mono-unsaturates appear to oppose this effect and could exert their cytoprotective actions, at least in part, by maintaining eIF2α in a de-phosphorylated state.

We are grateful to the Diabetes UK, Diabetes Research and Wellness Foundation and Boehringer Ingelheim for their financial support of these studies.

**References**


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Received 21 April 2008
doi:10.1042/BST0360959