

Malonyl-CoA Signaling, Lipid Partitioning, and Glucolipototoxicity

Role in β -Cell Adaptation and Failure in the Etiology of Diabetes

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β -Cells possess inherent mechanisms to adapt to over-nutrition and the prevailing concentrations of glucose, fatty acids, and other fuels to maintain glucose homeostasis. However, this is balanced by potentially harmful actions of the same nutrients. Both glucose and fatty acids may cause good/adaptive or evil/toxic actions on the β -cell, depending on their concentrations and the time during which they are elevated. Chronic high glucose dramatically influences β -cell lipid metabolism via substrate availability, changes in the activity and expression of enzymes of glucose and lipid metabolism, and modifications in the expression level of key transcription factors. We discuss here the emerging view that β -cell "glucotoxicity" is in part indirectly caused by "lipotoxicity," and that β -cell abnormalities will become particularly apparent when both glucose and circulating fatty acids are high. We support the concept that elevated glucose and fatty acids synergize in causing toxicity in islets and other organs, a process that may be instrumental in the pleiotropic defects associated with the metabolic syndrome and type 1 and type 2 diabetes. The mechanisms by which hyperglycemia and hyperlipidemia alter insulin secretion are discussed and a model of β -cell "glucolipototoxicity" that implicates alterations in β -cell malonyl-CoA concentrations; peroxisome proliferator-activated receptor- α and - γ and sterol regulatory element binding protein-1c expression; and lipid partitioning is proposed. *Diabetes* 51 (Suppl. 3):S405-S413, 2002

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ACC, acetyl-CoA carboxylase; CPT-1, carnitine palmitoyltransferase-1; FA-CoA, FFA-derived long chain acyl-CoA esters; FAS, fatty acid synthase; FFA, free fatty acid; GPAT, glycerol-palmitate acyltransferase; GSIS, glucose-stimulated insulin secretion; K_{ATP} , channel, ATP-sensitive potassium channel; MCD, malonyl-CoA decarboxylase; PPAR, peroxisome proliferator-activated receptor; SREBP, sterol regulatory element binding protein; TG, triglyceride.

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Type 2 diabetes is considered to be in most cases a polygenic disease with a strong involvement of environmental factors, such as diet and exercise (1). Glucose intolerance in association with hyperinsulinemia and insulin resistance are early hallmarks of the prediabetic phase (1–3). The traditional view of the pathogenesis of type 2 diabetes is that hyperinsulinemia occurs as a consequence of insulin resistance (1). In contrast, others have favored the concept that exaggerated insulin secretion will cause insulin resistance (3,4), possibly via fatty acid-mediated impairment of insulin action (5–7). To date, longitudinal studies in humans and animals have failed to determine whether insulin resistance precedes hyperinsulinemia or the reverse occurs (8,9). This may be because compensatory hyperinsulinemia is a fast inescapable defense of the organism against insulin resistance and hyperinsulinemia is rapidly compensated for by insulin resistance for the sake of maintaining fuel for the brain and glucose homeostasis. Thus the two defects and/or adaptive processes initially walk hand in hand in the progression toward overt diabetes. Nonetheless, what has recently emerged is that insulin resistance alone (in the non- β -cell target tissues of the hormone) cannot cause diabetes without β -cell failure (10). Indeed, although targeted disruption of the muscle insulin receptor causes a marked defect of insulin action in this tissue, the phenotype of such animals is modest with a lack of diabetes (11). Thus glycemia is a much defended variable by the organism, and if caloric intake is in excess of energy expenditure, normoglycemia can be maintained only at the expense of various compensatory changes, including hyperinsulinemia, hyperlipidemia, insulin resistance, and obesity.

The goal of this short study is to discuss the emerging view that glucose dramatically influences lipid metabolism in the β -cell and consequently that the so-called β -cell "glucotoxicity" has much in common with "lipotoxicity." The mechanisms whereby chronic elevated fatty acids impair insulin secretion are discussed. We also develop the concept that elevated glucose and free fatty acids (FFAs) synergize in altering β -cell function. Finally, we propose a testable model of β -cell "glucolipototoxicity" that implicates malonyl-CoA, peroxisome proliferator-activated receptor (PPAR)- α and - γ , sterol regulatory

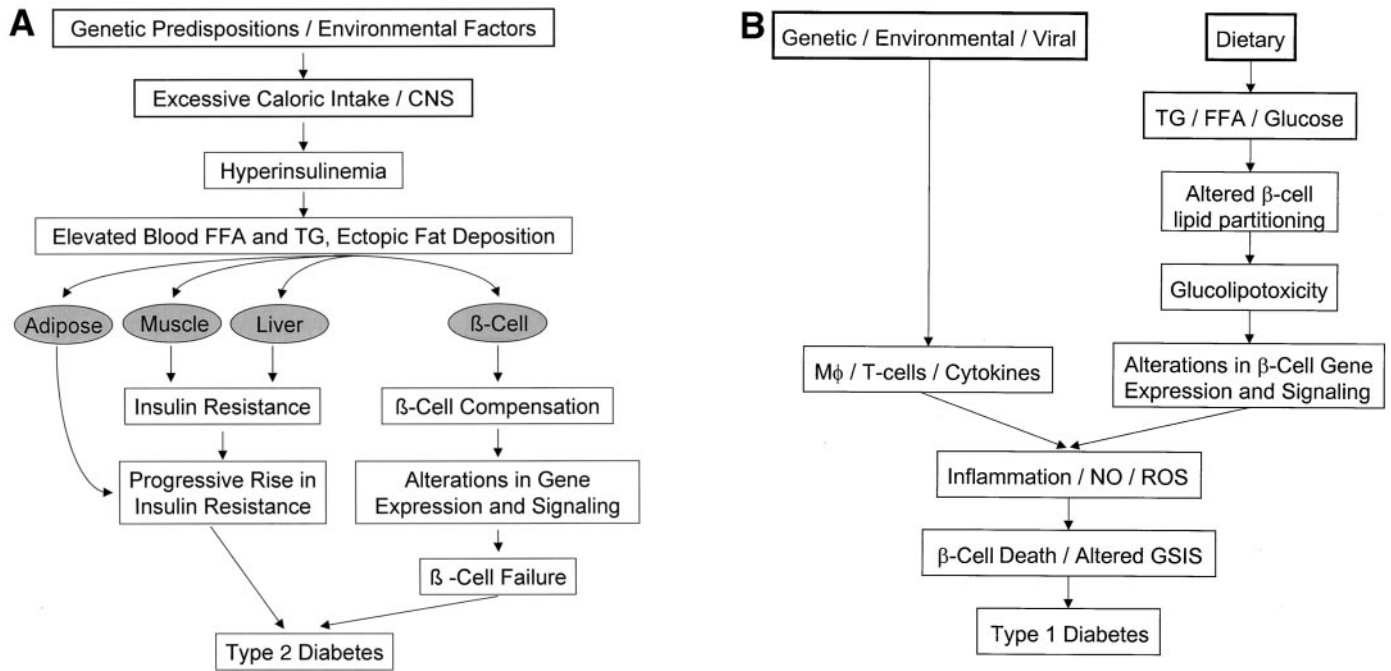


FIG. 1. Models illustrating the influence of calorigenic nutrients and overnutrition in the etiology of adipogenic type 2 diabetes (A) and type 1 diabetes (B). CNS, central nervous system; ROS, reactive oxygen species.

element binding protein (SREBP)-1c, and altered lipid partitioning.

THE DIABETIC MILIEU AND β -CELL DYSFUNCTION IN TYPE 2 AND TYPE 1 DIABETES

A nutritional model of the pathogenesis of type 2 adipogenic diabetes is illustrated in Fig. 1A. In this model, excessive carbohydrate and fat intake causes, in genetically susceptible individuals, hyperinsulinemia in association with increased hepatic lipoprotein secretion, adipose tissue growth, and elevated FFA levels. The latter, together with episodes of postprandial hyperglycemia, on the one hand cause muscle and liver insulin resistance and increase hepatic glucose production. On the other hand, the same calorigenic fuel stimuli allow β -cell compensation by promoting insulin secretion and biosynthesis as well as β -cell growth. However, at later stages, the progressive rise in insulin resistance together with alterations in β -cell gene expression and signaling induced by the rising FFA levels and the more prominent postprandial hyperglycemia and FFA levels will cause β -cell failure. As a result of this β -cell decompensation, possibly because of altered insulin secretion, biosynthesis, and apoptosis, overt diabetes occurs.

It should be made clear that the terms β -cell failure and β -cell decompensation in fact represent the loss of the curvilinear (hyperbolic) relation between insulin sensitivity and β -cell function that normally occurs in obese non-diabetes-prone individuals (12,13). In other words, it reflects a lack of the capacity of β -cells to cope with the progressively deteriorating obesity environment. This maladaptation of the β -cells is likely genetically determined and instrumental in causing overt diabetes (10).

Type 1 diabetes is characterized by severe β -cell dysfunction and an absence of glucose stimulated insulin secretion (GSIS) (14). After initial insulin treatment, many

patients demonstrate a “honeymoon” phase of reduced insulin requirement. This phase is variable in length, and sometimes insulin secretion is restored to the point that the patient no longer requires insulin treatment. This indicates that something in the diabetic milieu is toxic to the β -cell. Consistent with this view, transplantation of islets into streptozotocin-induced diabetic mice causes loss of GSIS by the islets in the absence of an immune reaction (15). This suggests that circulating factors normalized by insulin treatment, perhaps elevated FFA and glucose levels, contribute, in addition to autoimmune attack, to the development of type 1 diabetes (15).

The links between caloric dietary factors, elevated blood glucose and FFA levels, and the etiologies of types 1 and 2 diabetes are illustrated in Fig. 1A and B. Clearly, both nutritional models of β -cell “glucolipotoxicity” also apply to type 1 diabetic patients with a transplanted pancreas or islets who, with dietary indulgence, may become insulin resistant and eventually show β -cell failure.

ROLE OF ELEVATED GLUCOSE AND LIPIDS IN THE ETIOLOGY OF β -CELL DYSFUNCTION: GLUCOLIPOTOXICITY

The roles of glucose and FFA as “toxic” factors of the (pre)diabetic milieu influencing β -cell function and the etiology of diabetes are debated. It was first proposed that chronic hyperglycemia is progressively deleterious to the islet, which led to the concept of “glucotoxicity” (16) or β -cell “exhaustion” (17). However, more recent evidence suggests that elevated circulating and intracellular lipids, in addition to glucose, play an important role in the etiology of adipogenic diabetes (6,8). Studies in humans and animals have shown that obesity and type 2 diabetes are associated with hypertriglyceridemia and elevated

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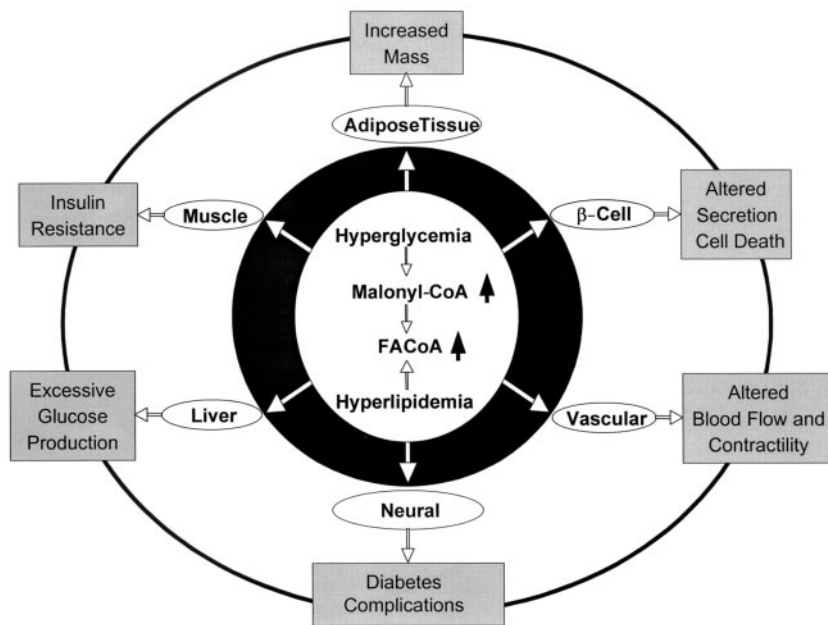


FIG. 2. Increased cellular levels of malonyl-CoA and FAcCoA as a common mechanism causing glucolipotoxicity in various tissues in obesity-associated type 2 diabetes. Glucose-derived malonyl-CoA reduces fat oxidation, thus causing FAcCoA accumulation in the cytosol and consequently the exaggerated production of various reactive complex lipid-signaling molecules that may lead to pleiotropic defects in various organs. These alterations may include insulin resistance in muscle, liver, and adipose tissue; defective insulin secretion; and β -cell death, as well as several of the complications of diabetes.

FFA levels (6,8). Elevated FFA levels were shown to be predictive of conversion from normal glucose tolerance and impaired glucose tolerance to diabetes (13). Patients with poorly controlled type 1 diabetes are ketotic because of altered lipid metabolism (8). Diabetes may therefore be considered as much a lipid disorder as a disease of glucose tolerance (6,8).

The endocrine pancreas is affected by hyperlipidemia, as chronic exposure of islet tissue to elevated FFA levels initially causes an elevated secretion at low glucose, which has been associated in many instances (18–20), but not all (21), with a reduced insulin release at high glucose. In addition, elevated FFA levels cause decreased insulin gene expression and proinsulin processing (21). Longer exposure of pancreatic islets to FFAs, particularly at high concentrations, can cause β -cell death (22).

To reconcile the roles of glucose and fatty acids in altering the function of various cell types in diabetes, in particular that of the β -cell, we first proposed the “glucolipoxia” concept (6), which was subsequently termed “glucolipotoxicity” (23). We suggested that either hyperglycemia alone or elevated circulating FFAs alone should not be so detrimental to a cell for the simple reason that when glucose levels alone are high, glucose is oxidized, and when FFAs alone are high, then they are oxidized instead of glucose. For example, FFAs are elevated during fasting, but are not toxic to cells under this low glucose condition. However, when both glucose and FFA levels are high, then the problem arises, and together they may progressively alter the function of various cell types (Fig. 2) (6). Thus, under this condition, FFA-derived long-chain acyl-CoA esters (FAcCoAs) are high, and furthermore they cannot be oxidized because glucose-derived malonyl-CoA is also elevated. Malonyl-CoA is a metabolic signaling molecule that regulates lipid partitioning (the relative fluxes of FFA oxidation and esterification) through its inhibitory action on carnitine palmitoyltransferase-1 (CPT-1), which catalyzes the rate-limiting step of the mitochondrial β -oxidation of fatty acids (8). As a result, FAcCoAs accumulate in the cytoplasm and could, for example,

either directly or indirectly via complex lipid or ceramide formation cause insulin resistance in muscle tissue, impair glucose induced secretion, or promote β -cell apoptosis and the progressive neural diabetes complications (Fig. 2). This integrated model of diabetes and the metabolic syndrome in which the accumulation of acyl-CoA compounds are detrimental to various cell types, particularly at high glucose, has received strong support from various studies in muscle tissues (7,24) and β -cells (25).

DIETARY FAT, GLUCOLIPOTOXICITY, AND TYPE 1 DIABETES

Caloric and fat restriction lower the incidence of diabetes in the NOD mouse model of autoimmune type 1 diabetes (26). In addition, the PPAR- γ agonist troglitazone, which redirects fat from different organs to adipose tissue (27) and reduces the β -cell content of triglycerides (TGs) (28), prevents diabetes in NOD mice (29). In the BB rat, a model of autoimmune diabetes, caloric restriction does not alter the progression toward diabetes, but the disease is prevented by essential fatty acid deficiency (30,31). With respect to humans, results from some studies have indicated that a high weight gain in infancy is associated with an increased risk of type 1 diabetes (32,33). A possible synergy between cytokines and lipids in causing β -cell cytotoxicity has been inferred from results demonstrating that the toxicity of interleukin-1 β is enhanced in TG-rich islets and reduced in fat-depleted islets after caloric restriction or leptin plus troglitazone treatment (34). Thus, to date, the role of lipotoxicity has been mostly studied in type 2 diabetes, but the evidence indicates that its role in type 1 diabetes may be more important than previously anticipated.

The role of caloric diets and lipids in the etiology of autoimmune diabetes has been poorly studied because, contrary to in type 2 diabetes, lipids are usually normal before the onset of hyperglycemia. However, once insulin deficiency occurs, fatty acids in addition to glucose are elevated, which may cause β -cell glucolipotoxicity. It

seems reasonable to hypothesize that this process might impair residual β -cell function, shorten the “honeymoon” phase in type 1 diabetes, and be particularly important in slow-onset type 1 diabetes.

GLUCOLIPOTOXICITY AND ISLET TRANSPLANTATION

With respect to transplantation in general, it is noteworthy that hyperglycemia and hyperlipidemia are common problems associated with the use of immunosuppressive drugs such as tacrolimus, sirolimus, cyclosporine, azathioprine, and steroids (35,36). Hyperlipidemia can be particularly severe with sirolimus (35,36). Post-transplant diabetes is more common with tacrolimus, probably because of a marked effect on pancreatic β -cells, resulting in a diminished GSIS (37). Increased TG levels are more frequently associated with cyclosporine treatment (38). Established complications of steroid treatment are insulin resistance, hyperglycemia, and hyperlipidemia (35,36). It has been suggested that reduction of hyperlipidemia may have a role in decreasing the incidence of chronic rejection of allografts (35,36). Thus, on the basis of the experience acquired with the transplantation of many tissues and various immunosuppressive regimens, the β -cell glucolipotoxicity concept can be extended to islet transplantation, both in terms of dietary and immunosuppression considerations. Perhaps β -cell glucolipotoxicity will provide an explanation to the fact that several of the patients that received the Edmonton protocol have returned to insulin treatment (39).

β -CELL “GLUCOAPOPTOSIS”

Depending on the experimental context, various effects of elevated concentrations of glucose have been reported on β -cell growth and death. Glucose-induced apoptosis, which may be termed “glucoapoptosis” by analogy with lipoapoptosis (28), was observed in β -cells of *ob/ob* mice and Wistar rats maintained in medium containing 10% serum (40). This study inferred that glucose-induced Ca^{2+} influx is involved in the toxic action of glucose, which could be mimicked by sulfonylureas (40). In contrast, high glucose promoted cell survival of purified β -cells cultured in the absence of serum (41), an experimental condition known to promote apoptosis. Islets from the gerbil *Psammomys obesus* cultured in the presence of serum responded to elevated glucose by increasing apoptosis, whereas no significant changes occurred in SD rat islets incubated under similar conditions (42). A possible explanation that may reconcile these apparently contradictory data is related to what was discussed above concerning the role of glucose in the control of lipid partitioning. Thus it is striking to note that elevated glucose is particularly pro-apoptotic in the context of high levels of intracellular lipids, as occurs in fat-laden *ob/ob* and *Psammomys* islets (28). This reinforces the hypothesis that will be discussed in more detail below, that elevated glucose and fat synergize in their toxicity toward the β -cell.

β -CELL “LIPOAPOPTOSIS”

Islets from diabetic ZDF *fa/fa* rats show elevated lipogenesis and a marked deposition of TG in association with a 10-fold increase in glycerol-palmitate acyltransferase (GPAT), the enzyme that catalyzes the rate-limiting step in

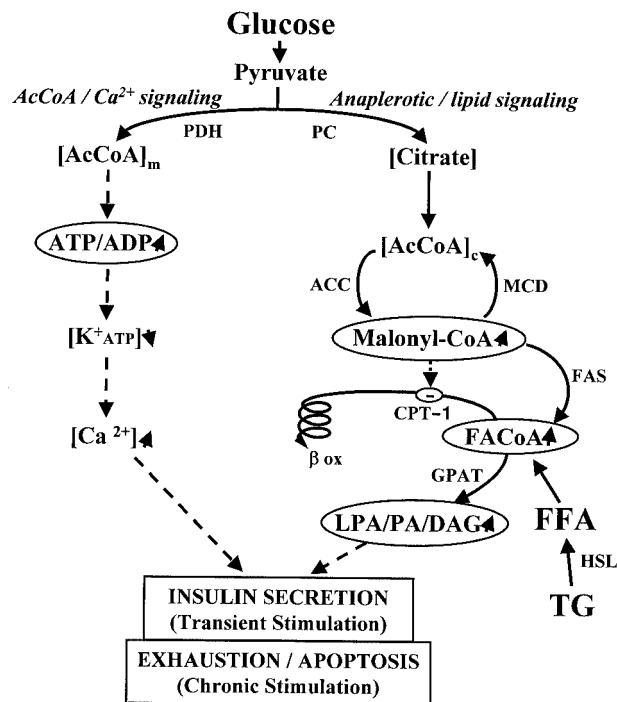


FIG. 3. Model illustrating the role of the anaplerotic/lipid-signaling pathway in β -cell metabolic signal transduction in health and diabetes. When transiently stimulated, the Ac-CoA/Ca²⁺ and anaplerotic/lipid-signaling pathways synergize to promote insulin secretion. Chronic stimulation of the same pathways results in β -cell dysfunction, exhaustion, and death. AcCoAc, cytosolic acetyl-CoA; AcCoAm, mitochondrial acetyl-CoA; DAG, diacylglycerol, LPA, lysophosphatidic acid; β ox, β oxidation of fatty acids; PA, phosphatidic acid; PC, pyruvate carboxylase; PDH, pyruvate dehydrogenase.

glyceride formation (43). Much evidence has been provided by Unger and colleagues (22) for the concept that cellular FFA-CoA and the induction of the ceramide pathway by elevated circulating FFAs may cause “lipoapoptosis,” in particular through NO synthase induction and NO cytotoxicity. It is interesting to note that leptin induces *in vivo* and *in vitro* rat islet CPT-1 and reduces the expression level of both acetyl-CoA carboxylase (ACC), the enzyme that catalyzes the formation of malonyl-CoA, and GPAT (34,44). These gene expression changes are found in association with decreased islet TG deposition and increased lipid oxidation. In obese ZDF rats overexpressing the leptin receptor, leptin causes changes with respect to lipid metabolism similar to those associated with reduced islet TG content and a reversal of the diabetic phenotype (28). Thus it is attractive to hypothesize that the ACC/malonyl-CoA/CPT-1 metabolic signaling network, which plays a key regulatory role in lipid partitioning (6,45), provides a link between glucolipotoxicity and β -cell apoptosis.

THE MALONYL-CoA/CPT-1 INTERACTION IN THE CONTROL OF INSULIN SECRETION

The role of acyl-CoA compounds as coupling factors linking glucose metabolism to insulin secretion has been reviewed extensively (6,45,46). We will briefly describe the lipid signaling model of insulin secretion (Fig. 3), as it has strong implications for our understanding of the long-term

actions of both glucose and FFAs on β -cell function and glucolipotoxicity.

After the rapid entry of glucose into the β -cell, glucose is metabolized to pyruvate. Subsequent acetyl-CoA production and increased mitochondrial metabolism activate the ATP-sensitive potassium (K_{ATP}) channel-dependent pathway of GSIS. Although the resulting increase in Ca^{2+} is necessary for secretion, it is clearly not sufficient (47). The further metabolism of glucose via the carboxylation of pyruvate by pyruvate carboxylase (anaplerosis) causes a direct rise in the level of Krebs cycle intermediates. Thus a rise in an external signal (glucose) is transduced into two key intracellular mitochondrial signals: accelerated acetyl-CoA production (for subsequent ATP synthesis) and a rise in citrate (for subsequent malonyl-CoA production). When abundant, citrate will escape the mitochondrion (cataplerosis) (48) to enter the cytoplasm, where it can be transformed to malonyl-CoA via two successive reactions catalyzed by citrate-lyase and ACC. In this model, malonyl-CoA plays a pivotal role, as this "signal of plenty" inhibits fatty acid oxidation through its allosteric interaction with CPT-1. Consequently, FACoAs accumulate in the cytoplasm to exert stimulation of insulin release, either directly on exocytosis (49) or indirectly via complex lipid formation (50,51), protein kinase C activation (52), or protein acylation (53).

This model has been challenged by two reports in which malonyl-CoA decarboxylase (MCD) has been overexpressed in INS cells, even though GSIS was unaltered (54,55). In our opinion, these study results were not conclusive for the following reasons. In the first study (54), very late passages of INS cells without a K_{ATP} -dependent pathway were used. Furthermore, the peroxysomal presequence of MCD was not removed from the MCD adenoviral construct. In the second study (55), overexpressing MCD in the cytosol of INS 832/13 cells did not alter lipid esterification processes nor malonyl-CoA levels at low glucose, indicating that the construct was relatively inefficient in altering malonyl-CoA and lipid signaling. We have recently overexpressed MCD in the cytosol of rat islets and stable clones of highly glucose-responsive INS cells and observed a profound reduction in malonyl-CoA levels, both at low and high glucose together with a 50% reduction of GSIS (R.R., M.P., unpublished observations). Additional molecular biology evidence for a role of malonyl-CoA and lipid signaling has been obtained by two recent reports (56,57). Thus overexpression of L-CPT-1 in INS-1E cells caused a 50% reduction of GSIS, an action that was overcome by exogenous FFA or the addition of the fat oxidation inhibitor etomoxir (56). Islets from hormone-sensitive lipase-deficient mice do not release insulin in response to glucose either in vivo or in vitro (57), and pharmacological inhibition of islet lipase with 3,5-dimethylpyrazole curtails GSIS (58). Genetic evidence in favor of the model depicted in Fig. 3 has recently been adduced by a report documenting a patient with hyperinsulinism and a mutation in the fat oxidation gene L-3-hydroxyacyl-CoA dehydrogenase (59).

The model shown in Fig. 3 does not oppose, but rather complements, other candidate mechanisms of metabolic signal transduction, including the cataplerotic output of

glutamate (60) and hydrogen shuttles (45), as well as pyruvate/malate (61) and pyruvate/citrate (48,62) cycling.

A ROLE FOR THE ACC/MALONYL-CoA/CPT-1 METABOLIC SIGNALING NETWORK IN THE CONTROL OF APOPTOSIS?

It is worth noting a report that has identified cell death-induced genes in a hematopoietic cell line LyD9 (63). Using a subtractive hybridization approach, those authors isolated a cDNA encoding CPT-1. Further work indicated that the addition of palmitate to cultured cells gives rise to de novo synthesis of ceramide followed by apoptosis. Inhibiting CPT-1 with etomoxir enhanced palmitate-induced apoptosis in association with more ceramide synthesis (63). With the yeast two hybrid system and a co-immunoprecipitation technique, it was shown that CPT-1 interacts with the anti-apoptotic protein Bcl-2 (64). Another study carried out in Hep2 cells provided further support for the emerging concept that CPT-1 plays a role in the regulation of apoptosis. Thus inhibiting CPT-1 in this system increased palmitate-induced apoptosis (65). We have observed that fatty acids cause a prominent induction of the CPT-1 gene in β (INS) cells (66) and decrease the expression level of ACC (67), an effect associated with reduced malonyl-CoA levels at low glucose and enhanced fat oxidation as well as NAD(P)H fluorescence and O_2 consumption (20). These changes may be part of a defense process against lipoapoptosis, as enhanced flux through CPT-1 is expected to redirect FACoAs from esterification and the ceramide pathway to their mitochondrial oxidation. As will be explained below, this lipid detoxification process is curtailed at elevated glucose because of the high level of glucose-derived malonyl-CoA. Further support for the idea that malonyl-CoA controls apoptosis was provided by a recent report (68) showing that inhibitors of fatty acid synthase (FAS) that cause a rise in malonyl-CoA induce apoptosis of human breast cancer cells. The apoptotic process was overcome by ACC inhibitors that caused a lowering of malonyl-CoA (68).

This series of observations suggest the working hypothesis that glucose-derived malonyl-CoA and FFA-derived FACoA act as synergistic pro-apoptotic signals linking glucolipotoxicity to β -cell death. It may appear at first sight paradoxical that malonyl-CoA and FACoA act as coupling factors in GSIS (45), whereas under other circumstances, they are implicated in β -cell death (6). However, there are numerous examples of signals that are implicated in both physiological transduction systems and the control of cell death. For example, the Ca^{2+} ion that plays a key role in many biological processes and GSIS is also an apoptotic signal in various cell types when its intracellular concentration is constantly elevated (69). In β -cells, a sustained Ca^{2+} influx, caused by chronic exposure to elevated glucose, is thought to contribute to glucoapoptosis (40). Thus we believe that the two transduction arms of glucose signaling shown in Fig. 3 are not only implicated in the control of insulin secretion in health, but are also involved in β -cell apoptosis when both glucose and lipid stimulation of the β -cell are excessive and chronic.

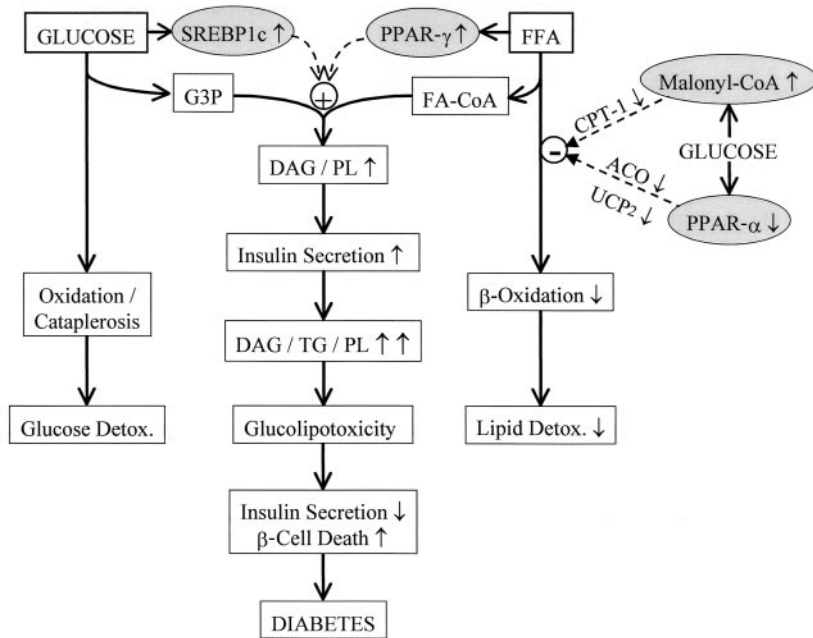


FIG. 4. Possible mechanism of β -cell glucolipotoxicity implicating malonyl-CoA, PPAR- α , PPAR- γ , SREBP-1c, and altered lipid partitioning. ACO, acyl-CoA oxidase; DAG, diacylglycerol; detox, detoxification; G3P, glycerol 3-phosphate; PL, phospholipid; UCP2, uncoupling protein 2.

GLUCOSE MARKEDLY INFLUENCES β -CELL LIPID METABOLISM: IS GLUCOTOXICITY IN PART LIPOTOXICITY?

It has long been known that glucose acutely reduces fat oxidation and promotes lipid esterification processes in β -cells (70). Glucose also rapidly increases the concentration of the candidate metabolic coupling factor malonyl-CoA (48,50). This key signal of glucose abundance regulates lipid partitioning by virtue of its inhibitory action on CPT-1, which catalyzes the limiting step of mitochondrial β -oxidation. Glucose also rapidly increases the mitochondrial and cytosolic concentrations of citrate (48), the carbon precursor of malonyl-CoA, and activates ACC (71), the limiting enzyme of fat biogenesis that forms malonyl-CoA. Finally, glucose acutely increases the formation of the C-kinase activator diacylglycerol (50,72,73) and the synthesis of various phospholipids, in particular phosphatidic acid (51,70) and phosphatidylinositol 4,5 bisphosphate (74).

The long-term actions of glucose on β -cell lipid metabolism are also prominent. Chronic elevated glucose markedly enhances β -cell ACC and FAS gene expression, both in vivo and in vitro (75-77). The sugar also causes a sustained enhancement of citrate and malonyl-CoA formation, an almost total inhibition of fat oxidation, and a pronounced increase in phospholipid and TG deposition in INS-1 cells (76). In addition, we recently reported that chronic exposure of rat islets to elevated glucose decreases the expression level of the FFA nuclear receptor PPAR- α , which controls numerous genes of fatty acid metabolism (78). The glucose-induced decrease of PPAR- α in islet tissue and INS cells was associated with a reduction in the expression of two major genes implicated in fat oxidation and lipid detoxification. These genes, containing PPAR-response elements, are fatty acyl-CoA oxidase (ACO) and UCP2, which catalyze the limiting steps of the peroxysomal β -oxidation and uncoupled mitochondrial fat oxidation pathways, respectively (78). Consistent with these results, Laybutt et al. (77) recently reported that

hyperglycemia in the 90% partial pancreatectomy rat model is associated with an induction of the ACC and FAS transcripts in islet tissue and a reduction in PPAR- α and ACO mRNA expression levels. Finally, the transcription factor SREBP-1c, whose induction is associated with a coordinate induction of lipogenic genes in various cell types, is induced by elevated glucose in cultured human islets (M.P., E.J, W.E.A, and R.R., unpublished observations) and is overexpressed in islets from hyperglycemic ZDF *fa/fa* rats (79).

Thus the available data underscore the finding that both short- and long-term exposure of β -cells to elevated glucose levels dramatically influences lipid metabolism and partitioning to cause enhanced conversion of glucose carbons into lipids, FFA esterification processes, and fat deposition. The implications of this β -cell feature for our understanding of the mechanism of β -cell glucotoxicity are far reaching, as they imply that glucotoxicity has much in common with lipotoxicity (6,25,80).

POSSIBLE MECHANISM OF β -CELL GLUCOLIPOTOXICITY: A ROLE FOR MALONYL-CoA, PPAR- α , PPAR- γ , SREBP-1c, AND ALTERED LIPID PARTITIONING

As a working model of β -cell glucolipotoxicity that implicates malonyl-CoA and key transcription factors of lipid metabolism, we propose the following (Fig. 4). Elevated glucose alone, at concentrations slightly above the normal range (<10 mmol/l), is not very toxic to islet tissue. The β -cell adapts via changes in gene expression, in particular to the induction of glycolytic and anaplerotic genes (76,81), allowing constantly elevated glucose oxidation and anaplerosis/cataplerosis (enhanced synthesis of Krebs cycle intermediates and their efflux from the cycle) for coupling factors production. This results in a leftward shift in the dosage dependence of GSIS and glucose detoxification because of the transformation of glucose into CO₂ and various metabolites, proteins, and lipids as well as the

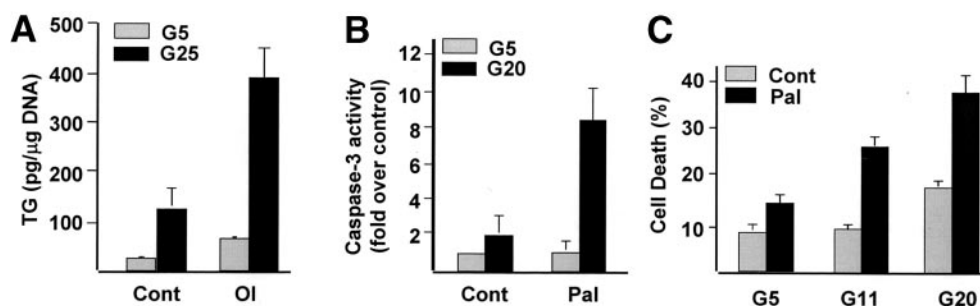


FIG. 5. Elevated glucose and fatty acids synergize in causing triglyceride deposition, caspase 3 activation, and β -cell death. **A:** INS(832/13) cells were incubated for 2 days at low or high glucose in the absence (Cont) or presence (OI) of 0.4 mmol/l oleate bound to BSA (0.5%). G5, 5 mmol/l glucose; G25, 25 mmol/l glucose; OI, 0.4 mmol/l oleate. **B:** INS cells were incubated for 24 h at 5 (G5) or 20 mmol/l (G20) glucose in the absence (Cont) or presence (Pal) of palmitate 0.4 mmol/l bound to BSA (0.5%). Caspase 3 was then measured in cell extracts. **C:** Same conditions as in B, except that cell death was assessed using the trypan blue method after a 48-h incubation time period. G5, 5 mmol/l glucose; G11, 11 mmol/l glucose; G20, 20 mmol/l glucose.

cataplerotic efflux of citrate from the cell (48). Elevated fatty acids alone, at reasonably elevated levels, also are nontoxic because at low glucose and in the absence of increased malonyl-CoA levels, FFAs will be oxidized in the β -cell, resulting in lipid detoxification. We would like to propose that the problem arises for the β -cell when, in the face of rising insulin resistance, there are long episodes of postprandial or sustained hyperglycemia together with elevated circulating levels of FFAs and TG-rich lipoproteins. This will markedly favor lipid esterification processes and fat deposition in islet tissue via the condensation of glucose-derived glycerol-3-phosphate with FFA-CoA. This can result initially in a further increase in insulinemia (β -cell compensation) through lipid-signaling molecules, such as some phospholipids or diacylglycerol, and FFA-CoA, which activates C-kinase enzymes (74). However, the “evil” action of FFAs would subsequently result from the progressive accumulation in the β -cell of FFA-CoAs and various lipid-signaling molecules (phosphatidic acid, lysophosphatidic acid, sphingolipids, ceramides, cyclo- and lipo-oxygenase products, and others). These will cause impaired glucose-induced insulin secretion and biosynthesis and promote apoptotic cell death. The accumulation of TGs may at least initially be just a defense mechanism, as TGs are likely to be inert in lipid droplets. However, massive accumulations may cause β -cell steatosis.

In this metabolic/nutritional hypothesis of β -cell failure implicating lipid partitioning, alterations in malonyl-CoA, PPAR- α , PPAR- γ , and SREBP-1c levels would play an instrumental role. Thus, at high glucose levels, malonyl-CoA will accumulate in the cytosol to curtail mitochondrial β -oxidation of FFAs through its interaction with CPT-1. The two other key pathways of lipid detoxification implicating UCP2 and peroxisomal β -oxidation will also be reduced because of the downregulation of PPAR- α by glucose (78). Consequently, the β -cell cannot escape excess fuel toxicity because all the different pathways of lipid oxidation (i.e., the one implicating β -oxidation in the mitochondrion via CPT-1, uncoupled mitochondrial fat oxidation via UCP2, and peroxysomal fat oxidation via ACO) will be curtailed. In addition, the lipogenic pathway and esterification processes will be favored because of glucose-induction of the SREBP-1c gene and fatty acid activation of PPAR- γ . Therefore, the only fate of FFAs will be esterification, causing progressive lipid accumulation

and β -cell failure. Consistent with this view, malonyl-CoA levels are markedly elevated and PPAR- α is decreased in INS cells and rat islets incubated for 3 days in the presence of both high glucose and oleate (78). In addition, elevated glucose and FFAs synergize in causing TG accumulation in β (INS) cells (Fig. 5A) and rat islets (82), as well as in activating the effector caspase 3 (Fig. 5B) and their apoptosis (Fig. 5C). Studies performed by Poitout and colleagues (83) support this view, as it was observed that a 72-h culture of rat islets in the presence of palmitate does not affect insulin mRNA and content at low glucose, but only at elevated concentrations of the sugar.

This lipid partitioning hypothesis in the etiology of β -cell failure in type 2 diabetes should not be seen as opposed to but as complementary to the oxidative stress hypothesis, which proposes that reactive oxygen species are implicated in both β -cell glucotoxicity and lipotoxicity (84). In this respect, it should be underscored that palmitate increases the production of reactive oxygen species in rat islets (85). Both hypotheses should be further tested in various animal models of diabetes as well as in human islets. The results of such studies may be revealing in understanding the biochemical basis of β -cell adaptation and failure in the etiology of adipogenic diabetes, and also may be relevant to type 1 diabetes and islet transplantation. Finally, it would be of interest to test the glucolipotoxicity concept and the possible role of malonyl-CoA and altered lipid partitioning in this process in other tissues in the contexts of insulin resistance and diabetes complications.

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