High Glucose Stimulates Early Response Gene c-Myc Expression in Rat Pancreatic \( \beta \) Cells*

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Glucose-induced insulin secretion from hyperglycemic 90% pancreatectomized rats is markedly impaired, possibly because of loss of \( \beta \) cell differentiation. Association of these changes with \( \beta \) cell hypertrophy, increased mRNA levels of the transcription factor c-Myc, and their complete normalization by phlorizin treatment suggested a link between chronic hyperglycemia, increased c-Myc expression, and altered \( \beta \) cell function. In this study, we tested the effect of hyperglycemia on rat pancreatic islet c-Myc expression both in vivo and in vitro. Elevation of plasma glucose for 1–4 days (glucose infusion/clamp) was followed by parallel increases in islet mRNA levels (relative to TATA-binding protein) of c-Myc and two of its target genes, ornithine decarboxylase and lactate dehydrogenase A. Similar changes were observed in vitro upon stimulation of cultured islets or purified \( \beta \) cells with 20 and 30 mmol liter\(^{-1}\) glucose for 18 h. These effects of high glucose were reproduced by high potassium-induced depolarization or dibutyryl-cAMP and were inhibited by agents decreasing cytosolic Ca\(^{2+}\) or cAMP concentrations. In conclusion, the expression of the early response gene c-Myc in rat pancreatic \( \beta \) cells is stimulated by high glucose in a Ca\(^{2+}\)-dependent manner and by cAMP. c-Myc could therefore participate to the regulation of \( \beta \) cell growth, apoptosis, and differentiation under physiological or pathophysiological conditions.

Besides being the major stimulus of insulin secretion, glucose exerts pleiotropic effects in pancreatic \( \beta \) cells, including stimulation of protein synthesis (1) and cell proliferation and cell growth (2). Stimulus-secretion coupling is largely under-stimulated of protein synthesis (1) and cell proliferation and differentiation under physiological or pathophysiological conditions. 

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1 The abbreviations used are: LDH, lactate dehydrogenase; bHLH-LZ, basic helix-loop-helix leucine zipper (bHLH-LZ) family that heterodimerizes with Max, another bHLH-LZ protein. The binding of Myc-Max heterodimers to E boxes (canonical consensus sequence CACGTG) activates the transcription of multiple genes implicated in the regulation of cell metabolism, protein synthesis, cell division, and apoptosis (10). c-Myc can also repress the expression of a number of genes and decrease cell differentiation by unclear mechanisms. On the other hand, Max binds to bHLH-LZ factors of the Mad family, and Mad-Max heterodimers can antagonize the effects of Myc/Max by a mechanism involving recruitment of histone deacetylase and prevention of gene transcriptional activation. Constitutive c-Myc expression because of gene activation is the cause of many human cancers, including lung carcinomas and Burkitt’s lymphomas (10). In differentiated cells, including fibroblasts, smooth muscle cells, and hepatocytes, c-Myc overexpression stimulates cell growth and proliferation, decreases cell differentiation, and sensitizes cells to apoptosis (11–14). Interestingly, these effects resemble those observed in \( \beta \) cells chronically exposed to hyperglycemia.
(loss of differentiation, proliferation, and/or hypertrophy depending on the model, apoptosis), which suggests that an increase in c-Myc expression could play a role in the deleterious effects of chronic hyperglycemia on pancreatic β cells. However, little is known about the regulation of c-Myc expression by glucose in pancreatic β cells (15). Therefore, we investigated whether and how glucose regulates β cell c-Myc expression by measuring changes in the mRNA levels of c-Myc, Max, Mad, and some of the putative Myc/Max target genes, ornithine decarboxylase (ODC), thymidine kinase (TK), LDH-A, and cyclin-dependent kinase 4 (CDK4) (10, 16, 17). The study was carried out with both in vivo and in vitro models of hyperglycemia.

**EXPERIMENTAL PROCEDURES**

The effect of hyperglycemia on c-Myc expression was investigated in islets isolated from glucose-infused rats (in vivo hyperglycemia) and in islets or purified β cells isolated from untreated rats and cultured at various glucose concentrations (in vitro hyperglycemia).

**Islet Isolation** — The rats were anesthetized by intraperitoneal injection of 100 mg kg⁻¹ pentobarbital (60 mg ml⁻¹ nembutal; Abbott Laboratories), and their pancreatic islets were isolated by collagenase digestion of the gland as described previously (7). They were then separated from the digest by density gradient centrifugation using Histopaque 1077, microdissected, and hand picked under a stereomicroscope to ensure high purity of the preparation.

**Glucose Infusions and Glucose Clamps** — Male Harlan Sprague-Dawley rats (~250 g) with indwelling jugular vein and carotid artery catheters were obtained from Taconic Farms (Germantown, NY). Following arrival at the animal facility, the rats were observed for at least 3 days to ensure general well being as assessed by weight gain and appearance. The rats were randomly assigned to a glucose-infused group (500 ml glucose per hour) with indwelling jugular vein and carotid artery catheters. The rats were randomly assigned to a glucose-infused group (500 ml glucose per hour) with indwelling jugular vein and carotid artery catheters. The rats were randomly assigned to a glucose-infused group (500 ml glucose per hour) with indwelling jugular vein and carotid artery catheters. The rats were randomly assigned to a glucose-infused group (500 ml glucose per hour) with indwelling jugular vein and carotid artery catheters. The rats were randomly assigned to a glucose-infused group (500 ml glucose per hour) with indwelling jugular vein and carotid artery catheters.

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**RNA Extraction and cDNA Synthesis** — Total RNA was extracted, quantified, and reverse transcribed into cDNA exactly as described (7), using Ultraspec (Biotec Laboratories, Houston, TX) and the kit Superscript II (Life Technologies, Inc.). Control reactions lacking the reverse transcriptase were run in parallel.

**Primers** — Except for the measurements of c-Myc and LDH-A mRNA levels in islets from glucose-infused rats that were performed as described previously (7), pairs of primers were designed for multiplex polymerase chain reaction using Hybimulator 4.0 software (Advanced Gene Computing Technologies, Irvine, CA). The sense and antisense primers were chosen in separate exons and their specificity was checked by BLAST search on the GenBank² data base (Table I).

**Semi-quantitative Radioactive Multiples PCR** — Polymerization reactions were performed with a PerkinElmer 7900 Thermocycler in a 25-µl reaction volume containing 1.5–3 µl of cDNA (10–20-ng RNA equivalents), 80 µM cold dNTPs, 1.25 µCi of [α-³²P]dCTP (3000 Ci mmol⁻¹), 1.25–15 pmol of appropriate oligonucleotide primers, GeneAmp Gold PCR buffer, and 1.25 units of AmpliTaq Gold DNA polymerase (PerkinElmer Life Sciences). The cycling conditions (Table I) were chosen to be in the exponential phase of amplification of each product in control islet cDNA (Fig. 1), as described previously (7). The thermal cycle profile was a 10-min denaturing step at 94 °C to release DNA polymerase activity followed by the amplification cycles (1 min at 94 °C, 1 min at the annealing temperature and 1 min at 72 °C, unless otherwise specified in Table I), and a final extension step of 10 min at 72 °C. In each set, the gene products of interest were amplified with an internal control gene (TATA box-binding protein (TBP)) to correct for experimental variations between samples (TBP mRNA levels remained constant under the different experimental conditions). The amplimers were then separated on a 6% polyacrylamide gel in Tris borate EDTA buffer, in parallel with a 100-bp pair DNA ladder. The gel was dried, and the amount of [α-³²P]dCTP incorporated in each amplimer was quantitated with a Cyclone Storage Phosphor System (Packard, Meriden, CT). The ratio of specific product/control gene was then calculated.

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**TABLE I**

| Gene name | Size | 5' oligonucleotide | 3' oligonucleotide | Primer | AT* | Cycle number |
|-----------|------|-------------------|-------------------|--------|-----|--------------|
| c-Myc     | 484  | CTC TCT CCA GCA   | GGG CAG GCC AAA   | 600    | 28  | 60           |
| Max 1     | 450  | CTC AGG TGA AGA   | GGG AC TGT TCC    | 200    | 24  | 60           |
| Max2      | 427  | CCA GCA         | 200    | 24  | 60  |
| Mad1      | 302  | GCA TCA ACT ACC   | GTC GCC TGG CAT    | 200    | 24  | 60           |
| ODC       | 297  | CCG GGT ACT AC    | 200    | 24  | 60  |
| CDK4      | 527  | CCT GTC GAA ATT   | 200    | 24  | 60  |
| LDH-A     | 518  | TTC TTC TTC        | 50      | 26  | 60  |
| HK         | 233  | CAA GGC CCA GTA   | 200    | 26  | 60  |
| TBP        | 190  | ACC CTC CAA TGA   | 40–200  | 24–28| 59–60| 60           |

* AT, annealing temperature.

* Oligonucleotide primer sequences are from Ref. 35.

* 2-min extension at 72 °C per cycle.
for each sample. Quintuplicate RT-PCR determination of the c-Myc/TBP ratio from the same RNA preparation gave a within-assay coefficient of variation of 5.5%.

The size of amplicons corresponded to the expected ones (published sequences), except for ODC that migrated at ~340 base pairs instead of 297 base pairs in PAGE. However, its size was correct in agarose gel electrophoresis, and its sequence (Eurogentec, Seraing, Belgium) corresponded to the published sequence of rat ODC (GenBank accession number J04792).

Western Blot—Equal batches of islets (~500) were cultured 18 h under various conditions, washed three times with ice-cold phosphate-buffered saline, and precipitated with trichloroacetic acid (100 g liter\(^{-1}\)). After double ether extraction, the pellet was dried and solubilized in modified Laemmli buffer (0.5 g liter\(^{-1}\) SDS, 1:10(v/v) glycerol, 1:40(v/v) β-mercaptoethanol, 62.5 mM liter\(^{-1}\) Tris, pH 6.8, and 50 mg liter\(^{-1}\) bromphenol blue). Total islet proteins were then separated on a 10% SDS-PAGE and electrophoretically transferred to polyvinylidene difluoride membranes (PerkinElmer Life Sciences). After Ponceau S staining to check transfer efficiency, the membrane was blocked for 2 h at room temperature with 10% nonfat dry milk in Tris-buffered saline (pH 7.4) containing 0.05% Tween-20 (NFDM-TBST). It was then incubated overnight at 4 °C in NFDM-TBST containing 0.05% Tween-20 (NFDM-TBST). It was then incubated overnight at 4 °C in NFDM-TBST containing 2 μg/ml anti-c-Myc (N-262) polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA), washed with TBST, and incubated for 1 h at room temperature with horseradish peroxidase-conjugated anti-rabbit IgG antibody (0.1 μg/ml NFDM-TBS). After six washes with TBST, detection of bound antibodies was carried out with the Supersignal West Femto kit (Pierce) using Kodak X-AR films.

Insulin Determinations—Insulin concentrations in the plasma and culture media were determined by radioimmunoassay using rat insulin as standard.

Data Analysis—The results are presented as the means ± S.E. for the indicated number of animals or batches of islets. Statistical significance of differences between groups was assessed by unpaired Student’s t test or by one-way analysis of variance (ANOVA) followed by the test of Newman-Keuls when more than two groups were compared. For mRNA half-life determination, mathematical analysis of the data was done using Prism 3.02 software (GraphPad Software, San Diego, CA).

RESULTS

Effect of Glucose Infusion and Glucose Clamp on Islet c-Myc mRNA Levels—The effect of glucose on islet c-Myc mRNA levels was tested in an in vivo model of hyperglycemia associated with β cell hypertrophy and impaired secretory function, the 50% glucose-infused rat (18, 19). Rats were infused with 500 g liter\(^{-1}\) glucose or 4.5 g liter\(^{-1}\) NaCl solution at a flow rate of 2 ml/h for 24 h. During saline infusion, plasma glucose and insulin concentrations remained stable at ~5.5 mmol liter\(^{-1}\) and ~1 ng ml\(^{-1}\), respectively. In contrast, glucose infusion was followed by large increases in plasma glucose and insulin concentrations up to ~20 mmol liter\(^{-1}\) and ~15 ng ml\(^{-1}\), respectively (Fig. 2A). Compared with control, noninfused or saline-infused rats, glucose-infused rats had an approximately 2-fold increase in islet c-Myc/TBP ratio without significant differences in Max1, Max2, and Mad1/TBP ratios (Table II). An approximately 2-fold increase in the mRNA levels of two c-Myc target genes, ODC, and LDH-A was observed, but two other putative c-Myc target genes, TK and CDK4, remained unaffected by glucose infusion (Table II).

When glucose was infused for 4 days at a constant flow rate, plasma glucose concentrations gradually returned to normal values, whereas a sustained increase in plasma insulin levels was observed (18) (not shown). At the end of that period, the islet c-Myc/TBP ratio was not different from that in control or saline-infused rat islets (n = 2; not shown). In contrast, when plasma glucose was maintained elevated at ~11 mmol liter\(^{-1}\) for 4 days by regularly adjusting the rate of glucose infusion (Fig. 2B), islet c-Myc/TBP ratio was increased ~2-fold compared with islets from matched saline-infused rats. There was a parallel rise in the ODC/TBP ratio but no change in Max1, Max2, Mad1, TK, or CDK4 mRNA levels (Table II).

Changes in Inlet c-Myc Expression Induced by Culture—The islets were cultured for 1 week in RPMI medium containing G10 or G30. Insulin secretion decreased by ~50% at both glucose concentrations over the first 3 days (p < 0.0001 for time effect by two-way ANOVA) but was consistently larger in the presence of G30 versus G10 (p < 0.0001 for glucose effect by two-way ANOVA), indicating good β cell viability (Fig. 3A).

The c-Myc/TBP mRNA ratio was low (0.7 ± 0.2, n = 4) in freshly isolated islets. After 18 h of culture in 10–30 mmol liter\(^{-1}\) glucose,
Levels in Islets Precultured for 1 Week and Reaggregated Purified Rat β Cells—The islet c-Myc/TBP ratio was low in islets precultured in G10 for 1 week (0.48 ± 0.07, n = 5). It did not change after a further 18 h of culture when the glucose concentration remained at 10 mmol/liter−1 (0.52 ± 0.09, n = 13) but increased ~5-fold in the presence of G30 (2.51 ± 0.36, n = 13, p < 0.0001 by unpaired t test). In a subset of five experiments in which the intermediate glucose concentration of 20 mmol/liter−1 was also tested, a clear concentration-dependent effect of glucose on insulin release and islet c-Myc mRNA levels was observed (Fig. 5). Under these conditions, c-Myc mRNA half-life was short and unaffected by glucose (35 ± 4 and 25 ± 1 min in the presence of G10 and G30 respectively, n = 3, no significant difference by unpaired t test) (Fig. 6). Similar to in vivo hyperglycemic conditions, the glucose-dependent rise in the c-Myc/TBP mRNA ratio in vitro was accompanied by parallel changes in c-Myc target genes ODC and LDH-A/TBP mRNA ratios, without changes in Max, Mad1, TK, or CDK4 mRNA levels (Fig. 5). To ascertain that the induction of c-Myc mRNA levels by high glucose occurred in β cells within the islets, β cells were purified, reaggregated, precultured for 3 days in the presence of G10, and further cultured 18 h in the presence of G10 or G30. Compared with β cells kept in G10, the normalized c-Myc/TBP ratio increased from 1.0 ± 0.06 to 1.81 ± 0.18 in the presence of G30 (p < 0.01, n = 4).

Role of Cytosolic Calcium Rise in the Induction of Islet c-Myc Expression by High Glucose—When the extracellular glucose concentration increases, islet glycosylation and mitochondrial metabolism are stimulated. There ensues a rise in the cytosolic ATP/ADP ratio, leading to the closure of ATP-dependent K+ channels, membrane depolarization, and opening of voltage-dependent Ca2+ channels. The subsequent elevation of cytosolic Ca2+ concentration ([Ca2+]i) triggers exocytosis of insulin granules (3). The ATP-dependent K+ channel opener diazoxide
and the voltage-dependent Ca\(^{2+}\) channel blocker nimodipine both inhibited insulin secretion in G10 and G30 and markedly reduced c-Myc and ODC mRNA levels in G30 (Fig. 7A). In contrast, stimulation of Ca\(^{2+}\) entry by high potassium-induced membrane depolarization in the presence of diazoxide to prevent any effect of glucose on β cell membrane potential (21) reproduced the effect of G30 on insulin secretion and islet c-Myc and ODC mRNA levels (Fig. 7B). Again, these effects were completely abolished by nimodipine, confirming their Ca\(^{2+}\) dependence. The effect of G30 on islet c-Myc expression was not mediated by insulin released in the culture medium, because islet c-Myc/TBP ratio and its induction by G30 remained unaffected by the addition of 1 μmol-liter\(^{-1}\) exogenous insulin to the culture medium (not shown).

**Effect of Clonidine and cAMP on Islet c-Myc Expression**—The inhibition of insulin secretion by α\(_2\)-adrenoreceptor agonists results from several mechanisms: a partial repolarization of the β cell membrane with a small decrease in [Ca\(^{2+}\)], (22, 23), a strong inhibition of adenylate cyclase (24), and a major distal inhibition on Ca\(^{2+}\)-induced exocytosis (22, 23). After 18 h of culture in the presence of 1 μmol-liter\(^{-1}\) clonidine, insulin secretion was inhibited by ~85% in G10, G30, and G10/K30/Dz (Fig. 8B). Under these conditions, the c-Myc/TBP mRNA ratio was unaffected in G10, slightly reduced in G30, and unaffected in G10/K30/Dz (Fig. 8A). These effects of clonidine cannot be ascribed to changes in [Ca\(^{2+}\)], because [Ca\(^{2+}\)] measured after overnight culture in G30 or G10/K30/Dz was unaffected by addition of clonidine during culture (data not shown). The membrane-permeable agent dibutyryl-cAMP markedly stimulated insulin secretion and c-Myc mRNA levels in G10 and G10/K30/Dz but was ineffective in G30 (Fig. 8). Under these conditions, clonidine partially inhibited insulin secretion but did not affect c-Myc mRNA levels. An increase in c-Myc protein level was observed in islets stimulated for 18 h with dibutyryl-cAMP in G10/K30/Dz medium versus G10 alone (Fig. 9).
High Glucose Stimulates Pancreatic β Cell c-Myc Expression

Letting previous observations of a rise in c-Myc mRNA levels in glucose-starved insulin-secreting RIN cells restimulated with glucose (15) and in islets from hyperglycemic 90% pancreactomized rats (7). Thus, high glucose increased c-Myc mRNA levels in rat islets in vivo for up to 4 days and in islets and purified pancreatic β cells in vitro. This increase is attributed to a higher transcription of c-myc gene, because c-Myc mRNA degradation was unaffected by glucose. It was accompanied by a rise in c-Myc protein and a parallel increase in the mRNA levels of two well characterized c-Myc target genes, ODC and LDH-A, both in vivo and in vitro. Consistent with these results, both ODC and LDH-A mRNA levels were found to increase in glucose-stimulated MIN6 cells (25), and a parallel increase in LDH-A and c-Myc mRNA levels was observed in islets from hyperglycemic 90% pancreatectomized rats (ODC was not tested in that study) (7). Two other putative c-Myc target genes involved in cell proliferation, TK and CDK4, were not affected by hyperglycemia. The fact that the mRNA levels of only two of four putative c-Myc target genes increased in parallel to c-Myc mRNA is not so surprising, because the list of putative c-Myc target genes largely depends on the methodology used, the cell type, and its degree of differentiation or transformation (10, 13).

It could be argued that G10 is not a basal condition to test the effect of high glucose on gene expression and that a lower glucose concentration should have been used in vitro. However, culturing islets at 5 mmol-liter⁻¹ glucose (G5) is associated with a large increase in β cell apoptosis (5, 26), a condition under which c-Myc mRNA levels are also increased (27). The mechanisms leading to this rise have been characterized and will be presented in another paper; they are clearly independent of changes in [Ca²⁺], or cAMP levels and are apparently related to the low rate of glucose metabolism. It is also important to note that fasting for up to 3 days was not accompanied by an increase in islet c-Myc mRNA levels, suggesting that the rise induced by culture at low glucose (G5) is specific to the in vitro situation (27). Because our in vitro model of hyperglycemia was developed to investigate the possible mechanisms by which hyperglycemia stimulates β cell c-Myc expression in vivo, it was important to choose as a reference the concentration of glucose that gave the minimal level of c-Myc expression in vitro. In this context, we would like to emphasize that the inverted bell-shaped effect of glucose concentration on islet c-Myc mRNA levels in vitro could lead to the erroneous conclusion that glucose does not affect c-Myc expression if only tested at 5 and 20–30 mmol-liter⁻¹ glucose (27).

Potential Mechanisms by Which High Glucose Stimulates c-Myc Expression in Islets—Our results firmly establish that the stimulation of islet c-Myc expression is due to the rise in [Ca²⁺], produced by glucose. Thus, the effect of high glucose on c-Myc expression was reproduced by high potassium-induced membrane depolarization and inhibited by [Ca²⁺]-reducing agents, without remaining effects of high glucose in the presence of fixed low or high [Ca²⁺]. The small stimulatory effect of G10 on c-Myc mRNA levels in the presence of diazoxide, but not nimodipine, was likely due to the incomplete blockade of glucose effect on ATP-dependent K⁺ channels at 50 μmol-liter⁻¹ diazoxide (28), as indicated by the persistence of a significant stimulation of insulin secretion by high glucose under this condition. It should be noted, however, that the reduction of β cell [Ca²⁺], in G10 by diazoxide or nimodipine (supported by the decreased insulin secretion) only caused a small decrease in islet c-Myc expression. These observations suggest but do not prove that c-Myc expression is only stimulated by a large and sustained elevation of [Ca²⁺], as that occurring in G30 or K30 but is not clearly Ca²⁺-dependent in G10, perhaps because [Ca²⁺], is lower or oscillates at that physiological concentration. A similar situation has been observed for the Ca²⁺-dependent increase in β cell apoptosis by G30 versus G10 (5).

How Does the Rise in [Ca²⁺], Lead to Increased c-Myc Expression in Islets?—It is unlikely that the increase in c-Myc expression by [Ca²⁺], results from the stimulation of insulin secretion and accumulation of β cell secretory products in the culture medium for two reasons. First, the addition of a saturating concentration of exogenous insulin was without effect on islet c-Myc expression at both G10 and G30. Second, the large inhibition of insulin secretion produced by clonidine in the presence of high glucose or high potassium was not accompanied by a parallel reduction in c-Myc mRNA levels. The small inhibitory effect of clonidine on the stimulation of c-Myc expression by high glucose probably results from an inhibition of adenylate cyclase and reduction in islet cAMP (24) rather than from an inhibition of [Ca²⁺], for several reasons. First, [Ca²⁺], was not affected by overnight culture in the presence of clonidine. Second, the inhibitory effect of clonidine was no longer observed when cAMP levels were elevated by use of membrane permeable dibutyryl-cAMP. The importance of cAMP for the regulation of islet c-Myc expression is further attested by the stimulatory effect of dibutyryl-cAMP in G10. The lack of effect in G30 seemed to suggest that the stimulatory

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effect of glucose was partly mediated by Ca\textsuperscript{2+} activation of adenylate cyclase (29), but this hypothesis is not supported by the synergistic effect of high potassium and dibutyryl-cAMP on islet c-Myc expression. It is, however, clear that like other early response genes in insulin-secreting cell lines, islet c-Myc expression is Ca\textsuperscript{2+}- and cAMP-regulated. The major difference resides in the fact that islet c-Myc expression lasted much longer than what has been reported for egr-1, c-fos, or nur77 induction (30–32).

Potential Role of c-Myc in Pancreatic β Cells under Physiological and Pathological Conditions—We recently suggested that the increase in c-Myc expression observed in islets from hyperglycemic 90% pancreatectomized rats could be the cause of β cell hypertrophy, loss of differentiation, and loss of function occurring in that model of diabetes (6, 7). The present study sets the basis of that hypothesis by demonstrating the stimulatory effect of high glucose on islet c-Myc expression and by partially identifying the mechanisms involved in that effect. It is also tempting to speculate that c-Myc expression could be involved in the regulation of β cell growth by glucose and cAMP-raising agents (33). Preliminary data about the effect of c-Myc overexpression in mouse pancreatic β cells (11, 34) and the actual view of c-Myc function in the control of cell growth, differentiation, and apoptosis (10) are in favor of our hypothesis.

Conclusion—The expression of c-Myc in rat pancreatic β cells is stimulated by high glucose in a Ca\textsuperscript{2+}-dependent manner and by cAMP. Because c-Myc overexpression has pleiotropic effects on cells (stimulation of growth [hypertrophy/proliferation], sensitization to apoptosis, and decrease of cell differentiation), this effect of glucose could be relevant to β cell mass adaptation to substrate supply under physiological conditions and to β cell loss of differentiation and altered secretory function in states of chronic hyperglycemia.

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