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Forum Review

Glycemic Control of Apoptosis in the Pancreatic Beta Cell: Danger of Extremes?

GEERT A. MARTENS and MARK VAN DE CASTEELE

ABSTRACT

Excessive formation of oxygen radicals is a well-established mediator of hyperglycemic damage in diabetes to a wide range of tissues, such as neurons, retinal cells, and vascular endothelium. Increased oxygen radical formation is generally considered a toxic side effect of excessive rates of mitochondrial oxidative metabolism and electron transport in high glucose-exposed cells. Along the same line, metabolic oxidative stress is currently also regarded as crucial mediator of beta cell dysfunction and apoptosis under hyperglycemic conditions. Here the authors argue that a healthy beta cell is well equipped to deal adequately with elevated glucose metabolic rates, and demonstrate that decreased glucose catabolism leads to ROS production and apoptosis. They therefore propose that adverse metabolic conditions in poorly controlled diabetes (hyperglycemia and/or dyslipidemia) or genetic defects could decrease the viability of beta cells by interfering with normal glucose sensing and metabolism, rather than by overactivating it. This view is supported by the fragmentary data currently available on the pathways for hypergycemic and hypoglycemic beta cell death. *Antioxid. Redox Signal.* 9, 309–317.

THE VICIOUS CIRCLE OF HYPERGLYCEMIA AND BETA CELL DEATH IN TYPE 2 DIABETES

PANCREATIC BETA CELLS continuously sense the prevailing blood glucose levels, and respond to it by appropriate secretion of insulin. Their adequate function is crucial for whole body glucose homeostasis throughout life, as loss of functional beta cell mass results in the chronically devastating condition of hyperglycemia. While in type 1 diabetes the loss of glucose homeostasis occurs rapidly when > 65% of the beta cell mass is destroyed through an autoimmune destruction (86), the hyperglycemia in the more prevalent type 2 diabetes develops more gradually. Currently, three main mechanisms have been proposed to explain the progressive loss of functional beta cell mass in type 2 diabetes. First: a failure of the beta cell mass to functionally adjust to the increasing metabolic load imposed by insulin resistance (41). Thus, in diabetes-prone subjects, the proliferation of beta cell mass that is normally observed when the body's insulin requirements increase, is defective, presumably on a genetic basis (3, 65, 70). Second: dysfunction of the remaining beta cells, by various possible mechanisms: impairment of glucose sensing, depletion of islet insulin content, and/or sustained beta cell activation leading to loss of glucose-inducible function (55, 58, 74, 95). Third: loss of beta cells via apoptotic cell death (18, 19, 71). Indeed, reduced beta cell numbers have been reported in type 2 diabetic humans as well as in animal models (10, 20, 48, 60, 78, 98).

Sustained exposure of beta cells to even modestly increased blood glucose levels is often cited as an important cause of both beta cell dysfunction and death, through interactions collectively known as *glucose toxicity* (19, 74). Additional toxic factors that potentially contribute to beta cell destruction in type 2 diabetes are the elevated levels of circulating fatty acids (18, 91), or increased islet amyloid aggregation (13). Finding out to which extent these pathological

Diabetes Research Center, Brussels Free University–VUB, Brussels, Belgium.
Partner of the Juvenile Diabetes Research Center for Beta Cell Therapy in Europe.

manifestations contribute to the increased cell death is not straightforward since their occurrence overlaps and their actions are probably synergistic, at least in human type 2 diabetes (71). In this work, we will focus on the complex role of glucose metabolism in beta cell survival; in particular, we wish to propose a novel hypothesis based upon the delicate balance between normal processes of glucose sensing, and their derangement under pathological conditions. Decreased glucose sensing refers here to a state of decreased glucose catabolism in glycolysis and subsequently in the Krebs cycle, which, in the beta cell, will ultimately affect beta cell-specific gene expression and specialized functions. Such a state can experimentally be induced *in vitro* by exposing beta cells to glucose concentrations below the Km of glucokinase (GK), but could occur under pathological conditions when expression and/or activity of glycolytic enzymes is decreased.

BOTH GENETIC BACKGROUND AND AGE DETERMINE SUSCEPTIBILITY OF BETA CELLS TO HYPERGLYCEMIA-INDUCED DYSFUNCTION OR DEATH

Studies *in vivo* in different rat models clearly support the concept of a progressive glucose-induced beta cell death (40, 52, 73, 75, 88, 96). In the desert gerbil, *Psammomys obesus*, a shift from its normal, low calorie diet to a high calorie intake rapidly elevates blood glucose, leading to a markedly increased beta cell apoptosis, and, importantly, this glucose effect can be reproduced *in vitro* using primary islet cultures of this animal (20). However, as pointed out by Donath and coworkers (19), beta cell preparations differ markedly in their susceptibility to glucose-induced beta cell apoptosis, which highlights the importance of genetic background: (a) islets from a diabetes-resistant line of *P. obesus* exhibit a blunted apoptotic response (20); (b) in rats, after 90% partial pancreatectomy, the incidence of beta cell apoptosis does not change despite increased glucose (95); (c) several *in vitro* studies on rat islets or isolated rat beta cells failed to detect apoptosis in high glucose (20, 34); (d) beta cell lines of human, mouse, or rat origin are routinely cultured and passaged at high glucose concentrations (11–25 m*M*) for many generations without increased apoptosis; e) in human islets, an increase in glucose concentration from 5.5 to 33 m*M* induces apoptosis (56), but with striking batch-to-batch variations in the magnitude of the response in beta cells (19). These findings suggest that when studies are performed using *in vivo* models or isolated islets and beta cells that do not carry the genetic background predisposing to diabetes, a lower susceptibility to glucose-induced apoptosis is found.

In addition, Maedler *et al*. (57) recently showed age to be an important determinant of beta cell susceptibility to hyperglycemia-induced beta cell death. Glucose, in the physiological range from 5.5 to 11 m*M*, suppressed apoptosis and induced proliferation in islets isolated from young rats (aged 2–3 months). In contrast, in islets from older rats (age 8 months), chronically increasing glucose concentrations from 5.5 to 33.3 m*M* induced a linear increase in beta cell death and a decrease in proliferation. Also in human islets, age correlated with beta cell vulnerability to hyperglycemia-associated apoptosis.

Together, these findings indicate that hyperglycemia does not necessarily result in irreversible beta cell dysfunction and death, but that the animal's beta cell mass can adapt through phenotypic changes (39, 40, 51). In line with current epidemiological data on type 2 diabetes in humans, such adaptation is critically dependent on genetic background, and might become less efficient with age. It remains to be determined whether the age effect represents a gradual loss of a beta/progenitor cell subset with intrinsically higher plasticity, or is caused via a progressive senescence of the whole beta cell population.

GLUCOSE-SENSING BETA CELLS ARE METABOLICALLY EQUIPPED TO COPE WITH HIGH GLUCOSE CATABOLIC FLUXES

When discussing any detrimental effects of chronic hyperglycemia on beta cell function or survival, it must be kept in mind that the normal beta cell is designed as a glucose-sensing device, and is equipped with multiple specialized pathways of intermediary metabolism to assure this function. It is now generally accepted that the stimulus–secretion coupling of beta cells is composed of two distinct signaling mechanisms, both involving mitochondrial metabolism of the glucose stimulus: the K_{ATP}-dependent and -independent pathways of GSIS (reviewed in Refs. 16, 32, 97). The K_{ATP}-dependent, or *triggering* pathway (32), was described 2 decades ago (2, 64) and forms the backbone of GSIS signaling. This pathway is activated by all nutrients that are mitochondrially oxidized and activate oxidative phosphorylation, leading to increased cellular ATP/ADP ratio. Increased cytoplasmic ATP/ADP ratio causes conformational changes and closure of ATP/ADP-gated K_{ATP} channels, reduced K^+ efflux leading to membrane depolarization, and opening of voltage-gated Ca^{2+} channels. The resulting Ca^{2+} influx then activates the exocytosis of primed insulin secretory vesicles. The tight concentration dependency between beta cell ATP/ADP ratio and ambient glucose level is explained by the extraordinary high glucose oxidation rates in beta cells, which are at least threefold higher in these cells than in most other cell types (80). Glucose is rapidly taken up by beta cells via facilitated diffusion through GLUT2 (rodents) or GLUT1 (humans) (15), and channeled into glycolysis by glucokinase (GK)-mediated phosphorylation. Unlike other hexokinase isoenzymes, GK is not inhibited by its own product, glucose-6-phosphate, assuring a low-affinity high-capacity system that does not saturate even under high glucose catabolic fluxes—the reason why this enzyme is often referred to as "the beta cell glucose sensor" (62). Glucose-derived pyruvate is efficiently transported into the mitochondria, where >90% of glucose carbon is oxidized to CO_2, associated with increased accumulation of reduced $FADH_2$ and NAD(P)H (59). As anaerobic lactate dehydrogenase-mediated fermentation is extremely low in normal beta cells (84), highly active hydrogen shut-

tles (glycerophosphate and malate/aspartate shuttles) provide re-oxidation of glycolytically-formed NADH to NAD^+, the necessary cofactor to maintain glyceraldehyde-3-phosphate dehydrogenase activity going.

In 1992, it was observed that glucose could still augment insulin secretion even when the role of K_{ATP}-channels was functionally suppressed (24, 79), which led to the definition of a K_{ATP}-independent or *amplifying* pathway. To date, the biochemical nature of the amplifying pathway is not clear. Importantly, the quest for its signaling mechanism has accelerated research into the unique metabolic properties of pancreatic beta cells and led to the description of various specialized features of beta cell intermediary metabolism (97). It is now clear that in beta cells active metabolic pathways branch off the mitochondrial Krebs cycle, and are activated when glucose metabolism accelerates. Efflux of mitochondrial citrate and malate activate cytosolic substrate cycles that generate pyruvate and NADPH—the main fuel for both ROS-scavenging enzyme systems and lipid biosynthetic pathways, and a likely mediator of glucose signaling towards insulin secretion. Differentiated beta cells also express high levels of ATP-citrate lyase (ACLY), the kick-off enzyme for the fatty acid synthesis pathway encompassing acetyl-coA carboxylase (ACC) and fatty acid synthase (FAS) complex. Also, up to 25% of glucose carbon entering the Krebs cycle is channeled into protein synthesis (80). Sustained glucose stimulation of beta cells has, in addition, been shown to stimulate the biosynthesis of complex lipids, such as cholesterol derivates and phospholipids—pathways that are incompletely characterized to date (23).

Collectively, the latter pathways are particularly important as they might represent, in addition to their possible function in stimulus–secretion coupling, "metabolic safety-valves" through which glucose carbon can leave the Krebs cycle under conditions of sustained glucose stimulation (76). Importantly, activation of ATP-generating pathways in beta cells also activate an array of endergonic ATP-consuming processes: (pro)insulin biosynthesis is mainly stimulated in the 5–10 m*M* glucose range, while further increasing glucose up to 20 m*M* further augments secretory vesicle exocytosis (83) and lipid synthetic pathways. Increased energy expenditure in glucose-stimulated cells can in part explain why ATP/ADP ratio in purified beta cells increases linearly with glucose oxidation levels between 0–10 m*M* glucose but does not increase further when glucose metabolism further accelerates between 10 and 20 m*M* glucose (17). Another explanation might come from the observation that in the mitochondria of healthy beta cells, proton leak, in part via uncoupling proteins (UCP), exerts a stronger control of ATP/ADP than in other tissues (1). The main take home message here is that caution is warranted when extrapolating the detrimental effects of sustained high glucose exposure observed in nonmetabolically specialized cells or tissues to the highly specialized beta cell, which, when healthy, is well equipped to maintain high rates of glucose catabolism. Of note, rodent beta cell function was typically studied in islet or beta cell preparations obtained from younger animals (3–5 months). In the future, it will be particularly important to investigate how metabolic and functional glucose responsiveness of beta cells evolves with increasing age of the animal. In particular, it should be examined if the observed higher susceptibility of beta cells from older animals to hyperglycemia-induced dysfunction is associated with an underlying alteration of their metabolic glucose responsiveness.

DECREASED GLUCOSE SENSING LEADS TO BETA CELL DEDIFFERENTIATION AND DEATH *IN VITRO*

Adequate glucose stimulation might also be necessary to maintain the beta cell in its differentiated, glucose-responsive state. Genome-wide expression profiling indicated that moderate glucose deprivation (3 m*M* glucose for 24 h) caused a marked downregulation of various genes that are typical for neuroendocrine cells, and beta cells in particular (81). Meal-to-meal oscillations in blood glucose levels, on the other hand, have been proposed to be pivotal to maintain the beta cell mass in a normally differentiated state (33).

It has been demonstrated in MIN6 insulinoma cells, purified rat beta cells, and mouse islets, that glucose dose-dependently (from 2 to 20 m*M*) suppresses beta cell apoptosis (34, 87, 92). This anti-apoptotic glucose effect is a characteristic feature of the beta cell and was shown to be dependent on the activation of protein kinase B/Akt, membrane depolarization, and Ca^{2+} influx (87). Thus, protective pathways appear to be active when beta cells are kept in normal glucose.

In virtually all nontransformed cell types, including beta cells, the absence of nutrients and growth factors triggers apoptosis. Conversely, the protective effect of growth factors is often dependent on the stimulation of cellular glucose metabolism, via receptor tyrosine phosphorylation and the PI3-K/Akt pathway (43, 69, 93). Glucose-mediated suppressive effects on apoptosis were shown to be dependent on glucose transport, the first committed step of glycolysis, and mitochondrial hexokinase (27, 69). In most cell types, anti-apoptotic effects of glucose are therefore maximal at 2 m*M* (6, 11). Differentiated adult beta cells are fundamentally different in that they do not express high-affinity hexokinase isoforms 1–3, but rely on high Km/low-affinity glucokinase activity for their glucose metabolic flux (82). In beta cells, the survival-stimulating effect of glucose is therefore maximal between 5 and 10 m*M* glucose, while beta cell viability over culture periods up to 1 week at 20 m*M* is not significantly different from that observed at 10 m*M* glucose (34).

In summary: (a) survival and function of the beta cell is optimal when its metabolism is fully activated by glucose; (b) glucose regulation of apoptosis occurs over a physiologically relevant glucose concentration range; (c) this control mechanism is beta cell intrinsic, does not involve growth factors (although insulin cannot be excluded), and remains intact even after the cells have been isolated and cultured.

Recent work in other cell types has identified many connections between glucose metabolism and apoptosis (31). In this context, both the glycemia and the peculiar glucose sensing by beta cells are likely to play a role in the control of beta cell mass, besides regulating beta cell function. However, the mechanisms that govern metabolic control of apoptosis in these cells are unknown.

IN VITRO EXPOSURE OF BETA CELLS TO LOW GLUCOSE DISCLOSES A MITOCHONDRIAL SUICIDE PROGRAM INVOLVING ELECTRON TRANSPORT CHAIN-DERIVED ROS AS EARLY SIGNAL

Excessive formation of oxygen radicals is a well-established mediator of hyperglycemic damage to a wide range of tissues, such as neurons, retinal cells, and vascular endothelium (9). The underlying mechanism is generally explained by the following sequence of events: hyperglycemia accelerates mitochondrial glucose breakdown, resulting in the accumulation of reduced NADH and $FADH_2$ cofactors, and associated acceleration of electron transport. As a result, the mitochondrial membrane potential $\Delta\varphi$ increases up to a point where its electrogenic potential tends to overcome the driving force of respiration-driven proton pumps—a state referred to as hyperpolarization. This is the moment where electron transport slows down, and electrons accumulate in particular redox centers within the respiratory chain—mainly at complex I and III—ready for univalent reduction of molecular oxygen to superoxide (85, 90). A similar slow-down can arise even more downstream in the electron transport chain when mitochondrial ADP becomes limiting, a condition referred to as state 4 respiration, which inhibits discharge of the mitochondrial H^+ gradient at the ATP synthase and thus also causes mitochondrial hyperpolarization and increased superoxide (49). In healthy cells such a disastrous scenario is prevented by control mechanisms at various steps. Examples include: (a) the inhibition of Krebs cycle dehydrogenases and diversion of glucose catabolic flux towards anaerobic fermentation when mitochondrial NADH/NAD^+ ratio is elevated; (b) regeneration of ADP by mitochondria-associated hexokinase activity (14); and (c) in beta cells, in particular, UCP-mediated or -independent proton leak, discharging the battery (8).

In view of their high rates of aerobic glucose metabolism, pancreatic beta cells were considered as an evident victim of this hyperglycemia-induced ROS scenario (73), even more than other cell types, as (cultured) beta cells were reported to express low levels of some classical ROS-scavenging enzyme systems (89). Hyperglycemia-induced ROS in beta cells was inferred mainly by indirect methods, by taking the expression levels of ROS scavenging enzymes as indicator for ongoing oxidative stress (51), or by cytoprotective effects of widely used scavengers such as *N*-acetylcysteine or SOD mimetics (38, 88). Few direct measurements of real-time ROS formation have been done in primary beta cells from rodents or humans. To date, only two groups reported that glucose stimulated the oxidation of dihydroethidine (DHE), a fluorescent probe that selectively detects cellular superoxide (4), in cultured rat islets. Bindokas *et al*. (5) used fluorescence microscopy to show higher superoxide formation at 10 m*M* as compared to 2 m*M* glucose, in cells of flattened islets that were cultured for 3 days in the presence of 10 m*M* glucose and serum. In the second study, Krauss *et al*. (50) used a similar methodology to show mitochondrial hyperpolarization and associated superoxide formation in dispersed islet cells following a sustained stimulation with 20 m*M* glucose, and implied uncoupling protein 2 (UCP2) in these glucose-induced alterations. Concurrent with these studies, we also quantified glucose-induced ROS in beta cells. Our method was substantially different: we used flow cytometry to directly quantify oxygen radicals in large populations (>5.10^4 cells/sample) of freshly isolated, intact (propidium-iodide negative) FACS-purified (>90% insulin positive) rat beta cells, taking islet non-beta cells as additional control population (59). In these cells, we measured the cellular oxidation of a) H_2-DCFDA, a fluorescent probe that is oxidized by both peroxide- and nitric oxide-reactive species (54), and b) superoxide-selective DHE. Simultaneously, we also measured glucose-associated accumulation of whole cell NAD(P)H and mitochondrial reduced riboflavin moieties ($FADH_2$/$FMNH_2$) as direct marker for glucose metabolic rate. To our surprise, we reproducibly ($n > 20$) found that glucose suppressed, rather than stimulated both mitochondrial H_2O_2 and superoxide formation, parallel to an increase in reduced electron carries NAD(P)H and $FADH_2$/$FMNH_2$. This suppressive effect was most prominent from 0–5 m*M* glucose, but was also seen in the 5 up to 30 m*M* glucose range; it was not observed in islet non-beta cells that also did not respond to glucose by a build-up of NAD(P)H and $FADH_2$/$FMNH_2$. When beta cells were first sorted on the basis of their intrinsic, stochastic glucose NAD(P)H responsiveness, beta cells that were most responsive to glucose—meaning that they generated more NAD(P)H over the whole glucose range than the low responders—showed lower overall superoxide formation. These observations were not an artifact of freshly isolated cells, as we also detected, in contrast with the findings by Krauss and Bindokas *et al*., lower superoxide levels and higher NAD(P)H/$FADH_2$/$FMNH_2$ levels in beta cells of islets that were chronically stimulated with high (20 m*M*) as compared to control (6 m*M*) glucose. We also showed that the increased ROS levels in beta cells with lower mitochondrial metabolic activity, when sustained (72 h in 5 m*M* glucose), directly contributed to the apoptotic processes initiated in glucose-deprived beta cells. A remaining question in our model is whether the lower detection of ROS in the higher glucose range reflects an actual suppression of mitochondrial ROS formation, or an increased ROS scavenging by the glucose-induced increase in NADPH (36). In fact, the relatively low expression level of ROS scavenging enzymes in beta cells might be correlated to their high levels of glucose-induced NADPH, the fuel that drives antioxidant engines. By providing NADPH, a normal glucose metabolic rate thus offers *ad hoc* metabolic protection to beta cells (68), decreasing whole cell oxidative stress, and enabling cells to survive with a relatively low expression level of ROS scavenging systems. Of note, we also detected increased ROS in glucose-deprived insulinoma cells. In these cells, as in other tumor cells (6, 11), glucose deprivation has to be more severe before mitochondrial ROS is firmly stimulated. The latter difference is presumably associated with the expression of high-affinity hexokinases in insulinoma-, but not primary insulin-producing cells (82). The marked contrast between our observations and the overwhelming body of evidence pointing directly or indirectly to a prime role of ROS in hyperglycemia-associated beta cell dysfunction and death, is intriguing and to date, unexplained. Glucose suppression of ROS was not critically dependent on the cell culture medium used, and was also ob-

served in beta cells purified from mice (unpublished work). One possible explanation could be that initial viability and glucose responsiveness of our beta cell preparations, isolated from younger rats (age 4 months), critically influences the period that the beta cells can cope with high glucose metabolic fluxes. Typical culture periods used in our studies—up to one week after isolation—might thus be too short to disclose glucotoxic alterations observed by others.

GLUCOSE CONTROLS TWO BETA CELL SUICIDE PROGRAMS

There are a few observations to support the idea that a hypoglycemic suicide program operates in beta cells: (a) As mentioned above, *in vitro* exposure of beta cells to glucose concentrations ≤ Km of glucokinase, induces their apoptosis (21, 34, 37, 57); (b) transplantation of a rat insulinoma was found to be associated with severe hypoglycemia (2.2 m*M*) and also with atrophy of the endogenous beta cell mass by a nonimmune mediated apoptosis (7, 12); (c) in MIN6 cells and rat beta cells, low glucose concentrations lead to a sustained ROS production and activation of the AMP-activated protein kinase (AMPK), which can sense the low metabolic state of a cell on the basis of the AMP concentration (77). The ROS production and AMPK activation were found to contribute to apoptosis of these cells (46, 59, 72). In hepatocytes and MIN6 cells, AMPK triggers activation of a Jun-N-terminal kinase (JNK)-mediated apoptosis pathway (45, 63).

It is far from clear whether apoptosis also plays a role in beta cell turnover *in vivo* during fasting. Sustained fasting (4 days) lowers blood glucose in rodents to 3 m*M* (12) but does not cause clearly detectable beta cell apoptosis, presumably because alternative mitochondrial substrates are available, such as fatty acids, ketone bodies, and amino acids, and the beta cell adapts to their metabolism (28). In addition, growth factors and intra-islet glucagon might be protective by increasing beta cell Akt/PKB kinase activity and cAMP levels. Indeed, amino acids, IGF-1, and cAMP-boosting isobutyl-methyl xanthine (IBMX) protect beta cells from low glucose-induced apoptosis (Fig. 1). However, in sustained insulin infusion-induced hypoglycemia, glucose is lowered even further and loss of beta cell-specific proinsulin mRNA occurs. True beta cell apoptosis by glycopenia *in vivo* takes only place in cases of extreme glucose deprivation (<2 m*M*), a situation that can be experimentally induced by implantation of insulinoma cells (7, 12).

The primary beta cell also has access to multiple other apoptosis programs (37). It appears that the hypoglycemia-induced pathway can, at least *in vitro*, be distinguished from a hyperglycemic pathway on the basis of the requirement for the cysteine-protease calpain-10, encoded by the type 2 diabetes susceptibility gene NIDDM1 (35). Normal glucose levels suppress calpain-10 activation through activation of RyR2-containing Ca^{2+} stores (which are closely associated with mitochondria), thus hypoglycemic beta cell death requires calpain-10 activation. However, the apoptosis induced in prolonged hyperglycemia does not require calpain-10 activation (37). Taken together, these findings demonstrate the existence of a cell death pathway that is activated at low glucose and is suppressed by normal glucose. A second pathway, the hyperglycemic pathway, is activated by high glucose and is inactive in normal glucose.

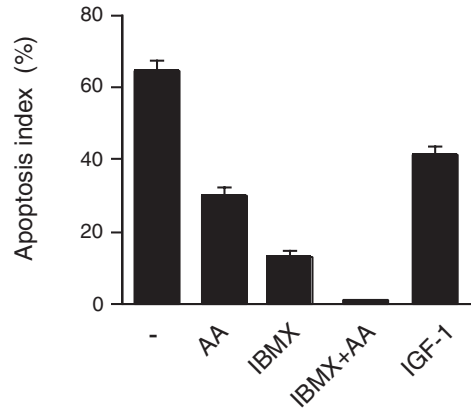

FIG. 1. Metabolic and growth factor signaling inhibit low glucose-induced apoptosis in beta cells. FACS-purified rat primary beta cells were cultured in Ham-F10 medium containing 3 m*M* glucose for 72 h in the absence (-) or presence of amino acids Leu and Gln (5 m*M* each; AA), iso-butyl-methyl-xanthine (50 μ*M*; IBMX), or IGF-1 (10 n*M*), as indicated. Apoptosis was quantified by fluorescence microscopy, as described (34).

LOW AND HIGH GLUCOSE-INDUCED BETA CELL DEATH PATHWAYS HAVE MULTIPLE FEATURES IN COMMON

The beta cell death in low glucose may be mediated through signals derived from, or targeting, mitochondria: it involves a dysregulation of specific BH-3-only members of the Bcl-2 family and activation of caspase-3, and it can be potently inhibited by Bcl-2 expression (10a). Similarly, in cultured human islets, sustained high glucose modulates the balance of mitochondrial pro-apoptotic and anti-apoptotic Bcl-2 family proteins toward apoptosis (22). In MIN6 cells and primary islet cells it was shown to result also from caspase-3 activation (47).

Another parallel between apoptosis pathways of beta cells in prolonged hypo- or hyperglycemia, is their increased production of mitochondrially-derived ROS, since in both conditions these molecules appear to enhance beta cell death.

In addition, both chronic hypo- and hyperglycemia cause an upregulation of the early response gene c-Myc in rat islets. In hyperglycemia, this effect was suggested to participate in regulation of beta cell differentiation, growth, and/or apoptosis (39, 53, 67). In low glucose concentrations (≤5 m*M*) beta cell c-Myc expression is chronically elevated as well (92), but it is unknown whether the c-Myc dysregulation contributes to a beta cell dedifferentiation, as it does in hyperglycemia (39, 53). However, there is evidence to suggest that beta cell apoptosis by prolonged culture in low glucose is in part mediated by the elevated c-Myc expression, and

that both phenomena are due to decreased nutrient-derived metabolic signals (92).

Furthermore, it has been shown that JNK activation is involved in the adverse effects of hyperglycemia or oxidative stress upon beta cell function and viability (44). Similarly, JNK activation may be a mediator of the beta cell death observed in low glucose: this condition leads to activation of both AMPK and JNK, and JNK was shown to mediate AMPK-induced apoptosis (45, 46, 63). Available data therefore suggest that c-myc, JNK, and mitochondrial signals involving Bcl-2 family proteins and increased ROS production, play a role in apoptosis in both low and high glucose. A mechanism underlying the common triggering of these multiple molecular events in low and high glucose, if it exists, is unknown to date. It is a reasonable starting point, however, to assume that in low glucose concentrations, inadequate glucose metabolism is the earliest trigger of the apoptotic chain.

STARVATION IN THE MIDST OF PLENTY: DECREASED BETA CELL GLUCOSE METABOLIC RATE AS TRIGGER FOR BOTH LOW AND HIGH GLUCOSE-INDUCED APOPTOSIS?

There is evidence that chronic hyperglycemia can result in *decreased* rather than *increased* glucose metabolic rate in beta cells. Chronic hyperglycemia has been shown to result in beta cell dedifferentiation with decreased expression and activity of key enzymes involved in glycolysis and downstream glucose metabolism. Sustained hyperglycemia has thus been shown to suppress the expression of GK, Glut2, and pyruvate carboxylase (40), and decreased Glut2 expression or GK activity hampers glucose metabolism in beta cells (29, 61). Kim and co-workers recently showed that chronic high glucose pretreatment of MIN6 cells causes GK and Glut2 downregulation and a decreased cellular ATP production in these cells (47). The same tendency is observed in beta cells from Zucker Diabetic Fatty rats. Loss of functional leptin receptors in this model causes multiple abnormalities in both lipid and glucose metabolism, resulting in lipid accumulation, associated with decreased Glut2 and GK expression. The latter was associated with decreased glycolytic and mitochondrial glucose metabolic rate (66), reversed upon reintroduction of the defective gene (94). Interestingly, and in apparent contradiction with their view that ROS in beta cells is simply associated with an acceleration of glucose metabolism, Bindokas *et al*. indeed observed higher rates of mitochondrial ROS in ZDF beta cells (5). Deficiency of glucose oxidation was also detected in islets isolated from spontaneously diabetic Goto–Kakizaki rats (26), as well as in beta cells isolated from adult rats that became glucose intolerant later in life after partial beta cell ablation using streptozotocin in the neonatal period (25). Direct comparisons of glucose metabolic rate in islets obtained from type 2 diabetic and healthy control humans are, to our knowledge, not available. Yet, Gunton *et al*. (30) recently showed that the expression of several key glycolytic enzymes was markedly suppressed in islets from type 2 diabetic human patients: mRNA levels of GK, glucose-6-phosphoisomerase, phosphofructokinase, and phosphoglucomutase were up to fivefold lower in diabetic islets, indicating a beta cell-intrinsic, severe impairment of the glucose-sensing machinery. These observations thus suggest that beta cell death by prolonged hyperglycemia is associated with a decreased glucose sensing and alterations in the preferential stimulation of mitochondrial oxidative metabolism by glucose. Thus, beta cell death by hyperglycemia may in part result from an *inadequate* rather than *excessive* glucose metabolism. Beta cell death induced by both glucose deprivation and sustained high glucose might thus share a partially common execution program, both initiated by an underlying decrease in glucose metabolism. In this context, it would be interesting to examine whether the low energy sensor AMPK is activated under chronic hyperglycemia.

In conclusion, our working hypothesis implies that primary changes in energy metabolism or glucose sensing can directly affect the susceptibility of a beta cell to the induction of apoptosis, either induced by exogenous aggression, or by activation of a beta cell endogenous suicide program. It further implies that the underlying apoptotic program would become active in conditions of decreased glucose metabolism, whereas increased metabolic activity would suppress it. In this view, adverse metabolic conditions in poorly controlled diabetes (hyperglycemia and/or dyslipidemia) could decrease the viability of beta cells by interfering with normal glucose sensing and metabolism, rather than by overactivating it. In

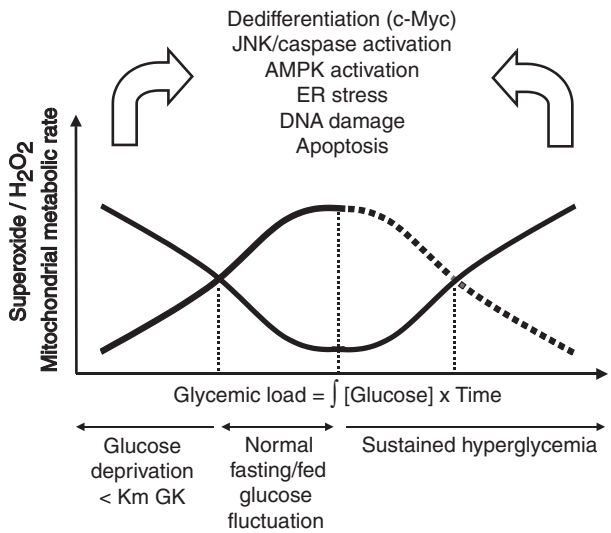

FIG. 2. Hypothetical relationship between glycemic load and mitochondrial glucose metabolism and associated ROS in beta cells. We use the term *glycemic load* here as a theoretical parameter, reflecting an integration of both duration and the intensity of glucose stimulation/deprivation. The scheme summarizes data and visualizes the concept that decreased glucose metabolism (by lowering the glucose concentration below the Km of GK) leads to increased ROS formation, which, when persistent, will induce beta cell apoptosis (*from middle to left*). The observation that persistent exposure to hyperglycemia (*from middle to right*) also increases ROS production and apoptosis is then interpreted on the same basis, namely an underlying inhibition of glucose metabolism (*dashed line, hypothetical*).

this view, the appearance of hyperglycemia-induced ROS formation could be considered as a marker for an underlying defect in mitochondrial nutrient metabolism, rather than being a consequence of excessively stimulated electron transport rate. This concept is schematically summarized in Fig. 2. We thus believe that it is mandatory to measure actual glucose oxidative metabolism when addressing the role of oxygen radicals in the presumed glucose toxicity.

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ABBREVIATONS

ACC, acetyl-coA carboxylase; Akt, protein kinase B; AMPK, AMP-activated protein kinase; H_2-DCFDA, dichlorodihydro-fluorescein-diacetate; DHE, dihydroethidine; FACS, fluorescence activated cell sorter; FAS, fatty acid synthase; GK, glucokinase; GSIS, glucose stimulated insulin secretion; IBMX, isobutyl-methyl-xanthine; IRS-1, insulin receptor substrate-1; JNK, c-Jun *N*-terminal kinase; *P. obesus, Psammomys obesus;* PI3-K, phosphoinositide-3–0H kinase; ROS, reactive oxygen species; RyR2, ryanodine receptor-2; SOD, superoxide dismutase; UCP, uncoupling protein; VDAC, voltage-dependent anion channel.

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Address reprint requests to:
Mark Van de Casteele
Diabetes Research Center
Brussels Free University–VUB
Laarbeeklaan 103
B-1090 Brussels, Belgium

E-mail: mvdcaste@vub.ac.be

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