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Glucolipotoxicity of the Pancreatic Beta Cell

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Summary

The concept of glucolipotoxicity refers to the combined, deleterious effects of elevated glucose and fatty acid levels on pancreatic beta-cell function and survival. Significant progress has been made in recent years towards a better understanding of the cellular and molecular basis of glucolipotoxicity in the beta cell. The permissive effect of elevated glucose on the detrimental actions of fatty acids stems from the influence of glucose on intracellular fatty-acid metabolism, promoting the synthesis of cellular lipids. The combination of excessive levels of fatty acids and glucose therefore leads to decreased insulin secretion, impaired insulin gene expression, and beta-cell death by apoptosis, all of which probably have distinct underlying mechanisms. Recent studies from our laboratory have identified several pathways implicated in fatty-acid inhibition of insulin gene expression, including the extracellular-regulated kinase (ERK1/2) pathway; the metabolic sensor Per-Arnt-Sim kinase (PASK); and the ATF6 branch of the unfolded protein response. We have also confirmed in vivo in rats that the decrease in insulin gene expression is an early defect which precedes any detectable abnormality in insulin secretion. While the role of glucolipotoxicity in humans is still debated, the inhibitory effects of chronically elevated fatty acid levels has been clearly demonstrated in several studies, at least in individuals genetically predisposed to developing type 2 diabetes. It is therefore likely that glucolipotoxicity contributes to beta-cell failure in type 2 diabetes as well as to the decline in beta-cell function observed after the onset of the disease.

Keywords

Fatty acids; Glucose; Islet of Langerhans; Diabetes; Insulin

I. Introduction

Over the last 20 years, the central role of pancreatic beta-cell dysfunction in the development of type 2 diabetes has become increasingly appreciated [1]. It is now generally accepted that when insulin resistance develops in response to environmental cues such as obesity, a subset of genetically predisposed individuals fails to adequately compensate for the increased insulin demand, and beta-cell failure ensues [2]. In addition, longitudinal studies in humans have

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clearly demonstrated that beta-cell function deteriorates during the years following diagnosis of type 2 diabetes, regardless of the therapeutic regimen [3,4]. Although the cause of this metabolic deterioration is unknown, several hypotheses have been proposed. Amongst them, chronic hyperglycemia (glucotoxicity [5]), chronic dislipidemia (lipotoxicity [6]), or the combination of both (glucolipotoxicity [7]), have been postulated to contribute to the worsening of beta-cell function over time, creating a vicious cycle by which metabolic abnormalities impair insulin secretion, which further aggravates metabolic perturbations, and so on. While elevated levels of glucose or fatty acids can, by themselves, be demonstrated to have detrimental effects on beta-cell function in many experimental systems, the combination of both nutrients is synergistically harmful, which has led to the concept of glucolipotoxicity [7,8]. However, despite years of investigation and significant progress made in the discovery of the underlying molecular and cellular mechanisms of glucolipotoxicity, its contribution to beta-cell failure in type 2 diabetes remains debated. We speculate that this uncertainty stems from several reasons. First, by nature of their long-term design, experiments to test cause-and-effect relationships between chronic metabolic perturbations and functional outcomes are plagued with confounding variables, and therefore difficult to interpret. Second, the inherent limitations of in vivo models have prompted the development of many in vitro systems to test the hypothesis and define its underlying mechanisms. As further discussed in this review, these systems also have important caveats. Third and perhaps most importantly, there is no clear consensus on the definition of the term glucolipotoxicity. While its root (toxicity) implies the presence of cell death, it is often employed more loosely to refer to the functional effects of the combination of high glucose and elevated lipids on the beta cell, for instance on insulin secretion or gene expression. Also, while the concept of glucolipotoxicity implicitly refers to a chronic situation, the notion of chronicity is variable, spanning from a few hours of ex vivo cell culture to many years in diabetic patients. This is particularly problematic since fatty acids have a dual and time-dependent effect on beta-cell function, acutely stimulatory but chronically inhibitory. Thus, there are virtually as many definitions of the term glucolipotoxicity as groups studying it, which has created confusion in the field. For the purpose of this article, we propose to define glucolipotoxicity as the combined, deleterious effects of elevated glucose and fatty acid levels on pancreatic beta-cell function and/or survival. This review focuses on recent developments in the field of glucolipotoxicity from both in vitro and in vivo studies.

II. Cellular and molecular mechanisms of glucolipotoxicity in the beta cell

Considering the complexity of designing mechanistic studies in vivo to investigate the chronic effects of fuel oversupply, a number of in vitro models, using insulin-secreting cells and isolated islets, have been employed to identify the cellular and molecular basis of glucolipotoxicity. In these systems, prolonged exposure to elevated levels of fatty acids is associated with inhibition of glucose-induced insulin secretion [9–12], impairment of insulin gene expression [13–18], and induction of cell death by apoptosis [19–28]. Importantly, several of these studies have provided evidence that lipotoxicity only occurs in the presence of concomitantly elevated glucose levels [15,16,28], an observation also confirmed in vivo [29, 30]. The biochemical basis for this permissive effect of glucose will be discussed first in this section, followed by a review of the mechanisms underlying the functional manifestations of glucolipotoxicity on the beta cell (insulin secretion, insulin gene expression, and cell survival).

A. Biochemical pathways and lipid intermediates implicated in glucolipotoxicity

The permissive effect of glucose on the deleterious actions of chronic fatty acids stems from its influence on intracellular metabolism of fatty acids [31,32]. Prentki and Corkey [7] first proposed that glucose determines fatty acid partitioning in pancreatic beta cells (Figure 1). At low glucose concentrations, fatty acids are transported into the mitochondria via the enzyme carnitine-palmitoyl transferase-1 (CPT-1) for beta-oxidation, which has essentially no

functional consequences. In contrast, when both glucose and fatty acid concentrations are elevated, intracellular metabolism of glucose leads to the formation of cataplerotic signals, such as citrate, and the generation of malonyl-CoA in the cytosol. Since fatty-acid synthase activity is lower than that of acetyl-CoA carboxylase in the beta cell [33], the predominant effect of malonyl-CoA is to inhibit CPT-1 activity, which in turn blocks fatty acid oxidation and leads to accumulation of long-chain acyl-CoA esters (LC-CoA) in the cytosol [7]. Accumulation of cytosolic LC-CoA, either directly or via generation of lipid-derived signals, adversely affects beta-cell function [8]. In addition to its metabolic effects directing fatty-acid partitioning into esterification, glucose coordinately activates the expression of genes involved in lipogenesis [34]. A key player in this mechanism is the enzyme AMP-activated protein kinase (AMPK), acting as a metabolic sensor that directs the beta cell into a “storage mode” in the face of nutrient oversupply [35], as it does in myocytes and hepatocytes [36]. Indeed, AMPK activity is inversely correlated with the glucose concentration [37] and is stimulated by palmitate [38] in beta cells. Downstream of AMPK, the transcription factor sterol-regulatory-element-binding-protein-1c (SREBP1c), which regulates the expression of genes controlling fatty acid synthesis [39], translates the metabolic signal sensed by AMPK into changes in gene expression, leading to enhanced lipogenesis. Glucose also increases the expression of liver X receptor (LXR) which then contributes to enhancing SREBP1c expression and lipid synthesis [40].

While it is now generally accepted that fatty acid partitioning towards esterification and cellular lipid synthesis underpins the cellular mechanisms of glucolipotoxicity in pancreatic beta-cells, the nature of the lipid-derived metabolites directly responsible for the deleterious effects of fatty acids is still elusive. It is unlikely that triglyceride accumulation itself might be the culprit, since triglycerides represent a relatively innocuous form of fat storage that can actually protect against lipotoxicity [41]. Studies have shown that monounsaturated fatty acids are less toxic and can actually protect from the detrimental effects of unsaturated fatty acids because they are more readily esterified into triglycerides [26,41]. Consistent with this notion is the observation that stearoyl CoA desaturase-1 (SCD1) protects from lipoapoptotic cell death induced by palmitate [42]. In fact, whereas deletion of SCD1 in mice improves insulin sensitivity [43], when introduced on the obese, leptin-deficient *ob/ob* background the SCD1 deletion leads to a worsening of diabetes associated with triglyceride and cholesterol overload in islets [44].

Prentki and colleagues [45,46] have proposed the elegant concept that increased glycerolipid/fatty acid cycling represents a mean by which the beta cell attempts to protect itself from nutrient oversupply while remaining fuel-responsive so as to be capable of releasing insulin in the face of increased demand. In turn, the unintended consequence of this fuel detoxification mechanism is the generation of harmful intermediates from increased flux through the cycle. The question remains that if triglyceride accumulation is merely a marker of enhanced esterification flux but does not cause glucolipotoxicity by itself, then what are the lipid-derived molecules directly responsible for the impairment of beta-cell function? The role of intermediates of the esterification pathway (e.g. lysophosphatidic acid, phosphatidic acid, diacylglycerols) has been suggested [2] but, to our knowledge, not formally demonstrated. De novo synthesis of ceramide has been shown to play a role both in fatty acid-induced beta-cell death [47] and fatty acid-inhibition of insulin gene expression [17], but not in the impairment of insulin secretion [48]. These observations illustrate an important point, which may in part explain why the lipid-derived intermediates mediating glucolipotoxicity have remained elusive: the mechanisms underlying the various functional manifestations of glucolipotoxicity are likely distinct. For example, accumulation of ceramide impairs insulin gene expression and, under certain circumstances, induces cell death, without affecting insulin secretion. Therefore, our view is that the full array of functional defects associated with glucolipotoxic

conditions is due to the generation of several intracellular metabolites acting on various signaling pathways and cellular functions rather than to a single intermediate.

While most studies investigating the mechanisms of glucolipotoxicity in the beta cell have focused on the esterification pathway and triglyceride synthesis, cholesterol metabolism has recently been shown to also play an important role. Exposure of beta cells to oxidized low-density lipoproteins (LDL) induces apoptosis [49] and decreases insulin gene expression [50], whereas native LDL particles have no effect and high-density lipoproteins (HDL) are protective. Beta-cell specific knock-out (KO) of the ATP-binding cassette transporter subfamily A member 1 (ABCA1), which mediates reverse cholesterol efflux, results in increase cellular cholesterol content and impaired insulin secretion downstream of glucose metabolism, probably at the level of insulin exocytosis [51]. In addition, the ability of the thiazolidinedione rosiglitazone to improve glucose tolerance in high-fat diet fed mice requires a functional ABCA1 in beta cells [51]. Finally, forcing cholesterol synthesis in beta cells by transgenic overexpression of SREBP2 under the rat insulin promoter results in a severe loss of beta-cell mass and a diabetic phenotype [52]. Since LXR regulates ABCA1 expression [51] and is itself directly regulated by glucose [53], glucose therefore coordinately increases fatty acid esterification and intracellular cholesterol synthesis.

The premise to the hypotheses described above that intermediates generated during triglyceride or cholesterol synthesis are mechanistically involved in glucolipotoxicity is that extracellular fatty acids are first transported across the plasma membrane and act intracellularly. This concept has been challenged by the deorphanization of the G-protein coupled receptor GPR40 [54,55]. GPR40 is specifically expressed in pancreatic beta cells and is activated by long-chain fatty acids, which raises the possibility that some of the functional effects of fatty acids on the beta-cell might be mediated by activation of a cell-surface receptor. Consistent with this possibility, a role for GPR40 in mediating fatty acid-inhibition of insulin secretion has been suggested by the observation that islets from GPR40 KO mice are insensitive to the inhibitory effects of prolonged fatty acids [56]. Using a different line of GPR40 KO mice, we were unable to reproduce these findings and found that deletion of the receptor does not protect islets from fatty acid-inhibition of glucose-induced insulin secretion [57]. In addition, subsequent studies also using whole-body KO found that GPR40 deletion did not protect mice from high-fat diet-induced glucose intolerance [58,59]. This conclusion was further supported by the observation that small molecule GPR40 agonists improved glucose tolerance in mice with high-fat diet-induced obesity [60]. Therefore, we do not favor the view that GPR40 plays a major role in the mechanisms of glucolipotoxicity in the beta cell.

B. Mechanisms underlying the functional manifestations of glucolipotoxicity

1. Fatty-acid impairment of insulin secretion—Prolonged exposure of beta cells to fatty acids in vitro inhibits glucose-stimulated insulin secretion [9–12], a phenomenon also observed in vivo in rats [61] and humans [62]. In recent years, several potential mechanisms have been investigated, including upregulation of uncoupling protein 2 (UCP2), activation of the novel isoform of protein kinase C PKCε, and late exocytotic events.

UCP2 is a ubiquitously expressed mitochondrial carrier which has been suggested to uncouple the respiratory chain from ATP synthesis [63], although its biological functions are still unclear [64]. Initial evidence suggested that UCP2 might modulate insulin secretion and thereby play a role in glucolipotoxicity. This was based on the observations that increasing UCP2 expression in beta cells impairs insulin secretion [65,66] and that UCP2 KO animals on a mixed genetic background have increased circulating insulin levels and are protected from diabetes [63,67]. This contention has been recently challenged by the observation that KO of UCP2 on 3 different congenic backgrounds in the mouse leads to oxidative stress and impaired insulin secretion [68]. Thus, the increase in UCP2 expression observed in islets after high-fat feeding in rodents

[30,66] or exposure to fatty acids in vitro [69,70] likely represents a cellular defense mechanism against fuel overload and oxidative stress rather than a deleterious response. Consistent with this possibility is the observation that transgenic overexpression of UCP2 does not alter mitochondrial function or glucose-induced insulin secretion but decreases reactive oxygen species production [71]. Overall, it appears unlikely that an increase in UCP2 expression in response to fatty acids represents a causal mechanism of the impairment of insulin secretion under glucolipotoxic conditions.

Activation of the lipid-regulated isoform PKCε has also been suggested as a possible candidate signaling molecule underlying the decrease in insulin secretion in glucolipotoxicity. Work by the group of Biden has shown that the normalization of glucose tolerance in PKCε KO mice under high-fat feeding was due to improved insulin secretion [72]. Further, they demonstrated that islets isolated from PKCε knockout mice were protected from the deleterious effects of fatty acids on insulin secretion in vitro, and that inhibition of PKCε was capable of restoring insulin secretion in islets from *db/db* mice [72]. More recently, this group has shown that the improvement in insulin secretion in PKCε knock-out islets in the face of glucolipotoxicity was due to selective restoration of the amplifying pathway of insulin release, probably due to the generation of a lipolytic intermediate [73]. Interestingly, this is consistent with the concept proposed by Prentki and colleagues that lipolysis-generated signals contribute to the regulation of insulin secretion [74] and that, more generally, glycerolipid/fatty acid cycling in the beta cell provides essential coupling factors for insulin secretion but becomes detrimental under conditions of fuel oversupply [45,46].

Finally, evidence suggests that fatty acids might alter one or more late steps of insulin exocytosis in beta cells. Kato et al. [75] have shown that expression of granuphilin, an effector of the small GTP-binding protein Rab27a, which plays a key role in the docking of insulin secretory granules to the plasma membrane, is increased in islets exposed to palmitate as a consequence of upregulation of SREBP1c. This in turn inhibits insulin secretion in response to fuel and non-fuel stimuli. In addition, Olofsson et al [76] demonstrated that prolonged exposure of mouse islets to glucose and fatty acids inhibited insulin secretion at a very late stage of exocytosis by interfering with the release of insulin at the fusion pore. These findings suggest that the mechanisms by which fatty acids affect insulin secretion might, at least in part, lie at the level of the exocytotic machinery and, consequently, impair insulin secretion in response not only to glucose but also to other secretagogues.

2. Fatty-acid impairment of insulin gene expression—We [15–18,77] and others [13,14] have shown that prolonged exposure to fatty acids impairs insulin gene expression in the presence of high glucose. The mechanisms whereby fatty acids affect insulin gene expression are distinct from those by which they impair insulin secretion. First, whereas both palmitate and oleate inhibit insulin secretion, only palmitate affects insulin gene expression [48]. This is due to the fact that only palmitate can serve as a substrate for de novo ceramide synthesis [17]. The transcriptional mechanisms by which palmitate inhibits insulin gene expression do not involve changes in insulin mRNA stability but, rather, inhibition of glucose-induced insulin promoter activity [17]. This is associated with decreased binding activity of the transcription factors pancreas-duodenum homeobox 1 (PDX-1) and MafA [18]. PDX-1 is affected in its ability to translocate to the nucleus, whereas MafA is affected at the level of its expression [18]. This is in contrast to the mechanisms of glucotoxicity, which involve post-translational modifications of MafA [78].

The mechanisms whereby ceramide generation from palmitate impairs PDX-1 subcellular localization and MafA expression are unknown, although recent studies have identified potential candidates. The c-jun NH2-terminal kinase JNK is a known target of ceramide [79] and can repress insulin gene transcription both via c-jun-dependent inhibition of E1-mediated

transcription [80,81] and c-jun independent inhibition of PDX-1 binding [82]. In addition, Solinas et al. [83] have shown that palmitate activates JNK in beta cells and that the resulting phosphorylation of insulin receptor substrates 1 and 2 at sites that impair insulin signaling decreases insulin gene transcription.

Recent studies in our laboratory have also attempted to identify the signaling mechanisms implicated in palmitate inhibition of insulin gene expression. First, we have shown that palmitate enhances glucose-induced phosphorylation of the extracellular-regulated kinases (ERK) 1/2, and that pharmacological inhibition of ERK1/2 partially restores insulin gene expression in insulin-secreting cells and isolated islets exposed to palmitate or ceramide [84]. Second, we have observed that palmitate blocks the induction of the Per-Arnt-Sim kinase (PASK) [84]. PASK is an evolutionarily conserved serine/threonine protein kinase, containing a PAS domain sensitive to the intracellular environment which regulates the kinase domain to transduce the signal [85]. In budding yeast, it coordinates sugar storage and protein synthesis with carbohydrate availability [86]. In mammals, it has been demonstrated to be an important regulator of glycogen synthase and cellular energy balance [87]. In pancreatic beta cells, PASK is required for glucose-induced insulin gene transcription [88]. In our recent study [84], we observed that overexpression of PASK prevents the inhibitory effect of palmitate on insulin mRNA and PDX-1 mRNA and protein expression in MIN6 cells. In addition, adenoviral-mediated overexpression of wild-type PASK increased, whereas a kinase dead mutant of PASK acting as a dominant negative decreased, insulin mRNA and PDX-1 protein expression in islets. Interestingly, the PASK pathway appears to be independent from the ERK1/2 pathway and to have no effect on MafA expression in our system, suggesting that at least 3 independent signalling arms contribute to the overall decrease in insulin gene expression [84] (Figure 2). Although our initial study revealed that palmitate mostly affects PDX-1 in its subcellular localization rather than its whole-cell expression levels [18], overexpression of a kinase dead mutant of PASK also reduces PDX-1 mRNA levels. This suggests that reduction of PDX-1 expression might also contribute to decreasing its binding activity under glucolipotoxic conditions. Whether PASK can directly phosphorylate PDX-1 and, thereby, alter its nuclear translocation is unknown and currently under investigation. Recently, expression the CAAT enhancer-binding protein β (C/EBPβ), a negative regulator of insulin gene transcription [89] has been shown to increase in beta cells in response to fatty acids [90]. Interestingly, we also observed a marked increase in C/EBPβ mRNA levels upon overexpression of the dominant-negative PASK mutant in MIN6 cells [84]. This raises the possibility that, as demonstrated under glucotoxic conditions [91], C/EBP-β binds to the transcription factor nuclear factor of activated T cells (NFAT) on the insulin promoter and thereby inhibits MafA binding activity.

A role for the unfolded protein response (UPR) and endoplasmic reticulum (ER) stress in beta-cell failure has received considerable attention in the past few years, in part because the beta cell's intense secretory activity makes it particularly susceptible to perturbations of ER homeostasis [92]. As discussed in more details in the next section, markers of ER stress have been shown to be induced by prolonged exposure to fatty acids in several studies [93–101]. In most cases, the strong induction of ER stress markers in response to fatty acids is associated with apoptosis. Under our culture conditions of isolated rat islets in the presence of glucose and palmitate, which are not associated with significant cell death [84,102], we have not been able to detect any activation of the inositol requiring ER-to-nucleus signal kinase (IRE) or protein kinase R-like ER kinase (PERK) branches of the UPR (unpublished data). In contrast, we have observed cleavage of the transcription factor ATF6 under these conditions. Since ATF6 is a negative regulator of insulin gene transcription [103], these preliminary results led us to hypothesize that an early activation of the ATF6 branch of the unfolded protein response upon exposure to fatty acids might represent a protective mechanism whereby the beta cell attempts to further decrease the load to the ER by inhibiting insulin gene expression. This would occur as part of the unfolded protein response, before overt ER stress and associated

apoptosis develops. In later stages of more severe ER stress associated with cell death, it is possible that alterations in PDX-1 function [96,104] or insulin mRNA stability [105] also contribute to the decrease in insulin gene expression.

Overall, available data regarding the mechanisms of fatty-acid inhibition of the insulin gene reveal a complex picture which appears to involve several independent pathways that all concur to decrease its expression, which is an early, and possibly protective, response of the beta cell in the face of nutrient oversupply (Figure 2). Importantly, the decrease in insulin gene expression under glucolipotoxic conditions is also observed in vivo ([77]; see section III below).

3. Fatty acid induction of beta-cell death—Saturated fatty acids can induce beta-cell death by apoptosis in the presence of high glucose [22,26,28], whereas unsaturated fatty acids are usually protective [21,22,28]. As mentioned above, this difference is likely due to the greater ability of unsaturated fatty acids to form intracellular triglycerides [21,41,42]. Several mechanisms have been implicated, including ceramide formation [20,23,26,47], oxidative stress [25,27,106,107], and inflammation [108]. Recently, as mentioned above considerable evidence has been provided in support of a role for the UPR and ER stress in saturated fatty-acid induced cell death ([93–101] and reviewed in [59]). The mechanisms by which saturated fatty acids such as palmitate induce ER stress are thought to involve depletion of ER calcium stores [99,101] and result in the activation of JNK [99,100], although JNK activation can, under some conditions, be detected prior to the appearance of ER stress [98]. Interestingly, palmitate was shown to induce a rapid degradation of carboxypeptidase E, which resulted not only in altered proinsulin maturation, but also in ER stress and apoptosis [109]. The changes in CPE levels were demonstrated to occur prior to the development of any sign of ER stress, and to require palmitate metabolism and calcium influx, although the precise mechanisms by which palmitate initiates CPE degradation remain to be clarified [109]. Of note, however, a study by Lai et al. [110] using insulin-secreting cells and isolated islets provided evidence that palmitate-induced apoptosis can also occur in the absence of detectable ER stress. Finally, markers of ER stress are increased in pancreatic sections of type 2 diabetic patients [111].

These observations raise the question as to whether fatty-acid induced apoptosis in beta cells is primarily mediated by ER stress or the mitochondrial death pathway. Intrinsic defects in mitochondrial function have been well documented under conditions of nutrient overload [112], and perturbations in mitochondrial permeability are observed early in the development of fatty-acid induced cell death in beta cells [113]. Luciani et al. [114] have recently shown that depletion of ER calcium stores under conditions of ER stress can lead secondarily to mitochondrial dysfunction, suggesting that perhaps under glucolipotoxic conditions ER stress is a primary event which leads to triggering of several proapoptotic pathways, including mitochondrial-mediated cell death.

Finally, a recent study by Lovis et al. [115] has shown that increased expression of the microRNAs miR34a and miR146 also contributes directly to palmitate-induced cell death in insulin-secreting cells and isolated islets, and the overall role of microRNAs in glucolipotoxicity will hopefully become clearer as progress towards understanding their implications in beta-cell function continues to be made.

C. Limitations of in vitro studies of glucolipotoxicity

While in vitro models using insulin-secreting cells and isolated islets have proven extremely valuable in dissecting the cellular and molecular mechanisms of glucolipotoxicity, they also have significant limitations which should be borne in mind when interpreting the results obtained in these systems. First, there appears to be species-related differences in the sensitivity to fatty acid-induced cell death [110]. For instance, whereas a 24-h exposure of human islets

to elevated glucose and palmitate is sufficient to observe apoptosis [28], we have not detected any cell death in rat islets after 72h of culture under similar conditions [17,48,84]. Second, the concentrations of fatty acids used in vitro vary amongst publications. The key determinant of fatty acid potency is the fraction that is unbound to BSA, which depends on the molar ratio of fatty acids to albumin as well as the mode of preparation. Using a fluorescent probe that specifically measures the unbound fraction of fatty acids [116], we observed that when palmitate at a total concentration of 0.5 mM was pre-complexed to bovine serum albumin with a molar ratio of fatty acid:albumin of 5:1, the unbound concentration is in the range of 200 nM (Figure 3), which represents approximately 3 times the unbound concentration measured in the plasma of lean individuals by the same method [117]. Finally, the concentrations of fatty acids in the vicinity of the beta-cells in vivo are unknown and are probably determined by several different factors, including the activity of lipoprotein lipase, which accounts for some of the local delivery of fatty acids to the cells [118]. In fact, it is likely that lipoprotein lipase activity is an important control point for fatty acid delivery to beta-cells, since both beta-cell specific deletion and overexpression of its gene in the mouse impairs glucose homeostasis and insulin secretion [119]. Thus, the results of in vitro experiments using fatty acids should be interpreted with caution, particularly when marked cytotoxicity is observed.

III. In vivo studies

A. Rodent models of glucolipotoxicity

For the reasons described above, the findings of in vitro studies should be confirmed in vivo before they can be extrapolated to physiological or pathological situations. In this regard, pioneering studies by the group of Unger in the Zucker Diabetic Fatty (ZDF) rat were instrumental in establishing the concept of lipotoxicity and identifying some of its basic mechanisms (reviewed in [120]). In particular, these studies first identified the key role for ceramide as an intracellular mediator of glucolipotoxicity. Thus, in this model accumulation of intra-islet ceramide is detected prior to beta-cell dysfunction [121] and inhibition of ceramide synthesis prevents beta-cell death [47]. In more recent studies the beneficial effects of pharmacological inhibition of sphingolipid synthesis on beta-cell function and diabetes progression has been confirmed not only in the ZDF rat but also in other rodent models [122–124]. However, since ceramide is also implicated in the mechanisms of insulin resistance [123], it is difficult in these in vivo studies to distinguish between the effects of the treatment on insulin sensitivity and those on beta-cell function.

Non-genetic models of glucolipotoxicity have been developed and most often use prolonged infusions of Intralipid, a soybean oil emulsion which generates a mixture of mostly unsaturated fatty acids [125] when co-injected with heparin. In these models, the effects of Intralipid or fatty-acid infusion on beta-cell function have been inconsistent, leading to either unaffected [77], enhanced [126,127] or reduced [9,61,128,129] insulin secretion. These discrepancies are likely due to differences in strain, sex, age, or infusion rates. For instance, Mason et al. [61] and Goh et al. [128] suggested that female Wistar rats are more susceptible to the deleterious effects of prolonged high fatty acid levels, and Steil et al. [127] have observed that a 96-h Intralipid infusion did not affect insulin secretion in male Sprague-Dawley rats. The influence of genetic predisposition on the insulin secretory response to excessive fatty acid levels is also illustrated by the observation that insulin secretion is impaired to a greater extent in heterozygous lean ZDF rats than in Wistar rats after Intralipid infusion [128]. Recent studies in our laboratory also highlight the importance of the age of the animals in the response to chronic fuel overload. In a first study we infused 8 week-old male Wistar rats alternatively with glucose for 4 h and Intralipid + heparin for 4h, for a total of 72 h [77]. Hyperglycemic clamps performed at the end of the infusion failed to detect any effects of the glucose + Intralipid infusion regimen on insulin secretion in vivo, as compared to control, saline-infused animals. Similarly, insulin secretion in response to glucose in isolated islets was unaffected.

In animals infused with glucose only, we observed an increase in insulin mRNA levels, PDX-1 nuclear localization, and PDX-1 binding to the endogenous insulin gene promoter in islets. In contrast, in islets from animals infused with glucose + Intralipid, insulin mRNA levels were reduced, PDX-1 localization was shifted towards the cytosol, and occupancy of the endogenous insulin promoter by PDX-1 was markedly diminished [77]. These results demonstrate that fatty acid inhibition of the insulin gene also occurs in vivo, and represents an early defect that can be detected prior to any alteration in insulin secretion. The lack of effect of the infusion on insulin secretion in 8-week old rats prompted us to assess whether older animals would be more susceptible to nutrient overload. To test this possibility, we recently conducted a second study in which glucose and Intralipid were infused simultaneously and continuously for 72h to either 8-week old or 6-month old Wistar rats (unpublished results). As in our first study, this infusion regimen did not alter insulin secretion in 8-week old rats, as assessed by hyperglycemic clamps at the end of the infusion. In marked contrast, infusion of glucose + Intralipid in 6-month old rats resulted in marked insulin resistance which was not adequately compensated for by a sufficient increase in insulin secretion in vivo, and in defective insulin secretion in vitro in isolated islets. The results from these two studies yield two important conclusions. First, defective insulin gene expression under glucolipotoxic conditions occurs in vivo and precedes abnormalities in insulin secretion. This confirms the physiological relevance of our previous in vitro findings [17,18] and suggests that impaired insulin gene transcription might represent an early defect in nutrient-induced beta-cell failure. Second, young rats are resistant to the effects of nutrient oversupply, and such studies are probably better conducted in older animals, which more closely resemble the typical setting of type 2 diabetes in humans. Whether or not this age-dependent susceptibility to nutrient oversupply is related to the reduced beta-cell proliferative capacity in older rodents [130,131] is unknown and currently under investigation.

B. Studies in humans

As in experimental animals, studies examining the effects of prolonged fatty acids on insulin secretion in humans have led to conflicting results. Initial reports from Boden and colleagues indicated that a 48-h lipid infusion induces an appropriate insulin secretory response in healthy subjects [132] but is defective in type 2 diabetic patients [133]. In contrast, Carpentier et al. [134] showed in non-diabetic individuals that an acute (90-min) lipid infusion elicits an increase in insulin secretion which disappears when the infusion is prolonged for 48h. The loss of insulin secretion is specific to the response to glucose, as the response to arginine remains normal [135]. The same group further showed that obese, but not diabetic, subjects are susceptible to the inhibitory effect of lipids on glucose-induced insulin secretion [136]. Importantly, the increase in insulin secretion observed in non-diabetic subjects in response to a 24-h glucose infusion does not occur if lipids are infused simultaneously with glucose [137]. Xiao et al [138] confirmed that fatty acids also alter beta-cell function in obese individuals when ingested orally, and observed interesting differences between saturated and polyunsaturated fatty acids. While polyunsaturated fatty acids impair insulin secretion directly, saturated fatty acids induce insulin resistance which was not adequately compensated for by an increase in beta-cell function [138]. The same group further observed that concomitant administration of the antioxidant taurine improved insulin resistance and beta-cell dysfunction induced by Intralipid infusion in vivo in humans, suggesting the possible contribution of oxidative stress [139].

Finally, the group of Cusi and De Fronzo has carried out a series of studies in non-diabetic subjects with and without family history of type 2 diabetes which clearly highlights the importance of genetic predisposition on the effects of chronically elevated fatty acids in humans. They showed that a 4-day Intralipid infusion enhances insulin secretion (taking into account insulin sensitivity) in control subjects but inhibits glucose-induced insulin secretion in individuals with a family history of type 2 diabetes [140]. This suggests that the genetic

predisposition to developing type 2 diabetes might be dependent, at least in part, on the ability of the beta cell to increase insulin secretion in response to elevated fatty acid levels. Importantly, treatment of susceptible subjects with Acipimox to decrease circulating fatty acid levels ameliorates insulin secretion [141].

IV. Conclusions

In recent years, major progress has been made towards a better understanding of the cellular and molecular mechanisms of glucolipotoxicity in the beta-cell. The biochemical basis for the permissive effect of elevated glucose on the deleterious actions of fatty acids is better delineated; the mechanisms by which the combination of excessive levels of fatty acids and glucose alter beta cell function are beginning to be unraveled; and it is becoming clear that the various functional effects of fatty acids (i.e. decreased insulin secretion, impaired insulin gene expression, and beta-cell death by apoptosis) have different underlying mechanisms. Despite significant progress, however, a number of important questions remain. While it is now clear that triglyceride accumulation is more a symptom than a cause of glucolipotoxicity, the nature of the lipid-derived intermediates directly responsible for the detrimental effects of fatty acids is still elusive. In that regard, a role for cholesterol accumulation is also likely. Amongst the several candidates recently proposed to explain fatty-acid inhibition of insulin secretion, the role of UCP2 has become unclear, while convincing evidence seems to implicate the novel isoform PKC ε as well as late exocytotic events. Regarding fatty-acid impairment of the insulin gene, a complex picture has emerged which includes prolonged activation of ERK1/2 via de novo ceramide synthesis, downregulation of PASK, and altered binding activities of the transcription factors PDX-1, MafA, and C/EBPβ. The role of the UPR under conditions of mild glucolipotoxicity (i.e., not associated with cell death) appears limited, although our current hypothesis is that early activation of ATF6 represses insulin gene transcription and thereby contributes to the reduction in proinsulin biosynthesis in an attempt to decrease the load to the ER. As conditions deteriorate, unresolved and sustained unfolded protein response likely leads to ER stress and, consequently, to beta-cell apoptosis under severe glucolipotoxic conditions. The necessity to confirm in vitro findings under physiological conditions has prompted several groups, including ours, to address these questions in in vivo models. Our studies have confirmed that the decrease in insulin gene expression is an early defect which precedes any detectable abnormality in insulin secretion, and have established that prolonged infusions of glucose and Intralipid impairs beta-cell function in old, but not young, animals, raising caution on the use of younger rodents to examine mechanisms of beta-cell failure. While still debated, the role of glucolipotoxicity in humans has been clearly demonstrated in several studies, at least in individuals genetically predisposed to developing type 2 diabetes.

We propose that the uncertainties regarding the role of glucolipotoxicity and its manifestations stem from the fact that it is being considered, as its name implies, as a deleterious phenomenon, while in fact the beta-cell's response to nutrient excess likely represents a continuum encompassing all stages of beta-cell compensation and beta-cell failure. In that sense, some of the early manifestations of glucolipotoxicity should actually be considered as a positive response and would be more appropriately named « glucolipoadaptation », as proposed by Prentki and Nolan [2]. Examples of such adaptive responses are the early decrease in insulin gene expression, as an attempt to protect the ER from overload [77], or the increase in UCP2 expression, as a defense mechanism against oxidative stress [68].

The hypothesis that glucolipotoxicity represents a continuum from an adaptative response to a deleterious outcome is illustrated in Figure 4. According to this view, in normoglycemic individuals experiencing weight gain, the beta cell mounts a compensatory response to counter insulin resistance associated with obesity. This response involves coordinated increases in beta-cell mass, insulin biosynthesis, and insulin secretion, and likely relies on an enhanced

responsiveness to fatty acids [142,143]. The magnitude of the compensatory beta-cell response is probably genetically determined and, in turn, is a major determinant of the long-term ability of an individual to maintain glucose homeostasis in the face of insulin resistance. In contrast, in genetically predisposed individuals beta-cell compensation eventually becomes insufficient and the beta cell is no longer able to sustain a secretory response that matches the demand imposed by insulin resistance. It is probably during this decompensation phase that glucolipotoxicity plays a major role, in that hyperglycemia is the permissive factor by which elevated fatty acids affect beta-cell function. Our data suggest that one of the first functional defect at this stage is a decrease in insulin gene expression, which likely contributes to eventual beta-cell failure since maintenance of adequate intracellular stores of insulin is necessary to sustain increased secretory demand [144]. Beta-cell decompensation evolves towards beta-cell failure when fasting hyperglycemia occurs. At this stage, it is likely that both glucotoxicity and glucolipotoxicity contribute to the decline in insulin secretion observed over time during the years following diagnosis of type 2 diabetes [3]. This model is based on extensive experimental evidence obtained in vitro and in rodents, but additional investigation is necessary to ascertain the precise contribution of glucolipotoxicity to the pathogenesis of type 2 diabetes in humans.

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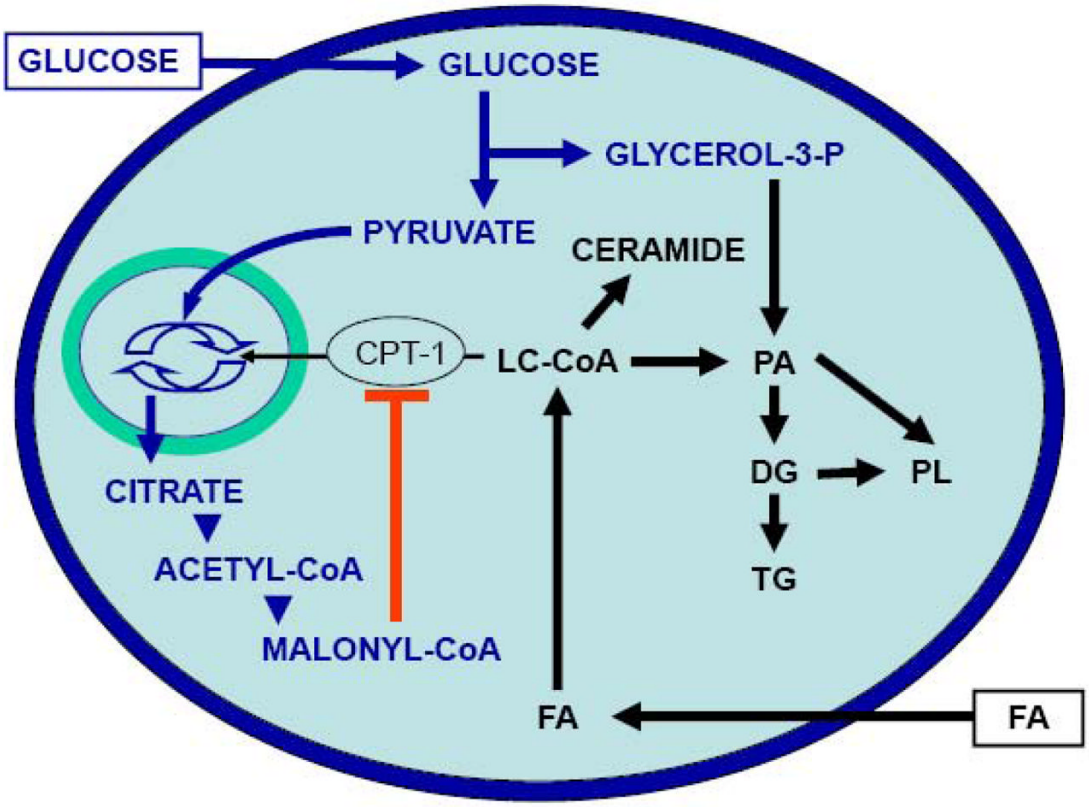

Figure 1. Effects of glucose on lipid partitioning in the beta cell

In the presence of simultaneously elevated levels of glucose and fatty-acid (FA), the increase in cytosolic malonyl-CoA resulting from glucose metabolism inhibits the enzyme carnitine-palmitoyl transferase-1 (CPT-1). Transport of long-chain acyl-CoA (LC-CoA) in the mitochondria is reduced, and the esterification pathway is preferentially activated, leading to cytosolic accumulation of lipid-derived signaling molecules such as ceramide, diglycerides (DG), phosphatidic acid (PA), phospholipids (PL), and triglycerides (TG).

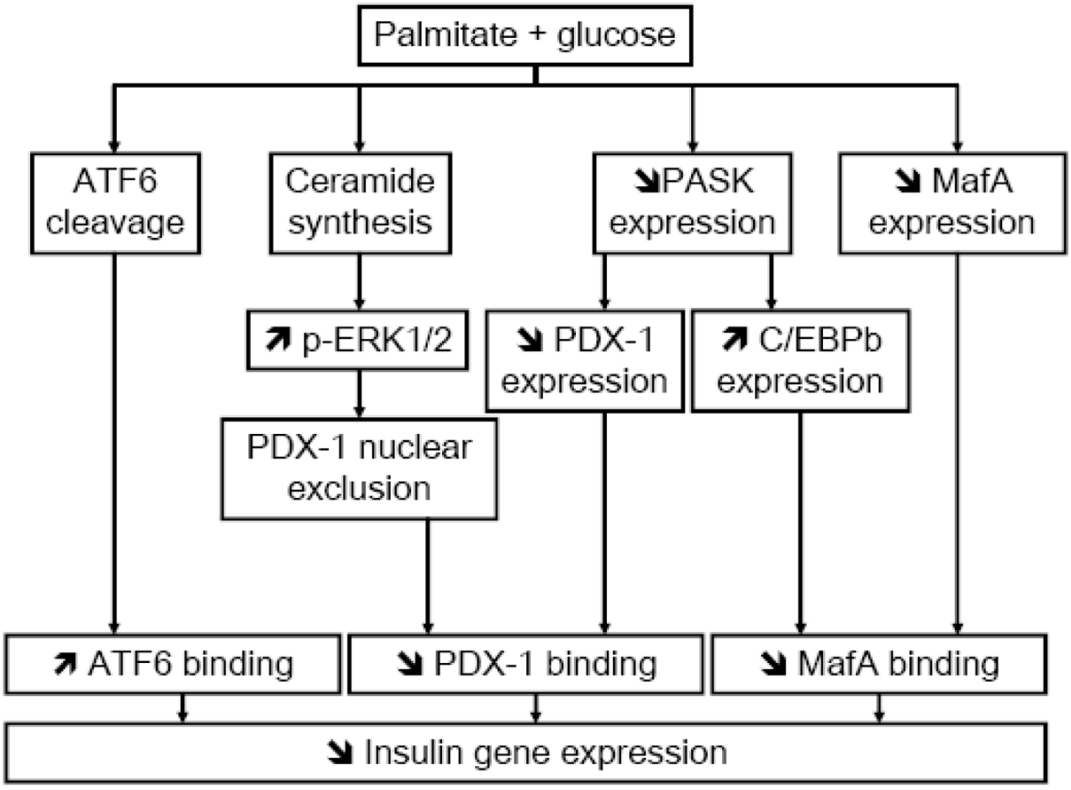

Figure 2. Working model of the mechanisms of fatty-acid inhibition of insulin gene expression

Several signaling pathways are activated in beta cells in the presence of simultaneously elevated levels of palmitate and glucose. First, de novo ceramide synthesis [17] leads to sustained activation of ERK ½ [82] and exclusion of PDX-1 from the nuclear compartment [18]. Second, palmitate blocks glucose-induction of PASK expression, which results in decreased PDX-1 expression and increased C/EBPβ expression [82]. Third, palmitate decreases MafA expression [18]. These 3 pathways result in decreased binding activities of PDX-1 and MafA on the insulin promoter. In addition, palmitate induces the cleavage of ATF6, which also represses insulin gene transcription (our unpublished data).

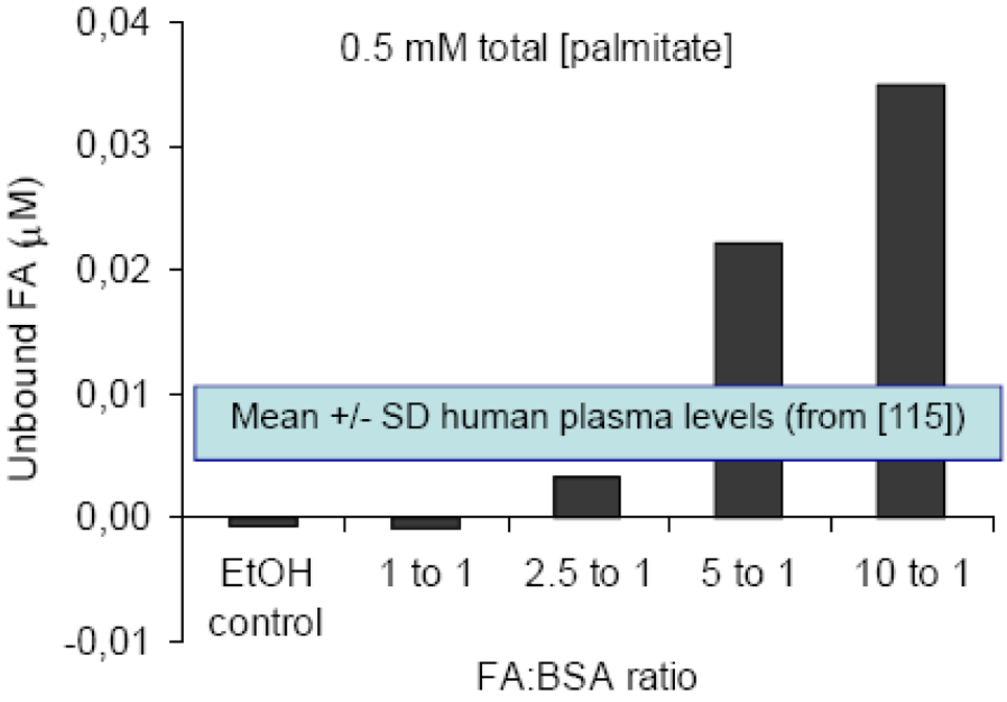

Figure 3. Concentrations of unbound fatty acids (FA) in solution as a function of the fatty acid: BSA ratio for a fixed total palmitate concentration of 0.5 mM

Unbound fatty acids were measured using the fluorescent probe ADIFAB [114]. Data are the average of 2 independent experiments. Also represented are the mean ± SD of unbound FA levels measured in human plasma using the same method, from [115].

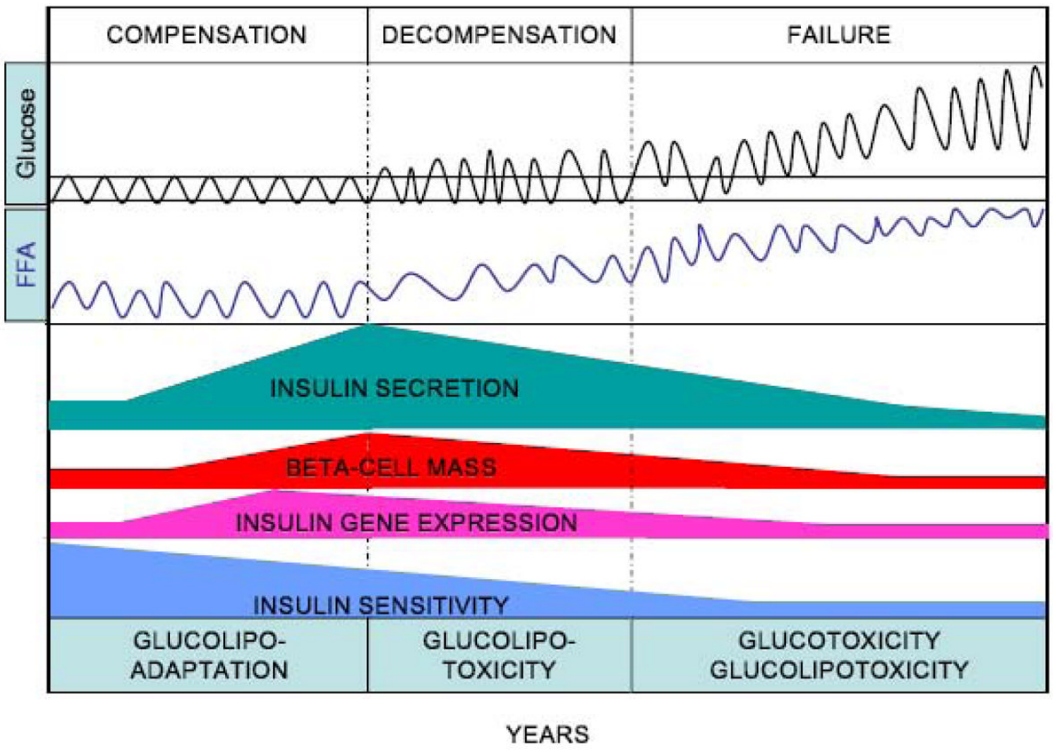

Figure 4. Hypothetical representation of the progression from beta-cell compensation to failure in the face of obesity-induced insulin resistance, and the role of glucolipotoxicity

According to this hypothesis, the decrease in insulin sensitivity is initially matched by a marked increase in insulin secretion, insulin gene expression, and beta-cell mass. At this stage the beta-cell adapts to nutrient oversupply by switching to preferential utilization of fatty acids, as part of the compensatory response (glucolipoadaptation [2]). In genetically predisposed individuals, the beta cell eventually becomes unable to further compensate and glucolipoadaptation evolves towards glucolipotoxicity, in which excursions of blood glucose levels outside of the normal range become permissive for the detrimental effects of elevated fatty acids. This phase is characterized by an early loss of insulin gene expression, decreased insulin secretion (relative to the degree of insulin resistance), and reduced beta-cell mass. Finally, beta-cell failure occurs when glucose levels are permanently in the hyperglycemic range. At that stage both glucotoxicity and glucolipotoxicity contribute to the continued deterioration of beta-cell function.