

NADPH Oxidase as Mediator of Glucolipototoxicity-Induced Beta Cell Dysfunction – Clinical Implications

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Abstract

An impairment of glucose-stimulated insulin secretion – reflecting decreased glucokinase expression – and a moderate decrease in beta cell mass attributable to increased apoptosis, constitute the key features of beta cell failure in type 2 diabetes. Oxidative stress, provoked by prolonged exposure to excessive levels of glucose and/or fatty acids (glucolipototoxicity), appears to be a key mediator of these defects. Oxidant-provoked JNK activation induces nuclear export of the PDX-1 transcription factor, required for expression of glucokinase and other beta cell proteins. Conversely, increases in cAMP induced by incretin hormones promote the nuclear importation of PDX-1, counteracting the diabetogenic impact of oxidant stress; this may explain the utility of measures that slow dietary carbohydrate absorption for diabetes prevention. The ability of oxidative stress to boost apoptosis in beta cells is poorly understood, but may also entail JNK activation. Recent work establishes a phagocyte-type NADPH oxidase as the chief source of glucotoxicity-mediated oxidative stress in beta cells. Since bilirubin is now known to function physiologically as an inhibitor of NADPH oxidase, and phycocyanobilin (PCB) derived from spirulina likewise can inhibit this enzyme complex, supplemental PCB may have utility in the prevention and control of diabetes, and Gilbert syndrome, associated with chronically elevated free bilirubin, may be associated with decreased diabetes risk.

Beta Cell “Failure” in Type 2 Diabetes

The beta cell “failure” intrinsic to type 2 diabetes appears to reflect two key phenomena – a cellular dysfunction characterized by a marked deficit of glucose-stimulated insulin secretion (GSIS), and a decrease in beta cell mass (relative to total pancreas mass) that reflects an increase in apoptosis rather than a loss of proliferative capacity.¹⁻⁴ The defect in GSIS is quite specific, since amino acid-evoked insulin secretion tends to be less notably impaired in this syndrome, and fasting insulin levels are often normal or elevated (relative to those in lean healthy controls). The failure of an increase in glucose to evoke an appropriate increase in insulin secretion may be primarily attributable to a reduction in beta cell expression of glucokinase, which has been observed in islets derived from

human type 2 diabetics as well as from diabetic rodents.¹ While hexokinase has a relatively low K_m for glucose, and flux through it is maximized at low physiological glucose levels, glucokinase has a higher K_m , such that flux through glucokinase increases as glucose levels increase within and beyond the normal physiological range. Adequate glucokinase expression is therefore crucial to the beta cell's ability to "detect" an increase in serum glucose, and indeed plays a rate-limiting role in this regard; thus, some authorities refer to islet glucokinase as the "glucose sensor."^{5, 6} The fact that insulin secretion by diabetic beta cells is comparable to that of healthy beta cell when these cells are incubated in low-physiological concentrations of glucose, presumably reflects the fact that glucose flux through hexokinase is well preserved in diabetic cells.

In vitro and in vivo, chronic exposure of beta cells to high-physiological levels of glucose and/or free fatty acids (palmitate) has been shown to lead to a decrease in beta cell GSIS associated with decreased glucokinase expression;⁷⁻¹⁴ this phenomenon is known as "glucolipotoxicity". This reduced expression, in turn, appears to reflect decreased function or expression of a beta-cell-specific transcription factor, PDX-1 (a.k.a. IDX-1, STF-1) required for transcription of the glucokinase gene.^{11, 15} This transcription factor also contributes in other ways to the characteristic differentiation of mature beta cells, boosting expression of the glucose transporter GLUT2 and of insulin;¹⁶ the reduced expression of these proteins observed in human diabetic beta cells may be a consequence of diminished PDX-1 activity.¹

Persistent glucolipotoxicity may also be at the root of the increase in beta cell apoptosis and decline in beta cell mass observed in diabetics. High physiological concentrations of glucose and saturated fatty acids have a synergistic impact on apoptosis in primary beta cells or beta cell-derived cell cultures in vitro.¹⁷

These considerations give rise to the hypothesis that type 2 diabetes involves a vicious cycle in which exposure of beta cells to excess levels of glucose and/or free fatty acids leads to decreased expression of glucokinase associated with a failure of GSIS, coupled with an increase in apoptosis that diminishes beta cell mass; the resulting deficit in physiologically appropriate insulin secretion tends to sustain the elevations of glucose and free fatty acids and thus complete the vicious cycle.¹⁸ Presumably, the cycle is triggered by factors that induce intermittent or chronic excesses of plasma glucose and free fatty acids, such as visceral obesity (characterized by insulin resistance of hypertrophied adipocytes), low muscle insulin sensitivity, and meals rich in high-glycemic index carbohydrates and saturated fats. The fact that beta cell GSIS of type 2 diabetics often recovers to a degree after a sustained period of normoglycemia during a prolonged fast or very-low-calorie dieting,¹⁹⁻²¹ suggests that diabetic beta cell dysfunction is at least partially reversible. Indeed, diabetes is often "cured" in obese patients who achieve profound weight loss following bariatric surgery.^{22, 23}

Oxidative Stress is a Key Mediator of Beta Cell Dysfunction and Death

How does exposure to excessive levels of glucose and/or palmitate precipitate the deficit of PDX-1 function? Numerous studies have shown that exposure of beta cells to high physiological levels of glucose and/or palmitate provokes an increase in oxidative stress. Kaneto and colleagues have demonstrated that induced oxidative stress can suppress the transcriptional activity of PDX-1 in beta cells.²⁴⁻²⁶ Thus, exposure of beta cells to oxidative stress causes a failure of GSIS and a reduction of nuclear expression of PDX-1 that is prevented by the antioxidants N-acetylcysteine and probucol.²⁶⁻²⁸ This work further shows that the impact of oxidative stress on PDX-1 is mediated by oxidant-induced activation of JNK, which in turn causes translocation of PDX-1 from the nucleus.^{24, 25} The effect of JNK in this regard is dependent on an intact leucine-rich nuclear export signal in PDX-1 as well as induced intranuclear transport of Foxo1.^{25, 29} Since PDX-1 stimulates its own transcription,³⁰ nuclear exclusion of PDX-1 may be at least partially responsible for the decline in total PDX-1 *expression* that has been noted in the islets of diabetic rodents.^{8, 31} However, *increased* expression of PDX-1 mRNA has recently been observed in the islets of human type 2 diabetics – even though reduced expression of glucokinase, GLUT-2, and insulin in these islets is suggestive of a decrease in PDX-1 activity. Thus, nuclear exclusion of PDX-1 may be the chief basis for reduced PDX-1 activity in human diabetes.

The likely relevance of these findings to beta cell function *in vivo* is supported by studies demonstrating that administration of certain antioxidants – N-acetylcysteine, probucol, aminoguanidine, and tempol – can retard the onset of beta cell dysfunction in rodent models of type 2 diabetes.^{27, 28, 32-34} Analogously, these antioxidants, or transfection of antioxidant enzymes, helps to preserve the insulin secretory capacity of in beta cell-derived cell lines exposed to high glucose and/or palmitate *in vitro*.^{32, 35} Likewise, perfusion of human diabetic islets *in vitro* with glutathione has been shown to normalize the GSIS of these islets.¹

The mechanisms by which glucolipotoxicity promotes apoptosis in beta cells do not yet appear to be well defined. However, there is suggestive evidence that evoked oxidative stress contributes to this phenomenon as well. Thus, antioxidant treatment tends to preserve beta cell mass in diabetic rodents, and, in the islets of human type 2 diabetics, beta cell mass tends to correlate inversely with an index of oxidative stress, 8-OHdG.^{3, 27, 28} The impact of oxidative stress on apoptosis in beta cells, may reflect, in part, a JNK-mediated diminution of Akt phosphorylation;²⁹ conversely, phosphorylation of Akt may be a key mediator of the anti-apoptotic impact of GLP-1 analogs, EGF, or insulin on fatty acid-exposed beta cells.^{36, 37}

The chief source of the beta cell oxidative stress evoked by sustained exposure to excessive glucose and/or fatty acids has recently been identified as a phagocyte-like NADPH oxidase; its activation is a downstream consequence of PKC activation in beta cells exposed to excess glucose/palmitate.³⁸⁻⁴² Thus, the NADPH oxidase inhibitor DPI, an oligonucleotide antagonist of p47phox, and a PKC inhibitor have been shown to alleviate oxidative stress in beta cells exposed to high glucose and/or palmitate. Increased expression of certain components of NADPH oxidase has also been reported in the islets of diabetic rats, and in beta cells exposed to glucolipotoxicity.^{41, 43} Angiotensin II, generated locally or systemically, boosts NADPH oxidase expression in beta cells, and administration of an AT1 receptor antagonist has been shown to help preserve beta cell function in diabetes-prone db/db mice;⁴³ this is possibly indicative of an up-regulation of the local renin-angiotensin system in human islets (as systemic generation of angiotensin II tends to be normal in diabetics). Moreover, there is clinical evidence that, in non-diabetic hypertensives, the use of angiotensin II antagonist drugs is associated with a significant decrease in diabetes risk.⁴⁴

Practical Measures for Restoring Appropriate Beta Cell Function

Recent research reveals that unconjugated bilirubin functions physiologically as a potent inhibitor of NADPH oxidase.⁴⁵⁻⁴⁹ Furthermore, phycocyanobilin (PCB), a biliverdin metabolite that constitutes about 0.6% of the dry weight of spirulina, is converted within cells to a close homolog of bilirubin, phycocyanorubin, that likewise can inhibit NADPH oxidase activity; this likely rationalizes the potent and wide-ranging anti-inflammatory and cytoprotective properties of oral spirulina or phycocyanin in rodent studies.⁵⁰⁻⁵² In light of the central role of NADPH oxidase activity in diabetic beta cell dysfunction, it is reasonable to suspect that administration of biliverdin (much more soluble than bilirubin, and converted to bilirubin in vivo) or of PCB could help prevent or postpone beta cell failure in pre-diabetics, and might aid restoration of normal beta cell function in patients with pre-existing diabetes. Moreover, it seems reasonable to predict that subjects with Gilbert syndrome, in whom plasma levels of free bilirubin are chronically elevated, may be at reduced risk for diabetes; this prediction should be readily testable.

Whereas oxidant stress promotes nuclear exclusion of PDX-1, cAMP, acting via PKA, has been shown to induce the nuclear uptake of PDX-1; thus, cAMP is a key functional antagonist of oxidant stress in beta cells.⁵³ GLP-1, an incretin hormone whose secretion is boosted by measures that increase the delivery of carbohydrate to distal portions of the intestine, functions physiologically to boost cAMP production in beta cells. This may help to explain why low-glycemic-index diets, acarbose, and high coffee intake have been linked to reduced risk for diabetes⁵⁴⁻⁵⁹ – although a reduction in postprandial glucose excursions no doubt contributes to this effect as well. (Coffee is a rich source of

chlorogenic acid, which may delay intestinal glucose absorption.^{60, 61}) The utility of the GLP-1 mimic exenatide in the management of type 2 diabetics is now well established.⁶²

Biotin, which in moderately supraphysiological concentrations can activate soluble guanylate cyclase, has been reported to boost expression of PDX-1 and glucokinase in cultured beta cells;^{63, 64} similarly, biotin administration increased glucokinase expression in the islets of diabetic rats.⁶⁵ Likely, this effect is mediated by cGMP, but there do not seem to be any reports regarding the impact of cGMP on glucokinase or PDX-1 activity in beta cells; however, cGMP (and biotin) have been shown to increase expression of glucokinase in insulin-deficient rat hepatocytes.^{66, 67} Since biotin is well tolerated in “mega-doses” as high as 100 mg daily, and has the potential to improve diabetic glycemic control by promoting glucokinase induction in both beta cells and hepatocytes, it merits consideration as an additional adjunctive measure for ameliorating the beta cell dysfunction of diabetics.⁶⁸ Biotin has shown utility in diabetic rodents, and the few clinical trials with biotin in diabetics have yielded mixed but mostly positive results.⁶⁹⁻⁷¹

Toward a Beta Cell Redifferentiation Therapy

In a previous essay, a strategy for “curing” type 2 diabetes by reestablishing proper differentiation of beta cells – dubbed “beta cell redifferentiation therapy” (BRT) – has been proposed.^{18, 72} First, the diabetic patient adopts a diet and lifestyle that likely would have prevented diabetes in the first place, and that enables him to lose a physiologically meaningful amount of excess adiposity. For example, a quasi-vegan diet low in saturated fats and high-glycemic-index starchy foods, and rich in structurally-intact whole grains, legumes, fruits and vegetables, coupled with effective aerobic exercise training, may be useful in this regard;^{73, 74} adjunctive measures which slow absorption of dietary carbohydrate – such as soluble fiber supplements, vinegar, chlorogenic acid, or the drug acarbose – may be useful complements to such a diet, since they would be expected to suppress postprandial hyperglycemia while boosting GLP-1 secretion. Supplements of PCB or biliverdin may also be useful for diabetes prevention, and thus would also be appropriate components of this regimen.

Once worthwhile weight loss has been achieved, preferably such that the patient weighs less than or no more than he did when his diabetes first manifested, he is then placed on a protein-sparing fast of about 7-10 days duration; the primary purpose of this fast is not to achieve further fat loss, but rather to achieve sustained normoglycemia, giving the beta cells a respite from the glucolipotoxicity that has induced and sustained their beta cell dysfunction. It is well documented that prolonged fasting or very-low-calorie dieting in diabetics induces an improvement in the beta cell function that is disproportionately large relative to the amount of fat lost during the diet.¹⁹⁻²¹

During and following the fast, adjunctive agents which promote proper beta cell differentiation – for example, PCB, exenatide, biotin, and angiotensin II antagonists – could also be administered, to amplify the redifferentiating benefit of normoglycemia. The patient then transitions back to his diabetes-preventive lifestyle. If beta cell function is sufficiently recovered by this regimen, if the preceding weight loss is of adequate magnitude, and if the patient is reasonably compliant with his new healthful lifestyle, it is conceivable that improved beta cell function will be maintained and glucotoxicity will not recur – in other words, the diabetes will be “cured”, at least so long as the compliance with the lifestyle persists. This strategy is an elaboration of a fasting-based regimen first described by Allen in the early twentieth century, and more recently revived by Fuhrman. The preceding discussion implies that measures which down-regulate NADPH oxidase activity – such as PCB or angiotensin II antagonists – should be useful components of such a strategy.

A potential flaw in this strategy is that normalization of beta cell function may be insufficient to maintain good metabolic control in patients whose beta cell mass is substantially diminished – as it seems unlikely that a brief period of normoglycemia would enable a complete restitution of beta cell mass. On the other hand, in patients whose beta cell mass is only modestly reduced, up-regulation of insulin secretion in beta cells whose normal function has been restored may succeed in normalizing metabolic control. Conceivably, partial alleviation of glucolipotoxicity during the preliminary “healthy lifestyle” phase could enable partial regrowth of beta cell mass, such that this would be sufficient to support good metabolic control following the fasting phase. Administration of agents such as PCB, exenatide, biotin, and angiotensin II antagonists during the preliminary healthy lifestyle phase might also help to suppress beta cell apoptosis and reconstitute a more adequate beta cell mass.

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