Regulation of the Insulin Gene by Glucose and Fatty Acids

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ABSTRACT The insulin gene is expressed almost exclusively in pancreatic β-cells. Metabolic regulation of insulin gene expression enables the β-cell to maintain adequate stores of intracellular insulin to sustain the secretory demand. Glucose is the major physiologic regulator of insulin gene expression; it coordinates the recruitment of transcription factors [e.g., pancreatic/duodenal homeobox-1 (PDX-1), mammalian homologue of avian MafA/L-Maf (MafA), Beta2/Neuro D (B2), the rate of transcription, and the stability of insulin mRNA. However, chronically elevated levels of glucose (glucotoxicity) and lipids (lipotoxicity) also contribute to the worsening of β-cell function in type 2 diabetes, in part via induction of insulin gene expression. The mechanisms of glucotoxicity, which involve decreased binding activities of PDX-1 and MafA and increased activity of C/EBPβ, are mediated by high-glucose–induced generation of oxidative stress. On the other hand, lipotoxicity is mediated by de novo ceramide synthesis and involves inhibition of PDX-1 nuclear translocation and MafA gene expression. Glucotoxicity and lipotoxicity have common targets, which makes their combination particularly harmful to insulin gene expression and β-cell function in type 2 diabetes. J. Nutr. 136: 873–876, 2006.

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Insulin is secreted uniquely from the islet β-cells of the pancreas and plays a major role in the maintenance of energy homeostasis. Insulin secretion is tightly regulated to maintain blood glucose levels within a narrow physiological range. Insufficient secretion of insulin from β-cells contributes to the chronic hyperglycemia characteristic of diabetes, a disease that affects >20 million Americans. Short-term regulation of insulin secretion, such as in response to a meal, occurs mainly at the level of exocytosis. However, the maintenance of adequate intracellular stores of insulin on a long-term basis relies on the transcriptional and translational regulation of insulin biosynthesis. The 2 most important nutrients in mammals, glucose and fatty acids, profoundly affect preproinsulin gene (hereafter referred to as "insulin gene") expression under physiological and pathological circumstances. This review briefly describes the key control elements and their cognate transactivating factors involved in the metabolic regulation of insulin gene transcription. We then describe the manner in which glucose normally regulates insulin gene expression, and how dysregulation occurs upon prolonged exposure to glucose (glucotoxicity) and fatty acids (lipotoxicity).

Structure of the insulin gene and key transcription factors involved in metabolic regulation. In adult mammals, expression of the insulin gene is essentially restricted to the pancreatic β-cell. A highly conserved region lying ~340 bp immediately upstream of the transcription initiation start, hereafter referred to as the insulin promoter, confers both tissue-specific expression and metabolic regulation of the insulin gene. Many transcription factors act upon this region, forming a highly sophisticated transcriptional network that ensures precise regulation. The most critical cis-acting DNA elements involved in transcriptional activation in vitro are referred to the A3, C1, and E1 sites (Fig. 1; reviewed in (1)).

Pancreatic/duodenal homeobox-1 (PDX-1) is a homeomain protein that binds to the A3 box of the insulin gene (2). PDX-1 is also essential in the normal development of the pancreas because its deletion results in complete agenesis (3). PDX-1 functionally interacts with proteins of the basic helix-loop-helix (bHLH) family that bind to the E1 box (4). The E1 activator is a heterodimer consisting of ubiquitous class A (E12/E47 and E2/5) and cell-restricted class B (B2) members of the bHLH family (5). The cooperation between PDX-1 and bHLH proteins also involves interactions with other DNA-binding proteins (6) and co-activators (7), thus forming a unique

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3 Abbreviations used: bHLH, basic helix-loop-helix; B2, Beta2/NeuroD; C/EBPβ, CCAAT/enhancer-binding protein β; JNK, c-jun N-terminal kinase; MafA, mammalian homologue of avian MagfA/L-Maf; PDX-1, pancreatic/duodenal homeobox-1; TG, triglycerides.
Glucose regulation of insulin gene expression. Glucose is the major nutrient regulator of pancreatic β-cell function and coordinately regulates insulin gene expression, insulin biosynthesis, and insulin secretion. Glucose controls all steps of insulin gene expression, including transcription, preRNA splicing, and mRNA stability. A3, E1, and C1 are the major glucose-responsive transcription control elements of the insulin gene. In addition, a more distal glucose-responsive element appears to bind a glucose-sensitive complex that is specifically present in primary islets but remains to be identified (9). Glucose promotes the binding of PDX-1 to the A3 site (2) and PDX-1 trans-activating potency (10). In addition, it is now recognized that PDX-1 stimulation of insulin gene transcription involves recruitment of co-activators, such as p300 (11), that affect chromatin structure through post-translational modifications of histones such as methylation (12) and/or acetylation (13).

The signal transduction mechanisms by which glucose increases PDX-1 binding to the insulin promoter have been studied extensively but remain controversial. Glucose appears to promote translocation and modification of a cytoplasmic, inactive 31-kDa form to the nuclear, active 46-kDa species (14). Although this transformation likely requires phosphorylation, the large increase in apparent molecular mass suggests that PDX-1 undergoes multiple post-translational modifications, possibly by O-linked N-acetylglucosamine (15) or small ubiquitin-related modifier 1 (16). A number of kinases were proposed to mediate PDX-1 phosphorylation, including p38 mitogen-activated protein kinase (17), phosphatidylinositol-3 kinase (18), and extracellular signal-regulated kinases (19).

MafA and B2/E47 are also stimulated by glucose (20). Although it is unclear how B2/E47 is regulated, MafA expression and binding are activated directly by glucose (21). Importantly, PDX-1, MafA, and B2 do not act in an isolated manner, but interact with each other to induce synergistic activation of insulin transcription (21). Therefore, glucose enhances insulin gene transcription by a number of complementary mechanisms that include recruitment of transcription factors to regulatory sites, histone modifications, and initiation of transcription. Importantly, our understanding of the control of the insulin gene has been gleaned principally from in vitro systems, and their relevance to the endogenous gene in vivo has yet to be established.

In addition to its major effects on the rate of transcription, glucose markedly stabilizes preproinsulin mRNA (22). Two elements located in the 3′-untranslated region of the mRNA molecule were proposed as mediators of this effect, i.e., the conserved UUGAA sequence (23) and a pyrimidine-rich sequence (24). Stabilization appears to involve glucose-regulated binding of a polypyrimidine tract-binding protein to the pyrimidine-rich sequence (24).

Dysregulation of insulin gene expression. As discussed above, glucose is the major physiologic regulator of the insulin gene. In contrast, when β-cells are exposed to elevated levels of glucose for prolonged periods of time, glucose becomes toxic to insulin secretion, gene expression, and β-cell survival. This phenomenon is referred to as glucotoxicity (25). Similarly, chronically elevated levels of fatty acids adversely affect pancreatic β-cell function through a process termed lipotoxicity (26). The relevance of nutrient-induced β-cell dysfunction in humans comes from the fact that chronic hyperglycemia and associated disorders of lipid metabolism are thought to contribute to the deterioration of pancreatic β-cell function observed in patients with type 2 diabetes (27).

Glucotoxicity. Exposure of insulin-secreting cells to elevated glucose levels for several weeks impairs insulin gene expression; this is associated with diminished binding activity of PDX-1 and MafA [reviewed in (28)]. The decrease in PDX-1 binding activity appears to involve post-transcriptional control (29), although the precise mechanism(s) remains to be established. In vivo, PDX-1 expression is also reduced in partially pancreatectomized, hyperglycemic rats (30) and in the diabetic gerbil Psammomys obesus (31), and its binding activity is decreased in islets from Zucker Diabetic Fatty rats (32). The reduction in MafA binding activity in the glucotoxic insulin-secreting HIT-T15 cell was shown recently to be due to a loss of protein expression without changes in mRNA expression, suggesting that glucose reduces MafA activity through a post-translational mode of action (33). Importantly, MafA expression is also reduced in mouse diabetic models (34). In addition, the C/EBPβ transcription factor may directly bind E47 and prevent formation of the B2/E47 activator complex under glucotoxic conditions (35). A recent study further proposed that CEBP/β prevents MafA binding to its cognate sequence under chronic exposure to elevated glucose and, in turn, prevents the cooperative induction of transcription by MafA and B2 (36).

Much progress has been made in recent years in understanding the biochemical mechanisms of glucotoxicity. Ample evidence supports the involvement of oxidative stress in this process, as a result of long-term exposure to elevated glucose [reviewed in (37)]. For example, the decrease in insulin gene transcription (38) and MafA protein expression (33) is prevented by antioxidants in glucotoxic insulin-secreting cells. Moreover, treatment of Zucker Diabetic Fatty rats with antioxidants normalizes plasma glucose levels and restores insulin secretion, insulin content, and insulin mRNA levels (38). In addition, activation of the hexosamine pathway decreases insulin gene expression and insulin secretion in isolated islets via generation of oxidative stress but not via O-linked glycosylation (39).

The signaling pathways mediating inhibition of insulin gene expression by oxidative stress appear to involve, at least in part, stress-activated kinases. Overexpression of a dominant-negative form of c-jun N-terminal kinase (JNK) prevents the decrease in PDX-1 binding activity in response to oxidative stress in a c-jun-independent manner (40). On the other hand, c-jun can directly inhibit insulin gene transcription by interfering with bHLH-mediated transcriptional activity (41). It is therefore likely that several interrelated pathways negatively affect insulin gene transcription under conditions of oxidative stress (Fig. 2). Another possibility, not exclusive with the previous one, is that glucotoxicity induces dedifferentiation of the β-cell, as suggested by the observed inhibition of genes associated with β-cell function and derepression of genes not normally expressed in differentiated β-cells (42). For example, the transcription factor c-myc is...
upregulated in islets from diabetic animals (43), and can inhibit insulin gene transcription by competing for B2 binding at the E-box (44) (Fig. 2).

**Lipotoxicity.** Disorders of lipid metabolism were proposed to contribute to β-cell dysfunction in type 2 diabetes (26). We (45–47) and others (48,49) showed that exposure of isolated islets and insulin-secreting cells to elevated levels of fatty acids in vitro impairs insulin gene expression when glucose concentrations are concomitantly elevated. Deleterious effects of fatty acids on insulin gene expression in isolated islets are associated with an increased accumulation of intracellular triglycerides (TG), suggesting a role for neutral lipid synthesis in this process (45). Nonetheless, elevated TG synthesis by overexpression of the enzyme diacylglycerol-acyltransferase inhibits glucose-induced insulin secretion but not preproinsulin mRNA levels (50), suggesting that TG accumulation is not directly involved in inhibition of insulin gene expression. Furthermore, because both palmitate and oleate inhibit insulin secretion, but only palmitate impairs insulin gene expression (51), we postulated that distinct mechanisms underlie these 2 functional effects. Because ceramide can be synthesized de novo from palmitate but not from oleate, we hypothesized that palmitate inhibition of insulin gene expression may be mediated by ceramide generation. In fact, exposure of islets to palmitate was shown to result in increased intracellular ceramide content that was previously shown to contribute to β-cell function (e.g., insulin gene expression). The combined and deleterious effects of glucose and fatty acids on insulin gene expression are likely to contribute to β-cell dysfunction in type 2 diabetes, although this hypothesis has yet to be tested in humans.

**Conclusions.** Regulation of insulin gene expression under normal circumstances is controlled chiefly by changes in glucose concentrations. Glucose coordinate recruits a highly sophisticated network of transcription factors and co-activators to the insulin promoter, and also prolongs the half-life of insulin mRNA. In vitro and in vivo studies in rodents have provided evidence that under circumstances of chronically elevated levels of glucose and fatty acids, insulin gene expression is greatly reduced. The mechanisms of glucotoxicity and lipotoxicity involve the PDX-1 and MafA transcription factors, with such a convergence having severe adverse consequences on β-cell function (e.g., insulin gene expression). The combined and deleterious effects of glucose and fatty acids on insulin gene expression are likely to contribute to β-cell dysfunction in type 2 diabetes, although this hypothesis has yet to be tested in humans.

**LITERATURE CITED**


42. Gao Y, Miyazaki J, Hart GW. The transcription factor PDX-1 is post-translationally modified by O-linked N-acetylglucosamine and this modification is correlated with its DNA binding activity and insulin secretion in min6 β-cells. Arch Biochem Biophys. 2003;415:155–63.


