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Recent insights into mechanisms of β-cell lipo- and glucolipotoxicity in type 2 diabetes

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Abstract

The deleterious effects of chronically elevated free fatty acid (FFA) levels on glucose homeostasis are referred to as lipotoxicity, and the concurrent exposure to high glucose may cause synergistic glucolipotoxicity. Lipo- and glucolipotoxicity have been studied for over 25 years. Here, we review the current evidence supporting the role of pancreatic β-cell lipo- and glucolipotoxicity in type 2 diabetes, including lipid-based interventions in humans, prospective epidemiological studies and human genetic findings. In addition to total FFA quantity, the quality of FFAs (saturation and chain length) is a key determinant of lipotoxicity. We discuss *in vitro* and *in vivo* experimental models to investigate lipo- and glucolipotoxicity in β-cells and describe experimental pitfalls. Lipo- and glucolipotoxicity adversely affect many steps of the insulin production and secretion process. The molecular mechanisms underpinning lipo- and glucolipotoxic β-cell dysfunction and death comprise endoplasmic reticulum stress, oxidative stress and mitochondrial dysfunction, impaired autophagy and inflammation. Crosstalk between these stress pathways exists at multiple levels and may aggravate β-cell lipo- and glucolipotoxicity. Lipo- and glucolipotoxicity are therapeutic targets as several drugs impact the underlying stress responses in β-cells, potentially contributing to their glucose-lowering effects in type 2 diabetes.

Keywords

pancreatic β-cell; islet; insulin; lipotoxicity; palmitate

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INTRODUCTION

It is well established that obesity is a critical risk factor for the development of type 2 diabetes (T2D). The worldwide prevalence of obesity has tripled since 1975 [1], driving in part the dramatic rise in T2D prevalence over the past few decades. Obesity is associated with elevated concentrations of circulating free fatty acids (FFAs), due to expanded adipose tissue mass and reduced FFA clearance [2]. The latter has been attributed to increased escape of FFAs from esterification in adipose tissue [3]. There is accumulating evidence that elevated FFAs may contribute to T2D pathogenesis and thus represent a mechanistic link between obesity and diabetes. A substantial body of data indicates that increased FFAs induce insulin resistance and pancreatic β-cell dysfunction, precipitating the two major defects underlying T2D pathophysiology. These deleterious effects of FFAs on glucose homeostasis are commonly referred to as lipotoxicity. The concurrent exposure to high glucose (glucotoxicity) after the development of impaired glucose tolerance is considered to exert synergistic toxic effects with FFAs, which led to the concept of glucolipotoxicity as first proposed by Prentki and Corkey [4]. In this review, we will focus on recent advances and current bottlenecks in the field of lipo- and glucolipotoxicity, taking a pancreatic β-cell perspective. The impact of FFAs on insulin action in insulin-sensitive tissues is outside the scope of this review and has been covered in several comprehensive reviews [5–7]. Here, we discuss current evidence supporting the concept of lipo- and glucolipotoxicity and experimental models utilized for its investigation in β-cells. In addition, we review the molecular mechanisms underpinning lipo- and glucolipotoxic β-cell dysfunction and death and the therapeutic modulation of glucolipotoxicity by currently used and potential future drugs.

EVIDENCE FOR LIPO- AND GLUCOLIPOTOXICITY IN HUMANS

The contribution of elevated FFA levels to β-cell dysfunction in human T2D remains debated [8]. An argument against the clinical relevance of lipo- and glucolipotoxicity is that insulin secretion is typically enhanced under conditions of elevated circulating FFA levels as seen in obese individuals, and that most studies showing deleterious effects of elevated FFAs on β-cell function were conducted *in vitro*. However, in this section we argue that several studies have clearly demonstrated an inhibitory effect of prolonged exposure to FFAs on insulin secretion, including in humans, at least in genetically predisposed individuals. In the following section, we review the experimental challenges of studying the impact of FFAs on the β-cell that explain some of the discrepancies reported in the literature.

Acute *versus* chronic effects of elevated FFAs on glucose homeostasis

In order to assess the effects of elevated FFAs on glucose metabolism in humans, most studies have used an intravenous lipid emulsion (Intralipid, a purified soybean oil emulsion, or Liposyn, a soybean and safflower oil emulsion) to experimentally raise FFA concentrations in participants. These commercially available lipid emulsions are composed mostly of polyunsaturated FFAs and they are usually administered together with heparin, to stimulate intravascular lipolysis. These studies demonstrated that acute and chronic elevations of FFAs by lipid infusion have differential effects on insulin secretion. Acute

exposure enhances glucose-stimulated insulin secretion (GSIS), compensating for lipid-induced insulin resistance [9, 10]. In contrast, a more prolonged elevation of FFAs (24–48h) causes β-cell function to deteriorate, impairing the ability of β-cells to compensate for the prevailing insulin resistance [9, 11, 12]. Significantly, when lipids are co-infused with glucose, the FFA elevation inhibits the stimulatory effect of hyperglycemia on β-cell function [13].

These effects of elevated FFAs may be more pronounced in genetically predisposed individuals. Subjects with a genetic predisposition to develop T2D have increased susceptibility to β-cell dysfunction induced by lipid infusion, compared to subjects without a family history of T2D [14, 15]. In individuals with established T2D, however, lipid infusion does not further worsen β-cell function [16]. Prolonged FFA exposure may no longer detectably affect insulin secretion in the presence of overt β-cell failure. Reduction of FFA levels with short-term administration of acipimox, an inhibitor of lipolysis in adipose tissue, ameliorates the insulin response in normal glucose-tolerant individuals at high risk of T2D [17, 18]. Because long-term acipimox use can cause rebound FFA elevation, it cannot be used as a chronic treatment [18]. In a recent study combining oral glucose tolerance tests with isoglycemic intravenous glucose tolerance tests, FFA elevation was shown to impair the potentiation of insulin secretion by incretins, both in non-diabetic and T2D individuals [19]. This impaired incretin effect could play an important role in the pathophysiology of T2D. These observations in humans seem concordant with *in vitro* observations in β-cell models. In rodent [20] and human islets [21, 22], acute exposure to FFAs exerts a stimulatory effect on insulin secretion. In contrast, prolonged exposure to FFAs enhances basal secretion and impairs GSIS [20–22].

Is FFA quantity or quality critical in lipo- and glucolipotoxicity?

FFA levels are positively correlated to body mass index (BMI) and inversely correlated to insulin sensitivity, independently of age and BMI [23]. Genetic factors and the progression to a pre-diabetic state also impact FFA levels. In individuals genetically predisposed to T2D who have normal fasting triglyceride levels and normal glucose tolerance, postprandial FFAs are elevated compared to BMI-matched controls [24]. Fasting and steady-state FFAs increase in individuals with impaired fasting glucose and impaired glucose tolerance compared to individuals with normal glucose tolerance, even after correction for age, BMI and insulin sensitivity [23].

As outlined in the previous section, studies employing lipid infusions for up to 4 days in humans suggest that increased FFA levels worsen β-cell function. However, longitudinal studies that have evaluated the association between FFA levels and the evolution of β-cell function or incidence of T2D have yielded inconsistent results. Certain studies have found a positive association between total FFA levels and declining β-cell function [25, 26], whereas other studies did not and altogether questioned the concept of lipotoxicity in β-cells [23]. These discrepancies could, at least in part, be explained by the fact that FFA quality, and not only quantity, may impact β-cell function. Most intervention studies have used the same lipid infusion, containing predominantly polyunsaturated fat. A short-term study in healthy individuals showed that oral ingestion of fat with different degrees of saturation resulted in

differential effects on insulin secretion and action: saturated palm oil caused insulin resistance without a compensatory increase in insulin secretion (pointing to β-cell dysfunction), polyunsaturated safflower oil reduced insulin secretion and monounsaturated olive oil was neutral [27].

Findings from prospective, epidemiological studies support the hypothesis that FFA composition rather than total levels mostly affects glucose homeostasis [28–33]. These studies evaluated fatty acid composition of plasma cholesterol esters and phospholipids at baseline as a predictor of incident T2D. Individuals who developed T2D had a higher baseline proportion of saturated palmitic acid [28–33], lower linoleic acid, the most abundant omega-6 polyunsaturated fatty acid [28, 29, 31], and a higher proportion of γ-linolenic acid [28, 29] (for FFA nomenclature and classification, see Fig. 1). The large-scale European EPIC-InterAct study identified a strong diabetes risk for a fatty acid combination of higher concentrations of saturated palmitic and myristic acid and lower concentrations of linoleic acid, very long-chain and odd-chain saturated FFAs. The top 20% of adults with this fatty acid profile had a 60% higher risk of T2D compared to the 20% with a fatty acid profile least like this combination [28]. These data were externally validated in the US-based NHANES cohort. This report [28] and other EPIC-InterAct analyses [33, 34] illustrate that even fatty acids belonging to the same subclass according to their degree of saturation (e.g. saturated or polyunsaturated) can be heterogeneous in their effects.

Palmitic acid is the most prevalent saturated FFA in humans. The association between palmitic acid and T2D appears very consistent across epidemiological studies, and among individual saturated FFAs, palmitic acid has the higher hazard ratio for diabetes development [33]. This is in keeping with data from *in vitro* studies. Palmitate is more toxic than monounsaturated oleate and polyunsaturated linoleate in rodent and human β-cells [35]. Combining palmitate with oleate confers protection from palmitate-induced apoptosis [36, 37].

Evidence for lipotoxicity from human genetic studies

The plasma fatty acid profile does not only reflect dietary intake; it is largely influenced by endogenous FFA metabolism. Saturated FFAs can be synthesized from acetyl-CoA *(de novo* lipogenesis) and FFAs can be desaturated or elongated through the action of desaturases and elongases, respectively (Fig. 2). In particular, SCD (stearoyl-CoA desaturase) catalyzes the formation of monounsaturated from saturated FFAs, by inserting a double bond. Two SCD isoforms exist in humans, SCD1 and SCD5. Δ^5- and Δ^6-desaturase, encoded by *FADS1* and *FADS2* respectively, are required for the synthesis of long-chain omega-6 and omega-3 polyunsaturated FFAs.

Recent genome-wide association studies (GWAS) have identified susceptibility loci for T2D related to desaturases. In a recent study aggregating data from 32 European GWAS, a T2D susceptibility signal was fine mapped to a coding variant of *SCD5* [38, 39]. Another signal was linked to lipoprotein lipase *(LPL),* but the fine mapping was of poor resolution [38]. A large-scale GWAS meta-analysis of continuous diabetes-related traits identified a locus near *FADS1* associated with fasting glucose [40]. A subsequent study using metabolic tests found

that the *FADS1* locus was associated with a lower insulinogenic index, a measure of GSIS, and had no effect on insulin sensitivity or proinsulin [41].

Mendelian randomization studies use genetic variants as an instrument to examine a causal link between an exposure (resulting from the genetic variants) and an outcome. As genotypes are distributed randomly, these studies are less susceptible to confounding or reverse causation than epidemiological studies. A Mendelian randomization study used a genetic variant associated with lower FADS1 and FADS2 activities to study the effect of desaturases on T2D risk. After adjustment for estimated enzymatic activities of FADS1 or FADS2, a genetically determined low FADS2 activity predicted lower T2D risk and low FADS1 activity tended to predict higher risk [42]. Low FADS1 activity would favor a low linoleic/γ-linolenic acid ratio. This appears to be in accordance with epidemiological studies showing that low concentrations of linoleic [28, 29, 31] and high concentrations of γ-linolenic acid [28, 29] are positively associated with T2D risk, as mentioned above. Taken together, these data provide further evidence for lipotoxicity as a contributor to T2D pathogenesis and highlight the importance of FFA composition.

EXPERIMENTAL APPROACHES TO STUDYING LIPO- AND GLUCOLIPOTOXICITY: CAVEATS AND LIMITATIONS

Since the early studies by the group of Grill in the 1990s [20, 43], a number of *in vitro* and *in vivo* systems have been used to study the mechanisms of lipo- and glucolipotoxicity, sometimes leading to conflicting results. Such discrepancies are at least in part related to methodological and experimental issues that are important to consider.

Methodological considerations for *in vitro* use of FFAs

Experiments aimed at investigating the impact of FFA exposure in isolated cells *ex vivo* attempt to mimic the concentrations present in the vicinity of the islets *in situ*. These concentrations are difficult to estimate as they depend not only on total circulating levels but also on factors influencing local delivery of FFAs, including LPL activity present in islets [44], as well as FFA release by islet cells [45].

FFAs are hydrophobic molecules with low solubility in aqueous solutions such as plasma or interstitial fluid, and are bound to albumin in the vascular and interstitial compartments [46, 47]. In healthy humans, on average 2 FFA molecules are bound to each albumin molecule in the circulation (range 1:1 to 3:1). The FFA/albumin molar ratio can rise to more than 5:1 in pathological conditions [48]. FFA transport and clearance from the circulation depend on FFA-albumin interactions. Thus, FFA accessibility for cellular uptake is a function of their unbound concentration at physiological concentrations of albumin [49] and investigating the effects of FFAs *ex vivo* in cultured cells requires previous complexation with albumin (usually bovine serum albumin, BSA) at a FFA:BSA molar ratio no greater than 5:1 [50, 51]. Because the biologically active fraction of FFAs depends on the FFA/BSA molar ratio, an increase in FFA concentration or decrease in BSA concentration will enhance their biological effects [50, 52]. Different methods exist to prepare FFA-BSA solutions. FFAs can be used with charcoal-absorbed BSA (to remove endogenous bovine FFAs) or FFA-free

BSA, either directly or after precomplexing. FFAs used in the presence of 1% charcoal-absorbed BSA or 0.75% FFA-free BSA, or precomplexed FFAs used in the presence of 0.67% FFA-free BSA resulted in similar unbound FA concentrations [52], suggesting that the FFA-BSA precomplexing process results in lower FFA concentrations due to aggregation. Different FFA-free BSA preparations of variable purity are commercially available. As BSA contaminants can impact FFA binding, albumin used in *in vitro* experiments must be of high-grade purity. Albumin (2%) present in the fetal bovine serum that is often used in cell culture will also bind FFAs, thereby decreasing unbound FFA concentrations.

To complicate things further, the affinity of FFAs for albumin varies with the length of the carbon chain and the degree of saturation. For example, stearate has higher affinity for albumin than arachidonate, and therefore more stearate is needed than arachidonate to obtain the same of unbound fraction for the same total concentration [46]. This must be taken into account in experiments aimed at comparing the potencies of different FFAs. Although unbound FFA concentrations can be measured using a fluorescent probe (Acrylodan-Labeled Intestinal Fatty Acid Binding Protein) [51, 53, 54], this is not very practical and rarely reported in the literature.

In conclusion, given the number of experimental parameters that impact the biologically active, unbound fraction of FFAs and the possibility for non-specific detergent effects, it is essential that such parameters be precisely reported in manuscripts including total FFA concentration, type of BSA, molar ratio, and complexation protocol.

In vivo models for the study of lipo- and glucolipotoxicity

Pioneering studies by the Unger group in Zucker Diabetic Fatty (ZDF) rats established the concept of lipotoxicity [55]. ZDF rats carry a loss-of-function mutation (fa) in the leptin receptor leading to massive obesity, hyperlipidemia and insulin resistance. Male animals develop hyperglycemia due to β-cell dysfunction and a progressive loss of β-cell mass. β-Cell damage in this model is mediated by increased flux of the *de novo* ceramide biosynthesis pathway [56]. A pathogenic role for sphingolipids in β-cells was confirmed in other models, where ceramide induces β-cell apoptosis [57–59] and inhibition of insulin gene expression [60]. Because mutations in the leptin receptor gene profoundly perturb intracellular FFA metabolism and are extremely rare in humans [61], alternative rodent models have been developed that are more relevant to human physiopathology.

In an *in vivo* model of prolonged intravenous infusion of Intralipid with heparin in rats, GSIS was either unchanged [62], increased [63, 64] or decreased [43, 65–68]. These discrepancies may be explained by differences in strain, sex, age, duration/rate of lipid infusion and glucose levels. A 48-hour Intralipid infusion impaired GSIS in male Wistar rats [66], while a 96-hour infusion did not in male Sprague-Dawley rats [64]. Glucose and Intralipid infusion decreased insulin secretion in 6-but not in 2-month-old Wistar rats [69]. Genetic predisposition to diabetes also influences the rodent β-cell response to FFAs. Insulin secretion is impaired to a greater extent in lean heterozygous ZDF rats than in Wistar rats after Intralipid infusion [68].

Species differences exist between rodent and human β-cells with dissimilarities in islet architecture [70, 71] and β-cell metabolism [72], plasticity [73–77] and lifespan [72, 77–79]. Human islet transplantation into immunodeficient mice represents an attractive approach to study human β-cell pathophysiology in a long-term *in vivo* system. Gargani et al [80] transplanted human islets into immunodeficient mice that were subsequently placed on high-fat diet. Human islets from non-diabetic donors adapted to the high-fat obese environment with an increase in β-cell volume, although no increase in β-cell proliferation was detected. β-cell function (corrected for insulin sensitivity) declined in high fat-fed mice compared to chow-fed mice. Human islets from two T2D donors did not change in mass or function with high-fat feeding [80]. This transplantation model allows to study both the human islet graft and murine islets, thereby shedding light on differences between murine and human β-cells.

IMPACT OF LIPO- AND GLUCOLIPOTOXICITY ON β-CELL INSULIN PRODUCTION AND SECRETION

Insulin release by the β-cell results from a highly coordinated process that encompasses insulin gene transcription, proinsulin biosynthesis, and insulin secretion. Glucose, the major insulin secretagogue, positively regulates all these steps so as to ensure that adequate intracellular stores are available to meet the secretory demand. Lipo- and glucolipotoxic conditions have been shown to alter several key functions along the insulin factory (Fig. 3).

Insulin gene expression and proinsulin biosynthesis

Studies by our group [60, 81–84] and others [85–89] have demonstrated a detrimental impact of elevated saturated FFAs on insulin gene transcription. In isolated islets, we found that combined exposure to high levels of glucose and palmitate impairs the expression of the transcription factor MafA and the nuclear translocation of the transcription factor PDX-1 [81], a finding that was confirmed in an *in vivo* model of glucolipotoxicity [62]. This results in diminished insulin promoter activity [60]. Kim et al observed that the increased expression of PGC1-α under glucolipotoxic conditions suppresses the activity of the transcription factor BETA2/NeuroD [89], while Plaisance et al [86] showed that palmitate-induced C/EBPβ expression reduces insulin promoter activity. These transcriptional changes are mediated, at least in part, via intracellular generation of ceramide [60, 90, 91] and activation of stress kinases including c-Jun N-terminal kinase (JNK) [88], extracellular-regulated kinases (ERK)1/2 and Per-Arnt-Sim (PAS) kinase [83] (Fig. 4). Interestingly, rare nonsynonymous mutations in the PAS kinase gene cause maturity-onset diabetes of the young [92].

The impairment of insulin gene transcription under lipo- and glucolipotoxic conditions is accompanied by a loss of glucose regulation of proinsulin biosynthesis both *in vitro* [93] and *in vivo* [69], thereby contributing to a dramatic decrease in intracellular insulin content.

Stimulus-secretion coupling

Glucose triggers insulin secretion through the generation of various coupling factors including a change in the ATP/ADP ratio; closure of ATP-sensitive potassium channels; membrane depolarization; and calcium influx which stimulates insulin exocytosis. Although

mechanisms affecting β-cell function during chronic FFA exposure are not fully elucidated, different levels of the insulin secretion machinery are affected.

Prolonged exposure to FFAs, particularly palmitate, interferes with glucose metabolism at several levels. Intracellular FFAs are activated into long-chain CoAs and, at low or normal glucose levels, are readily oxidized by the β-cell. However, if glucose levels rise, FFA oxidation is blocked by the generation of malonyl-CoA, which inhibits carnitine-palmitoyl transferase 1, thereby diverting intracellular FFA metabolism towards esterification and the generation of complex lipids including sphingolipids (Fig. 5). In the “tricycling” model described by Prentki et al [45, 94, 95], the Krebs, pyruvate and glycerolipid/FFA cycles are interlinked. They generate essential metabolic coupling factors in the β-cell to amplify signals for insulin secretion, but become detrimental under fuel oversupply conditions.

Lipo- and glucolipotoxicity are associated with mitochondrial dysfunction [96, 97] and disturbed calcium handling via upregulation of neprilysin, a plasma membrane protein that regulates Ca^{2+} influx in β-cells [98]. Given its classical function as a mitochondrial uncoupler, many studies have investigated the role of uncoupling protein-2 (UCP2) in insulin secretion and suggested a role in glucolipotoxicity [99]. UCP2 expression is increased *in vitro* by chronic high glucose and FFA exposure, and in some *in vivo* studies it was associated with impaired insulin secretion, hyperglycemia and T2D [100–105]. Acute inhibition of UCP2 by genipin increases GSIS in isolated islets [106] and, conversely, overexpression of UCP2 impairs insulin secretion [100, 102]. Whole-body UCP2 knockout mice were shown to have enhanced GSIS, higher ATP content and increased intracellular cytotoxic reactive oxygen species (ROS) levels in islets [107–109]. In a β-cell specific UCP2 knockout (βUCP2KO), Robson-Doucette et al observed that UCP2 does not act as a classical uncoupling protein in β-cells, with no changes in ATP levels or mitochondrial coupling in βUCP2KO islets, while ROS production and GSIS were increased [110]. This suggests that UCP2 negatively regulates GSIS by reducing mitochondrial ROS production and not through a defect in ATP production. The precise contribution of UCP2 in as a mediator of glucolipotoxicity is not fully understood.

Finally, FFAs have recently been shown to modulate post-translational modifications of intracellular proteins in the β-cell including acetylation [111], palmitoylation ([112], see below), and glycosylation [113].

Insulin exocytosis

In T2D patients, the first phase of insulin secretion is almost abolished, suggesting a defect in priming and/or fusion of insulin-containing granules with the β-cell plasma membrane. Andersson et al [114] observed a significant decrease in the expression of exocytotic genes and proteins in islets from T2D donors. Furthermore, in T2D β-cells insulin secretory granules docked at the plasma membrane do not cluster to voltage-gated Ca^{2+} channels [115]. This loss of proximity between the immediately releasable granule pool and high Ca^{2+} influx microdomains could contribute to the lost first-phase insulin secretion in diabetic patients. Similarly, exposure of mouse islets to palmitate does not decrease the number of release-competent granules but alters the tight complexes of Ca^{2+} channels with secretory granules [116, 117]. Loss of this organization leads to insufficient Ca^{2+}

concentrations close to the secretory granule to produce exocytosis in response to brief depolarizations. Interestingly, in mouse and human islets treated with palmitate, the defect in insulin secretion is reversed by the K^+ channel blocker tetraethylammonium that pharmacologically prolongs β-cell action potentials [116, 117]. In the same manner, clustering of Kv2.1 voltage-gated K^+ channels with insulin secretory granules promotes exocytosis. Overexpression of these channels rescues insulin exocytosis from T2D β-cells [118]. Localized influx due to very close proximity of ion channel clusters and insulin-containing granules thus seems to be crucial for GSIS, and FFA-induced impairment in insulin secretion could be due to their structural disruption. Johnston et al recently identified a subset of specialized β-cells within islets, called hub cells, which coordinate calcium dynamics, electrical activity and insulin secretion, and the activity of which is disrupted under glucolipotoxic conditions [119].

Another link between FFA metabolism and the amplifying pathways of GSIS is the lipid-regulated protein kinase C (PKC) ε. Inhibition of PKCε increases insulin secretion and improves glucose homeostasis in rodent models of T2D [120]. PKCε knockout islets exposed to palmitate upregulate the amplification pathway of GSIS [121]. This enhanced GSIS, occurring selectively after lipid exposure, depends on glucose-induced hydrolysis of triglyceride stores. PKCε knockout islets have enhanced lipolysis rates and triglyceride lipase activity and pharmacological lipase inhibition abrogates GSIS.

MOLECULAR MECHANISMS OF LIPO- AND GLUCOLIPOTOXIC β-CELL DYSFUNCTION AND DEATH

Exposure of β-cells to palmitate recapitulates key features of β-cell failure in T2D, since it induces apoptosis, raises basal insulin secretion and inhibits GSIS. For this reason, it has been the most extensively used *in vitro* model of β-cell lipotoxicity. Mechanistic studies have revealed that palmitate with or without high glucose has pleiotropic effects on β-cell function and survival/death pathways. *In vitro* studies have provided good insight into the cell death process triggered in β-cells upon palmitate exposure. The detection of β-cell apoptosis *in vivo* is much more difficult as apoptotic cells are rapidly cleared. In this section, we summarize current knowledge on the molecular mechanisms underlying lipo- and glucolipotoxicity, focusing on the effects of palmitate (Fig. 6).

ER stress

The endoplasmic reticulum (ER) has a central role in lipid biosynthesis, Ca^{2+} storage and the synthesis and folding of secreted proteins. The accumulation of unfolded or misfolded proteins in the ER lumen is defined as ER stress and activates the ER stress response, also known as the unfolded protein response. This set of intracellular signaling pathways is initiated by three ER stress sensors and transducers, namely protein kinase RNA (PKR)-like ER kinase (PERK), activating transcription factor-6 (ATF6), and inositol-requiring enzyme-1 (IRE1). The signal transduction aims to restore ER homeostasis by attenuating global protein translation, upregulating ER folding capacity and degrading misfolded proteins. When this cannot be attained, apoptosis is triggered.

β-cells are susceptible to ER stress due to the high demand for insulin synthesis placed on the ER, particularly in the face of insulin resistance. There is now compelling evidence for a role of ER stress in diabetes pathogenesis. Islets from T2D patients show increased expression of ER stress markers and distended β-cell ER, an ultrastructural hallmark of ER stress [122]. Mutations affecting components of the ER stress response cause monogenic diabetes (reviewed in [123]).

Palmitate triggers ER stress through perturbations that affect ER folding capacity and cause misfolded protein overload in the ER (Fig. 7). Palmitate depletes ER Ca^{2+} stores, compromising the folding capacity of Ca^{2+}-dependent chaperones [36, 124]. Exposure to high glucose enhances palmitate-induced ER Ca^{2+} depletion. High glucose downregulates the sarcoendoplasmic reticulum Ca^{2+}-ATPase (SERCA) that pumps Ca^{2+} into the ER [125]; Ca^{2+} depletion by FFAs is not mediated by transcriptional modulation of SERCA [36]. Palmitate may also affect protein folding by aberrant protein palmitoylation [112], a post-translational modification that occurs in cysteine residues of a protein. Palmitoylation increases protein hydrophobicity and this can alter protein activity, stability and trafficking [112]. Palmitate causes alteration of ER lipid species that affect the composition and distribution of ER lipid rafts [126]. These specialized membrane microdomains regulate the entry of certain types of proteins into the secretory pathway. The alteration of ER lipid rafts by palmitate causes reduced budding of ER-derived vesicles [126] and disrupts ER-to-Golgi trafficking [127, 128]. The perturbed protein trafficking promotes ER luminal protein overload, triggering ER stress. Furthermore, palmitate induces a rapid degradation of carboxypeptidase E [129], an enzyme required for insulin processing. This enhances ER stress possibly due to buildup of unprocessed proinsulin in the secretory pathway. Islet amyloid polypeptide (IAPP) is a peptide hormone co-secreted with insulin by β-cells. Accumulation of unprocessed IAPP can initiate the formation of amyloid deposits. FFAs induce IAPP expression in mouse and human β-cells and its aggregation contributes to ER stress and inflammation [130–132].

The accumulated unfolded proteins activate the ER sensors PERK and IRE1 through their luminal domains, either by engaging directly these domains [133] or by inducing their dissociation from the chaperone BiP [134]. Interestingly, the transmembrane domains of PERK and IRE1 can also sense increased ER membrane lipid saturation and directly activate the ER stress response, as mutant PERK and IRE1 proteins lacking the luminal stress-sensing domain remain responsive to palmitate [135].

Unresolved lipo- and glucolipotoxic ER stress leads to apoptosis, through the induction of the PERK-dependent pro-apoptotic transcription factor CHOP [36]. Lipotoxic ER stress induces the pro-apoptotic BH3-only proteins PUMA and DP5 and causes loss of the anti-apoptotic proteins Bcl-2 and Mcl-1 [136]. The activation of these effectors downstream of ER stress culminates in mitochondrial permeabilization and engagement of the mitochondrial pathway of apoptosis.

Oxidative stress and mitochondrial dysfunction

Physiological levels of ROS are a requisite for normal glucose sensing and GSIS [137]. Imbalance between ROS formation and cellular antioxidant defenses can lead to oxidative

stress. FFA and excess glucose are potent inducers of ROS through different mechanisms. Long-chain FFAs (C>14), being poor substrates for mitochondrial β-oxidation, are shortened by peroxisomal β-oxidation and subsequently transported to mitochondria for further degradation. Peroxisomal β-oxidation of palmitate generates H_2O_2 [138]. Given that β-cells lack the H_2O_2-inactivating enzyme catalase in peroxisomes, peroxisomal H_2O_2 formation contributes to palmitate-induced oxidative stress [138].

Excess glucose generates ROS through the shunting of its metabolites into alternative metabolic pathways, such as dihydroxyacetone and diacylglycerol formation, glucosamine and hexosamine metabolism, and sorbitol metabolism [139]. Moreover, high glucose and palmitate lead to enhanced superoxide formation via activation of NADPH oxidase [140]. The latter is dependent on the activation of PKC, possibly through increases in diacylglycerol [140].

Oxidative stress impairs mitochondrial function and integrity. Mitochondrial DNA (mtDNA) carries 37 genes, encoding tRNAs, rRNAs and polypeptides of the electron transport chain complexes. mtDNA is more vulnerable than nuclear DNA to oxidative stress-related damage due to the lack of protective histones and low repair mechanisms [141]. Being at close proximity to the source of ROS generation, mitochondrial components are at high risk of oxidative injury [141]. FFAs cause a dose-dependent damage in mtDNA, contributing to apoptosis [142]. In addition to DNA damage, exposure to a combination of glucose and FFA disrupts mitochondrial dynamics. Under glucolipotoxic conditions, β-cell mitochondria lose their ability to undergo fusion and become fragmented [143].

Autophagy

Autophagy is a physiological process that regulates the balance between synthesis, degradation and recycling of cellular components. It serves as a quality control mechanism, removing damaged or redundant organelles, protein aggregates and lipids, and protects cellular homeostasis under conditions of nutrient deprivation. Autophagy starts with the sequestration of cytoplasmic constituents or organelles and the formation of a double-membrane structure, called the autophagosome. Autophagosomes fuse with lysosomes, in which the contents of the autophagosome are degraded by lysosomal enzymes. The significance of autophagy in β-cell physiology and metabolic adaptation has been highlighted by studies in transgenic mice deficient in autophagy. Mice with β-cell-specific ablation of Atg7, a gene essential for autophagosome formation, develop glucose intolerance due to decreased insulin secretion [144]. Furthermore, these mice exhibit a lack of compensatory increase in β-cell mass following high fat diet [144].

β-cells from T2D donors show signs of dysregulated autophagy, with more abundant autophagic vacuoles and autophagosomes and lower lysosomal associated membrane protein 2 and cathepsin expression compared to β-cells from non-diabetic donors [145]. Interestingly, these features could be induced by *in vitro* exposure of human islets to FFAs (oleate and palmitate) [145]. Autophagy is often measured by the conversion of LC3 I to its lipidated form (LC3 II), a necessary step for autophagosome biogenesis. Palmitate (and high glucose) increases the LC3 II to LC3 I ratio [132, 146–152] and inhibits expression of a number of genes involved in autophagy and lysosomal function [132]. Consistent with this

gene expression signature in palmitate-treated human islets, studies using pH-sensitive LC3 expression vectors and measuring p62 degradation showed that palmitate (and high glucose) suppresses autophagic turnover, due to impaired fusion of autophagosomes with lysosomes and impaired lysosomal acidification [149, 151, 153]. Re-acidification of lysosomes by photoactivatable nanoparticles restored autophagic flux in (gluco-)lipotoxic conditions [152]. Treatment with carbamazepine or rapamycin (that both stimulate autophagic activity) confers protection against palmitate and glucose toxicity [132, 147, 149, 151, 154], while blocking autophagy exacerbates lipotoxicity [154]. These data support the concept that inhibition of autophagic flux in human β-cells by palmitate is a mediator of lipo- and glucolipotoxicity.

Inflammation

Saturated FFAs can trigger inflammatory pathways in β-cells, both via direct activation and secondarily to oxidative and ER stress [155]. Palmitate induces cytokine (IL-1β, IL-6 and IL-8) and chemokine (CCL2, CXCL1) production in β-cells, mediated by IL-1 receptor signaling [156, 157]. However, whether these pro-inflammatory changes *per se* induce β-cell glucolipotoxicity is controversial [155–157].

Interactions between β-cells and immune cells mediate inflammation-mediated lipo- and glucolipotoxicity. Palmitate induces the production of chemokines by β-cells via the Toll-like receptor 4 (TLR4), recruiting M1-type proinflammatory macrophages/monocytes to the islets [158]. Depletion of M1 macrophages/monocytes before ethyl palmitate infusion protected mice from β-cell dysfunction and preserved expression of key β-cell genes [158]. Palmitate and high glucose also synergistically trigger islet secretion of the S100 calcium-binding protein A8 (S100A8), a damage-associated pattern molecule that activates macrophages in a TLR4-dependent manner [159]. The subsequent cytokine production by macrophages elicits β-cell apoptosis [159].

Crosstalk between stress pathways

The above described stress pathways may operate simultaneously or synergistically, generating feed-forward mechanisms in lipo- and glucolipotoxicity. For example, oxidative stress caused by H_2O_2 induces ER Ca^{2+} depletion, leading to ER stress [125]. ER stress induces pro-inflammatory gene expression, through the activation of the transcription factor NF-κB and JNK [160]. Mild ER stress sensitizes β-cells to the cytokines IL-1β and TNF-α, amplifying the inflammatory response [161]. ER stress also stimulates autophagy, through the JNK pathway [146, 148, 153]. Crosstalk between stress pathways thus exists at multiple levels and may aggravate β-cell lipo- and glucolipotoxicity.

Differential effects of FFAs on stress pathways

As discussed above, FFAs are not homogeneous in their effects. *In vitro* β-cell studies of palmitate and oleate, the two best studied FFAs, have revealed significant differences. Palmitate activates more potently than oleate the PERK branch of the ER stress response, considered as the most pro-apoptotic branch, probably through more marked ER Ca^{2+} depletion [36]. Palmitate also preferentially activates the IRE1 branch, whereas ATF6 signaling is activated by both FFAs to an equal extent [36]. Contrary to palmitate, oleate

does not induce peroxisomal H_2O_2 generation [162]. Oleate improved the fusion of autophagosomes to lysosomes and stimulated autophagic flux more effectively than palmitate [163]. Collectively, these differences may explain the mild to absent toxicity of oleate [35, 164]. In keeping with this, enhanced SCD expression in β-cells confers resistance against lipoapoptosis [165] and, conversely, SCD inhibition increases ER stress and apoptosis [166].

Potential role of (gluco-)lipotoxicity in β-cell dedifferentiation

There is mounting evidence that β-cells can revert to progenitor-like cells (β-cell dedifferentiation) or transdifferentiate to other endocrine cell types. This process may contribute to the loss of functional β-cell mass, in addition to apoptosis [167–169]. As described in the section on insulin gene expression, glucotoxicity decreases insulin gene expression, due to reduced expression and binding of key transcription factors to the insulin promoter. Glucotoxicity downregulates other genes critical for the maintenance of the differentiated β-cell phenotype. These include transcription factors, such as NKX6.1, HNF1α and HNF4α, glucose sensing and metabolism genes, and secretory pathway genes (for detailed description, see [170]).

Conversely, hyperglycemia results in the upregulation of genes with absent or very low expression in mature β-cells under physiological conditions. Those ‘forbidden’ genes are involved in metabolic pathways, transcriptional regulation and stress responses in β-cells [170]. In animal models of diabetes, hyperglycemia has been associated with the expression of transcription factors typically expressed in progenitor cells at the embryonic stage, such as neurogenin 3 (NGN3) and OCT4 [170]. High glucose [171], as well as FFA exposure [172], upregulate the inhibitor of differentiation 1 (ID1), another transcriptional regulator. ID1 upregulation has been shown to contribute to the suppression of insulin secretion and the development of glucose intolerance under high-fat diet [172].

Foxo1, a transcription factor integrating nutrient and hormone-driven signals [167], has been shown to play a role in β-cell dedifferentiation. Palmitate treatment *in vitro* induces Foxo1 activation by nuclear translocation [173]. Foxo1 activation is initially an adaptive response to maintain the balance of mitochondrial glucose versus lipid utilization [167]. However, prolonged Foxo1 activation leads to its degradation [167]. Foxo1 depletion has been associated with preferential oxidation of lipids rather than glucose for energy generation and with suppressed expression of β-cell identity transcription factors [174]. Using immunostaining for Foxo1 and ALDH1A3, a β-cell dedifferentiation marker [169], a recent report found that islets from mice chronically exposed to a high-fat diet showed signs of β-cell dedifferentiation (suppression of Foxo1 and ALDH1A3 expression) [175]. No such signs were observed in islets from low-fat fed mice. The above data raise the hypothesis that glucolipotoxicity may act as a driver for β-cell dedifferentiation.

LIPO- AND GLUCOLIPOTOXICITY: THERAPEUTIC TARGETS?

Metformin

Metformin is recommended as first-line medication for T2D treatment. Besides acting as an insulin sensitizer, it has beneficial effects in β-cells under (gluco-)lipotoxic conditions. Metformin prevents the impairment in glucose-stimulated secretion induced by FFAs and high glucose in rat [176] and human islets [177]. This protective effect is, at least in part, mediated by the restoration of glucose utilization and oxidation by metformin [176, 177]. Metformin further prevents lipotoxic β-cell apoptosis, through attenuation of the ER stress response with decreased pro-apoptotic PERK/CHOP signaling [178, 179].

Glucagon-like peptide 1 analogs

Glucagon-like peptide 1 (GLP-1) receptor agonists are more recently developed glucose-lowering medications. GLP-1 engages a G-coupled protein receptor, which activates adenylate cyclase and augments GSIS, through cAMP-induced activation of protein kinase A. The GLP-1 analog exendin-4 protects against lipotoxic β-cell dysfunction/apoptosis via a number of mechanisms. Exendin-4 protects β-cells from palmitate by increasing cellular defenses via the induction of the ER chaperone BiP and the anti-apoptotic protein JunB [180]. Exendin also abrogates the palmitate-induced activation of the pro-apoptotic stress kinases JNK and p38 MAPK [181]. Both protective mechanisms are cAMP/protein kinase A-dependent [180, 181]. In addition, exendin restores lysosomal function and prevents the inhibition of autophagic flux by glucolipotoxicity [153].

With regard to function, exendin corrects the palmitate-induced impairment in insulin release in mouse β-cells [182]. In high fat-fed mice treated or not with exendin, islets isolated from exendin-treated mice preserved glucose responsiveness, in contrast to islets from non-treated mice. In this study, islets were isolated after the disappearance of exendin from the circulation, suggesting durable effects of GLP-1 receptor agonists on insulin secretion [183]. Liraglutide, another GLP-1 analog, abolished the downregulation by palmitate of the transcription factors Pdx-1, MafA and NeuroD, through activation of the PI3K/Akt pathway [184]. The preservation of these key β-cell transcription factors may contribute to the improvement of β-cell function under lipotoxic conditions by GLP-1 receptor agonists.

Thiazolidinediones

Thiazolidinediones (or glitazones) are ligands of peroxisome proliferator-activated receptor γ (PPARγ), a transcription factor regulating lipid storage and metabolism. Use of these antidiabetic drugs has declined over the past years, after controversy sparked by studies suggesting an association of rosiglitazone use with cardiovascular events and of pioglitazone with increased risk of bladder cancer. Nonetheless, *in vitro* studies have demonstrated protective effects of the two drugs against β-cell glucolipotoxicity. Rosiglitazone prevented FFA-induced downregulation of PPARγ and impairment of insulin release in human islets [185]. Pioglitazone attenuates β-cell oxidative stress [186], inflammation and ER stress in FFA-treated β-cells [125, 187], through the repression of NF-κB activation [187] and restoration of SERCA pump expression [188]. However, β-cell-specific overexpression of

PPARγ in a mouse model led to worse glucose tolerance and β-cell loss upon high fat diet [189]. Thus, the potential of PPARγ agonism as anti-lipotoxic therapy remains uncertain.

Chaperones

Chaperones are low molecular weight compounds known to stabilize protein conformation and improve ER folding capacity. The two chaperones most extensively studied are 4-phenyl butyric acid (PBA) and the taurine-congugated derivative of ursodeoxycholic acid TUDCA. Both chaperones have good safety profiles [190]. In rat β-cells, PBA [191] and TUDCA [192] decreased palmitate-induced ER stress and ameliorated GSIS [191]. In a small study of obese or overweight non-diabetic individuals, participants were pretreated for 2 weeks with PBA before a lipid infusion over 48h. PBA was found to partially prevent the decrease in disposition index induced by the lipid infusion, pointing to a protective effect of PBA against β-cell lipotoxicity [190]. It is unknown whether this was mediated by alleviation of ER stress or by ER stress-independent mechanisms.

Caloric restriction and exercise to reduce pancreatic fat

The DiRECT trial showed that T2D was reversible in almost half of the patients that received a very low-calorie diet over 3–5 months [193]. In a smaller, earlier study of acute weight loss with a very low-calorie diet, recovery of the first-phase insulin secretion was associated with a decrease in pancreatic fat [194]. A further study evaluating normal glucose tolerant and T2D patients undergoing bariatric surgery found that the decrease in pancreatic fat was specific to T2D patients and did not occur in non-diabetic individuals, despite a similar weight loss [195]. This suggested that pancreatic triacylglycerol accumulation is not a mere reflection of obesity and may be associated with T2D-related metabolic abnormalities. However, in a subgroup of DiRECT study participants who underwent detailed metabolic testing, the decrease in intrapacreatic fat was similar in patients who had diabetes remission and in non-responders [196]. Fat accumulation in human pancreas mostly occurs in intrapancreatic adipocytes that can occupy up to 20% of pancreas area [197]. Local lipolysis could induce β-cell lipotoxicity in a paracrine manner. Whether a causal relationship exists between pancreatic fat deposition and declining β-cell function remains an open question (reviewed in [198]).

Two weeks of exercise training decreased pancreatic fat in non-diabetic, prediabetic (impaired fasting glucose and/or impaired glucose tolerance) and T2D subjects, especially in those with fatty pancreas, with some improvement in measures of β-cell function [199].

Novel therapies

In search for new therapeutic targets against glucolipotoxicity, a high-throughput screening study was performed on rat β-cells exposed to palmitate. Applying computational methods to the initial hits pointed to mitogen-activated protein kinase 4 (MAP4K4) inhibition as a β-cell protective mechanism. Genetic variants in MAP4K4 have been associated with prediabetes. Pharmacological and siRNA-mediated inhibition of MAP4K4 partially reverted lipotoxic cell death in human islets [200]. MAP4K4 inhibition has been shown to protect β-cells against TNF-α-induced suppression of insulin secretion *in vitro* [201]. In adipocytes, MAP4K4 is a negative regulator of PPARγ [202]. Whether the protection from lipotoxicity

by MAP4K4 inhibition is secondary to anti-inflammatory effects or other mechanisms is unclear.

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Abbreviations

ACC	acetyl-coA carboxylase
BMI	body mass index
BSA	bovine serum albumin
ER	endoplasmic reticulum
FFA	free fatty acid
GSIS	glucose-stimulated insulin secretion
GWAS	genome-wide association study
JNK	c-Jun N-terminal kinase
LPL	lipoprotein lipase
PBA	4-phenyl butyric acid
PKC	protein kinase C
PPARγ	peroxisome proliferator-activated receptor γ
ROS	reactive oxygen species
T2D	type 2 diabetes
UCP2	uncoupling protein-2
ZDF	Zucker Diabetic Fatty

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PERSPECTIVES

In conclusion, there is substantial evidence (including human studies) that lipo- and glucolipotoxicity contribute to β-cell demise in T2D. In experimental models, lipo- and glucolipotoxicity adversely affect many steps of the insulin production and secretion machinery (Figs. 3 & 4). The underlying mechanisms are still unclear but likely involve the generation of intracellular lipid metabolites (Fig. 5) and several interrelated and stress-induced pathways (Fig. 6). While much progress has been made in identifying the molecular basis of lipo- and glucolipotoxicity in experimental systems, whether or not this phenomenon actually plays an important role in human T2D remains debated. There are several reasons for this controversy. First, as discussed in this review, mimicking *in vivo* FFA levels in *in vitro* systems is technically challenging. Second, the changes brought about by lipo- and glucolipotoxic conditions are, by definition, progressive and chronic, which precludes their direct assessment in short-term clinical studies. Third, the difficulty to accurately quantify β-cell function and our inability to quantify β-cell mass in humans represent major limitations for glucolipotoxicity studies. With the development of *in vivo* β-cell imaging methods [203], it will hopefully become possible to longitudinally assess β-cell mass in the future and therefore assess the impact of fuel surfeit on human β-cells.

Highlights

- Prolonged exposure to elevated FFA and glucose levels contributes to β-cell demise
- Human and genetic studies support the relevance of lipotoxicity for T2D
- Mimicking prevailing *in situ* islet FFA levels in *in vitro* experiments is challenging
- Glucolipotoxicity impairs key steps in insulin biosynthesis and release
- FFAs elicit ER and oxidative stress and impair autophagy, causing β-cell apoptosis

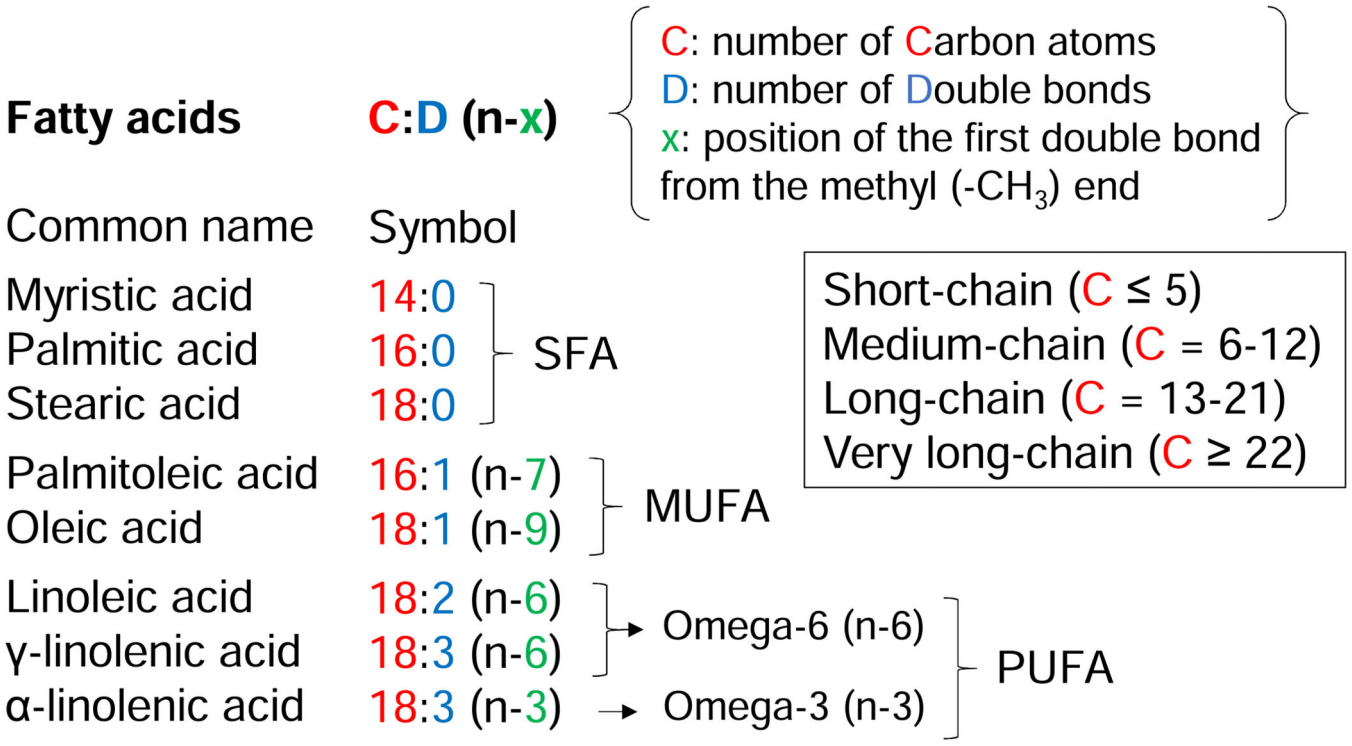

Figure 1: Types of FFAs and nomenclature.

FFAs can be categorized according to the length of the carbon chain (box), the presence of double bonds and the position of the first double bond. Depending on the presence of one or more double bonds, FFAs are classified into saturated (SFA), monounsaturated (MUFA) and polyunsaturated FFAs (PUFA). Depending on the position of the first double bond counting from the methyl end of the chain, FFAs are classified into omega-3, omega-6 etc. This figure does not provide an exhaustive list of common FFA names and symbols, but illustrates the FFAs mentioned in the text. Additional systems of FFA nomenclature exist.

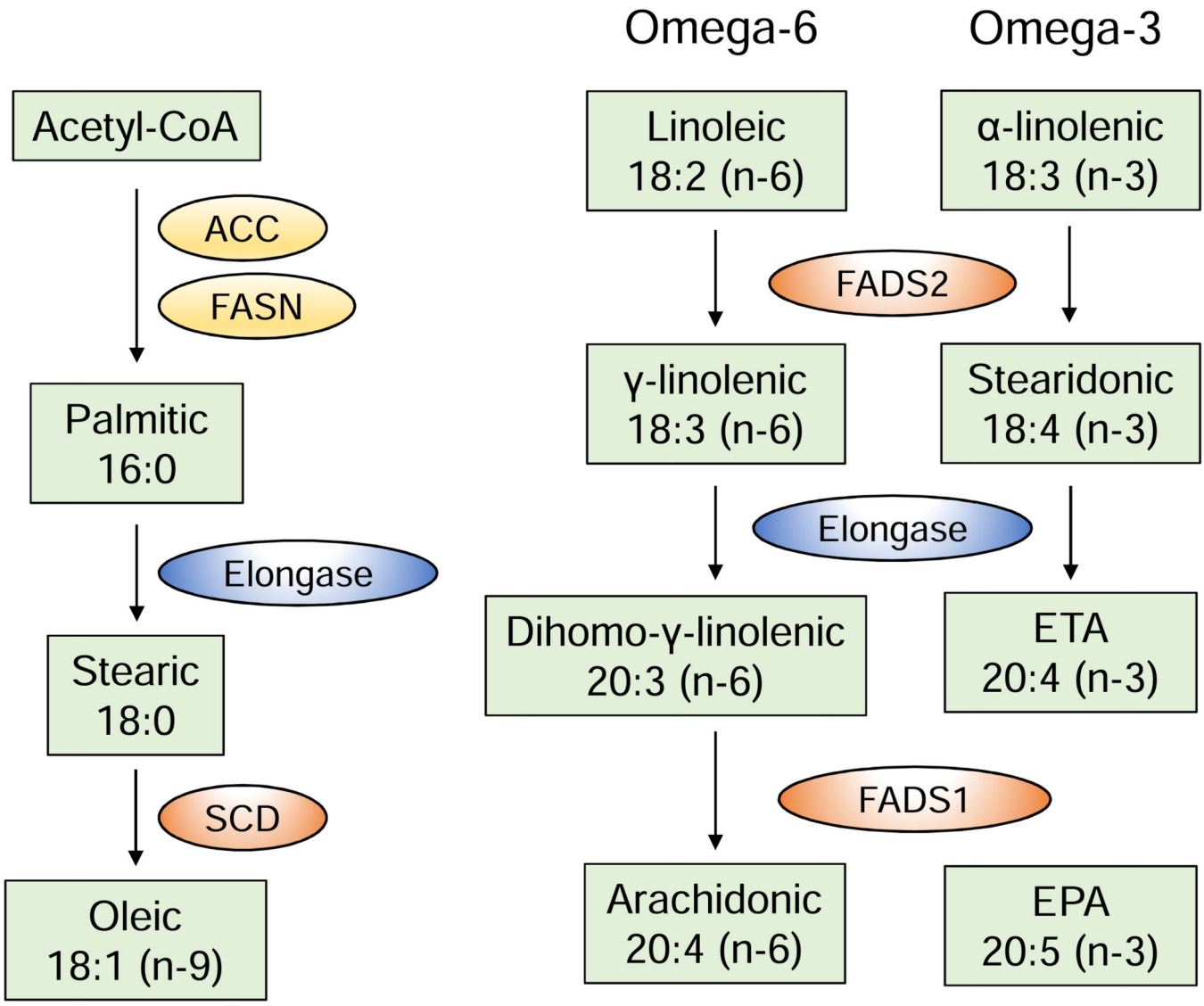

Figure 2: FFA metabolic pathways.

Examples of FFA metabolic pathways, highlighting the action of elongases and desaturases, adapted from [204]. Elongases extend the chain of FFAs with two carbons. Stearoyl-coA desaturases (SCD) insert a double bond in saturated FFAs at position 9, counting from the carboxylic acid end (Δ9). Δ5- and Δ6-desaturases, designated as fatty acid desaturase 1 (FADS1) and 2 (FADS2) insert double bonds at positions Δ5 and Δ6, respectively. ACC: acetyl-CoA carboxylase; FASN: fatty acid synthase; ETA: eicosatetraenoic acid; EPA: eicosapentaenoic acid.

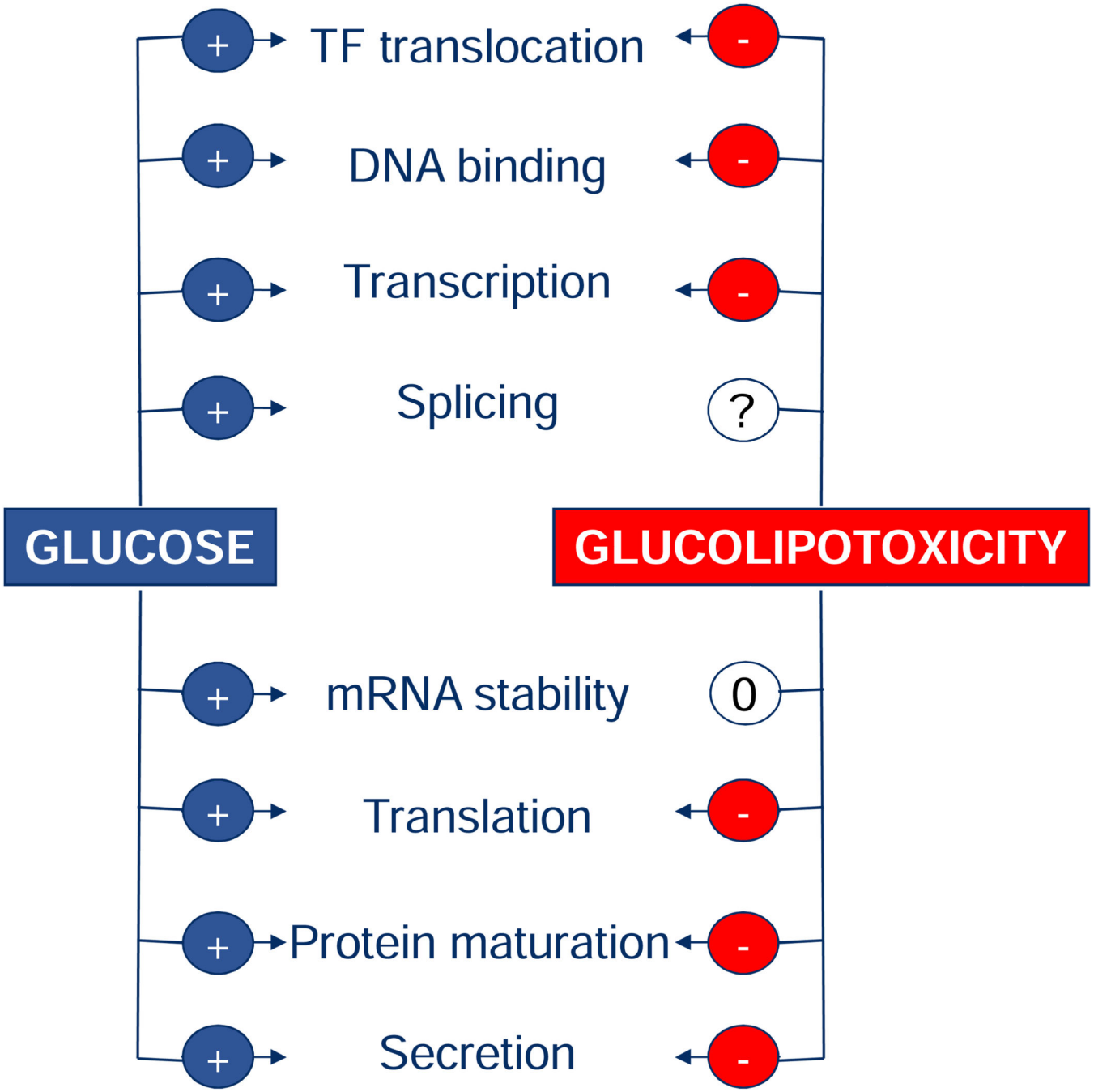

Figure 3: Impact of glucolipotoxicity on insulin production and secretion.

Glucose stimulates transcription of the insulin gene, pre-mRNA splicing, mRNA stability, proinsulin translation, protein maturation, and exocytosis. In contrast, lipo- and glucolipotoxic conditions have been shown to impair several of these steps. TF: transcription factor; +: stimulation; −: inhibition; 0: no effect; ?: not studied.

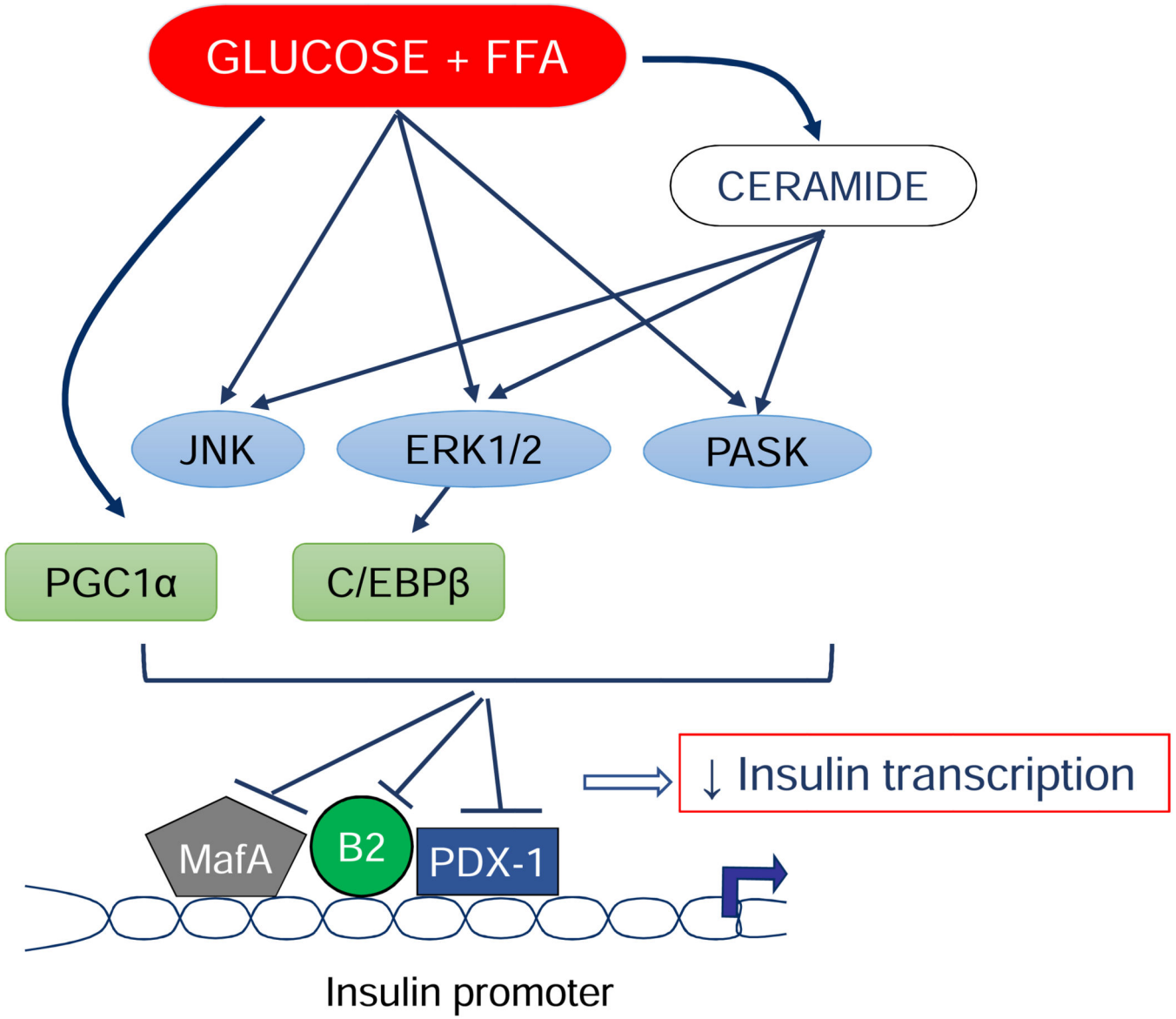

Figure 4: Signaling leading to the glucolipotoxic impairment of insulin gene transcription.

High glucose and the FFA palmitate stimulate ceramide production and activate the stress kinases JNK, ERK1/2 and PASK. These suppress the binding of the transcriptional regulators MafA, PDX1 and BETA2/NeuroD (B2) to the insulin promoter, in part through the activity of the transcriptional factors PGC1α and C/EBPβ. This results in decreased insulin transcription.

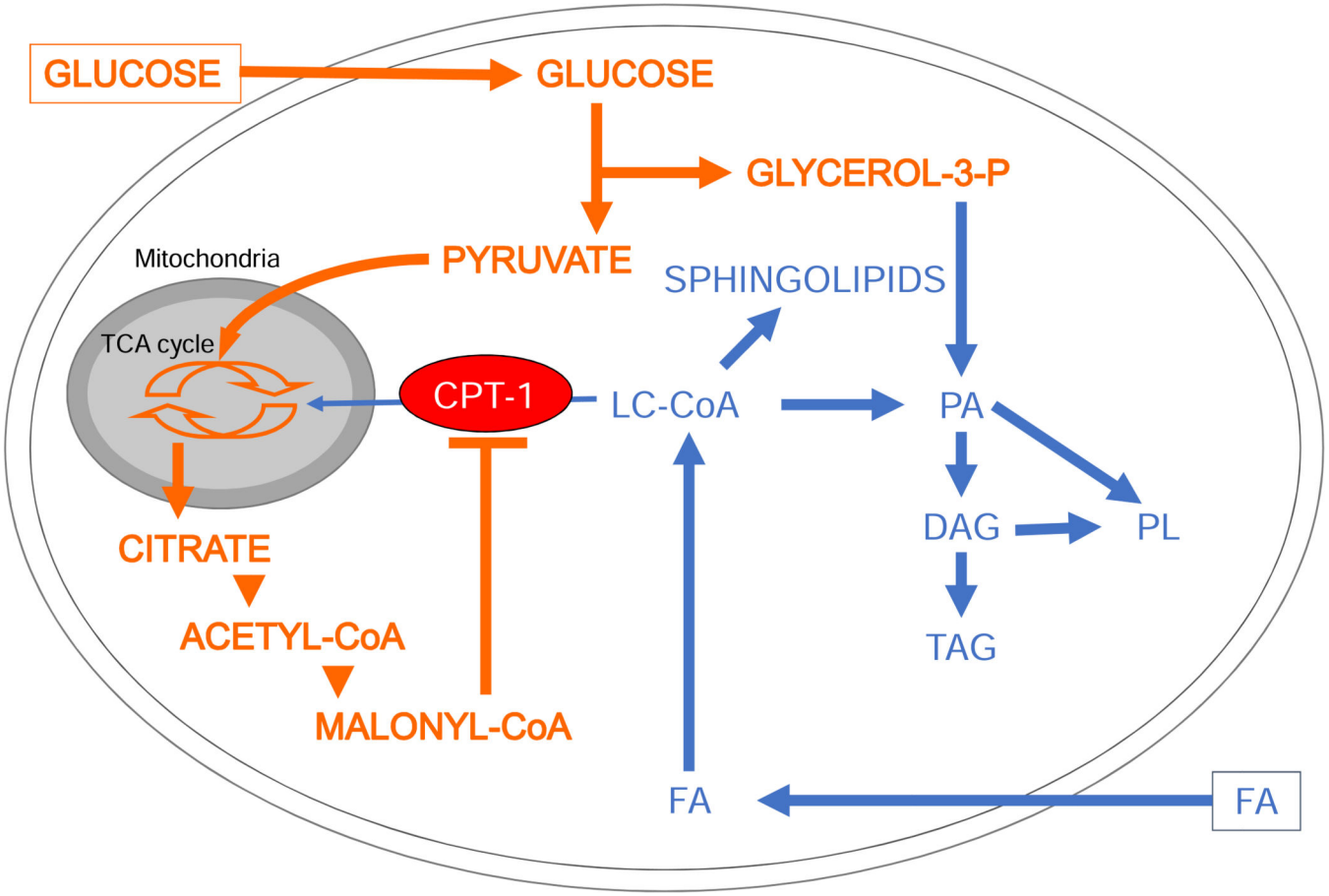

Figure 5: Effects of glucose on intracellular lipid metabolism in the β-cell.

In the presence of simultaneously elevated levels of glucose and FFAs, the increase in cytosolic malonyl-CoA resulting from glucose metabolism inhibits carnitine-palmitoyl transferase 1 (CPT-1). Transport of long-chain acyl-CoA (LC-CoA) in the mitochondria is blocked, and FFA metabolism is diverted towards the synthesis of lipid-derived signaling molecules such as sphingolipids, diacylglycerols (DG), phosphatidic acid (PA), phospholipids (PL) and triacylglycerols (TG). Adapted from [51] with permission.

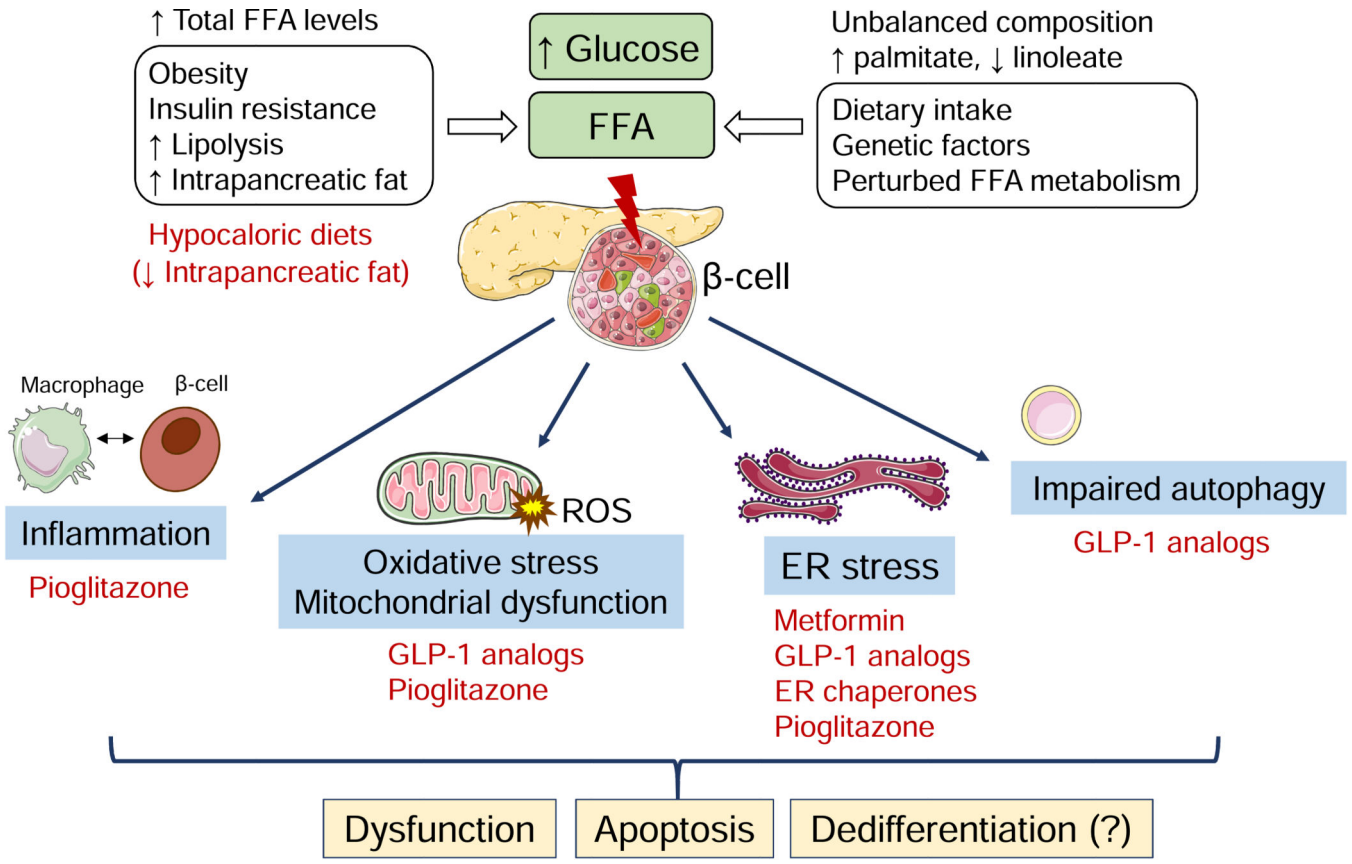

Figure 6: Molecular mechanisms of lipo- and glucolipotoxic β-cell demise and potential treatments.

A prolonged increased FFA supply and/or unbalanced FFA composition, alone or in combination with high glucose, elicits stress responses in pancreatic β-cells. These include ER stress, oxidative stress with excessive ROS production, mitochondrial dysfunction, inflammation and impaired autophagic flux. Crosstalk between these pathways may give rise to feed-forward mechanisms, aggravating glucolipotoxic stress. Collectively, these phenomena culminate in β-cell dysfunction, apoptosis and possibly dedifferentiation. *In vitro* data suggest that metformin, GLP-1 analogs, thiazolidinediones and ER chaperones mitigate lipo- and glucolipotoxicity. These therapies (shown in red) target distinct stress pathways. The graphic illustrations used in this figure are from Servier Medical art (https://smart.servier.com).

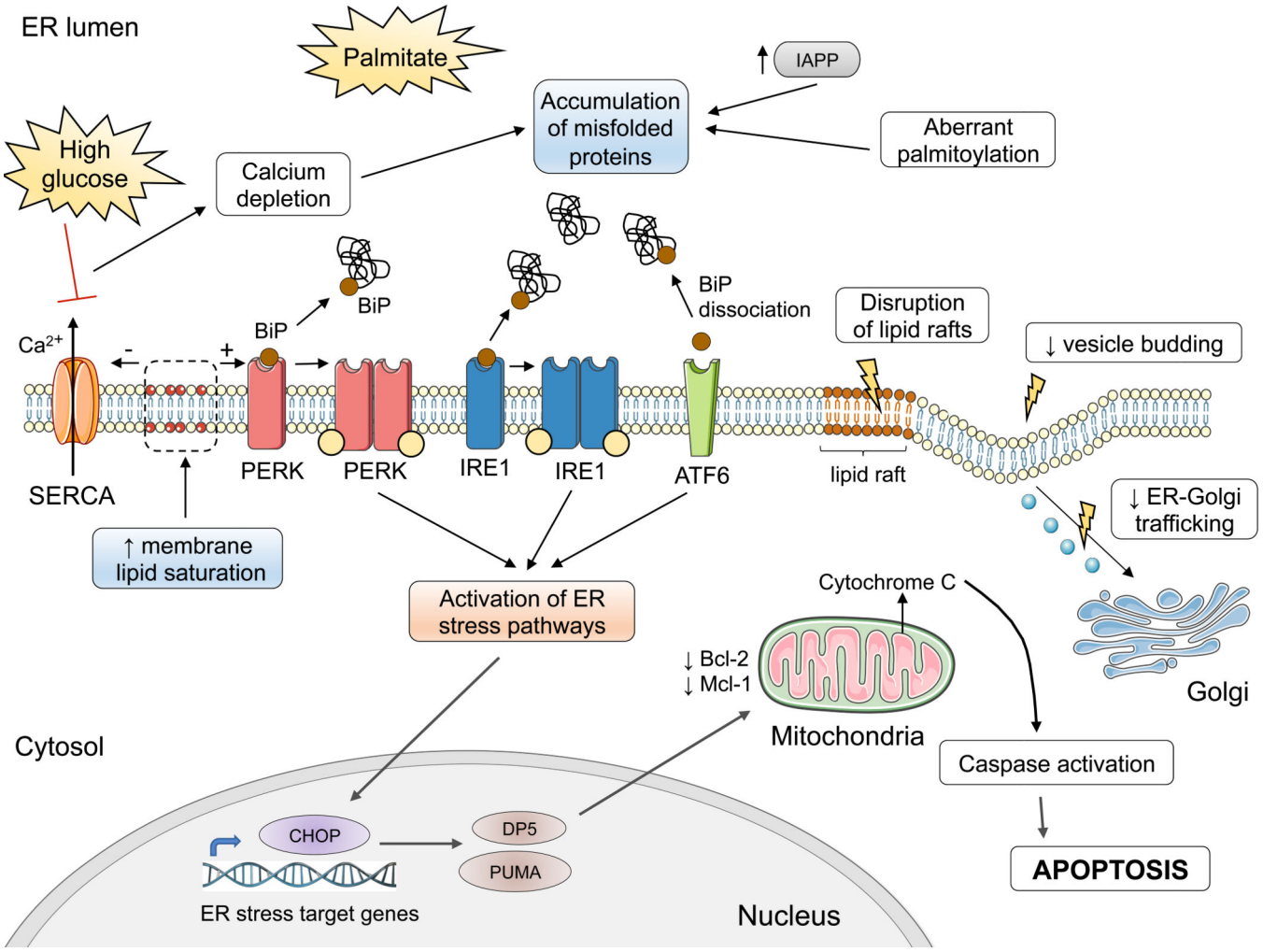

Figure 7: Activation of ER stress pathways by palmitate.

Exposure of β-cells to palmitate induces aberrant protein palmitoylation and Ca^{2+} depletion in the ER, affecting ER folding capacity. The depletion of Ca^{2+} stores is aggravated by the downregulation of the sarcoendoplasmic reticulum Ca^{2+}-ATPase (SERCA) pump in high glucose conditions. In parallel, palmitate disrupts the export of cargo from the ER and trafficking to the Golgi, contributing to the buildup of unfolded or misfolded proteins. The misfolded proteins recruit the ER chaperone BiP, causing its dissociation from the luminal domain of the ER stress transducers PERK, IRE1 and ATF6. This, together with increased ER membrane lipid saturation, results in the activation of the ER stress transducers, eliciting downstream ER stress signaling. This in turn leads to the induction of the proapoptotic proteins CHOP, PUMA and DP5, the latter inhibiting anti-apoptotic members of the Bcl-2 family. These events culminate in mitochondrial permeabilization, cytochrome C release and mitochondrial apoptosis. Graphic elements used in this illustration come from Servier Medical art (https://smart.servier.com).