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Nutrient toxicity in pancreatic β-cell dysfunction

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Nutrients, such as glucose and fatty acids, have a dual effect on pancreatic β-cell function. Acute administration of high glucose concentrations to pancreatic β-cells stimulates insulin secretion. In addition, short term exposure of this cell type to dietary fatty acids potentiates glucose-induced insulin release. On the other hand, long-term exposure to these nutrients causes impaired insulin secretion, characterized by elevated exocytosis at low concentrations of glucose and no response when glucose increases in the extracellular medium. In addition, other phenotypic changes are observed in these conditions. One major step in linking these phenotypic changes to the diabetic pathology has been the recognition of both glucose and fatty acids as key modulators of β-cell gene expression. This could explain the adaptative response of the cell to sustained nutrient concentration. Once this phase is exhausted, the β-cell becomes progressively unresponsive to glucose and this alteration is accompanied by the irreversible induction of apoptotic programs. The aim of this review is to present actual data concerning the development of the toxcity to the main nutrients glucose and fatty acids in the pancreatic β-cell and to find a possible link to the development of type 2 diabetes.

Key words: Diabetes, Pancreatic β-cell, Glucotoxicity, Lipotoxicity.

Pancreatic β-cells possess an unique stimulus-response coupling system that requires nutrient metabolism in order to induce electrical membrane events that trigger insulin secretion. Metabolism of the main β-cell fuels (glucose and fatty acids) generates signals that provoke changes at the level of intracellular messengers which elicit Ca^{2+} entry, allowing insulin release (21, 23, 25, 27, 40-42).

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In contrast, sustained elevation of circulating glucose and fatty acids (typical of diabetic states) results in an impaired β-cell function, characterized by an altered pattern of insulin secretion, such as loss of first phase of hormone secretion (11, 35), accompanied by additional phenotypic changes. This general phenomenon has been called toxicity to nutrients: glucotoxicity for glucose or lipotoxicity for fatty acids (35, 43). Therefore, the term toxicity implies irreversible cell alterations caused by chronic exposure to elevated concentrations of the corresponding nutrient. The development of gluco- or lipotoxicity is preceded by an adaptation phase which becomes reversible when physiological nutrient concentrations are restored. In this adaptation phase, insulin hypersecretion, loss of first phase of insulin release and increased glucose sensitivity are displayed as compensatory mechanisms in response to the excess of demand, which in turn leads to refractoriness of the β-cell to glucose stimulatory concentrations (figs. 1 and 2) (21, 27). Sustained hyperglycemia and hyperlipidemia renders the β-cell progressively unresponsive to glucose and this alteration is strongly related to the extracellular nutrient concentration and the duration of the challenge. Finally, once the adaptation phase finishes, β-cell passes a "point-of-no-return", leading to overt toxicity, which is characterized by depletion of insulin stores, impaired insulin biosynthesis and dramatic phenotypic changes (4, 12, 24, 35, 43, 45).

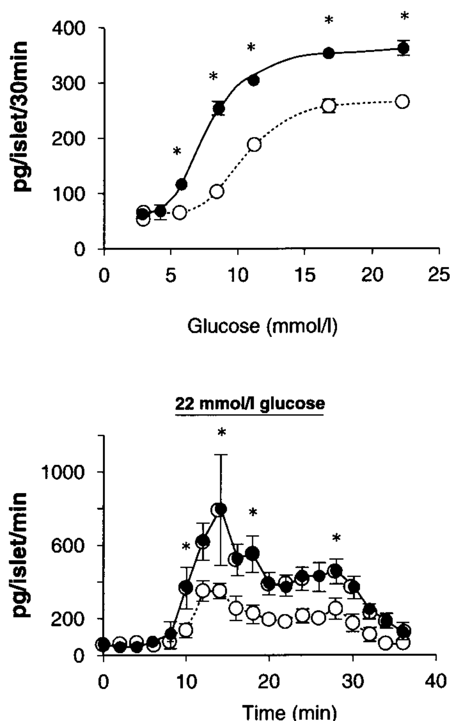

Fig. 1.- *Effect of 60% pancreatectomy (Px) on glucose-induced insulin secretion.*
Top: Islets from control (○) and 60% Px (●) mice were incubated in triplicate for 30 min at 37° C in 1 ml of fresh modified KRB with 1% BSA plus different glucose concentrations. Bottom: Batches of ten islets from control (○) and 60% Px (●) mice were perfused for 10 min with 3 mM glucose, then for 20 min with 22 mM glucose, and finally for 10 min with 3 mM glucose at a flow rate of 1 ml/min at 37 °C. The perfusion medium was fresh modified KRB with 1% BSA. Insulin was assayed by RIA and determinations were run in triplicate. Values are expressed as mean ± SE for seven experiments; $^{*}p < 0.05$ compared with control group. Adapted from 21 with permission.

Glucotoxicity

Elevated circulating glucose has been recognized as a potential factor contributing to β-cell dysfunction, and subsequent development of non-insulin-dependent diabetes mellitus (NIDDM) (17, 43, 47). Pancreatectomized animals and cell culture models have served to unravel the main features of the adaptation process of pancreatic β-cells to hyperglycemia which is characterized by increased glucose sensitivity and augmented insulin secretion, cell hypertrophy and hyperplasia (5, 17, 21).

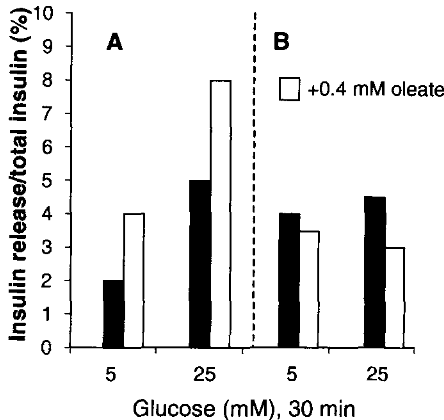

Fig. 2.- *Short (A) and long term (B) effects of glucose (Glc) and oleate on basal and induced insulin secretion.*
Insulin secretion is determined during 30 min by radioimmunoassay (RIA). In A, INS-1 cells were cultured for 3 days at 5 mM basal glucose and insulin secretion was measured during 30 min in the presence of 5 or 25 mM Glc only (■) or with 0.4 mM oleate (□).In B, cells were cultured for 3 days in the presence of high nutrient concentration: 25 mM glucose (■) or 0.4 mM oleate in basal glucose concentration (□) and after, insulin was determinated during 30 min at 5 or 25 mM Glc (■) without or with 0.4 mM oleate (□). Cells incubated in basal conditions at 5 mM glucose for 3 days exhibited a dose-dependent insulin secretion which is potentiated by oleate during the 30 min of secretory stimulus (A). However, cells incubated either at 25 mM glucose or 0.4 mM oleate for 3 days showed an increased basal insulin release and absence of response to high glucose concentrations (B).

Multiple glucose responsive events must be considered in this respect, such as modulation of gene expression and control of diverse enzymatic activities which include not only glycolysis, but other metabolic pathways. Studies performed in animal models and in the β-cell line INS-1 have revealed the main features of β-cell adaptation to extracellular high glucose concentrations (21, 32, 34). These include:

1. Increased glycogen deposition that may provide glucose during glycogenolysis contributing to the generation of metabolic coupling signals that increase glucose sensitivity and trigger insulin secretion, even at low glucose concentrations.

2. Increased glucokinase activity or changes in the hexokinase I/glucokinase proportion, whereas no modification at the level of mRNA is observed. This supports a post-transcriptional modulation mechanism of the enzyme that remains to be identified.

3. Coordinated induction of genes coding for strategic enzymes of glycolysis, anaplerosis and lipogenesis. The glycolytic enzymes include 6-phosphofructo-1-kinase (PFK-1), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and L-pyruvate kinase (L-PK). Increased expression of PFK-1 and L-PK may be required for accelerated glycolytic flux resulting in increased insulin secretion and cell growth. On the other hand, the NADH generated in the step catalyzed by GAPDH may fuel mitochondrial production of ATP and diadenosine polyphosphates, linking the action of glucose on the K_{ATP} channel and Ca^{2+} influx (9, 22).

The chronically elevated levels of citrate and malate at elevated glucose indicate sustained anaplerosis. This is accompanied by an increase in the activities of the key enzymes pyruvate carboxylase and malic enzyme. Anaplerosis should favour mitochondrial metabolism and ATP production, stimulating consequently insulin secretion. Finally, the lipogenic enzymes acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) display a dramatic increase in cells chronically exposed to high glucose concentrations. This increase is related to lipid generation and accumulation, which may be casually linked to lipotoxic events (see point 6).

4. Increased glycolytic flux, glucose utilization and glucose oxidation as a result of the augmented enzyme activities. This might produce subsequent increase in acetyl-CoA which may be used for

energy demand, ATP and diadenosine polyphosphate (22) production or biosynthetic purposes.

5. Inhibition of fatty acid oxidation due to an increase in malonyl-CoA content as a result of an increase in ACC protein expression (7). High levels of malonyl-CoA can inhibit carnitin-palmitoyl transferase I (CPT-I), the key enzyme in mitochondrial lipid β-oxidation. Therefore, malonyl-CoA is used for lipid biosynthesis through the increased activity of FAS. This augmented production of lipids may activate certain protein kinase C (PKC) isoforms or acylate proteins involved in the secretory machinery (1, 46).

6. Finally, the shift of acyl-CoA to esterification processes may be linked to fatty acid deposition leading to a possible link to lipotoxicity. It can be speculated that an increase in lipid esterification could be related to more distal effects. These may include secretory granule generation and cell membrane turnover, processes which could be dramatically exaggerated in hyperglycemic conditions (2).

Therefore the biochemical basis of the β-cell adaptation to hyperglycemia involves, at least in part, activation of main metabolic pathways as a result of activation of key enzymes and the deposition of the two major forms of energy stores: glycogen and triglycerides. However, the metabolic dysfunction described is unable to completely explain the molecular basis for the selective unresponsiveness of β-cells to glucose *in vivo*. This may suggest the involvement of the autonomic nervous system in the *in vivo* development of glucotoxicity (26).

If it can be extended to the *in vivo* situation (21), these phenotypic changes observed during the adaptation period could be related to the hyperinsulinemia described in the first stages of NIDDM in several animal models. This is also accompanied by errors in insulin processing as confirmed by the increased levels of circulating hormone precursors (4, 12, 35). In this period, elevated glucose stimulates β-cell proliferation, although *in vivo* models have demonstrated that progression to glucotoxicity severely affects β-cell turnover. A progression to hypoinsulinemia is observed in these conditions together with an exhaustion of insulin stores and inhibition of cell proliferation (8).

The transcriptional regulation of insulin gene requires the expression of transcription factors which are also instrumental in development and differentiation of endocrine pancreas. Islets from an *in vivo* model of severe hyperglycemia, such as 90% pancreatectomized mice, displayed reduced expression of insulin gene. This observation correlates with selective decreased levels in transcription factors, such as PDX-1, RIPE3b1, HNF3β, HNF1α, HNF4α, Pax6 and Nkx6.1. This is accompanied by an increased expression of c-Myc (16), a transcription factor involved in cell cycle regulation and growth (13). Interestingly, the consensus sequence recognized by c-Myc is identical to the glucose responsive element described in several glucose-modulated genes (44). In addition, key genes coding for proteins involved in control of insulin secretion (including enzymes of glucose metabolism and proteins modulating ion channel activity) displayed gradual decreased expression in 90% pancreatectomized mice. Furthermore, islets from the same animal model present an induced expression of lactate dehydrogenase-A (LDH-A) and hexokinase-I (HK-1), two enzymes barely detectable in differentiated β-cells (16, 37). Altogether, these data support the idea that chronic hyperglycemia triggers the development of an undifferentiated phenotype in pancreatic

β-cell, leading to β-cell dysfunction, impaired insulin biosynthesis and subsequent decrease of secretory function.

In addition to β-cell dysfunction, persistent hyperglycemia correlates with a reduced number in β-cell mass. Apoptotic mechanisms seem to be involved in this process as stated in experiments performed in pancreatic islets from ob/ob mice and Wistar rats. Long term incubation at high glucose concentrations of islets isolated from these animals displayed DNA fragmentation, indicating the induction of apoptosis (29). This cell death process is Ca^{2+}-mediated, because the pharmacological blockade of $[Ca^{2+}]_i$ entry inhibits apoptosis (10).

Altogether, these data support a possible model to explain the development of glucotoxicity. An important point is that glucose toxicity is preceded by an adaptation process (32, 34), which usually fails if high glucose concentration persists. This period is characterized by increased glucose sensitivity, insulin hypersecretion, and β-cell proliferation (28). Return to euglycemic conditions resolves the defect. Subsequently, persistent hyperglycemia leads to the establishment of a "point-of-no-return" characterized more likely by an increase in β-cell apoptosis (8, 29). The remaining cells undergo hyperplasia in order to support increasing insulin demand. Finally, high glucose induces changes in transcription factors implicated in maintaining the differentiated phenotype of the β-cell and provoking severe defects in insulin biosynthesis, processing and secretion (16).

Lipotoxicity

The main accepted hypothesis in lipotoxicity is that increased levels of circulating fatty acids may cause irreversible β-cell alterations that result in the development of obesity-dependent type 2 diabetes (24, 30, 43). Several laboratories have demonstrated that long-term exposure of rat islets to high concentration of fatty acids displayed elevated insulin secretion at low glucose concentration, low levels of proinsulin biosynthesis, exhaustion of insulin stores and impaired ability of β-cell to respond to high sugar concentrations. These features were consistent with the alterations observed in type 2 diabetic animal models and human patients (24, 30, 43).

The Zucker diabetic fatty rat has been extensively used as an adequate model to study the effects of high concentration of non-esterified circulating fatty acids on β-cell function (30, 43). This animal model displays elevated levels of circulating lipids in the prediabetic phase leading to a progressive increase in triglyceride content in β-cells. When overt hyperglycemia appears (9-11 weeks of age), islets present massive triglyceride deposition, lower β-cell mass despite enhance proliferation rate and hyperplasia. This observation suggests that functional and phenotypic changes are operating at the level of β-cell in order to adapt insulin secretion to increased insulin resistance. Finally, this situation arrives to a "point-of-no-return" where β-cell is unable to sustain insulin demands for peripheric tissues, leading to instauration of overt hyperglycemia (24, 30). This β-cell failure appears to be mediated by increased levels of apoptosis instead to reduced levels of β-cell proliferation or neogenesis (38, 39). The excess of lipids is the main factor causing β-cell defects because severe restriction of lipids in diet delays the apparition of overt diabetes in this animal model. Altogether, these data mean that individuals genetically predisposed and exposed during years to high concentrations of circulating non-esterified fatty acids can develop β-cell abnormalities and obesity-associated NIDDM (30).

Development of lipotoxicity is also related to important phenotypic changes which involve the modulation of genes which must be identified. At early stages, fatty acids are very efficient in inducing immediate early response genes (IEGs) which include key transcription factors such as c-Fos, Nur-77 and Zif-268 as well as genes coding for metabolic enzymes (3, 33). Proteins of c-Fos family are capable of heterodimerising with proteins from c-Jun family to form the pleiotropic AP-1 transcription factor (15, 31). The targets for Nur-77 transcription factor are still elusive, although its expression is dramatically increased in T-cell apoptosis (18). Zif-268 is a zinc finger transcription factor that recognizes GC-rich sequences in the promoter of several secondary genes, such as α-myosin heavy chain (15). Products of IEGs regulate secondary genes which are implicated in the modulation of cell proliferation, cell cycle progression and apoptosis.

The transduction mechanism of *c-fos* and *nur-77* induction by the dietary fatty acids in β-cell seems to implicate the participation of intracellular Ca^{2+} increases and PKC activation. Indeed, the phorbol ester PMA (phorbol 12-myristate 13-acetate), which is a strong activator of certain PKC isoforms, induces both IEGs to a similar extent compared with palmitate and oleate (33). If extended to other cell types, this observation suggests that under circumstances of elevated concentration, fatty acids may act as tumor promoters, inducing dramatic phenotypic changes in mutated cells.

Concerning the β-cell and taking into account that the target genes for c-Fos and Nur-77 are still poorly characterized, the accumulated experimental evidence may suggest a possible link between fatty acids and apoptosis. Previous reports indicate that fatty acids induce β-cell apoptosis in Zucker diabetic rats, through the upregulation of nitric oxide synthase (iNOS), overproduction of NO and induction of programmed cell death (38). This observation is consistent with the increase in AP-1 activity which is present and necessary for the induction of iNOS genes (20). An additional study in the same animal model indicates an increased production of the apoptotic intermediate ceramide (39). This observation indicates that palmitate can be incorporated into the biosynthetic pathway of this compound. However higher levels of apoptosis can be also observed in β(INS-1)cells incubated at elevated oleate concentrations (JORDÁN *et al.*, unpublished observation). Taking into account that oleate does not participate in ceramide biosynthesis, it is possible to hypothesize that, aside from ceramide production, a general mechanism is involved in β-cell apoptosis induced by fatty acids. It is possible to postulate a role of mitochondrial dysfunction in the programmed cell death process. Indeed, no alterations at the main cytosolic pathways of β-cell metabolism were noticed when β(INS-1)cells were incubated at high oleate concentrations. However, this observation excludes mitochondria which displays enhanced metabolic activity and an exacerbated rate of respiration under hyperlipidemic conditions (36). This result, together with the instrumental role of mitochondria in the secretory process (19, 30) may explain the altered pattern of insulin secretion in cells incubated for long periods of time at high fatty acid concentrations.

In this context CPT-I gene is transcriptionally induced by elevated fatty acids in β-cell (3). The CPT-I protein is considered as a key enzyme in acyl-CoA partitioning between mitochondrial oxidation and cytoplasmic accumulation as complex lipids and triglycerides. Interestingly the CPT-I gene behaves in β-cells as an IEG, displaying a rapid kinetics of induction (3

h for maximal induction) and robust messenger accumulation in the presence of the protein synthesis inhibitor cycloheximide, both features being characteristic of IEGs. The elevation of CPT-I mRNA was followed by an increase in CPT-I enzymatic activity and FA-oxidation. This observation together with that of the induction of acyl-CoA oxidase (48) may be linked to detoxification processes, in an attempt of the β-cell to degrade toxic fatty acids.

At long-term, sustained exposure of β-cells to elevated concentrations of fatty acids reduces the expression levels of the glucose transporter Glut-2 and lipogenic enzymes ACC and FAS (6, 14). This reduction is linked to a decrease in the metabolic rate of the cell in an attempt to diminish the accumulation of toxic lipids derived from glucose metabolism. Altogether, the data presented from different studies suggest that high concentrations of the main circulating lipids are instrumental in the development of apoptosis and the establishment of type 2 diabetes.

Conclusion

Nutrients have a dual effect on β-cell function. Acute administration of glucose stimulates insulin secretion. This effect is potentiated by dietary fatty acids. However long term exposure of β-cells to either glucose (sustained hyperglycemia) or fatty acids (sustained hyperlipidemia) is related to β-cell dysfunction and death by apoptotic mechanisms. The molecular components of this process are still poorly characterized (figs. 3 and 4). The main caveat concerns the fact that major points of this mechanism have been established

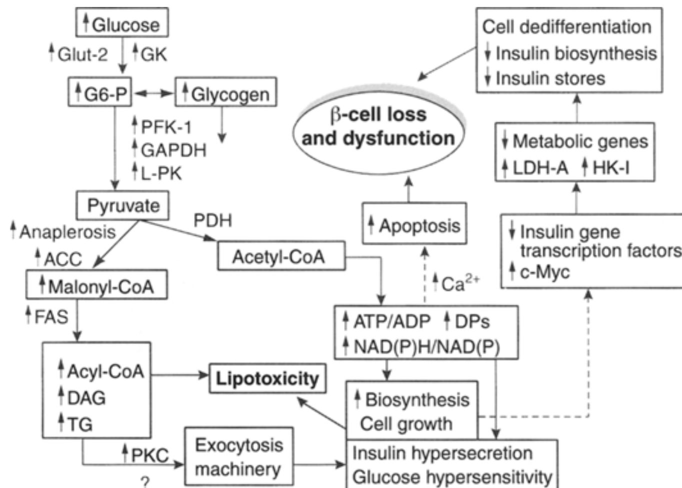

Fig. 3.- *Model illustrating the metabolic events and gene inductive processes contributing to glucotoxicity.* The right half of the figure represents the adaptive process to sustained glycemia. Only the glucose-induced genes coding for glycolytic, anaplerotic and lipogenic enzymes are shown as well as glucose modulated enzymatic activities. The discontinued line represents the "point-of-no-return" from which toxic effects of glucose lead to loss of β-cell differentiation and apoptosis. See the text for more details. ACC: acetyl-CoA carboxylase, DAG: diacylglycerol, DPs: diadenosine polyphosphates, FAS: fatty acid synthase, G6P: glucose-6-phosphate, GAPDH: glyceraldehyde-3-phosphate dehydrogenase, GK: glucokinase, HK-1: hexokinase-1, LDH-A: lactate dehydrogenase-A, PDH: pyruvate dehydrogenase, PFK-1: phosphofructokinase-1, L-PK: L-pyruvate kinase, PKC: protein kinase C, TG: triglycerides, (↑): increased levels, increased activity, (↓): decreased levels, decreased activity, (?) non-well established step.

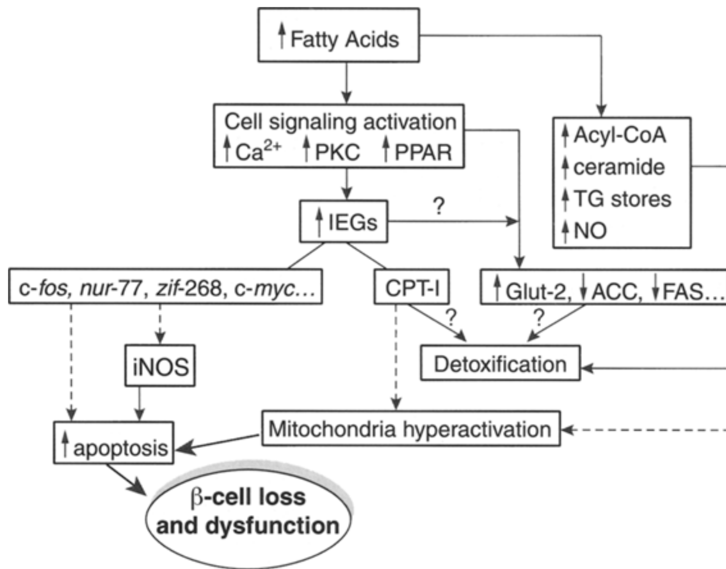

Fig. 4.- *Model illustrating the metabolic events and gene inductive processes contributing to lipotoxicity.* The halfupper part of the figure represents the adaptive process to sustained lipidemia. Only the fatty acid-induced genes as well as fatty acid modulated enzymatic activities are shown. The discontinued line represents the "point-of-no-return" from which toxic effect of fatty acids lead to β-cell dysfunction and apoptosis. See the text for more details. ACC: acetyl-CoA carboxylase, CPT-I: carnitine palmitoyltransferase-I, FAS: fatty acid synthase, IEGs: immediate early genes, iNOS: inducible nitric oxide synthase, PKC: protein kinase C, PPAR: peroxisome proliferator-activated receptor, TG: triglycerides, (↑): increased levels, increased activity, (↓): decreased levels, decreased activity, (?) non-well established step.

in cell culture and animal systems. Therefore, it remains to be determined whether the nutrient toxicity hypothesis applies to human NIDDM. It is very likely, because managing hyperglycemia or using caloric restriction to control plasma levels of fatty acids are very effective in controlling diabetes. In this respect, this knowledge will help to understand the molecular mechanism operating in previous steps before the setting of overt NIDDM and to establish dietary and pharmacological strategies in order to prevent and cure this disease.

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E. ROCHE, I. MAESTRE, F. MARTÍN, E. FUENTES, J. CASERO, J. A. REIG y B. SORIA. *Toxicidad de nutrientes en la disfunción de la célula beta pancreática* (minirrevisión). J. Physiol. Biochem., **56** (2), 119-128, 2000.

Tanto la glucosa como los ácidos grasos tienen un doble efecto sobre la función de la célula β pancreática. La administración aguda de elevadas concentraciones de glucosa a la célula β estimula la secreción de

insulina. Además, una corta exposición de esta célula a los ácidos grasos de la dieta potencia la liberación de insulina inducida por glucosa. Por otra parte, la exposición prolongada a estos nutrientes causa alteraciones en la secreción de insulina, caracterizada por una elevada exocitosis a bajas concentraciones de glucosa y una falta de respuesta cuando la glucosa incrementa en el medio extracelular. Además, otros cambios fenotípicos aparecen en estas circunstancias. Un paso importante a la hora de relacionar estos cambios fenotípicos con la patología diabética ha sido la identificación de la glucosa y los ácidos grasos como controladores esenciales en la expresión génica en la célula β. Esto podría explicar la respuesta adaptativa de la célula a las elevadas concentraciones de nutrientes. Una vez esta fase se ha agotado, la célula β se vuelve progresivamente insensible a la glucosa y esta alteración viene acompañada por una inducción irreversible de programas apoptóticos. El objetivo de esta revisión es presentar datos actuales concernientes al desarrollo de la toxicidad a los principales nutrientes glucosa y ácidos grasos en la célula β pancreática y encontrar una posible relación con el desarrollo de la diabetes tipo 2.

Palabras clave: Diabetes, Célula β pancreática, Glucotoxicidad, Lipotoxicidad.

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