Life and death decisions of the pancreatic \( \beta \)-cell: the role of fatty acids

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ABSTRACT

Both stimulatory and detrimental effects of NEFAs (non-esterified fatty acids) on pancreatic \( \beta \)-cells have been recognized. Acute exposure of the pancreatic \( \beta \)-cell to high glucose concentrations and/or saturated NEFAs results in a substantial increase in insulin release, whereas chronic exposure results in desensitization and suppression of secretion, followed by induction of apoptosis. Some unsaturated NEFAs also promote insulin release acutely, but they are less toxic to \( \beta \)-cells during chronic exposure and can even exert positive protective effects. Therefore changes in the levels of NEFAs are likely to be important for the regulation of \( \beta \)-cell function and viability under physiological conditions. In addition, the switching between endogenous fatty acid synthesis or oxidation in the \( \beta \)-cell, together with alterations in neutral lipid accumulation, may have critical implications for \( \beta \)-cell function and integrity. Long-chain acyl-CoA (formed from either endogenously synthesized or exogenous fatty acids) controls several aspects of \( \beta \)-cell function, including activation of specific isoenzymes of PKC (protein kinase C), modulation of ion channels, protein acylation, ceramide formation and/or NO-mediated apoptosis, and transcription factor activity. In this review, we describe the effects of exogenous and endogenous fatty acids on \( \beta \)-cell metabolism and gene and protein expression, and have explored the outcomes with respect to insulin secretion and \( \beta \)-cell integrity.

METABOLISM OF GLUCOSE AND FATTY ACIDS IN THE \( \beta \)-CELL

In writing this review, we are fully aware that most of the studies cited have utilized rat-, mouse- or hamster-derived insulinoma \( \beta \)-cell lines to study function in vitro. This is due to the inherent difficulty in maintaining primary rodent islet \( \beta \)-cell mass and function for more than a few days in vitro and, of course, the scarcity of human islets for research purposes. There are as yet no suitable human \( \beta \)-cell lines available for unrestricted in vitro studies; however, the major rodent \( \beta \)-cell lines have provided substantial data and insights into cell function in normal or pathogenic situations. The most widely used cell lines include INS-1, MIN-6, RINm5F, and BRIN-BD11. To stimulate insulin secretion from

Key words: acetyl-CoA, apoptosis, fatty acid toxicity, glucose metabolism, insulin secretion, non-esterified fatty acid (NEFA), pancreatic \( \beta \)-cell.

Abbreviations: ACC, acetyl-CoA carboxylase; AMPK, AMP-activated kinase; CPT-1, carnitine palmitoyltransferase-1; DAG, diacylglycerol; ER, endoplasmic reticulum; GLP-1, glucagon-like peptide-1; GSIS, glucose-stimulated insulin secretion; HMG-CoA reductase, 3-hydroxy-3-methylglutaryl-CoA reductase; HNF-4\( \alpha \), hepatocyte nuclear factor 4\( \alpha \); HSL, hormone-sensitive lipase; iNOS, inducible form of NO synthase; IRS-2, insulin receptor substrate-2; MPTP, mitochondrial permeability transition pore; mTOR, mammalian target of rapamycin; NEFAs, non-esterified fatty acids; NF-\( \kappa \)B, nuclear factor \( \kappa \)B; OGG1, 8-oxoguanine DNA glycosylase/apurinic lyase; PI3K, phosphoinositide 3-kinase; PKB, protein kinase B; PKC, protein kinase C; PPAR, peroxisome-proliferator-activated receptor; SREBP1c, sterol-regulatory-element-binding protein 1c; TAG, triacylglycerol; UCP, uncoupling protein.

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primary islets and β-cell lines, glucose is transported into the pancreatic β-cell by the non-insulin-dependent glucose transporter GLUT2 in rodents and by both GLUT1 and GLUT2 in humans. Once inside the cells, glucose is phosphorylated by the low-affinity (K_m = 6–11 mmol/l) hexokinase IV (glucokinase). The glycolytic flux in pancreatic islet β-cells is therefore regulated by a combination of the rate of glucose uptake and the glucokinase activity, although it is unlikely that glucose uptake is rate-limiting under most conditions. Therefore glucokinase has been referred to as the β-cell's glucose sensor [1]. The flux of metabolites through the pentose phosphate pathway is low and glycogen synthesis accounts for approx. 1–7% of the glucose utilized by β-cells. Furthermore, the β-cell is metabolically distinct from almost all other mammalian cell types in several respects; namely because (i) it can utilize glucose in the physiologically relevant range (2–20 mmol/l); (ii) it displays low lactate dehydrogenase and plasma membrane monocarboxylate pyruvate/lactate transporter activity and correspondingly high activity in the mitochondrial malate/aspartate shuttle, so ensuring mitochondrial oxidation of NADH; (iii) it has a high activity of both pyruvate dehydrogenase and pyruvate carboxylase, ensuring that both anaplerotic and oxidative metabolism of glucose/pyruvate can co-exist. Acetyl-CoA formed from pyruvate can condense with oxaloacetate forming citrate for metabolism in the tricarboxylic acid cycle (Krebs cycle), leading to NADH and FADH_2 production [2].

All these specific metabolic adaptations are geared to enhancing mitochondrial tricarboxylic acid cycle activity, oxidative phosphorylation and efficient ATP production. An enhancement of the ATP/ADP ratio results in closure of ATP-sensitive K^+ channels, depolarization of the plasma membrane, opening of voltage-activated Ca^{2+} channels [1], influx of Ca^{2+} and, finally, fusion of insulin-containing granules with the plasma membrane [3,4]. In addition to this metabolic complexity, the β-cell can metabolize a number of key amino acids, which, via mitochondrial metabolism, can generate further stimulus-secretion 'coupling' factors [5]. Indeed, additional signals which 'amplify' the initial glucose-dependent stimulation of insulin secretion may be generated independently of ATP-sensitive K^+ channel activity. These, as yet, unidentified signals appear to increase the efficacy of Ca^{2+} on the exocytosis of insulin granules [6].

Pancreatic β-cell and islet glucose metabolism results in increased levels of cytosolic long-chain acyl-CoA compounds, TAG (triacylglycerol) and phospholipid turnover as a consequence of increased citrate and malonyl-CoA production and subsequent inhibition of β-oxidation of NEFAs (non-esterified fatty acids) [3,7]. Malonyl-CoA produced from glucose plays a role in this shift from NEFA to glucose as an oxidative fuel, since it inhibits CPT-1 (carnitine palmitoyltransferase-1), found on the outer mitochondrial membrane, thereby reducing long-chain CoA entry into the mitochondria [8].

Long-chain acyl-CoA, phosphatidate and DAG (diacylglycerol) contents are elevated in response to increased glucose metabolism, leading to a subsequent elevation in intracellular Ca^{2+}, activation of PKC (protein kinase C) isoforms [9] and changes in the acylation state of key proteins involved in regulating ion channel activity and exocytosis [9–11]. On the other hand, long-chain CoA may also stimulate insulin release by a more direct effect on exocytosis, as it facilitates the fusion of insulin-secretory granules with the β-cell plasma membrane [10].

The ability of the pancreatic islets to convert glucose into fatty acids by de novo synthesis has been demonstrated directly by determination of the content and composition of fatty acids from rat pancreatic islets and culture medium after incubation for 1 and 3 h in the absence and presence of 5.6, 8.3 or 16.7 mmol/l glucose using HPLC analysis [12]. The total lipid content of pancreatic islets was substantially reduced after incubation for 1 h in the absence of glucose; however, the lipid content was restored by incubation in the presence of 5.6 mmol/l glucose and was increased further by incubation with 8.3 or 16.7 mmol/l glucose. Pancreatic islets exported a substantial amount of fatty acids to the medium either in the absence or presence of glucose. After incubation for 1 h, the addition of 5.6 mmol/l glucose raised the medium content of palmitate (3.4-fold) and stearate (5.4-fold) compared with islets incubated in the absence of glucose. Martins et al. [12] concluded that the synthesis and release of saturated and, to a lesser extent, unsaturated fatty acids from glucose-exposed islets represents a novel mechanism for modulating GSIS (glucose-stimulated insulin secretion) from pancreatic β-cells.

**REGULATION OF FATTY ACID METABOLISM IN β-CELLS**

Hyperlipidaemia is frequently associated with insulin resistance, a condition that is associated with hyperglycaemia and hyperinsulinaemia, as found in Type 2 diabetes and obesity [13–15]. A direct effect of the fatty acids on insulin secretion in vivo may explain the co-existence of hyperlipidaemia and hyperinsulinaemia. Although it has been accepted that fatty acid metabolism is necessary for fatty acid stimulation of insulin secretion [16,17], the precise mechanism(s) by which fatty acids stimulate insulin secretion remain unknown.

Long-chain fatty acids may be transported into the cell by free diffusion with no requirement for active transport [18]. Extracellular NEFAs can be rapidly distributed in lipid bilayers and move rapidly from cell to cell,
Role of non-esterified fatty acids in β-cell function and integrity

Figure 1  Potential pathways of fatty acid metabolism in the pancreatic β-cell

Fatty acids are ‘activated’ on entry into the β-cell to become acyl-CoA. This acyl-CoA may be oxidized in the mitochondrial matrix, or may be esterified in the cytosol to become TAG. TAG/NEFA cycling may occur in the β-cell. Acyl-CoA may also give rise to lipid-based signalling molecules such as DAG. Glucose metabolism may also give rise to cytosolic citrate via cataplerosis, which subsequently can be converted into acetyl-CoA and oxaloacetate by ATP-citrate lyase, and then conversion of acetyl-CoA into malonyl-CoA via the action of ACC, the ‘committed’ step for fatty acid synthesis. Endogenously synthesized fatty acids may be incorporated into TAG, signalling lipids or may be exported. NEFAs may also bind to GPR40 receptors and stimulate unknown signal transduction pathway(s). G3P, glucose 3-phosphate.

In addition, the presence and action of HSL (hormone-sensitive lipase) in β-cells reinforces the concept that endogenous lipolysis participates in the regulation of insulin secretion through the generation of NEFAs or other lipid signalling molecules [21]. Hence the islet TAG store, via HSL-mediated lipolysis, may play an important role in the stimulus–secretion coupling mechanism of GSIS (Figure 1). As discussed above, fatty acids, their CoA derivatives or complex lipids formed from fatty acids, play a key role for nutrient signalling in the β-cell. Lipolysis of β-cell TAG also plays an important role in the action of incretins, such as GLP-1 (glucagon-like peptide-1), due to the mechanism of potentiation of GSIS, which involves an increase in intracellular cAMP content [22,23]. cAMP can activate PKA (protein kinase A), which is a potent stimulator of HSL. In fact, HSL-knockout mice have reduced GSIS both in vivo and in isolated islets [21]. Additionally an increase in glucose concentration induces HSL (LIPE) gene expression in the β-cell, resulting in a 2-fold increase in HSL protein and enzymatic activity [24]. This occurs concomitantly with an increase in basal insulin secretion [25], corroborating the proposition that lipid signalling molecules are essential to GSIS [26,27].

Long-chain acyl CoA may additionally be esterified to TAG in the β-cell in the presence of glycerol 3-phosphate provided by glucose metabolism, or alternatively may be oxidized when rates of glucose uptake and metabolism are low. The key metabolic ‘sensor’ AMPK (which is activated by a fall in the ATP/AMP ratio) may provide a unique switching mechanism to allow β-cells to oxidize fatty acid when glucose oxidation (and ATP
IMPACT OF NEFAs ON β-CELL GLUCOSE METABOLISM

In pancreatic β-cells, cytoplasmic NEFAs are converted into long-chain acyl-CoA by acyl-CoA synthase. Under basal conditions, the long-chain acyl-CoA molecules are transported into the mitochondria via CPT-1, where β-oxidation takes place. High levels of glucose inhibit this process (via inhibition of AMPK and subsequent ACC activation) and induce a marked rise in the cytoplasmic content of long-chain acyl-CoA [29]. Malonyl-CoA inhibits CPT-1 activity, allowing the accumulation of long-chain acyl-CoA in the cytosol [30,31]. Thus malonyl-CoA acts by switching β-cell metabolism from fatty acid oxidation to glucose oxidation. An important consequence of this switching is a marked increase in cytosolic long-chain acyl-CoA, which acts as an effector molecule in the β-cell signalling, as discussed above [32] (Figure 1).

Randle et al. [14] proposed the glucose/fatty acid cycle in their studies with rat heart muscle and rat diaphragm muscle. In principle, a rise in plasma NEFA levels decreases glucose uptake and oxidation by many tissues, but the mechanism of this inhibition was unknown before the work of Randle, Garland, Hales and Newsholme [14]. The key steps of this hypothesis are co-ordinated in the following way. The rise in plasma NEFA induces β-oxidation with increased production of acetyl-CoA, inhibiting pyruvate dehydrogenase and thus oxidation of pyruvate. At the same time, the production of citrate and ATP inhibits phosphofructokinase activity and thus glycolysis, resulting in accumulation of glucose 6-phosphate. The increased level of glucose 6-phosphate, in turn, inhibits muscle hexokinase activity with a reduction in the glucose transport/phosphorylation activity. However, the β-cell, due to the necessity of operating a high glycolytic rate (in the presence of elevated glucose concentrations) in the presence of long-chain acyl-CoA, does not appear to be subjected to the same regulatory principles. For example, as a consequence of elevated glucose metabolism, resulting in high cytosolic ATP and citrate concentrations, the β-cell glycolytic rate is not impeded. β-Cell-specific expression of key regulatory enzymes, such as glucokinase (which is not subjected to allosteric inhibition by the product glucose 6-phosphate), allows high rates of glycolysis in an ‘energy rich’ environment.

In contrast with the acute effects of glucose and fatty acids, the chronic inhibitory effect of NEFAs on GSIS may be due to a metabolic effect. Reduced glucose oxidation may result from decreased conversion of pyruvate into acetyl-CoA, a consequence of a decline in islet pyruvate dehydrogenase activity, due to the inhibitory action of increased NADH production via NEFA β-oxidation [33], an increase in pyruvate dehydrogenase kinase activity [34] or via changes in expression of key metabolic genes or transcription factors. Adiponectin, an adipose-tissue-derived peptide hormone, may additionally regulate fatty acid metabolism in the β-cell, mainly through activation of AMPK [35–41]. Adiponectin is required for normal insulin action in the primary target tissues and regulation of energy homoeostasis [42,43].

MODULATION OF INSULIN SECRETION BY NEFAs

In vivo studies
Insulin secretion is influenced, at any given time, predominately by the blood glucose concentration and by the prevailing fatty acids in the circulation [44]. Gut-derived incretins, other hormones and neurotransmitters may also exert an influence. Many groups have investigated the
effect of fatty acids on the process of GSIS (for example, [7,45–49]). The rapid effect of NEFAs to potentiate GSIS in vitro, while having little effect on secretion at non-stimulatory glucose concentrations, would suggest that they act to amplify metabolic stimulus–secretion coupling mechanisms [50,51]. The potency of fatty acids to promote glucose-induced insulin release increases with the chain length and decreases with the degree of unsaturation [52,53]. Long-chain fatty acids (such as palmitate, linoleate and α-linolenate) potentiate insulin secretion in response to basal glucose concentrations [54].

Acute lowering of plasma fatty acids levels is associated with a decreased insulin response to glucose [45,55], but full secretory function can be restored by inclusion of fatty acids during perfusion of pancreas from fasted rats [48,49,56]. GSIS by perfused islets of rats fasted for 24 h is reduced when compared with the response of islets from fed rats [48,49,56,57]. In rats deprived of food for 18–24 h, the ability of the β-cell to secrete insulin in response to a glucose load is fully dependent upon the elevated levels of circulating NEFAs characteristic of the fasted state [48]. In fact, circulating fatty acids are essential for an efficient glucose stimulation of insulin release after prolonged fasting in humans and rats [58,59].

A recent advance in the understanding of the mechanism(s) by which NEFAs modulate insulin secretion in vivo is the discovery of high levels of expression of the membrane-bound G-protein-coupled receptor GPR40, a putative NEFA receptor in human and animal islet β-cell preparations [60]. The GPR40 mRNA level was positively correlated with the insulinogenic index [60]. The potential signalling mechanism(s) by which GPR40 regulates insulin secretion are still under investigation, but are likely to involve changes in intracellular Ca2+ mobilization [61–63].

**In vitro studies**

Pancreatic islets exposed to high concentrations of NEFAs for periods of 24–48 h [64,65] display enhanced basal insulin secretion, decreased insulin synthesis, depletion of stored insulin and an impaired response of the β-cell to stimulation by glucose; all of which are characteristics seen in Type 2 diabetes. Other studies using pancreatic islets have demonstrated that palmitate increased cytosolic Ca2+ [50]. Rat and human islets exposed to fatty acids for 48 h demonstrated increased insulin release at basal glucose concentration (3 mmol/l), but decreased release at an elevated (stimulatory) glucose concentration (27 mmol/l) [33].

The effects of high concentrations of NEFA on isolated islets and clonal pancreatic insulin-secreting cells are dependent on the period of exposure during cell culture. Acute exposure (1–3 h) of pancreatic islets to NEFAs enhances insulin secretion [66,67] and plays a critical role in modulating the stimulatory effect of glucose on insulin release [50,68]. Acute removal of exogenous NEFAs may, however, result in excessively high rates of GSIS. After incubation of rat pancreatic islets for 4 h with a high concentration of fatty-acid-free BSA, they responded to glucose with extraordinarily high rates of secretion, without changing the typical biphasic pattern of the response [69]. The latter results may argue in favour of a buffering role for NEFAs, such that they siphon off some glucose (in the form of a downstream metabolite, glycerol 3-phosphate) for formation of TAG (see later discussion) so as to ensure that excessively high rates of insulin secretion do not occur. The TAG stores may subsequently release NEFAs upon appropriate stimulation via specific TAG lipases. Expression of UCP-2 (uncoupling protein-2) in β-cells is increased by NEFAs. UCPs are located in the inner mitochondrial membrane and act as proton channels. They uncouple the electrochemical gradient produced by the respiratory chain from ATP synthesis [70,71]. Up-regulation of UCP-2 or overactivity of UCP-2 leads to inhibition of ATP synthesis. Fatty acids increase UCP-2 mRNA and protein levels in several cells including β-cells. As a result, ATP synthesis is reduced and GSIS is blunted. UCP-2−/− mice have lower fasting blood glucose and elevated insulin levels when fed a high-fat diet compared with wild-type mice [72]. Exposure to palmitate reduced GSIS in wild-type islets, whereas UCP2−/− islets had enhanced GSIS.

Importantly, few studies have attempted to explore the interplay between glucose, amino acids and fatty acids with respect to β-cell mass and functional integrity in vitro. In a key recent study [73], culture of clonal BRIN BD11 β-cells for 24 h with the polyunsaturated fatty acid arachidonate increased β-cell proliferation and enhanced amino-acid (alanine)-stimulated insulin secretion. Conversely exposure to the saturated fatty acid palmitate for 24 h was found to decrease β-cell viability (by increasing apoptosis) and increase the intracellular concentration of TAG, while inhibiting alanine-stimulated insulin secretion.

**INTRODUCTION TO THE EFFECT OF NEFAs ON β-CELL APOPTOSIS**

In addition to the acute effects of fatty acids on the insulin secretory function of pancreatic β-cells described above, it has become increasingly clear that fatty acids can also exert complex effects on β-cell viability during more chronic periods of exposure. This response is often perceived as primarily a detrimental effect leading to compromised viability (a condition that has often been described as 'lipotoxicity') (reviewed in [74]). However, this is an oversimplification, as it is increasingly evident that both the chain length and the degree of saturation of fatty acids can exert a dramatic influence on their capacity to affect β-cell viability and that not all fatty acids are intrinsically toxic. In addition, the ambient glucose
concentration also plays a role, leading some authors to suggest that the toxic effects should be described as ‘glucolipotoxicity’ rather than simply as lipotoxicity [75].

**DOES LIPOTOXICITY (OR GLUCOLIPOTOXICITY) OCCUR IN β-CELLS IN VIVO?**

It is important to consider whether (gluco)lipotoxicity proceeds *in vivo* and if it might contribute to β-cell loss under conditions such as those found in Type 2 diabetes, as some workers have proposed that fatty-acid-mediated β-cell toxicity may be primarily an *in vitro* phenomenon that is unrepresentative of the situation found *in vivo* [76].

Robertson et al. [77] have considered this matter in some detail and have argued that the primary alteration associated with Type 2 diabetes is increased oxidative stress that is imposed on pancreatic β-cells by virtue of the prevailing hyperglycaemia. However, when hyperlipidaemia is superimposed on top of hyperglycaemia, this leads to a still more rapid demise of the cells due to the attendant cytotoxic effects of fatty acids operating in the context of elevated glucose.

It is known that elevated circulating NEFAs occur both under fasting conditions and post-prandially in patients with Type 2 diabetes and, therefore, it must be accepted that the potential for lipotoxic effects at the level of the β-cell clearly exists. However, it must also be acknowledged that it is very difficult to obtain conclusive proof to confirm the involvement of lipotoxicity in the progression of Type 2 diabetes in man, although evidence from animal studies is abundantly supportive [78–80]. Moreover, there is strong evidence that net β-cell apoptosis persists in patients with long-term Type 2 diabetes [81] and it is not imprudent, therefore, to deduce that at least part of this is likely to be caused by the chronic elevation of NEFAs.

In considering the implications of these various conclusions it is clear that, *in vivo*, the combined effects of elevated glucose and fatty acids may be more relevant to the long-term effects on β-cell viability than the effects of fatty acids alone. Moreover, it is also evident that any reduction in viability associated with these stimuli occurs over a long timescale. Therefore it is must be recognized that any effects of fatty acids on β-cell viability seen during exposure *in vitro* cannot be fully representative of the situation *in vivo*, but this does not mean that the mechanisms involved are irrelevant or that the effects are purely artefactual. Rather, the *in vitro* system should be viewed as a model in which the pathological processes are increased in speed, but which can still yield important information.

What, then, of the suggestion that lipotoxicity is essentially an artefact of culturing pancreatic β-cells in the presence of exogenous fatty acids *in vitro*? This suggestion has been made most forcibly by Moffitt et al. [76] based on their studies of TAG formation during incubation of a β-cell line INS-1 with exogenous fatty acids. These authors observed that exposing β-cells to exogenous fatty acids leads to increased TAG formation and, as such, they have confirmed earlier results obtained by several other groups [73,82–85]. However, Moffitt et al. [76] have extended their analysis to show that the fatty acid composition of the TAG generated in the cells reflects the chemical structure of the predominant fatty acid species present in the incubation medium, and they noted further that, in the case of long-chain saturated fatty acids (e.g. palmitate), this leads to the formation of TAG species that are essentially solid at physiological temperatures. As a result, these lipid species accumulate in the cell and effectively cause a physical disruption of cellular integrity by virtue of their inflexible physical state. Hence, Moffitt et al. [76] argue that lipotoxicity may be an artefact of the *in vitro* incubation of β-cells with single species of long-chain saturated fatty acids which are not representative of the fatty acid composition of the extracellular fluids encountered by β-cells *in vivo*.

The latter proposition may be correct, but the idea that fatty acid toxicity elicited *in vitro* does not represent a relevant mechanism from which important mechanistic information can be deduced is more contentious. In fact, the implications derived by Moffitt et al. [76] sit at the heart of a, still raging, debate about the functional role of TAG in fatty acid toxicity in β-cells. On one side of the argument are those who consider that fatty-acid-induced TAG formation is beneficial to β-cell survival (by sequestration of potentially toxic fatty acid derivatives) [78,84] whereas, on the other, there are groups who suggest that it is causative to β-cell death (by promoting the physical disruption of the cells) [85–89]. All agree that β-cell TAG levels are increased following exposure to fatty acids, but they diverge dramatically on the interpretation of this finding.

One possibility that might explain the functional impact of TAG accumulation in β-cells is enhanced TAG/fatty acid cycling. This can be expected to consume considerable amounts of glucose (as a supplier of glycerol 3-phosphate for esterification) and ATP, and the diversion of ATP to TAG/fatty acid cycling and release of potentially high intracellular concentrations of NEFAs may negatively impact on β-cell function and viability.

**POTENTIAL MEDIATORS OF β-CELL FATTY ACID TOXICITY**

NEFAs are rapidly transported into cells and the bulk of these are then esterified to form acyl-CoA for subsequent metabolism by β-oxidation or storage as neutral lipids. There is general agreement that long-chain saturated (rather than shorter chain saturated or long-chain
unsaturated) fatty acids are the most toxic to β-cells and that the initial esterification step appears to be critical, as agents such as Triacsin-C, which block this step, effectively prevent β-cell death [75,90,91]. Thus it is not the NEFA itself which causes cell death, but a derivative generated as a result of acyl-CoA synthesis. In support of this, the methyl derivative of palmitate in which the carboxy group is unavailable for esterification to CoA is not toxic (H. J. Welters and N. G. Morgan, unpublished work). Moreover, bromopalmitate, which can be esterified to CoA, but cannot then be oxidized further, is, in our hands, also non-toxic to β-cells, although this has not been universally observed [84]. Moreover, we find that bromopalmitate attenuates the toxicity associated with exposure to palmitate. The simplest explanation for this is that bromopalmitate sequesters the pool of free CoA thereby reducing the rate of esterification of palmitate and minimizing its toxicity, although it cannot be excluded that bromopalmitate may also influence other aspects of palmitate utilization (e.g. protein acylation).

The specificity of fatty acid toxicity in β-cells has not been studied very extensively and most investigators have employed palmitate and/or oleate as the fatty acids of choice. This is probably because these two fatty acids represent the major species present in human serum and, therefore, they are the principal molecules to which β-cells might be exposed in vivo [92,93]. However, a consideration of the specificity of the effects of fatty acids on β-cell viability is informative, as it has been shown that the C14 saturated molecule myristate is tolerated to a much greater extent than palmitate [94,95], implying that some important feature of the way these two molecules are handled determines their relative toxicity in β-cells. Myristate oxidation has not been extensively studied in β-cells and it remains to be confirmed that this proceeds at an equivalent rate to that of palmitate. Arachidonate is also relatively well-tolerated by β-cells and has even been reported to exert beneficial effects on insulin secretion and β-cell mass [73,96]. This may reflect the fact that arachidonate occurs with relatively low abundance in β-cells (approx. 2% of total) and that provision of exogenous arachidonate leads to exchange of more ‘toxic’ species (e.g. palmitate) for arachidonate, thereby exerting beneficial effects.

Whatever the nature of the metabolite that is responsible for initiating the loss of viability caused by palmitate, it seems probable that a succession of additional steps are required to cause the final demise of the cell. A variety of signals have been implicated in this respect; the most studied of which include NO, ceramide formation, inhibition of PKB (protein kinase B)/Akt activity [97,98], activation of calpain-10 [99] or activation of PKCδ [53,97,100].

NO is widely accepted to play a role in the death of β-cells exposed to pro-inflammatory cytokines implicated in the development of Type 1 diabetes, and some investigators have considered NO as a common signal that might also be elevated by fatty acids [101]. Indeed, NO formation has been reported in fatty-acid-treated β-cells by several different groups and this has been correlated with the induction of iNOS (inducible form of NO synthase) in some cases [102–106]. By contrast, a number of other studies have failed to find any direct evidence that fatty acids promote a rise in NO formation in β-cells or in other cell types [84,94,107–109]. The hypothesis of a cell death mechanism involving activation of NF-κB (nuclear factor κB; which regulates the transcription of iNOS) in β-cells exposed to fatty acids has been proposed [101,110,111], but it was shown recently that NF-κB-dependent genes are not induced in response to fatty acids [110]. Thus there is no firm consensus as to whether NO formation represents a common factor that might mediate fatty acid toxicity and it remains enigmatic why some studies report iNOS induction and NO formation in fatty-acid-treated β-cells while others do not.

The sphingolipid ceramide is a second candidate that has been proposed as a mediator of palmitate-induced β-cell toxicity, and this idea sits well with some experimental observations; notably the finding that the rate-limiting step in its biosynthesis shows a marked preference for saturated fatty acids having a chain length of C16 (compared with C14) [112]. Thus ceramide levels are expected to increase in cells exposed to palmitate, whereas they are less likely to be changed when myristate is provided. This difference could provide a convenient explanation for the differential specificity of these two fatty acids as mediators of β-cell death if ceramide formation is involved. The precise mechanisms by which changes in ceramide might cause β-cell death are not entirely clear, although it seems likely that alterations in the disposition of plasma membrane survival and/or death receptor signalling complexes could be involved.

Palmitate has been shown to promote ceramide formation in β-cells [113], and treatment of islet cells with ceramide analogues can lead to loss of viability [102,114], although the magnitude of this effect is often rather modest [115]. In addition, an inhibitor of ceramide synthesis, fumonisin-1B, has been reported to attenuate palmitate-induced cytotoxicity in both rodent [102,116] and human [117,118] islet cells, suggesting that ceramide formation might be involved in fatty-acid-induced death under some circumstances. However, this agent was not effective in pure populations of β-cells [94,107], which implies that ceramide formation may not account completely for the cytotoxic response to palmitate.

One hypothesis that has been considered in some detail is that activation of a specific isof orm of PKC (PKCδ) may underlie the ability of palmitate to promote β-cell death. This is supported by evidence that PKCδ is translocated from the cytosol to the nucleus as an early
event in palmitate-treated cells [53,97,100] and that PKCδ is required for apoptosis in various cell types [119–121]. This may be mediated, at least in some cells, by PKCδ-induced phosphorylation and activation of caspase 3 [122]. Caspase 3 activity is increased in cells treated with palmitate which would be consistent with such a mechanism [75,97,123]; however, much of the evidence implicating PKCδ in palmitate-induced β-cell apoptosis has come from experiments conducted with rottlerin, a purportedly selective PKCδ inhibitor [124], which has been shown to suppress palmitate toxicity very effectively [97,125]. Taken at face value, this would be consistent with a central role for PKCδ in mediating the response to palmitate, but this conclusion may be premature. For example, studies in BRIN-BD11 β-cells suggest that PKCδ can be successfully down-regulated by treatment with PMA, but that this manoeuvre fails to alter their susceptibility to palmitate toxicity or their sensitivity to rottlerin [125]. Although it is known that rottlerin can affect other intracellular signalling components [126], so far none of these has been directly correlated with palmitate toxicity.

In addition to rottlerin, we have also shown [127] that inhibitors of gene transcription and mRNA translation can markedly attenuate the ability of saturated fatty acids to cause β-cell death, suggesting that early changes in gene expression may be instrumental in the process. Indeed, exposure of β-cells to palmitate is associated with a range of alterations in gene expression and some of the candidates have been identified [118,128–131]. Among these is the nuclear receptor Nur77, which has been suggested as a possible regulator of lipotoxicity in response to fatty acids in β-cells, although its precise role has not been defined [132]. In some cells, Nur77 serves to regulate lipid metabolism [133] and it is possible, therefore, that its up-regulation in β-cells represents principally an adaptive response to fatty acid availability.

One potential means to identify the pathway by which saturated fatty acids cause a loss of β-cell viability is to grow cells in medium containing fatty acids and to use this as a means to exert selection pressure such that long-term survivors can be propagated. Such cells can be expected to exhibit a variety of underlying mechanistic alterations, but some may display specific changes in the pro-apoptotic pathway by which fatty acids induce apoptosis. This approach has recently been taken by Busch et al. [134], who succeeded in identifying MIN-6 β-cell clones that are resistant to palmitate toxicity. The resistant cells display elevated rates of palmitate oxidation which implies that oxidation itself is not the means of toxicity, but they also have enhanced expression of a desaturase enzyme involved in cholesterol ester synthesis. As a consequence, the cells also have elevated ratios of oleate to palmitate and this may be related to their resistance to lipotoxicity, since certain unsaturated fatty acids are often protective (see below). However, it is also possible that the diversion of palmitate metabolism towards cholesterol ester formation forms part of the protective mechanism, which supports the concept that palmitate utilization (other than to generate cholesterol esters) is normally important in causing its lipotoxic effects.

A further pathway that should be considered as a potential mediator of lipotoxicity is the ER (endoplasmic reticulum) stress response that is known to be associated with increased apoptosis in a number of situations. ER stress leads to the altered expression of a variety of characteristic genes involved in the regulation of protein synthesis and may also promote the generation of ceramide. It is primarily a protective response designed to minimize cell damage by inhibition of protein synthesis in the face of unfavourable conditions, but prolonged ER stress is ultimately detrimental (reviewed in [135]). In various cell types, including β-cells, exposure to palmitate induces a pattern of protein alterations that is consistent with long-term ER stress [136–138], and imposition of ER stress may, therefore, be a contributory factor to fatty-acid-induced apoptosis in β-cells.

One of the changes in gene expression that may be indicative of a state of ER stress is increased expression of the transcription factor SREBP-1c, which acts as a lipogenic molecule in β-cells [139]. Overexpression of SREBP-1c in the β-cells of transgenic mice leads to increased TAG accumulation and a reduction in β-cell mass [140], and this may recapitulate some of the features of lipotoxicity as SREBP-1c is also elevated in isolated β-cells exposed to fatty acids. Additionally, Yamashita et al. [141] overexpressed SREBP-1c in INS-1 cells, which resulted in enhanced TAG accumulation, blunted GSIS and enhanced expression of UCP-2. siRNA (small interfering RNA) to UCP-2 increased the ATP/ADP ratio and partially rescued GSIS but it did not affect the TAG content. This would argue in favour of increased TAG/fatty acid cycling under conditions of SREBP1c overexpression which, due to elevated cytosolic NEFA concentrations, would activate UCP-2 activity, thus decreasing mitochondrial efficiency. An alternative target for SREBP-1c is the promoter region of IRS-2 (insulin receptor substrate-2) [139], a tyrosine kinase substrate that regulates survival pathways controlled by PI3K (phosphoinositide 3-kinase) and PKB/Akt. Indeed, increased expression of SREBP-1c leads to reduced transcription of IRS-2 in β-cells (probably be displacing certain forkhead transcription factors that are required to mediate transcription), suggesting that loss of IRS-2, and thereby attenuated signalling through the PI3K survival pathway, might be one consequence of exposure of β-cells to fatty acids. This hypothesis warrants further investigation since it has also been proposed that control of IRS-2 levels might represent a common step at which multiple pro-apoptotic pathways converge in the β-cell [142] and blockade of the PI3K pathway may
enhance fatty-acid-induced death in β-cells under some circumstances [107]. Sustained expression of SREBP-1c (perhaps initiated by the ER stress response) may therefore prove to be a critical factor that causes the ultimate loss of viability in β-cells exposed to saturated fatty acids.

Increasing evidence suggests that another key organelle that can regulate the entry of cells into apoptosis is the mitochondrion [143]. It is well established that the inner mitochondrial membrane contains a group of proteins that are regulated to form a pore [the MPTP (mitochondrial permeability transition pore)] through which certain, critical, pro-apoptotic proteins can exit under specific conditions [144]. Among these is cytochrome c which is normally resident within the inner mitochondrial membrane and does not access the cytosol. However, under pro-apoptotic conditions cytochrome c can be released, via the MPTP, to form a cytosolic complex with Apaf-1 (apoptotic protease-activating factor-1) and caspase 9, leading to the activation of the latter enzyme and thereby promoting the effector arm of apoptosis.

It has been reported that exposure of β-cells to saturated fatty acids leads to loss of mitochondrial membrane potential and the release of cytochrome c, suggesting that the mitochondrial pathway may be important for fatty-acid-induced cytotoxicity [105,116,118]. If this is the case, then additional alterations at the level of the mitochondria are likely to precede the release of cytochrome c, as this molecule is normally anchored at the inner face of the lipid bilayer of the mitochondrial inner membrane by cardiolipin. Reduction in the levels of cardiolipin have been implicated in palmitate-induced apoptosis in other cell types [90,145], but has received very little attention as a possible mediator of fatty-acid-induced apoptosis in β-cells. Cardiolipin could, though, play a significant role since its composition will be altered by the prevailing fatty acid milieu. This is because cardiolipin contains four fatty acid molecules in its structure and, normally, these are primarily unsaturated molecules as cardiolipin synthase displays a marked preference for unsaturated fatty acids. If these are replaced with saturated species, then the binding properties of cardiolipin are altered and its affinity for cytochrome c is markedly reduced. This will tend to promote dissociation of cytochrome c from cardiolipin which, in turn, will allow it to exit from the mitochondrion via the MPTP. Thus alterations in the binding of cytochrome c to cardiolipin, arising from changes in the fatty acid composition of its side chains, may account for the increased propensity of saturated fatty acids to induce mitochondrial cytotoxic c release (reviewed in [146]). This mechanism could also explain, in part, why unsaturated fatty acids are better tolerated by β-cells than their saturated counterparts.

In a recent study [147], mitochondrial DNA damage was reported to occur in INS-1 β-cells exposed to fatty acids, and this effect could be overcome by targeted expression of the DNA repair enzyme OGG1 (8-oxoguanine DNA glycosylase/apurinic lyase). Moreover, increased expression of OGG1 minimized the induction of apoptosis in the fatty-acid-treated cells, implying that damage to mitochondrial DNA might be a critical component of the cytotoxicity. The protective effects of OGG1 expression were also accompanied by reduced cytochrome c release from the mitochondria, implying that mitochondrial damage could be causative factor in mediating the lipotoxic response under the conditions employed.

Further evidence for the involvement of mitochondria in NEFA-induced apoptosis comes from studies of the expression of Bcl-2 and related proteins. Bcl-2 is a member of the large family of apoptosis-regulator proteins that either facilitate cell survival (e.g. Bcl-2, Bcl-XL, Bcl-w and others) or promote cell death (Bax, Bak, Bad etc.). The relative amounts of these proteins plays a key role in determining cell fate [148,149], and apoptosis of islet cells in response to saturated fatty acids is accompanied by a marked reduction in Bcl-2 expression [75,83,118,131] and up-regulation of Bax [105].

**PROTECTIVE EFFECTS OF FATTY ACIDS AGAINST β-CELL APOPTOSIS: THE LONG-CHAIN FATTY ACID PARADOX**

One disconcerting feature that emerges from an examination of the literature covering effects of fatty acids in β-cells is that there is often very little consensus about the net outcome as reported by different sets of investigators. This frustration is compounded by the fact that it appears to bear no obvious relationship to the model under investigation (whether a cell line or isolated islets; one species compared with another) and the differential effects reported are frequently diametrically opposed (rather than being subtle alterations of response). This failure to achieve consensus can be extremely unsettling but it should not be readily dismissed. One factor that almost certainly contributes (but does not provide a full explanation) is that fatty acids are intrinsically difficult molecules to work with; not least because they are readily altered in culture (e.g. by peroxidation) and because they can bind in different proportions to many components that are typically present in cell cultures, including proteins (especially albumin and other serum proteins) and even to tissue culture plastics. Thus experiments that are apparently performed under identical conditions in different laboratories may not be equivalent. Indeed, in our hands, even batches of the same albumin formulation purchased from a given supplier can give rise to quite different responses when fatty acids are employed *in vitro*.

However, despite these differences, one common and striking theme that emerges from the literature...
is that saturated and monounsaturated fatty acids of equivalent chain length exert distinct effects on β-cells. As discussed in preceding sections, there is little debate that saturated long-chain molecules are toxic to β-cells during chronic exposure but, equally, an area of reasonably strong consensus is that monounsaturated (and most polyunsaturated) molecules are well tolerated \[84, 94, 107, 116, 118, 150, 151\]. Thus there appears to be a critical difference in response that derives from the introduction of a single double bond into a long-chain fatty acid molecule. It might be easy to dismiss this as the inevitable outcome of a change in a parameter such as membrane fluidity; however, this would be an oversimplification and there may be a much more significant explanation that has important implications for the understanding of β-cell biology.

This is illustrated most clearly from studies in which both saturated and monounsaturated molecules are provided to β-cells in combination. Thus, whereas palmitate is toxic to β-cells when used in isolation, its toxicity is dramatically attenuated (even abolished) by the simultaneous presence of a monounsaturated molecule, such as palmitoleate or oleate \[105, 116, 118, 150\]. Hence these latter species are not ‘inert’ as far as their effects on β-cell viability is concerned; rather they are actively protective. This is an important distinction that has critical implications for an understanding of their actions.

Perhaps the most obvious factor that might influence the toxicity of a saturated fatty acid is the ability of another agent (including an unsaturated analogue) to alter some aspect of its metabolism. This could occur at any one of several levels ranging from a change in the rate of uptake into the cell (e.g. by competing for a common transporter) to competition for esterification to CoA and/or an alteration in the ultimate metabolic fate. These various possibilities have only been investigated to a limited extent in β-cells, but evidence has been obtained \[94\] which supports the view that the anti-apoptotic protection exerted by monounsaturated fatty acids does not require their activation or subsequent metabolism in the β-cell. Among these data are studies with etomoxir and poorly metabolized fatty acid derivatives \[94\]. For example, etomoxir completely failed to alter the ability of palmitoleate to protect β-cells against serum-withdrawal-induced apoptosis, suggesting that mitochondrial entry and subsequent β-oxidation of the monounsaturated molecule is not a pre-requisite for its protective action \[94\]. Moreover, the non-metabolizable analogue methyl-palmitoleate attenuated β-cell cytotoxicity very effectively \[152\], thereby providing additional evidence that formation of fatty acyl-CoA is not required for the protective response, since the presence of the methyl group will mitigate against acyl-CoA formation. Thus the protective mechanism must involve a process that is selectively activated by (mono)unsaturated fatty acids, but which does not directly require their metabolism within the cell.

The validity of this conclusion is illustrated most dramatically by experiments in which the time of addition of saturated and monounsaturated fatty acid to cultured β-cells was varied to eliminate early changes in metabolic fate arising from the combined presence of both molecules. Under such conditions, it was established that the unsaturated molecule palmitoleate could be introduced as late as 6 h after the addition of palmitate without dramatically altering its ability to protect β-cells against the cytotoxic effects of the saturated molecule \[94\]. This result has several implications. First, it confirms that the toxicity of palmitate is not mediated by an instant ‘lytic’ effect (perhaps due to an action on membrane permeability), as this would not be preventable by an intervention occurring as much as 6 h later. Secondly, it implies that the protective action of palmitoleate is likely to occur rapidly since, in the absence of this molecule, the toxicity of palmitate is already becoming evident within 6–8 h and cell death is increasing at this time during incubation in vitro. When considered in the context of the data (outlined above) showing that the non-metabolizable derivative of palmitoleate, methyl-palmitoleate, is equally effective as palmitoleate at protecting β-cells against palmitate toxicity, this suggests very strongly that altered fatty acid metabolism does not underlie the protective response.

Taken together, these observations suggest that the monounsaturated fatty acids may control β-cell viability in a more fundamental way and data to support this have now been provided. Arguably, the most convincing evidence in this regard has come from the unexpected demonstration that monounsaturated molecules retain their ability to exert protective actions when β-cell viability is reduced by alternative means that do not involve incubation with saturated fatty acids. For example, the loss of viability associated with withdrawal of serum from cultures of β-cells is prevented by the addition of palmitoleate, and the well-known cytotoxic actions of pro-inflammatory cytokines are similarly attenuated by the monounsaturated fatty acid \[94\]. Neither of these responses would be expected to involve an alteration in the metabolism of saturated fatty acids and, therefore, they point to the conclusion that palmitoleate controls β-cell viability in an entirely different way.

One obvious possibility is that monounsaturated fatty acids may exert their protective effects by interaction with either a cell-surface or intracellular receptor, rather than by acting at an intracellular site. Consistent with this idea is the fact that the protective actions occur at very low concentrations of NEFAs, which are likely to be in the low (or even sub) nanomolar range and are at least 10-fold less than the concentration of saturated molecules required to initiate cell death. (Note that the total concentration of fatty acid is always much
Role of non-esterified fatty acids in β-cell function and integrity

Figure 2  Potential cytoprotective effects of NEFAs

Fatty acids (FFA) may enter the β-cell and become activated by conversion into acyl-CoA. The fatty acids may then become incorporated into TAG, be oxidized in the mitochondrial matrix, be incorporated into cardiolipin or bind to and activate specific transcription factors or receptors. NEFA may also bind to GPR40 receptors and stimulate relevant signal transduction pathway(s). All or some of these mechanisms may help protect the β-cell from potentially damaging nutrient or inflammatory factors. AA, arachidonate; MUFA, monounsaturated fatty acid.

higher than the free concentration since most is not biologically active due to its binding by other components present in the incubation medium.) It is well known that fatty acids can interact with the class of intracellular PPARs (peroxisome-proliferator-activated receptors), and there are reports that other agonists of these molecules (including some thiazolidinedione PPARγ agonists) can protect β-cells against NEFA-induced damage [80,83,153], although this has not been seen in all studies [127].

The idea that NEFAs may exert some of their effects by interaction with cell-surface receptors has emerged only relatively recently, but is becoming increasingly accepted with the description of at least two previously designated ‘orphan’ G-protein-coupled receptors (GPR40 and GPR120), whose endogenous ligands appear to be long-chain fatty acids (others with specificity for short-chain fatty acids also exist) [61,154,155]. One of these, GPR40, is known to be expressed in β-cells and has been implicated in mediating the acute regulatory effects of long-chain fatty acids on insulin secretion [61–63,154,156]. However, this receptor appears to bind both saturated and unsaturated molecules with approximately equal affinity and, for this reason, appears unlikely to be responsible for mediating the highly selective effects of unsaturated molecules on cell viability. The possible role of GPR120 has not yet been studied in β-cells, although, in the original report describing it as a NEFA receptor, it was suggested to be absent from the β-cell line MIN-6 [157]. These findings suggest that GPR120 may not be a prime candidate in mediating the protective effects of unsaturated fatty acids in β-cells, but this conclusion still requires verification in other β-cell lines and in normal islets. This is especially true since GPR120 has recently been implicated as the mediator of fatty-acid-induced protection during serum-withdrawal-mediated apoptosis in the intestinal cell line STC-1 [155].

Given the (still hypothetical) possibility that monounsaturated fatty acids may protect β-cells via ligation of a cell-surface receptor, this raises a question about the second messenger system that might underlie the response. One well-described receptor-mediated anti-apoptotic signalling mechanism in β-cells is exemplified by GLP-1, which appears to act by raising cAMP levels (reviewed in [158,159]). Thus it is possible that cAMP could represent a common anti-apoptotic signalling molecule in the β-cell whose level might be regulated by monounsaturated fatty acids. We have examined this possibility directly [160] and have shown that cAMP is not elevated in BRIN-BD11 β-cells exposed...
to palmitoleate under conditions when its potent anti-apoptotic effect is evident. On this basis, we do not consider that CAMP represents a strong candidate to fulfill the requirements of the second messenger mediating the protective actions of monounsaturated fatty acids. In support of this, we have noted further that, when \( \beta \)-cells are treated with saturated fatty acids under conditions favouring a rise in cAMP, their viability is not improved significantly [160]. This lack of protection occurs despite a reduction in caspase 3 activity in cells having elevated cAMP and appears to reflect a switch from a primarily apoptotic mode of death, to necrosis, under these conditions. This situation stands in marked contrast with that seen when monounsaturated fatty acids are employed as the protective agents, where cell viability is maintained at a high level and secondary necrosis does not occur [160].

Alternative intracellular pathways that could be involved in the cytoprotection mediated by monounsaturated fatty acids abound, but none has yet emerged as a strong candidate. We have summarized some of the possible cellular protective pathways in Figure 2. Indeed, few protective mechanisms have been investigated in depth, although Beeharry et al. [107] have reported that inhibitors of the PKB/Akt pathway (acting at the level of PI3K) can minimize the protective effects of unsaturated fatty acids in \( \beta \)-cells. This could imply that PI3K and thus pro-survival signalling is regulated by monounsaturated fatty acids, but this conclusion remains to be confirmed. Even if it proves to be the case, then this still leaves open the question as to how unsaturated fatty acids initiate the apoptotic effect is evident. On this basis, we do not consider that CAMP represents a strong candidate to fulfill the requirements of the second messenger mediating the protective actions of monounsaturated fatty acids. In support of this, we have noted further that, when \( \beta \)-cells are treated with saturated fatty acids under conditions favouring a rise in cAMP, their viability is not improved significantly [160]. This lack of protection occurs despite a reduction in caspase 3 activity in cells having elevated cAMP and appears to reflect a switch from a primarily apoptotic mode of death, to necrosis, under these conditions. This situation stands in marked contrast with that seen when monounsaturated fatty acids are employed as the protective agents, where cell viability is maintained at a high level and secondary necrosis does not occur [160].

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105 Maestre, L., Jordan, J., Calvo, S. et al. (2003) Mitochondrial dysfunction is involved in apoptosis induced by serum withdrawal and fatty acids in the β-cell line INS-1. Endocrinology 144, 335–345


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