Nutrient-Induced Metabolic Stress, Adaptation, Detoxification, and Toxicity in the Pancreatic β-Cell

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Paraphrasing the Swiss physician and father of toxicology Paracelsus (1493–1541) on chemical agents used as therapeutics, "the dose makes the poison," it is now realized that this aptly applies to the calorigenic nutrients. The case here is the pancreatic islet β-cell presented with excessive levels of nutrients such as glucose, lipids, and amino acids. The short-term effects these nutrients exert on the β-cell are enhanced insulin biosynthesis and secretion and changes in glucose sensitivity. However, chronic fuel surfeit triggers additional compensatory and adaptive mechanisms by β-cells to cope with the increased insulin demand or to protect itself. When these mechanisms fail, toxicity due to the nutrient surplus ensues, leading to β-cell dysfunction, dedifferentiation, and apoptosis. The terms glucotoxicity, lipotoxicity, and glucolipotoxicity have been widely used, but there is some confusion as to what they mean precisely and which is most appropriate for a given situation. Here we address the gluco-, lipo-, and glucolipo-toxicities in β-cells by assessing the evidence both for and against each of them. We also discuss potential mechanisms and defend the view that many of the identified "toxic" effects of nutrient excess, which may also include amino acids, are in fact beneficial adaptive processes. In addition, candidate fuel-excess detoxification pathways are evaluated. Finally, we propose that a more general term should be used for the in vivo situation of overweight-associated type 2 diabetes reflecting both the adaptive and toxic processes to mixed calorigenic nutrients excess: "nutrient-induced metabolic stress" or, in brief, "nutri-stress."

Excessive nutritional intake triggers chronically elevated insulin secretion and insulin resistance, which contribute to type 2 diabetes (T2D). The elevated insulin secretion, whether upstream of insulin resistance or a compensatory response to it, eventually declines, and β-cell failure ensues resulting in T2D due to altered glucose sensing, depletion of insulin stores, and dedifferentiation, with β-cell death likely playing a role in the progression of the disease (1–6).

What are the factors and nutrients causally implicated in β-cell dysfunction? It has long been known through the work of G. Weir, J. Leahy, R.H. Unger, and others that high glucose concentration per se can exert "toxic" effects on β-cells by influencing their phenotype independently of elevated free fatty acids (FFA) or hyperlipidemia, a phenomenon named "glucotoxicity" (7). Also, chronic markedly elevated FFA can cause β-cell dysfunction at normal glucose levels in a process termed "lipotoxicity" (8,9). We proposed in 1996 that elevated glucose and lipids synergize in causing β-cell dysfunction and death as well as multiple tissue defects and coined the term "glucolipoxia" (10) that was subsequently renamed "glucolipotoxicity" (11). Considering that circulating FFA as well as triglycerides (TG) within lipoprotein particles are present in the pericellular milieu, and that FFA (including from TG hydrolysis by lipoprotein lipase) as well as VLDL and LDL via endocytosis can be taken up by β-cells (12) and aggravate glucotoxicity (13), we believe that it is not optimal terminology to attribute the in vivo toxic effects of nutrient excess to β-cells exclusively to glucose (glucotoxicity). It is also known that glucose stimulates lipolysis of intracellular glycerolipids in β-cells, leading to FFA production in situ (1,14). Observations that increased ectopic pancreatic fat in rodents and humans is associated with β-cell dysfunction and that the reversal of pancreatic TG accumulation correlates with the restoration of β-cell

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function and the reversal of T2D support the idea that excess in both blood lipids and glucose contributes to β-cell dysfunction in T2D (15). However, the terms glucotoxicity, lipotoxicity, and glucolipotoxicity are often used interchangeably, and there is some confusion as to what they mean precisely and which term is most appropriate for a given situation. In addition, doubts have been raised with regard to the in vivo significance of β-cell glucolipotoxicity (16), particularly in the accompanying review by Gordon C. Weir in this issue of *Diabetes* (17).

In this Perspective, we address controversies regarding the gluco-, lipo-, and glucolipo-toxicities of pancreatic β-cells by assessing the evidence both for and against each of them. In addition, we review potential mechanisms and defend the view that many of the so-called "toxic" effects of nutrient excess observed on the β-cell are in fact beneficial adaptive processes. Also, candidate fuel-excess detoxification pathways are evaluated. Finally, we propose that a more general term should be used for the in vivo situation of overweight-associated T2D reflecting both the adaptive and toxic processes related to the positive energy balance with mixed nutrients excess: "nutrient-induced metabolic stress" or, in brief, "nutri-stress" (Fig. 1).

FACETS OF NUTRIENT EXCESS–INDUCED β-CELL TOXICITY

Glucotoxicity

Concept, Definition, and the Frequent Misuse of the Term

Even slightly but chronically elevated glucose can alter the sensitivity of β-cells in their glucose responsiveness and cause various phenotypic changes. A key issue is to distinguish those actions of elevated glucose like cell death and mitochondrial dysfunction that are truly "toxic" from those that are adaptive processes (e.g., changes in glucose sensitivity or insulin gene expression). To our knowledge the term "glucotoxicity," used over decades, has never been clearly defined, leading to some confusion. Also, depending on the elevated glucose levels and the time of exposure, toxic versus adaptive processes can be differentiated.

Evidence for Glucoadaptation and Glucotoxicity

In Vitro Studies. While the short-term adaptation of the β-cell to an elevated glucose supply is dependent on a rapid increase in insulin biosynthesis, long-term adaptation requires changes in the expression of genes coding for transcription factors such as MafA, PDX-1, and NeuroD1 as well as the insulin gene, enzymes involved in mitochondrial metabolism, cholesterol biosynthesis

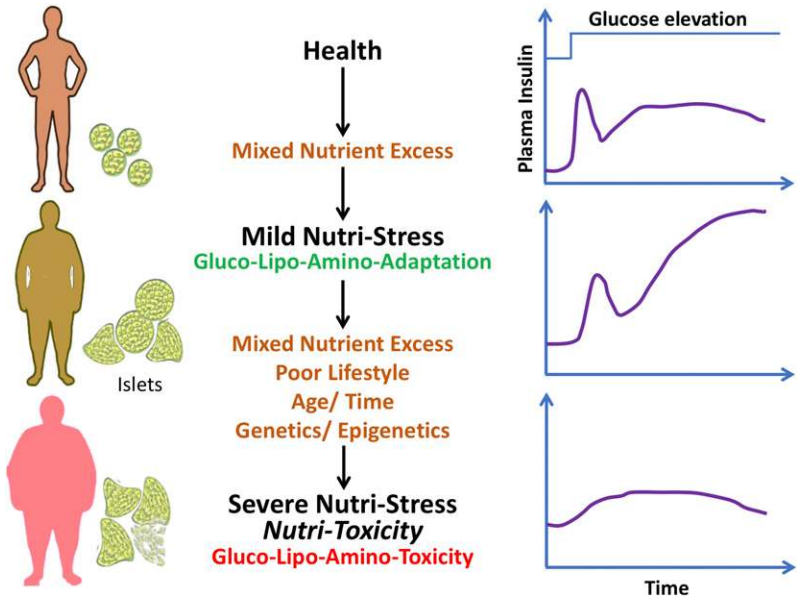

Figure 1— Development of pancreatic β-cell dysfunction due to prolonged nutri-stress. Pancreatic islets in healthy individuals respond to the nutritional cues by secreting insulin in a biphasic manner, with a sustained amplification phase. However, excessive availability of fuel secretagogue nutrients such as glucose, lipids, and amino acids initially causes mild nutri-stress on the β-cells, leading to elevated basal secretion and enhanced amplification second phase, thus resulting in hyperinsulinemia that drives obesity and insulin resistance. During this stage, β-cells undergo hyperplasia resulting in larger islets, and there is a leftward shift in the glucose dose dependence of insulin secretion due to multiple molecular changes. There is no cell death, and β-cells cope with the metabolic stress via gluco-lipo-amino–adaptative processes that include fuel-excess detoxification pathways. Islet architecture begins to alter, and eventually first-phase GSIS is reduced. This mild nutri-stress stage, which often is accompanied by poor lifestyle, with prolonged fuel surfeit, time, and aging, exerts chronic and sustained insult on β-cells that is aggravated by an individual's genetic/epigenetic makeup. Severe nutri-stress ensues due to gluco-lipo-amino–toxicity, which can be named more simply nutri-toxicity, that culminates in β-cell dysfunction and failure with the marked reduction of β-cell secretory response, leading to T2D.

enzymes, and hormone receptors (18). Also, several studies showed that chronic in vitro exposure to mildly elevated glucose levels renders the β-cells hypersensitive to glucose, reducing the threshold for insulin secretion (19–21). These adaptive actions would be better termed glucoadaptation than glucotoxicity.

By contrast, long-term in vitro exposure of β-cell to very high glucose (15–30 mmol/L) can induce deleterious processes, and in that case the term glucotoxicity is appropriate. For example, many studies using β-cell lines or rat or human islets have shown that high glucose can induce β-cell apoptosis and death (7,22). Also, incubation of rodent or human islets at high glucose for long periods of time leads to decreased glucose-stimulated insulin secretion (GSIS), due to β-cell overstimulation causing exhaustion of insulin stores (19).

A very diverse number of mechanisms of glucotoxicity, which can also apply to glucolipotoxicity (Fig. 2), have been proposed, making it difficult to distinguish the key causal players in the process. Some that have received much attention are indicated below.

Endoplasmic Reticulum, Mitochondrial, and Oxidative Stress. Maintenance of β-cell function for insulin biosynthesis requires efficiently functioning unfolded protein response (UPR) machinery. UPR components, including Txnip, Myc, and Chop, were found to be elevated in β-cells following chronic high glucose exposure, indicative of endoplasmic reticulum (ER) stress (19). Suppression of particular UPR components such as Atf6 in β-cells can prevent the high glucose–induced decline in insulin gene expression (7). Due to the poor expression of antioxidant enzymes, β-cells have low capacity to scavenge reactive oxygen species, and this makes them prone to oxidative damage (23). Expression of oxidative stress markers, including heme oxygenase-1, was found elevated in rodent islets and INS1 β-cells chronically exposed to high glucose (7,19). Also, chronically elevated glucose, either with or without FFA present, alters mitochondrial fission and fusion dynamics that can affect energy metabolism and impact on β-cell apoptosis (24).

β-Cell Dedifferentiation and Identity. Elevated glucose can alter the expression of transcription factors and β-cell differentiation markers, including MafA (25), HIF1α, HIF2α, PDX1, NeuroD1, NKX6.1, and Pax6 as well as Glut2 and glucokinase (7,26). In addition, chronic high glucose induces

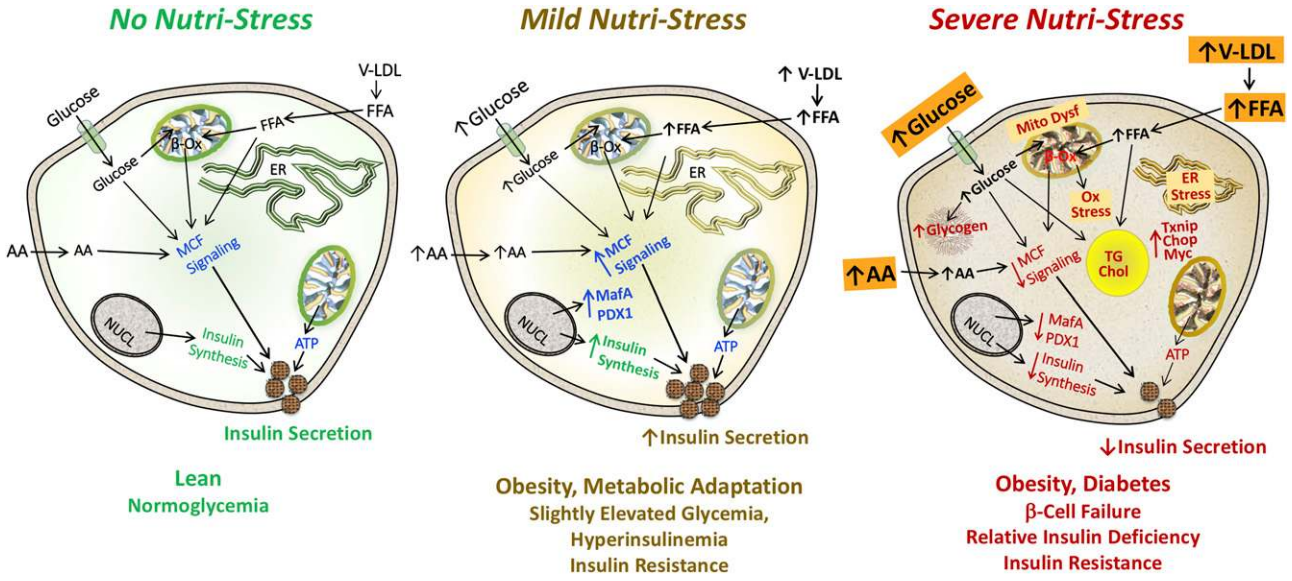

Figure 2— Biochemical basis of β-cell adaptation and failure in relation to increasing nutri-stress. Pancreatic β-cell responds to glucose, FFA, and amino acid (AA) stimulation by producing metabolic coupling factors (MCF) through various pathways in cytosol, ER, and mitochondria, and these MCF play a critical role in optimal insulin secretion to suffice the needs of the body. While glucose and AA are supplied from dietary carbohydrates and proteins, FFA are supplied mostly by the action of lipoprotein lipase on lipoproteins (VLDL), locally, within the islets. VLDL may also be taken up by the β-cells through the corresponding receptors and generate FFA inside the cells. Nuclear transcriptional pathways are activated to produce insulin, in response to glucose, which is packed in the insulin granules and released through exocytosis that is triggered by a rise in the ATP/ADP ratio and additional MCF. Supply of normal levels of the nutrient secretagogues results in insulin secretion that is sufficient to maintain glucose homeostasis. However, a mild nutri-stress, as seen in overweight/obesity conditions, leads to augmented nutrient secretagogue stimulation of the β-cell, resulting in increased MCF production and insulin synthesis and elevated basal and stimulated insulin secretion. Under these conditions, there is a metabolic adaptation of the β-cell to the nutrient-excess environment and slightly elevated glycemia, insulin resistance, and hyperinsulinemia become progressively evident. Obesity with β-cell failure and diabetes will result when there is severe chronic nutri-stress that overwhelms the β-cell metabolic machinery. Under these conditions, there is mitochondrial and ER dysfunction causing oxidative and ER stress, accompanied by reduced MCF production and decreased insulin synthesis and secretion. Severe nutri-stress also lead to the accumulation of TG, cholesterol (Chol), glycogen, and unfolded proteins due to ER dysfunction. In addition, there are lower expression of β-cell markers such as MafA, PDX1, and NeuroD1 and increased levels of stress markers TxniP, Chop, and Myc, with β-cell dysfunction due also to dedifferentiation, and in later stages with diabetes progression, possibly β-cell death. β-Ox, β-oxidation.

the expression of genes normally disallowed in the β-cell such as lactate dehydrogenase, glucose 6-phosphatase, and hexokinase (27). It should be considered that β-cell dedifferentiation in T2D is an adaptive process that allows these cells to cope with the excess glucose and thus escape irreversible damage. Support for this possibility is a study in EndoC-βH1 human pancreatic β-cells, where it was observed that dedifferentiation induced for 3 days through FGF2/ FGFR1c signaling could be reversed by incubating the dedifferentiated cells for 11 days in the absence of FGF2 (28).

Voltage-Dependent Anion Channel 1. The ATP-conducting mitochondrial outer membrane voltage-dependent anion channel-1 (VDAC1) is upregulated in islets from T2D organ donors (29). Exposure of human islet cells to 20 mmol/L glucose for 3 days led to ChREBP-dependent overexpression of VDAC1 and its mistargeting to the plasma cell membrane. It was postulated that the overexpression and altered localization of VDAC1 is another mechanism of β-cell glucotoxicity (29), as VDAC1 allows the movement of ATP and causes its cellular loss when present in the cell membrane.

In Vivo Studies. Results of several studies in which there were correlations between glycemia and reduced insulin secretion have been taken as argument in favor of glucotoxicity as a key player in β-cell dysfunction in T2D.

Glucose Infusion in Animals and Humans. In several animal studies, infusion of glucose after partial pancreatectomy led to β-cell dysfunction and diabetes (30), indicating a toxic effect of high glucose. By contrast, numerous studies showed adaptive effects of the β-cell to hyperglycemia in terms of β-cell proliferation, mass, and glucose sensitivity (5,7,19,31,32). Interestingly, these adaptive effects can be modulated by circulating lipids such that the glucoadaptation process is prevented. Thus, the increase in β-cell mass by glucose infusion in mice was shown to be blunted by simultaneous infusion with FFA (33). Also, it was observed that in obese people without diabetes, insulin sensitivity as well as the disposition index were maintained at a higher level, following prolonged (24 h) infusion of glucose, but both parameters declined if glucose infusion was combined with Intralipid. Thus, the circulating levels of lipids appear not only to influence the toxic actions of elevated glucose but also the β-cell adaptation to hyperglycemia.

Experiments With Phlorizin and Other SGLT2 Inhibitors. Studies showing protection of β-cell function in diabetes, in animal models and patients, by using phlorizin, a nonspecific inhibitor of sodium–glucose cotransporters (SGLTs), and more recently SGLT2 inhibitors to decrease blood glucose levels through promoting glycosuria have provided support for the β-cell glucotoxicity concept (34–36).

Rodent Models and Humans With Partial Pancreatectomy. Employing the 90% pancreatectomy diabetic rat model, it was shown that the residual β-cells lose their ability for GSIS in vivo (30,37,38). This was found to be associated with reduced expression of Glut2 and Pdx-1 and elevated expression of c-Myc in the remaining islets. Because the abnormalities of β-cell function in this model are better related to glucose than FFA (22,39,40), this was taken as support for glucotoxicity being responsible for the noted defects. By contrast, a recent study in patients with partial pancreatectomy showed that higher levels of intrapancreatic fat determine the increased likelihood of insulin dependence and higher postprandial glucose excursion, probably due to reduced β-cell function (41), and this was attributed to detrimental effects of elevated FFA release on β-cells. These results favor the view that glucolipotoxicity is a better term to use in this context than glucotoxicity.

Correlations of Hyperglycemia With β-Cell Dysfunction. As stated by Weir (17), there is a tight correlation between loss of first-phase GSIS and rising glucose levels in humans, and this is most clearly seen as individuals progress from normal glucose tolerance to T2D (42,43). The abnormalities in GSIS are reduced when glucose levels are returned to the normal range, as this has been particularly well documented in gastric bypass surgery patients (44). Do these insulin secretion changes truly reflect a toxic action of excess glucose if they are largely reversible? Bariatric surgery most often results in the reversibility of diabetes itself, and therefore the situations described above likely reflect adaptive changes that should be better named mild nutri-stress, gluco-adaptation, or nutri-adaptation rather than glucotoxicity. However, such reversibility of the excess nutrient and glucose environment is likely dependent on the level and duration of the hyperglycemia and genetic susceptibility of the organism. Thus, prolonged hyperglycemia was shown to induce irreversible β-cell apoptosis and decreased proliferation, leading to diabetes in the gerbil *Psammomys obesus* but not in normal rats (45).

How Relevant Is Glucotoxicity Per Se In Vivo and Is It a Good Term?

Observations of concurrent hyperglycemia and β-cell failure in animal models and in patients without a concomitant rise in plasma FFA led to the proposition that elevated glucose concentration per se is toxic to β-cells and that the term "glucotoxicity" versus others like lipotoxicity or glucolipotoxicity is the best to use (16,17,22). This reasoning suffers from several caveats. First and most important, it does not make the distinction between cause and consequence between hyperglycemia and β-cell dysfunction/toxicity. Clearly, if secretion is impaired, glycemia will rise and both will correlate. Second, lack of close correlation of circulating FFA levels with β-cell dysfunction is not a strong argument either, as the FFA are primarily delivered to cells not via blood FFA but via lipoprotein lipase action on various circulating TG-containing lipoproteins (46). In addition, the role of lipids other than FFA

that can be introduced into β-cells via lipoprotein endocytosis (e.g., LDL) has not been taken into account (12,47). LDL can cause β-cell death by their cellular uptake and oxidative modifications (13). Also, it has never been argued, unlike stated in the accompanying review (17), that the glucolipotoxicity concept is referring only to FFA and not to other lipids and in particular circulating TG. Considering that glucose-mediated effects either on insulin secretion and biosynthesis, β-cell growth and response to various stresses, or β-cell metabolism are markedly modulated by other fuels (e.g., various lipids and amino acids) and other players such as hormones, ligands of certain cell surface receptors, that can change greatly under various circumstances, the term glucotoxicity appears quite restrictive for the in vivo situation of fuel excess, and therefore other terms should be considered.

Lipotoxicity, Glucolipotoxicity, and Gluco-lipo Adaptations

The deranged regulation of lipid homeostasis in T2D leads to elevated production and release of different lipids, including FFA, ceramides, TG, cholesterol, and various bioactive lipids, into plasma and the interstitial space, which then exert adaptive or toxic effects on the cells within the exposed tissues (8,9). In vitro studies revealed that FFA, particularly saturated FFA, in unphysiological high doses, can exert truly toxic effects (lipotoxicity) including induction of apoptosis in isolated islets or β-cells in culture. Thus, it was shown that while palmitic acid (0.5 mmol/L) induced apoptosis through the formation of ceramide in rat islet cells, under the same conditions palmitoleic acid, which is the monounsaturated form of palmitate, had no such effects and even promoted β-cell proliferation at low glucose (5.5 mmol/L) (48). However, also in vitro, FFA only caused adaptive effects such as elevated basal insulin secretion if used at a lower concentration (49–52).

With respect to the in vivo situations, Intralipid infusion studies in rodents and humans revealed overall modest effects in terms of β-cell function, gene expression, and GSIS, with no sign of true toxicity (53). As Intralipid contains predominantly unsaturated fatty acids and these are much less toxic at high levels than saturated fatty acids, this could also be an explanation of its benign effects on β-cells in these experiments (54). In some instances, insulin secretion was found to be reduced only if insulin resistance was taken into account (55–57). Excellent reviews have covered the results obtained in vitro and in vivo in these β-cell lipotoxicity studies and the mechanisms implicated (56–58). However, because the in vivo studies, in which chronic experimentally induced elevations of TG and FFA revealed no apparent toxic effects, lipoadaptation in our opinion would be a better and less misleading term to use to describe them. Thus, we would agree with Weir (17) that, at normal blood glucose levels, β-cell lipotoxicity defined as the toxic action of excess blood lipids, in particular FFA, does not really exist in the in vivo context.

The term "glucolipotoxicity" was first defined to describe the toxic effects of the combination of both hyperglycemia and hyperlipidemia in vivo or elevated glucose and FFA in vitro. Since it was first coined (11), it was meant to describe not only the additive action of glucotoxicity and lipotoxicity, but the synergistic toxic action of the combined presence of elevated glucose and lipids. The initial rationale behind the use of this term is that glucose inhibits fat oxidation and promotes lipogenesis and when intracellular glucose and FFA are concomitantly elevated, various metabolic pathways (e.g., mitochondrial metabolism, cholesterol synthesis, etc.) would become altered in the β-cells (10). Below we consider the evidence supporting the view that glucolipotoxicity contributes to the initiation and progression of fuel excess–associated T2D and is a reasonable term to use in that context.

In Vitro Studies

Multiple in vitro studies provided the initial evidence for the synergistic detrimental effects of elevated glucose and FFA on the function or survival of β-cells using either cell lines (INS, MIN6, or HIT cells) or isolated rodent and human islets. Considering that the in vitro occurrence of glucolipotoxicity in cell cultures is widely accepted, this will not be further discussed and there are excellent reviews covering this (8,9,57,59).

In Vivo Studies

The relevance of glucolipotoxicity in vivo has been questioned due to the unsuccessful attempts to relate plasma levels of FFA to β-cell dysfunction, unlike glucose, even though increased plasma glucose as well as TG and FFA levels are present in obese individuals with insulin resistance (60,61). Gordon C. Weir, in his *Diabetes* Perspective (17), states: "Evidence supporting the importance of glucotoxicity is strong because there is such a tight correlation between defective insulin secretion and rising glucose levels. However, there is virtually no convincing evidence that the alterations in FFA levels occurring during progression to diabetes are pathogenic." One of the main points Weir makes is that the interstitial space concentrations of glucose reflect that present in circulation. However, it is important to realize that there are no reliable methods to assess the FFA levels in the interstitial space in the vicinity of β-cells in islets, and unlike glucose, this does not follow a simple equilibrium between plasma and the pericellular space (57). Thus, absence of direct correlation between circulating FFA/lipids and β-cell dysfunction cannot be construed as lipids do not contribute to T2D pathogenesis. In the context of nutrient excess–associated T2D, in both humans and rodent models, there is defective lipid homeostasis with associated hyperlipidemia, hypercholesterolemia, and often elevated plasma FFA that precedes the onset of T2D. Also, with associated insulin resistance, glucose intolerance and fasting glycemia gradually increase in the prediabetic phase, before they reach levels related to the definition of diabetes. Thus, both elevated circulating glucose and

lipids precede obesity-associated T2D, and it is reasonable to think that both classes of nutrients in excess are implicated in the pathogenesis of T2D.

Rodent Studies. Several rodent studies favor the glucolipotoxicity concept.

a) In Zucker diabetic fatty (ZDF) rats, circulating FFA rise and reach 1.9 mmol/L prior to the onset of diabetes and loss of GSIS, and this β-cell dysfunction can be prevented by pair-feeding 6-week-old prediabetic ZDF rats with lean littermates until the age of 12 weeks, which inhibits the FFA increase (58,62).
b) Inhibition of ceramide synthesis pharmacologically in glucose-intolerant ZDF rats and in dihydroceramide desaturase 1 knockout mice prevented β-cell apoptosis and dysfunction and diabetes (63–66).
c) Effects of lipids plus glucose infusion in vivo on β-cell function have been inconsistent, possibly due to differences in rodent strains, age, infusion rates, or type of lipid infused. Infusion of mice with the fat emulsion liposyn II increased blood levels of FFA and prevented glucose-induced β-cell proliferation, without inducing β-cell death (33). By contrast, in rats, glucose infusion modestly stimulated β-cell proliferation and oleate-enriched lipid emulsion ClinOleic alone had no effect, but glucose plus ClinOleic infusion markedly stimulated β-cell proliferation (67). This can be considered a manifestation of a glucolipoadaptive process.
d) We studied the biochemical basis of β-cell dysfunction in obesity-associated T2D rodent models with altered glucose tolerance in association with hyperlipidemia and/or hypercholesterolemia (high-fat diet–fed mice, ZDF rats, and partially pancreatectomized Zucker fatty [ZF] rats) (68–73). It was found that reduced GSIS in vivo and ex vivo is associated with an altered glycerolipid/fatty acid cycle (lipogenesis and lipolysis) with a lack of an enhancement of this futile and cellular signaling cycle, as found in compensating obese nondiabetic ZF rats (1,69,71). In all these models, there was no evidence of apoptosis or reduced β-cell mass indicating that β-cell dysfunction, not cell death, contributes to the initiation of diabetes.
e) Poitout and colleagues (70) provided direct evidence of glucolipotoxicity in vivo, but interestingly it was found to be age dependent. Thus, alternate infusion of glucose and Intralipid (4 h cycle, each), for 72 h, resulted in no apparent changes in 8-week-old rats. By contrast, 6-month-old rats became insulin resistant and showed reduced insulin secretion in vivo and ex vivo, despite an increase in β-cell mass. This was associated with reduced second-phase GSIS, lowered islet insulin content, and β-cell dedifferentiation.
f) Expression of stearoyl-CoA desaturase (SCD) isozymes and also Elovl6 (very long-chain fatty acid elongase 6) is higher in prediabetic ZDF rat islets and declines markedly at the onset of diabetes, supporting the view that monounsaturated fatty acid synthesis protects β-cells from elevated saturated FFA-induced ER stress during prediabetes (74). Similarly, deletion of SCD1 in obese glucose-intolerant *leptin*$^{ob/ob}$ mice (75) or Elovl6 in *db/db* mice (76) accelerated the progression to severe diabetes.
g) Semenkovich and colleagues (46) demonstrated that lipoprotein lipase delivers FFA via TG hydrolysis in the vicinity of the β-cell surface, within a specific concentration range that is optimal for GSIS, and that either increased or decreased activity of lipoprotein lipase in β-cells by genetic manipulations leads to disturbed glucose homeostasis and β-cell dysfunction, without concomitant changes in plasma levels of FFA, cholesterol, and TG. This study shows the significance of FFA in the vicinity of the β-cell in controlling β-cell function and favors the view that plasma FFA level is not the best component to be considered to evaluate the "lipo" aspect of the glucolipotoxicity concept.

Human Studies. The significance of β-cell glucolipotoxicity in humans is a matter of debate, but the balance is in favor of both glucose and lipids playing a role in β-cell dysfunction in T2D.

A 3-year follow-up study (77) in individuals without diabetes and in subjects with impaired glucose tolerance observed an association of circulating FFA with insulin resistance but not with β-cell dysfunction in terms of GSIS, in vivo. By contrast, a recent Canadian 6-year follow-up study indicated a strong negative correlation between plasma total FFA levels and β-cell function (78). Similarly, another study revealed that elevated plasma FFA in both adults and children strongly associated with reduced β-cell function, in particular more with insulin secretory capacity than insulin sensitivity (79). In addition, another two studies indicated a strong positive correlation between elevated blood FFA or TG and β-cell dysfunction and T2D (80–82).

Studies of lipid infusion for 48 h in normal individuals without diabetes revealed loss of the acute incretin effect of FFA to promote insulin secretion (55). Also, the compensatory increase in insulin secretion observed in obese individuals at risk for T2D was found not to occur if lipids were infused together with glucose (83–85). A prolonged increase of plasma FFA abolished the stimulatory effect of moderate hyperglycemia on insulin sensitivity and β-cell function in obese humans, indicating that impaired β-cell function caused by FFA also applies to the hyperglycemic state (85). Thus, a series of studies by Carpentier, Giacca, Lewis, and colleagues (55–57,83–85) support a role for glucolipotoxicity in human β-cell failure.

In a positron emission tomography and magnetic resonance imaging study, it was noticed that unlike glucose uptake, pancreatic FFA uptake was on average 68% greater in obese as well as T2D subjects than in healthy participants, and this was associated with β-cell dysfunction as well as five-times higher pancreatic fat content (86).

Collectively, the above evidence strongly implicates the significance of both excess lipids and glucose in

contributing to β-cell dysfunction and failure in fuel surfeit–associated T2D in both rodents and humans.

Amino Acids and Nutri-stress

Nutri-stress in β-cells may arise not only from glucose and lipids but also from amino acids, in particular branched-chain amino acids (BCAA), including leucine, isoleucine, and valine. It was reported half a century ago by Felig, Marliss, and Cahill (87) that the BCAA valine, leucine, and isoleucine, as well as tyrosine and phenylalanine, were increased, and glycine decreased, in obese hyperinsulinemic subjects compared with matched control subjects. The concentration of each of the amino acids elevated in obesity correlated directly with serum insulin. Subsequent studies have identified a correlation between elevated plasma BCAA levels and also aromatic amino acids (tyrosine and tryptophan) and obesity, insulin resistance, and susceptibility to T2D, probably through sustained activation of mTOR and also deranged mitochondrial function (88–90). Also, the results from the Framingham Offspring Study indicated a significant predictive association between the plasma amino acid levels, specifically BCAAs and aromatic amino acids, and future diagnosis of diabetes (91) as well as cardiovascular diseases (92,93). Overall, the evidence suggests that elevated BCAA levels may cause changes in β-cell function and insulin sensitivity leading to diabetes, but the mechanism is not known. Because we now know that initial hyperinsulinemia can drive obesity, insulin resistance, and T2D (6), it can be hypothesized that elevated BCAA levels in obesity contribute to signal the β-cell to secrete more insulin. Whether it is a compensatory mechanism for insulin resistance or it can drive insulin resistance is uncertain.

Glutamine via its metabolism promotes insulin secretion and through IGF2/IGF1 receptor signaling has positive effects on β-cell proliferation (94). Plasma glycine levels positively associate with GSIS and glucose homeostasis via central and peripheral mechanisms (95). A recent study (96) showed that enhancing plasma amino acids by a high-protein nutrient preload leads to improved β-cell function and GLP-1 secretion, suggesting possible beneficial effects of amino acids in T2D patients and in some rodent models of diabetes. Thus, available studies indicate overall positive effects of elevated amino acids in obesity on β-cell function in terms of enhancing insulin secretion. This could be part of an adaptive process related to mild nutri-stress (elevated glucose, lipids, and amino acids) but could also contribute to β-cell exhaustion. However, it remains to be examined whether chronically elevated levels of various amino acids under some situations synergize with hyperglycemia or hyperlipidemia to negatively impact various β-cell functions.

IN SEARCH OF FUEL-EXCESS DETOXIFICATION PATHWAYS

The actions of excess glucose and lipids to cause β-cell dysfunction and toxicity have been widely studied. However, little is known about the mechanisms used by the β-cell to protect itself against fuel-excess toxicity. Many cells are endowed with defense mechanisms against nutrient-induced toxicity. For example, in muscle tissues chronically elevated fuel supply induces insulin resistance, thus limiting glucose entry (97), a likely beneficial process for the prevention of tissue dysfunction from fuel overload (6,98). Storage of excess glucose or FFA as more "inert" as glycogen and TG in liver and TG in adipose tissues are well-known mechanisms to cope, at least transiently, with fuel excess (99). β-Cells, however, have to constantly sense glucose levels in the blood and rapidly equilibrate glucose across the plasma membrane to release insulin. They cannot protect themselves by reducing glucose entry, and they have limited capacity to store excess fuels (2,3,57). However, they can divert excess glucose and FFA carbons toward some metabolic pathways that allow to continuously maintain glucose metabolism for insulin secretion and to protect from nutrient excess (Fig. 3). What are these fuel-excess detoxification processes that may be termed glucodetoxification, lipodetoxification, or glucolipodetoxification pathways?

The Glycerolipid/FFA Cycle

This pathway is not only involved in fuel signaling for insulin secretion (1,2,14) but may also provide a means for fuel-excess detoxification because it is an ATP-consuming futile cycle (1,2,14). Of relevance, we estimated that glucose carbon flux through the glycerol backbone of glycerolipids accounts for 25% of the total islet glucose utilization in ZF rat islets (71). This cycle was found to be elevated in compensating ZF islets but altered in various rodent models of T2D (69). Thus, this pathway can divert significant quantities of glucose entering the cell away from mitochondrial oxidation to prevent reactive oxygen species production or to limit the buildup of potentially harmful lipids such as lysophosphatidate. Thus, this cycle is likely implicated in glucolipodetoxification.

Glycerol Release

Release of this glucose-derived metabolite from the β-cell is another means of "glucodetoxification." Thus, rodent islets and tumoral β-cells surprisingly release very large amount of glycerol, amounting to about 25% of glucose carbon equivalents, entering glycolysis at elevated glucose (100). We found that at elevated glucose a newly identified metabolic enzyme, a glycerol-3-phosphate phosphatase (gene name *pgp*), directly hydrolyses glycerol-3-phosphate (Gro-3-P) to release glycerol. Interestingly, knockdown of this enzyme enhances glucotoxicity and glucolipotoxicity in vitro, whereas its overexpression reduced the toxic actions of glucose and excess FFA in terms of apoptosis.

Fatty Acid Oxidation

Enhanced β-oxidation occurs in rodent models of obesity and T2D and inhibitors of carnitine palmitoyltransferase 1 (CPT1) that catalyzes the rate-limiting step of this pathway enhancing β-cell apoptotic glucolipotoxicity in vitro (51). In β-cells PPARα signaling is important for maintaining fatty

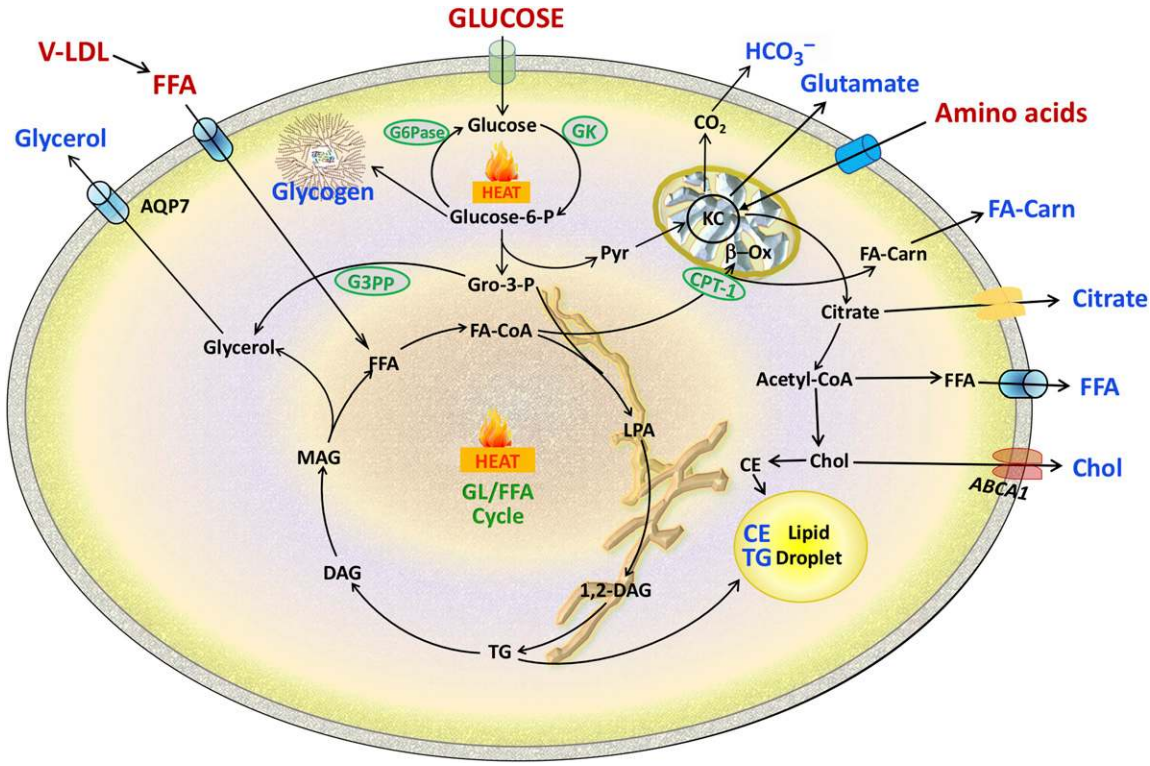

Figure 3—Nutrient detoxification pathways in the β-cell. In order to cope with toxic levels of fuel surplus, β-cells employ multiple detoxification pathways. Glucose entering the β-cell is converted by glucokinase (GK) to glucose 6-phosphate (glucose-6-P), which enters the glycolytic pathway. However, when excess glucose becomes available, some of the glucose-6-phosphate is hydrolyzed by glucose-6-phosphatase (G6Pase), resulting in a futile cycle of net ATP hydrolysis and heat generation. In addition, some of the glucose-6-P also is sequestered as glycogen, which is a relatively inert form of stored energy, at least when the accumulation is reasonable. Glycolysis-derived pyruvate through its participation in Krebs cycle (KC) generates citrate, a significant portion of which enters into cytosol (cataplerosis). Citrate, when produced in excess, may leave the β-cell or is converted to acetyl-CoA, which is the precursor for fatty acids (FFA) and cholesterol (Chol). Cholesterol, being toxic to the cell, is also transported out of the cell via ABCA1 transporter or is converted to cholesterol esters (CE) and stored away in lipid droplets. Part of the glucose carbons are also oxidized to CO_2 in KC and leave the cell as HCO_3^-. The Krebs cycle also generates α-ketoglutarate, which is transaminated to glutamate that can exit from β-cells. FFA entering the cells after conversion to fatty acyl-CoA participate in the GL/FFA cycle and through the action of lipogenic enzymes generate TG, which is sequestered in lipid droplets. Sequential hydrolysis of TG gives rise to FFA, which either leave the cell or recycle into the futile GL/FFA cycle, and glycerol, which can leave the cell through aquaporin-7 (AQP7). Gro-3-P, formed during glycolysis, and fatty acyl-CoA are the starting substrates for GL/FFA cycle, which, when fully operational, results in a net hydrolysis of 7 ATP molecules per turn and heat production and leads to the elimination of glucose carbons as glycerol and sequestration of TG into lipid droplets. A significant proportion of Gro-3-P, when produced in elevated levels at high glucose concentrations, is directly hydrolyzed by Gro-3-P phosphatase (G3PP), producing glycerol, which leaves the β-cell and thus helps in detoxifying excess glucose. When the β-oxidation pathway in mitochondria is flooded with excess availability of FFA, not only is there enhanced CO_2 production and HCO_3^- efflux, but fatty acylcarnitines produced by CPT1 can also leave the cell, thereby detoxifying excess FFA. ABCA1, ATP-binding cassette transporter-A1; β-Ox, β-oxidation; DAG, diacylglycerol; FACarn, fatty acylcarnitine; LPA, lysophosphatidic acid; MAG, monoacylglycerol; Pyr, pyruvate.

acid oxidation and high glucose exposure reduces PPARα expression, leading to disturbed lipid metabolism in β-cells (101). Enforced expression of PPARα in β-cells retards lipotoxicity due to chronic FFA exposure (102). By contrast, pharmacological inhibition of CPT1 enhances β-cell glucolipotoxicity in vitro (51). Thus, PPARα, CPT1, and β-oxidation appear to be involved in β-cell lipodetoxification.

FFA Release

It is surprising that at high glucose, β-cells not only release large amounts of glycerol but also FFA, in particular in their saturated form (~10% of glucose carbons equivalent entering glycolysis) (103). What is the significance of FFA release? It could be implicated in an autocrine/paracrine lipid amplification pathway via FFA binding to FFAR1 but also in glucolipodetoxification, particularly because FFA release is glucose-dependent and does not show saturation at maximal levels of glucose for insulin secretion (103).

Formation of TG, Cholesterol, Cholesterol Esters, and Glycogen

Our recent work in rat islets documented that the fate of approximately 15%, 10%, and 5% of glucose carbons entering glycolysis at elevated glucose is conversion to TG, cholesterol esters, and glycogen, respectively (103). In other cell types, TG and cholesterol esters are known as a relatively inert form of excess lipid deposition, and similarly glycogen is a relatively nontoxic form of glucose storage. This does not discount the

possibility that true TG steatosis and marked deposition of glycogen in β-cell in poorly controlled T2D could contribute to β-cell dysfunction and death (58,104). Cholesterol efflux also appears to be a component of the fuel-excess detoxification pathways. Thus, loss of the ABCA1 and ABCG1 cholesterol transporters in β-cells results in the accumulation of islet cholesterol, inflammation, and impaired β-cell function (105).

Futile Cycles and Mitochondrial Uncoupling

A futile glucose cycle (glucose phosphorylation/glucose-6-phosphate dephosphorylation) has been well documented by Efendić and colleagues (107) in islets and has been found to be elevated in various animal models of diabetes (106). A polymorphism within the glucose-6-phosphatase catalytic subunit 2 (G6PC2) gene is associated with fasting plasma glucose levels. G6PC2 is an islet-specific enzyme that can form a futile cycle with glucokinase to modulate GSIS.

FFA rapidly induce the uncoupling protein 2 (UCP2) gene in β-cells (108), and this could cause some uncoupling contributing to fuel-excess detoxification. However, the role of UCP2 as an uncoupling protein per se is debated (109). Thus, identification of additional futile cycles and uncoupling pathways in the β-cell is a promising avenue of research with potential therapeutic implications.

Release of Citrate and Various Krebs Cycle Intermediates

Citrate release from INS-1 cell was found to account for 20% of glucose carbon entering glycolysis at elevated glucose (110). Various tissues such as the heart (111) release a significant amount of various citric acid cycle intermediates, the significance of which is uncertain.

The study of fuel-excess detoxification processes is emerging. Additional work, particularly in vivo studies, is required to directly test the significance and relative importance of these and other pathways to protect the β-cell from fuel-surfeit toxicity.

IS β-CELL DYSFUNCTION IN EARLY DIABETES BENEFICIAL AND THE PRICE TO PAY FOR ITS SURVIVAL?

Hundreds of studies, including ours, in search of the etiology of β-cell failure in diabetes and novel drug targets, have documented many changes in the β-cell phenotype in various in vitro studies, in models of T2D, and in human islets. In the vast majority of these studies, such changes were interpreted as defects that could possibly explain β-cell dysfunction, reduced GSIS and insulin biosynthesis, dedifferentiation, loss of identity, or impaired proliferation and apoptosis. However, we would like to suggest that in most cases these were overall beneficial adaptive changes. Indeed, it has been shown that overweight-associated T2D (<10 years) in humans is reversible following major weight reduction due to a hypocaloric diet, possibly via reduction of intrapancreatic TG content and reversible dedifferentiation of pancreatic β-cells (112).

A given cell, depending on its environment, is programmed for particular functions like secretion, proliferation, and detoxification that are often largely incompatible. Thus, if proliferation is favored, then secretion and detoxification processes will be less efficient. For example, we found that in several models of obese T2D, β-cell fatty acid oxidation is enhanced in association with reduced GSIS (1,69,71). However, enhanced β-oxidation allows lipodetoxification but at the same time is associated with reduced GSIS because lipid signaling amplification in-volving the glycerolipid (GL)/FFA cycle will be reduced due to the removal of lipid signaling molecules such as monoacylglycerol that promotes exocytosis (113). Is this change in fat oxidation a defect, or is it beneficial for the β-cell? We would favor the view that it is beneficial since it should help in the removal of nutrient excess in the β-cell to prevent ER stress and apoptosis at the cost of reduced GSIS. This reasoning is not only valid for the survival of the β-cell in the toxic fuel-excess environment but also for the whole organism as reduced secretion should prevent more hyperinsulinemia that would otherwise favor the obesity. Thus, some rethinking of the so-called β-cell dysfunction processes, as to what they exactly are, is mandatory.

CONCLUSION

For decades we had a glucocentric view of diabetes, and hence the term glucotoxicity was much in favor. With the realization that obesity-associated T2D is a disease of overall nutrient homeostasis with derangements in both carbohydrate and lipid metabolism (114), the terms lipotoxicity and glucolipotoxicity were subsequently introduced. We now know that amino acid metabolism is also deranged in obesity (99), and therefore T2D and its associated β-cell defects cannot be related to the excess of a single nutrient or class of nutrient. Therefore, more general terms should also be used to describe adaptive and toxic effects of the combined excess of nutrients.

In recent years the term metabolic stress has been widely used. Although it is a good term, it encompasses stresses due to both fuel excess and shortage and in fact it was initially used for the latter. Thus, there is a need to use appropriate and precise terms to describe particular situations and stresses related to fuel excess. For example, in the past glucotoxicity and lipotoxicty were often used to describe both adaptive and truly toxic effects, leading to confusion not only in the β-cell field. Thus, for the in vivo situation of overweight-associated T2D reflecting both the toxic and adaptive processes related to the positive energy balance with mixed nutrients excess, "nutrient-induced metabolic stress" or, in brief, "nutri-stress" would be appropriate. Other terms like glucotoxicity, glucoadaptation, and glucolipotoxicity can still be used to describe some situations, particularly in the in vitro context, but they should be used only for what they mean and not more generally as they are currently. Although not covered here, there is a large variety of both intrinsic and extrinsic factors

besides nutrients that influence the response to nutri-stress such as the plasticity in islet vasculature (115), the levels of various gluco-incretins like GLP-1 that may be protective for the β-cell (116), inflammatory cells that could favor toxicity or be beneficial depending on the context (117), or factors that are present in the serum from calorie-restricted animals (118). Finally, we need to learn more about the early defects or adaptive changes that occur in the natural history of the β-cell in diabetes to better distinguish causes from consequences of diabetes and have more studies with older animals since T2D largely occurs in aging individuals. We should also increase our understanding of the pathways of fuel-excess detoxification, a field which until recently has been largely overlooked.

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