Lipid-induced pancreatic β-cell dysfunction: focus on in vivo studies

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OBESITY, in particular central distribution of body fat, is a major predisposing factor for type 2 diabetes. Obesity is associated with insulin resistance, which is in part due to elevated circulating free fatty acids (FFA) and ectopic fat storage in nonadipose tissues. The mechanisms of lipid-induced insulin resistance have been discussed before and are beyond the scope of this review. Insulin resistance may be compensated for by increased insulin secretion, thereby maintaining normal glucose homeostasis. However, in individuals predisposed to developing type 2 diabetes, insulin secretion fails to fully compensate for insulin resistance, leading to impaired glucose tolerance and eventually type 2 diabetes. This review describes how chronically elevated FFA impair β-cell compensation, thus contributing to the pathogenesis of type 2 diabetes. A number of comprehensive reviews have outlined the mechanisms of the effects of FFA on the β-cell at the molecular level (62, 71). The present review instead focuses on findings from in vivo studies performed in animals and humans, including our own work over the past decade.

Effects of Chronic Elevation of Plasma FFA on β-Cell Function

Acute exposure to elevated plasma FFA enhances glucose and non-glucose-stimulated insulin secretion in vitro (84, 90) and in vivo in animals (21) and humans (16, 26, 37, 67). The molecular mechanisms of this effect have been extensively reviewed elsewhere (62, 71).

Prolonged exposure to FFA selectively impairs glucose-stimulated insulin secretion (GSIS) in vitro (28, 71, 98); however, the in vivo data are less consistent. With prolonged FFA elevation, absolute GSIS (uncorrected for insulin resistance) was found to be increased (6, 57), unchanged (16–18, 45, 53, 82, 86, 91, 93, 94), or decreased (15, 67) in humans and increased (56) or decreased (59) in rats. A summary of the studies in humans is shown in Table 1.

There are several explanations for these discordant results. First, differences in experimental protocols (i.e., glucose levels, duration of fat infusion, assessment of first-phase vs. steady-state insulin secretion) may influence the results of the...
Role of Genetic Predisposition in Lipid-Induced β-Cell Dysfunction

Individuals with normal glucose tolerance were found to differ in their response to prolonged elevation of circulating FFA according to their positive (FH+) or negative (FH-) family history of type 2 diabetes (45). GSIS increased in the various studies. Second, several human studies have shown that genetic predisposition to diabetes greatly affects the β-cell response to FFA, as reviewed in the section below. Third, and most importantly, prolonged elevation of FFA impairs insulin sensitivity (7), which confounds the interpretation of insulin secretion in the majority of the studies discussed above. The relationship between insulin sensitivity and insulin secretion in vivo is hyperbolic (4, 18, 42), so that the disposition index (DI), which is the product of insulin sensitivity and insulin secretion, is constant. Since FFA induce insulin resistance, which would be expected to lead to compensatory insulin hypersecretion, even an unchanged or mildly elevated absolute GSIS that is not at the level anticipated for that degree of insulin resistance indicates β-cell dysfunction, and it is the DI, not the absolute GSIS, that correctly evaluates β-cell function (Fig. 1).

Accordingly, in all of our studies in humans, apart from one (15), we have found that a 48-h intravenous fat infusion did not affect absolute GSIS. However, 16- to 48-h lipid infusion impaired DI, i.e., the ability of β-cells to compensate for the lipid-induced insulin resistance (15–18, 53, 91, 93, 94). Furthermore, in our studies that used a graded intravenous glucose infusion rather than a hyperglycemic clamp (16), we observed a small but significant elevation of plasma glucose after prolonged fat infusion, which is additional proof of impairment of β-cell function. Similarly, we have consistently demonstrated reduced glucose infusion (G_{inf}) rate during a hyperglycemic clamp after prolonged lipid infusion. Since G_{inf} is an integrated measure of glucose homeostasis, the reduction in G_{inf} during hyperglycemic clamp studies supports our interpretation of pancreatic β-cell failure. In rats, where FFA induce less insulin resistance than in humans (59), we have more consistently shown absolute rather than only relative impairment of insulin secretion, as demonstrated by absolute reductions in C-peptide concentrations (59, 63). In summary, acute FFA elevation enhances insulin secretion, whereas chronic elevation of FFA induces β-cell dysfunction, as evidenced in most cases by failure to compensate for FFA-induced reduction in insulin sensitivity.
FH− but decreased in the FH+ subjects. When insulin secretion was adjusted for FFA-induced insulin resistance, β-cell dysfunction became even more evident in FH+. Glucose-intolerant first-degree relatives of type 2 diabetic patients were also more sensitive to the impairing effect of lipid infusion on β-cell function than glucose-intolerant controls (86). In contrast, in our study of nondiabetic subjects from the Sandy Lake Oji-Cree community of Ontario, Canada, a population with the third highest prevalence of type 2 diabetes in the world, 2-day lipid infusion decreased β-cell function (DI) to a lesser degree compared with non-Oji-Cree subjects (17). Although it may appear that Oji-Cree individuals were less susceptible to lipid-induced β-cell dysfunction, they already had impaired DI prior to the lipid infusion. In summary, there is evidence that at least some genetically predisposed individuals have increased susceptibility to lipid-induced β-cell dysfunction. Some genetically predisposed individuals may already have impaired β-cell function, as lowering of circulating FFA has been shown to improve insulin secretion in this population (3, 23, 68). It is also possible that β-cell exposure to FFA is higher in FH+ during intravenous fat administration, as we showed that FFA appearance during intravenous Intralipid and heparin infusion is increased in Caucasian FH+ due to enhanced spillover of fatty acids (10). With the recently identified genetic variants that are associated with increased risk of type 2 diabetes (81), future studies are needed to examine the susceptibility to lipotoxicity of individuals carrying those specific variants. Indeed, genetic variants of the K\textsubscript{ATP} channel associated with type 2 diabetes have been shown to have greater sensitivity to opening by various FFA in vitro (73).

**Effect of Fatty Acid Saturation on Lipid-Induced β-Cell Dysfunction**

The only safe intravenous lipid infusions for human studies are commercially available sterile triglyceride mixtures containing mostly polyunsaturated fat such as Intralipid or Lyposyn, to which heparin is added to release lipoprotein lipase into the circulation and thereby hydrolyze the triglycerides of Intralipid. However, a number of studies have shown that the impairment of β-cell function induced by Intralipid + heparin is relatively mild both in animals (59) and in humans (53). In humans, Intralipid + heparin induces an approximately fourfold increase in linoleate (the most prevalent n-6 polyunsaturated fatty acid, PUFA) but only about 2- and 1.7-fold increases in oleate (the most prevalent monounsaturated fatty acid, MUFA) and palmitate (the most prevalent saturated fatty acid, SFA), respectively (19). MUFA (either oleate or olive oil infusion) appears to be the most effective to decrease β-cell function after intravenous infusion in rats (27, 59, 63). We have performed studies in humans in which an oral fat emulsion of predominantly PUFA, SFA, or MUFA was ingested over 24 h (92). Only PUFA ingestion, which caused the least insulin resistance, resulted in an absolute reduction in GSIS. The calculated DI (unpublished data not reported in that paper) did not differ between types of fat. In summary, MUFA may exert a greater impairing effect on β-cell function in rats, whereas there are currently no convincing data in humans that demonstrate greater impairment with MUFA.

**Glucolipotoxicity In Vivo**

Individuals with type 2 diabetes usually have elevation of both FFA and glucose concentrations; therefore, the combined effects of lipotoxicity and glucotoxicity on the β-cell are potentially of great clinical importance. Despite the extensive in vitro literature about glucolipotoxicity (71), very few studies have examined glucolipotoxicity in vivo. Combined 48-h infusions of Intralipid and glucose had an additive effect in decreasing GSIS in the perfused pancreas of dexamethasone-treated rats (74). However, cyclic and alternating infusion of glucose and Intralipid decreased insulin gene transcription in normal rats, whereas neither glucose nor Intralipid alone had a significant effect, suggesting a synergistic effect of glucose and fat in vivo in a model of mild β-cell dysfunction (35). Although prolonged elevation of plasma glucose induces β-cell dysfunction in humans (8), experimental hyperglycemia due to prolonged glucose infusions is not well tolerated by humans (due to iv site inflammation, nausea, symptoms of generalized swelling, and electrolyte imbalance). In our study, 24-h glucose infusion achieving plasma glucose levels of 7.5 mM actually enhanced β-cell function (DI), and this increase was abolished by coinfusion of Intralipid and heparin (53). We cannot refer to this effect as true glucolipotoxicity, since DI did not decline to levels lower than those observed with Intralipid alone. These data are in accord with Cusi et al., in which a low-dose infusion of lipid, glucose, or both together was administered to FH+ healthy subjects. In this study, only elevated FFA (but not hyperglycemia) decreased GSIS, suggesting the primacy of lipotoxicity over glucotoxicity in genetically predisposed individuals (22). In summary, although glucolipotoxicity has been amply demonstrated in vitro (71), and in initial studies in animals (35), perhaps due to limitations in experimentally raising blood glucose concentrations, true glucolipotoxicity has not been demonstrated in humans.

**Lipid-Induced β-Cell Dysfunction in Obese and Diabetic Animals and Humans**

Lipid-induced β-cell dysfunction is accentuated in islets of fat-fed or fructose-fed insulin-resistant rats (20) as well as in obese rodents studied in vivo (33) and obese vs. lean humans (15). In our study in obese humans, not only DI but also the absolute GSIS were decreased by Intralipid (15). The latter finding applied only to glucose-stimulated insulin secretion, whereas arginine-stimulated insulin secretion was not affected (14). Nonobese diabetic GK rats also show increased susceptibility to β-cell dysfunction induced by high-fat diet (11). Moreover, the Intralipid-induced impairment in β-cell function was accentuated in obese subjects with recent onset diabetes or glucose intolerance and mild hyperglycemia (Hb A\textsubscript{1c} 6.2%) compared with obese controls. The hyperglycemic subjects had a greater increase in FFA with Intralipid + heparin (18). An element of reversibility in the hyperglycemia-induced accentuation of lipotoxicity is suggested by the fact that the β-cell function was similar in Intralipid-infused controls and previously diabetic subjects following normalization of glycemia by biliopancreatic diversion (18). Intralipid-induced β-cell dysfunction was not evident, however, in subjects with established type 2 diabetes (15), presumably because of the preexisting profound β-cell impairment. Consistent with these results, other authors have found that lowering of fatty acids improved...
GSIS only in diabetic patients having the lowest levels of HbA1c (72). In our study (15), those with established diabetes actually showed an increase in absolute GSIS vs. controls, which is similar to our finding in prediabetic ZDF rats with mild hyperglycemia (33).

Recently, studies have emphasized possible similarities in the pathogenesis of type 1 and type 2 diabetes, as islet inflammation has been implicated in both (9). As will be discussed below, we have found that the effect of Intralipid to decrease β-cell function is accentuated in normoglycemic prediabetic BB rats with preexisting insulitis (unpublished observations), which raises the question of a possible role of lipotoxicity in the pathogenesis of late-onset type 1 diabetes.

In summary, obese animal models and humans and other prediabetic states appear to be more susceptible to lipid-induced impairment of β-cell function than are healthy animals and humans, whereas frankly diabetic animals and humans do not demonstrate lipid-induced impairment, perhaps because their β-cell function is already profoundly impaired at the time of study.

Effects of High-Fat Diet on β-Cell Function and Mass

High-fat diet is a convenient but nonselective model of chronic lipid elevation in animals. High-fat-fed rodents do develop glucose intolerance, suggesting that eventually, compensation for fat-induced insulin resistance becomes inadequate because of a defect in β-cell function or mass. Lipid-induced β-cell dysfunction (1, 41) precedes the decrease in β-cell mass (56, 83). β-Cell mass is still increased in response to insulin resistance after 16–20 wk of high-fat diet in rodents (41, 75) and, similar to β-cell function, the most common finding is failure of β-cell mass to appropriately adapt in response to insulin resistance rather than an absolute decline in β-cell mass. The presence of apoptosis (38, 41, 75, 80) and β-cell senescence (80), however, implies reduction of effective β-cell mass. The induction of apoptosis in the in vivo high-fat diet model is very important evidence for β-cell lipotoxicity, as models of FFA-induced apoptosis in vitro can be questioned, because in vitro cells are exposed to fatty acid albumin complexes that cannot mimic the physiological binding of albumin to fatty acids in vivo and often result in toxic levels of unbound fatty acids (71).

β-Cell mass is reduced in type 2 diabetes (60) due to increased β-cell apoptosis (12). Numerous factors likely contribute to β-cell apoptosis in type 2 diabetes in addition to lipotoxicity (glucotoxicity, amyloid toxicity, etc.). Interestingly, studies in transgenic rodent models of islet amyloid deposition show that amyloid toxicity is increased by fat (39, 61).

Mechanisms of Lipid-Induced β-Cell Dysfunction and Death: Focus on In Vivo Studies in Animals

The mechanisms of the impairing effect of a prolonged FFA elevation on insulin secretion have been addressed by numerous in vitro studies (71), but until recently the relevance of many of these mechanisms had not been tested in vivo, and very few data are available from human studies. The mechanisms of lipotoxicity and glucolipotoxicity that have received some support from in vivo data are summarized in Fig. 2. As can be seen, some of the mechanisms that were proposed in in vitro studies are not included in this figure because the relevant in vivo data are lacking or controversial. In particular, there are no in vivo data regarding the direct effects of FFA on glucose-metabolizing enzymes and ion channels affecting glucose-induced β-cell depolarization. The role of upregulation of UCP2 in decreasing glucose-induced ATP production and GSIS has been addressed in vivo using high-fat diet in genetic mouse models of UCP2 knockdown, but the results are controversial (41, 70). Equally controversial results have been obtained with high-fat diet studies in mice with deficiency of GPR40 (52, 85), perhaps due to differences in mouse genetic background. However, a role for FFA-induced activation of PKCε has been suggested on the basis of high-fat diet studies in PKCε-null mice (26). The mechanism might include changes in insulin gene transcription, as reduced insulin gene transcription was found in islets of rats infused with cyclic and alternating infusions of Intralipid and glucose (35).

The mechanisms proposed to explain FFA-induced apoptosis in part overlap with those involved in FFA-induced β-cell dysfunction. Information is generally derived from in vitro models, which have intrinsic limitations as discussed above. Data from in vivo studies are limited; however, transgenic mice overexpressing a kinase-negative PKCε were found to be protected from high-fat diet-induced apoptosis (38), and in vivo treatment with ceramide inhibitors (77) prevented β-cell apoptosis in ZDF rats. In vivo treatment with iNOS inhibitors (78) or antioxidants prevented β-cell dysfunction and apoptosis in obese diabetic rodents (43). Similarly, β-cell-specific overexpression of glutathione peroxidase prevented loss of β-cell volume and insulin granulation and ameliorated hyperglycemia.
in db/db mice (36). Similar results were obtained by β-cell-specific overexpression of thioredoxin (95).

Our group has focused on the role of oxidative stress in β-cell dysfunction selectively induced by fat by using in vivo, ex vivo, and in vitro models. MIN6 β-cells or rat islets exposed to FFA for 48 h showed decreased GSIS, an effect prevented by the antioxidant taurine. We found that the antioxidants taurine, N-acetylcysteine (NAC), and tempol prevented the impairment in β-cell function induced by prolonged exposure to FFA in vivo during hyperglycemic clamps and ex vivo in isolated islets of olate-treated rats (63). Other authors have found NAC to prevent impairment of GSIS in vivo and in the perfused pancreas of 96-h Intralipid-infused rats (97).

Oxidative stress is a known activator of IKKβ (2), which, by phosphorylating the inhibitor IκBα, activates NFκB. Our preliminary studies show that the IKKβ inhibitor salicylate prevents fat-induced impairment in GSIS in vitro, ex vivo and in vivo in rats (manuscript submitted). In addition to oxidative stress, IKKβ may be directly activated by FFA via Toll-like receptors (46) and ER stress (44) which can be induced by FFA independent of oxidative stress (34). There is a reciprocal link between oxidative stress, ER stress and inflammation, as one can cause the others and vice versa (13, 50, 96). Islets of type 2 diabetic subjects show oxidative stress (24), ER stress (58) and inflammatory macrophage infiltration (30).

Consistent with the notion that inflammation plays a role in fat-induced β-cell dysfunction, we found that this is accentuated in normoglycemic prediabetic BB rats with preexisting insulinitis and prevented by in vivo treatment with antioxidants (NAC) (unpublished observations) and interleukin-1 receptor (IL-1R) antagonist (88). IL-1R antagonist (75) or anti-IL-1 antibody (65) also decreased apoptosis in high-fat-fed rodents. Also, Toll-like receptor 2-deficient mice were protected from β-cell dysfunction induced by high-fat diet (29). Inflammatory kinases such as IKKβ and JNK may be activated by FFA directly, via oxidative stress/endoplasmic reticulum stress or via PKC (32, 54). PKC may be activated by oxidative stress (49), and PKC can also induce oxidative stress via activation of NADPH oxidase (40). Indeed, we have recently shown that in vivo treatment with apocynin, an inhibitor of NADPH oxidase, partially prevents fat-induced β-cell dysfunction in rats (47).

Activation of inflammatory kinases and PKC can induce β-cell dysfunction and apoptosis partly by impairing β-cell insulin signaling (48). Accordingly, a role for fat-induced decrease in Akt has been proposed in lipotoxicity based on the insulin-secretory defect observed in high-fat-fed mice overexpressing dominant negative Akt (5). These studies are in line with our preliminary results showing that the tyrosine phosphatase inhibitor bisperoxovanadium prevents fat-induced β-cell dysfunction in vitro, ex vivo, and in vivo (64).

Mechanisms of Fat-Induced β-Cell Dysfunction: Studies in Humans

Many of the findings from animal studies discussed above have been confirmed by in vivo clamp studies in humans. A recent study from our group suggested that oxidative stress is implicated in fat-induced β-cell dysfunction in humans (93). Two antioxidants, taurine or NAC, were administered orally. Taurine at 3 g/day was administered orally for 2 wk prior to and during a 48-h Intralipid infusion. NAC was administered orally with a loading dose of 140 mg/kg followed by 70 mg/kg every 4 h during the 48-h Intralipid infusion. Oral treatment with taurine, which reduced plasma markers of oxidative stress, improved the FFA-induced decrease in insulin sensitivity and β-cell function. NAC had no such effect, except for an improvement in insulin clearance, presumably because of its effect in the liver and low systemic bioavailability due to high first-pass hepatic clearance after oral administration.

Our positive results with intravenous salicylate in rats (unpublished observations) contrast with the negative results we obtained with 4.5 g of oral salicylate for 1 wk in obese humans (94), although in another recent study performed in young obese subjects salsalate (salicylate dimer) improved GSIS (31). Treatment with IL-1R antagonist improved insulin sensitivity and β-cell function in type 2 diabetic patients (51). Our studies suggest that the chemical chaperone 4-phenyl butyric acid (PBA), shown previously to reduce endoplasmic reticulum stress in mice (66), improves both insulin sensitivity and β-cell function in Intralipid-infused humans (91).

Conclusions and Future Directions

Given the adverse consequences of prolonged elevation of FFA on insulin sensitivity and secretion in humans, it would appear that pharmacological reductions of elevated fatty acids may be beneficial. Currently available antilipolytic treatments, however, are not very effective due to the short half-life of drugs, FFA rebound and independent adverse effects of the drugs on insulin sensitivity. Furthermore, at least one theoretical pitfall of any therapy that primarily targets adipose tissue fatty acid mobilization without reducing net energy balance is the exacerbation of obesity and its consequent adverse inflammatory and metabolic sequelae. We have shown in several small, proof-of-concept, mechanistic studies that therapies directed toward diminishing oxidative stress and ER stress prevent lipid-induced β-cell dysfunction in animals and humans, but these therapies need to be assessed in larger, well controlled, prospective, and randomized clinical trials before they can be adopted widely for prevention and treatment of type 2 diabetes. Several antidiabetic drugs and nonpharmacological weight loss have been shown to improve β-cell function in addition to their main recognized mechanism of action. For example, metformin and thiazolidinediones (TZDs) have multiple effects, being insulin sensitizers, antioxidant, anti-inflammatory agents as well as activators of AMP kinase, which should deplete islet fat (25). Thus, both metformin and TZDs improve insulin secretion in fat-exposed rat (69, 79) and human islets (55, 89). A combination of these properties may be clinically advantageous, as β-cell dysfunction induced by lipotoxicity is likely a mixture of oxidative, ER, and inflammatory stress. However, whether there is a role for other more specific “stress inhibitors” in the prevention and/or treatment of type 2 diabetes remains to be determined.

In conclusion, lipid-induced impairment of β-cell function has been well established both in vitro and in vivo in animal models and humans. It is still not known, however, what the relative contribution of lipotoxicity is to what undoubtedly will turn out to be a complex array of inherited and acquired defects in β-cell function in type 2 diabetes. Some of the mechanisms linking chronically elevated fatty acids to β-cell dysfunction are beginning to be elucidated. These mechanisms may be
attractive targets for therapies aimed at preventing and correcting the functional defect of β-cells, although one wonders whether this approach to pharmacotherapy of type 2 diabetes can ever be truly effective in the face of uncorrected and persistent net positive energy balance of the organism.

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DISCLOSURES

No conflicts of interest are reported by the authors.

REFERENCES


