Glucose Toxicity

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INTRODUCTION

The term â€œglucose toxicityâ€ was originally coined to describe the adverse effects of chronic exposure of pancreatic Î²-cells to high concentrations of glucose (1,2). First suggested in Î²-cells by the observations of Haist in 1940 (3), the notion that high glucose exerts multiple pathological effects on many cells and tissues has been established by abundant evidence for its causative role in the chronic microvascular complications of diabetes (4-6), its effects on insulin action in metabolic target tissues (7,8), and in several other adverse outcomes noted in people with diabetes, such as frequent fungal infections (9) and an increased frequency of congenital birth defects (10). This chapter will review the contribution of high glucose to Î²-cell dysfunction and to insulin resistance, two key components in the pathogenesis of type 2 diabetes. The cellular and molecular mechanisms will be outlined, and these mechanisms compared to those thought to contribute to the chronic complications of diabetes. Since these mechanisms appear to overlap to a considerable extent, the term â€œglucose toxicityâ€ can be, and is currently used more broadly, to describe the pathogenic role of high glucose on multiple organ systems (Table 1).

Table 1: The spectrum of glucose toxicity	
Eyes	Retinopathy (microaneurysms, hemorrhages, exudates, neovascularization)
Kidneys	Nephropathy (albuminuria, nephrotic syndrome, hyporeninemic hypoaldosteronism, end stage renal disease)
Nerves	Neuropathy (distal sensory Â± motor neuropathy, mononeuritis multiplex, autonomic neuropathy, amyotrophy, chronic demyelinating immune polyneuropathy)
Skin/Mucous Membranes	Microvascular lesions, necrobiosis lipoidica diabeticorum, staphylococcus/streptococcus infection/cellulitis, fungal infections
Fetus	Macrosomia, congenital anomalies (neural tube defects), shoulder dystocia

Pancreas	Endocrine – decreased insulin secretion, Î²-cell failure Exocrine – decreased digestive enzyme synthesis and secretion
Insulin target tissues	Insulin resistance in fat, muscle and liver
Vascular system	Atherosclerosis, endothelial cell dysfunction (decreased vasodilatation), restenosis

PANCREATIC Î²-CELLS

The hypothesis that hyperglycemia may contribute to defects in insulin secretion and potentially, to the well documented deterioration of Î²-cell function in type 2 diabetes (T2DM) commonly referred to as Î²-cell exhaustion, resulted from clinical observations. Thus, it was noted that improvement of glycemic control in subjects with T2DM by either lifestyle changes (diet and exercise) (11), oral hypoglycemic agents (eg. sulfonylurea) (12), or even short term insulin therapy (13), resulted in an improvement in insulin secretion. The effects of high glucose on Î²-cell function, gene expression and survival have now been extensively investigated both in animal models and cell culture.

One very useful rodent model in the study of glucose toxicity has been the partial pancreatectomized rat (14,15). After 90% pancreatectomy rats develop mild fasting hyperglycemia and glucose intolerance. Insulin secretion in response to glucose becomes severely impaired. Treatment of these rats with phloloridzin, an inhibitor of renal tubular glucose reabsorption which results in normoglycemia, completely restored normal insulin secretion expressed per gram of pancreatic mass (16) (Fig. 1).

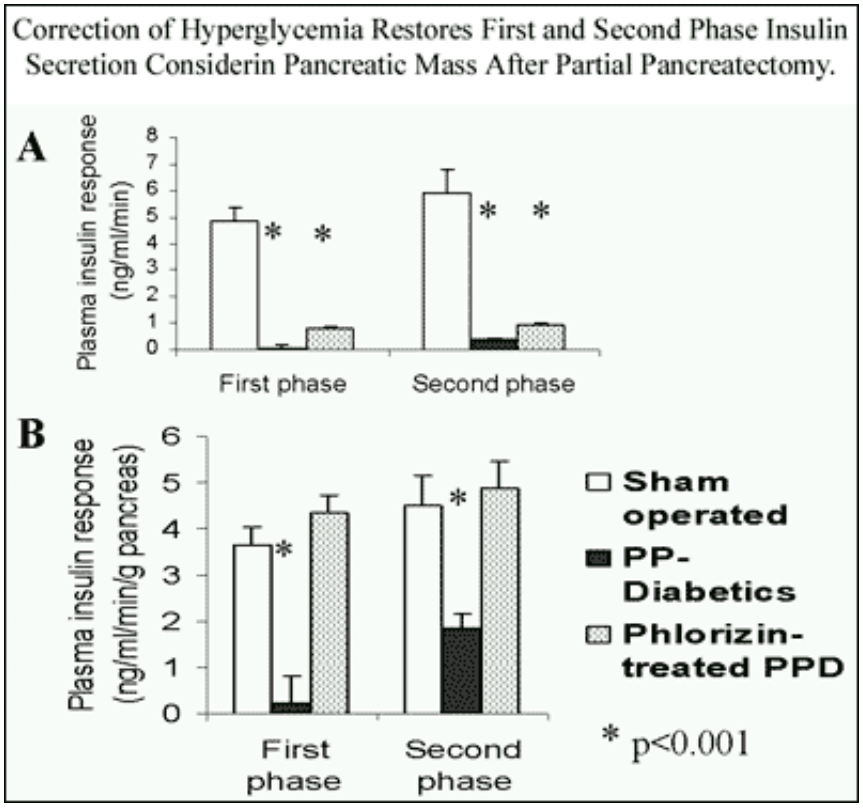

Figure 1.To determine the effects of hyperglycemia on insulin secretion the 90% partial pancreatectomized rat model has been studied. In this model significant hyperglycemia occurs by 2 weeks. The insulin secretory responses were assessed at 6 weeks by a glucose infusion designed to maintain a constant level of hyperglycemia after fasting (~216 mg/dl, 12 mmol/L) and both first phase (first 10 min) and second phase (10-60 min) responses were expressed as the mean increment in insulin concentration above basal. The drug phlorizin, administered from 2-6 weeks, normalized plasma glucose in the partially-pancreatectomized diabetic (PPD) rats [fed plasma glucose (mg/dl), control 143 Â± 2, PPD 284 Â± 13, Phlorizin-treated PPD (142 Â± 6]. In panel A, it is noted that insulin secretion in absolute terms was markedly decreased in PPD and phlorizin-treated PPD rats. However, Panel B demonstrates that when corrected for residual pancreatic mass, maintenance of normoglycemia with phlorizin prevented the impairment in insulin secretion indicating Î²-cell â€œglucose toxicityâ€. (Adapted with permission from ref. 16).

Apart from its direct stimulatory action to release insulin, glucose potentiates Î²-cell insulin secretion in response to nonglucose stimuli. The amino acid arginine (Arg) has been frequently used to study this phenomenon. In the 90% pancreatectomized hyperglycemic rat, early on, the response to Arg was increased, i.e. potentiated as expected, but returned to normal upon treatment with phloridzin. These observations have been confirmed in rats subjected to neonatal streptozotocin+ injection which causes mild to moderate insulin deficiency and hyperglycemia at 6-8 weeks of age. In this model maintenance or restoration of normal glucose levels by insulin supplementation improved both the acute insulin response to glucose and the potentiated insulin response to nonglucose stimuli (17,18).

+Streptozotocin is a Î²-cell toxin

In 60% pancreatectomized rats, glucose levels remain normal (19), confirming the observation that > 85 â€“ 90% of pancreatic islet function must be lost in a â€œnormalâ€ rat or human before diabetes is observed. However, feeding these rats 10% sucrose in their water caused mild hyperglycemia and a subsequent 75% reduction in insulin secretion (19). The minimal elevation (average 0.83 mmol/L, 15 mg/dl) of glucose required to produce this response demonstrates the marked sensitivity of the Î²-cell to desensitization, at least in the presence of reduced Î²-cell mass. In intact rats, as short as 48 h exposure to high glucose, average 14 mM, by glucose infusion, impaired subsequent glucose-induced insulin release (20). Thus sustained hyperglycemia, namely elevation longer than that used to stimulate an acute insulin response, over a relatively short period of time, 2 days to ~ 6 weeks, results in impaired, but reversible, insulin secretion. However, in the absence of glucose normalization irreversible changes in Î²-cell function follow. For example, 50 â€“ 80% partially pancreatectomized dogs did not develop diabetes over 8 â€“ 9 months. Additional exposure to 2 weeks of glucose infusion irreversibly impaired insulin secretion and induced diabetes (21). This model is reminiscent of the progressive decline in Î²-cell function seen in human T2DM.

Pathophysiology and Phases of Hyperglycemia-induced Î²-cell Dysfunction

To characterize the physiology and molecular changes caused by long term exposure to high glucose, Weir and colleagues have used the partial pancreatectomized rat. Based on studies of: 1) glucose-stimulated insulin secretion (GSIS), 2) gene expression of transcription factors, metabolic enzymes and proteins involved in the stress response and apoptosis (programmed cell death), and 3) morphological changes, it has been proposed that the Î²-cell response to increased insulin demand and hyperglycemia can be classified into 4 phases of adaptation (Fig.2) (22). Although these phases exist as a continuum with overlapping features and a variable time course, the classification is useful to investigate and identify the cellular and molecular basis of Î²-cell dysfunction. Briefly, phase 1 is characterized by successful adaptation to increased demand which appears to be mediated by increasing the residual Î²-cell mass by hypertrophy and to a lesser extent, hyperplasia and by a lower set point of insulin secretion in response to glucose. Î²-cell function otherwise remains normal. Phase 2 is classified as mild decompensation, in which GSIS is impaired while response to other secretagogues is relatively preserved. This phase is observed at minimally elevated glucose, eg. fasting glucose of 5.6 mmol/L (100 mg/dl), i.e. before the development of overt diabetes. Insulin stores and insulin mRNA levels are preserved, suggesting that the defect is at the level of secretion.

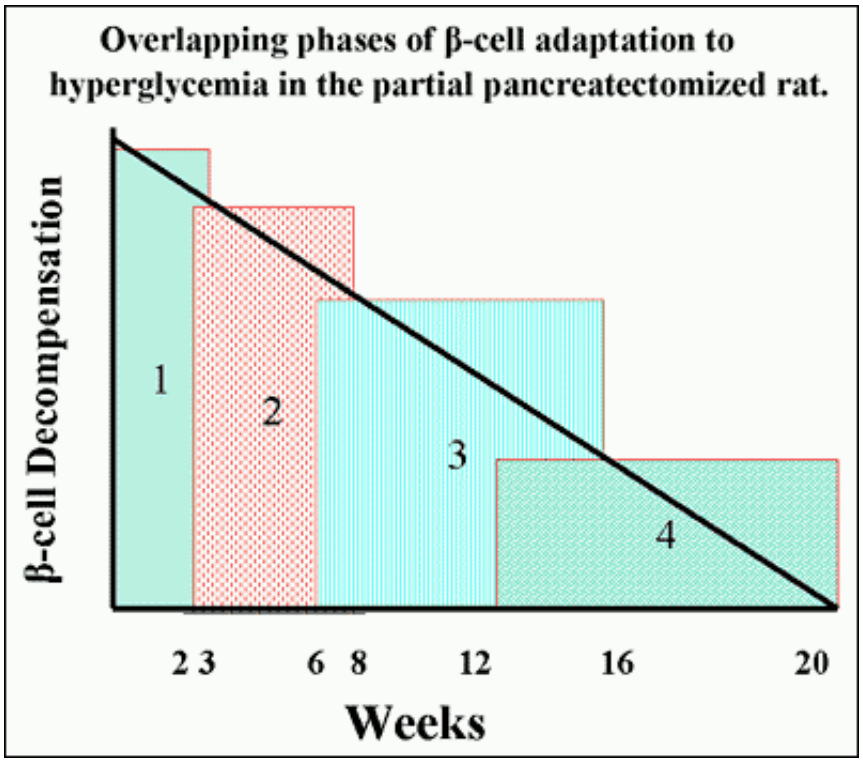

Figure 2.The adverse effects of high glucose on pancreatic Î²-cell function have been described in the partial pancreatectomy rat model. The changes in function and structure have been divided into 4 phases. Phase 1 is â€œsuccessful adaptationâ€ and occurs from 0 â€“ 3 weeks post-pancreatectomy. Phase 2 is â€œmild decompensationâ€ and occurs between week 2 and 8. Phase 3 is â€œsevere decompensationâ€ and is observed between week 6 â€“ 16. Phase 4 is â€œÎ²-cell failure with structural damageâ€ and occurs between week 12 â€“ 30. (See text

for details).

The mechanism by which high glucose desensitizes the Î²-cell to subsequent stimulation by glucose in phase 2 is not clear. Most studies have documented that glucose uptake and metabolism/oxidation by the Î²-cell, at least in the earliest phases of desensitization, remain normal (2,23). Since insulin stores are maintained, it has been proposed that coupling between glucose signaling and the secretory process is impaired. A key element here is the ATP-dependent K+ channel which is regulated by the cellular ratio of ATP/ADP. Thus, closure of the channel in response to glucose is dependent on ATP generation (see Chapter 3), which is regulated not only by the flux of glucose through the oxidative pathway but also by the efficiency of the electron transport chain and the coupling of oxidative phosphorylation. It has been found that uncoupling protein-2 (UCP-2) is upregulated in Î²-cells in rodent models of diabetes (24), and that elevated UCP-2 impairs GSIS (25). Another proposed mechanism of impairment of oxidative phosphorylation is the relative depletion of NADH by increased conversion of pyruvate to lactate (22,26). This may result from upregulation of LDH (lactate dehydrogenase) as part of a generalized Î²-cell dedifferentiation program initiated by exposure to high glucose (see below) (27). Oxidation of NADH would reduce electron transport and ATP generation. Another possibility is that rather than (or in addition to) decreased generation of ATP, increased consumption of ATP is a consequence of hyperglycemia. In the GK (Goto-Kakizaki) diabetic rat, a model of type 2 diabetes, glucose oxidation is elevated in the presence of diminished insulin release (28). It has been found that glucose cycling, i.e. the phosphorylation of glucose to glucose-6-phosphate (G6P) by glucokinase followed by dephosphorylation back to glucose by glucose-6-phosphatase (G6Pase), is significantly elevated in the islet of GK rats. Glucose cycling which has been termed a â€œfutile cycleâ€, consumes one molecule of ATP with each cycle. This increase of glucose cycling was associated with elevated G6Pase activity. Correction of hyperglycemia with phloridzin in the GK rat normalized G6Pase activity associated with significantly improved insulin secretion (29).

Two other hypotheses previously proposed to explain high glucose â€“ mediated desensitization are impaired membrane phosphoinositide hydrolysis (30), now considered unlikely (31), and elevated PGE2 (prostaglandin E2) which has been documented to inhibit insulin secretion (33). The contribution of this mechanism requires further investigation.

Phase 3 occurs in response to a longer duration of high glucose exposure and is termed severe decompensation. At this stage not only is GSIS impaired, but responses to nonglucose stimuli are also decreased. Î²-cell insulin mRNA is now decreased, degranulation is evident and a host of changes in gene expression are observed. Some of these changes are postulated to reflect Î²-cell dedifferentiation since a large subset of the downregulated genes code for proteins which regulate the specific insulin secretory function of the Î²-cell, e.g. the uptake, metabolism and response to glucose and the synthesis of insulin (Table 2) (34). Another set of genes which are affected in this setting are classified as â€œstressâ€ genes. These include genes coding for pro- and anti-oxidant enzymes and pro- and anti-apoptotic proteins. The relevance of the stress genes relates to the known induction of cellular oxidative stress by high glucose which likely

contributes to the pathogenesis of glucose toxicity (see below). In the final or 4th phase, Î²-cell decompensation is accompanied by structural damage. Microscopic changes may include amyloid deposits, glycogen and/or lipid deposits, and in some cases, fibrosis. The relative importance of apoptosis versus loss of Î²-cells by limited replication to the loss of Î²-cell mass is not clear. The role of amyloid as a mediator or consequence of Î²-cell dysfunction remains controversial (35). The frequent observation of lipid (triglyceride) accumulation in Î²-cells in rodent models of obesity-associated T2DM, along with the documentation of a Î²-cell â€œtoxicâ€ effect of excess circulating FFA (free fatty acids), has given rise to the term lipotoxicity (36).

Table 2. A partial list of islet expressed genes altered by hyperglycemia (adapted for 34)

	Downregulated Expression	Upregulated Expression
Transcription Factors	PPARÎ± SREBP-1c Transcription Factor Beta 2 PDX-1 HNF1Î±, HNF3Î², HNF4Î± NkX6.1/PaX 6	PPARÎ³ PPARÎ´ C/EBP-Î² c-Myc
Lipid Metabolism/Transport	Acyl CoA Oxidase Malonyl CoA decarboxylase (NS)	Acetyl CoA Carboxylase Fatty Acid Synthase Hormone Sensitive Lipase Carnitine Palmitoyl Transferase-1
Lactate Production/Transport		Lactate Dehydrogenase (A) Monocarboxylate Transporter â€“ 1 Monocarboxylate Transporter â€“ 2 Monocarboxylate Transporter – 3
Mitochondrial Proton Transport/ATP Synthesis	ATP-Synthase Î± (NS) ATP-Synthase Î² (NS) mGPDH (mitchrondrial	Uncoupling protein – 2

	glycerol phosphate dehydrogenase)	
Islet Hormone/Metabolism Enzymes	Insulin Glucagon (NS) GLUT2 Glutamate Dehydrogenase (NS) Glucokinase Kir6.2 (ATP dependent K+ channel) Islet amyloid polypeptide (IAPP) SERCA3, SERCA2B (Sarcoendoplasmic reticulum Ca++ – ATPase Voltage â€“ dependent Calcium Channel a1D Inositol phosphate 3 receptor	Glucose-6-phosphatase Fructose-bisphosphatase-1 Fructose-bisphosphatase-2 12-lipoxygenase Cycloxygenase-2 Hexokinase
Stress/Apoptosis Genes	Bcl-2 (NS)	Inducible Nitric oxide synthase Heme oxygenase-1 Cu/Zn superoxide dismutase (NS) Mn superoxide dismutase Fas Antiapoptotic A20

Lipotoxicity

Since it is beyond the scope of this chapter to discuss lipotoxicity in detail the reader is referred to recent reviews (36â€“38). It is important to mention that elevated FFA, in the short term, appears to stimulate insulin secretion (39), while in the longer term impairs GSIS, both in vivo

and in vitro (40-42). This has been termed lipotoxicity and appears to relate to an impairment of lipid oxidation and shunting of FFA to the esterification or synthesis pathway. Thus, Prentki and Corkey have proposed that the LC-CoA (long chain fatty acyl Co-enzyme A) and/or one of its metabolic products, e.g. DAG (diacylglycerol), generate signals to impair insulin secretion (36,38). Although these intermediates are known to activate PKC (protein kinase C), the precise mechanism of lipotoxicity is not clear. Another metabolic product of FFA, specifically of the saturated FA, palmitate, is ceramide, an activator of phosphatases, kinases and a pro-apoptotic factor (42,43). It is significant that in the gene expression studies of Weir, high glucose resulted in increased Î²-cell expression of PPARg (peroxisome proliferator-activated receptor-g), which is a transcription factor which stimulates the expression of genes involved in fatty acid and triglyceride synthesis and storage, while downregulating PPARa, a transcription factor which promotes expression of genes encoding proteins involved in FFA oxidation. The net result would be an intra-islet metabolic state consistent with lipotoxicity and enhanced FA esterification. In this context treatment of islets with leptin, the adipocyte-derived satiety hormone, stimulated FA oxidation, reduced Î²-cell triglyceride content and improved insulin secretion in rodent models of obesity and diabetes (44).

The relative importance of glucotoxicity versus lipotoxicity in T2DM remains controversial. In a recent attempt to resolve this issue Robertson treated ZDF (Zucker diabetic fatty) rats, which are mildly hyperglycemic and hyperlipidemic and develop overt diabetes between 6 and 12 weeks of age, with either phloridzin to restore and maintain euglycemia or bezofibrate to treat the hyperlipidemia. Despite remaining hyperlipidemic, the phloridzin-treated rats were protected from Î²-cell decompensation, while the fibrate treatment, which did not alter glucose levels, was without effect (45). These results indicate that Î²-cell toxicity is mediated primarily by glucose but can be exacerbated by increased FFA or high fat diet. However, elevated FFA in the presence of low glucose (such as might occur during starvation) would not result in Î²-cell toxicity, presumably because FA oxidation remains active (46). In humans, hyperlipidemia, specifically hypertriglyceridemia and low HDL, is commonly associated with T2DM. Thus, the extent of the contribution of FFA to Î²-cell dysfunction and failure in the common form of T2DM in humans is not completely defined.

Genetic Contributions

It has long been appreciated that not all individuals with obesity and insulin resistance develop diabetes and that a positive family history of the disease indicates a genetic predisposition. A role for Î²-cell genetic defects in the etiology of diabetes is best demonstrated by the MODY (maturity-onset diabetes of the young) syndromes (47) (see Chapter 10). Although the most common of these is caused by a mutation in the gene encoding glucokinase (MODY-2), the major regulator of glucose metabolic rate in the Î²-cell, a number of the other forms are associated with mutation of transcription factors. One such transcription factor is IPF1/PDX-1 (pancreatic duodenal homeobox-1) which is a key regulator of insulin gene expression (48), and associated with MODY4 (49). In an animal model of type 2 diabetes, the gerbil or sand rat, Psammomys obesus, diabetes develops rapidly when the animal is switched from its native low energy diet to standard rat show (50,51). The time course of the phases of adaptation outlined above is greatly contracted in this model, and recent studies indicate that an absence of PDX-1

is responsible (52). When exposed to acute hyperglycemia, P. obesus islets do not increase insulin gene expression and insulin depletion follows fairly rapidly (50-52). In addition, these defects are reversed in isolated islets by PDX-1 gene transfer (52). The relevance of this exaggerated model, namely, total lack of PDX-1 and marked sensitivity to hyperglycemia-induced Î²-cell failure, is illustrated by the diminished activity of PDX-1 observed in other rodent models, e.g. exposure to FFAs (53), partial pancreatectomy (54), and in vitro in cultured insulin-secreting HIT-T15 cells exposed to chronic high glucose (55).

In human subjects, the best illustration of the influence of genetic predisposition to pancreatic Î²-cell decompensation has been documented in the population of Oji-Cree natives in Northern Ontario, Canada (56). In this genetically relatively homogeneous population, T2DM has increased over the past 2 generations from < 5% to almost 50%. This has been associated with obesity, high fat diet and sedentary lifestyle (57). A search for inherited traits revealed that a proportion of the population were heterozygous or homozgous for a mutation in the HNF-1a gene, the same gene associated with MODY-3 in other populations (47). The effect of harbouring the mutation was not to cause, but rather to accelerate the onset of diabetes. Thus heterozygotes developed T2DM about 7 years earlier than those without a mutation, while homozygotes did so ~ 14 years earlier (Fig.3) (56). Similar to PDX-1, HNFs are downregulated by hyperglycemia in the partial pancreatectomized rat (Table 2). These data support the concept that the sensitivity of the Î²-cell to decompensation in the presence of chronic mild hyperglycemia, for example in a state of impaired glucose tolerance (IGT), is influenced by genetic and likely, environmental factors. Unraveling these will ultimately lead to new therapeutic targets to prevent Î²-cell decompensation.

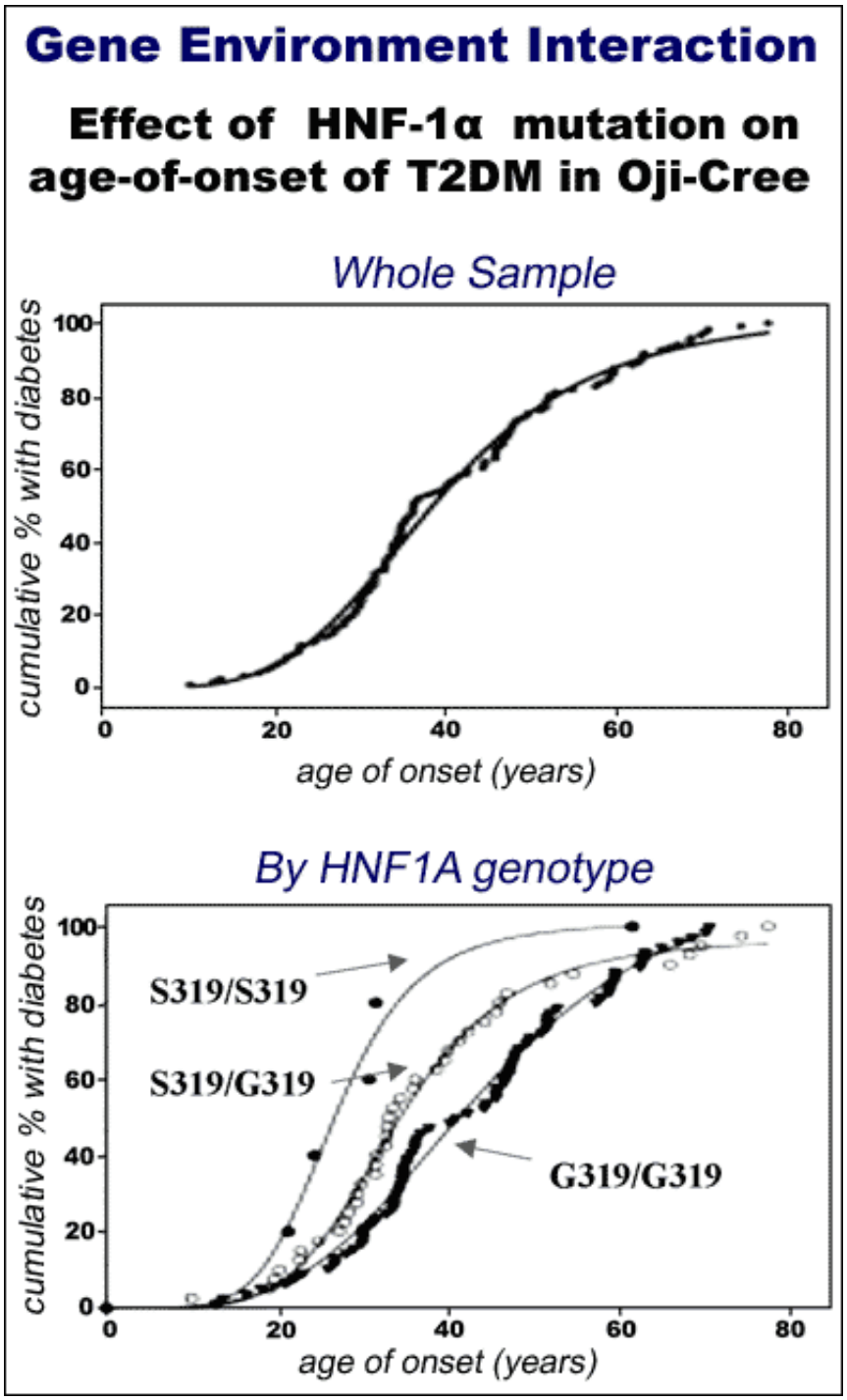

Figure 3.An example of gene-environment interaction in the pathogenesis of diabetes. The incidence of obesity and type 2 DM has increased dramatically in the native Oji-Cree population in Northern Canada. A glycine to serine (G/S) mutation in the transcription factor HNF-1Î±, which regulates insulin gene expression, was found in a proportion of this population. The effect of this diabetes susceptibility gene is to accelerate the onset of diabetes caused by lifestyle factors. In A, the age of onset of DM is plotted for the entire diabetic population, while in B, the population is divided into those with wild-type (G/G) HNF-1Î±, heterozygotes (G/S) HNF-1Î±, and homozygous mutant (S/S) HNF-1Î±. Each copy of the mutant HNF-1Î± accelerates the onset of diabetes by ~ 7 years. (Reproduced with permission from ref 56).

Time course of Î²- cell glucose toxicity

As outlined above there are few formal studies of the time course of hyperglycemia-induced Î²-cell toxicity. In vivo models differ, with progression to Î²-cell failure in the partial pancreatectomy model occurring over weeks to months, in the ZDF rat over 1 â€“2 months (58) and in P. obesus over days. In vitro studies of cultured HIT-T15 cells (a hamster insulin-secreting cell line) revealed a continuous, slow and progressive diminution of insulin secretion and insulin content over months upon exposure to high glucose. This deterioration was reversible until passage 92 but became irreversible at passage 99 (59). (Passaging of cells refers to the subculturing of a confluent dish or flask of cells into equal aliquots, e.g. 1:4, and then feeding these subcultures to allow growth and proliferation until each again reaches confluence. This can generally vary from 3 â€“ 10 days). The precise signal which determines irreversible damage is not clear but likely involves failure of Î²-cell replication and/or the induction Î²-cell death. In humans, the UKPDS (United Kingdom Prospective Diabetes Study), which followed subjects with T2DM over 12-14 years clearly demonstrated deterioration of Î²-cell function over time, and apparently, this was similar in the intensively managed and conventionally treated groups (60). The fact that the HbA1C was lower by 0.12% in the intensively treated group could be interpreted to mean that glucose toxicity did not contribute to this progressive Î²-cell failure. However, this difference in HbA1C hides the fact that the absolute HbA1C values in both groups were still significantly elevated. The median HbA1C rose above 7% after approximately 3 years follow-up in the conventional group and after 5 years in the intensively treated group. Furthermore, these values continued to increase over time. As discussed above, the data in rodents and humans indicate that the Î²-cell is extremely sensitive to very small, sustained increases in circulating glucose concentrations. Thus, one would speculate that the dose-response of Î²-cell glucose toxicity is shifted to the left (i.e. more sensitive), when compared to that demonstrated for the microvascular complications (see the Diabetes Control and Complications Trial) (5).

PERIPHERAL INSULIN TARGET TISSUES

Insulin resistance is a well described characteristic of individuals with T2DM and is present prior to the onset of overt hyperglycemia (1,2 and see Chapter 9). However, it was reported in 1977 that induction of diabetes in the dog model with alloxan, a Î²-cell toxin, resulted in insulin resistance (61). Over the next decade it became apparent that insulin resistance was not only a precursor, but also a consequence of diabetes (62, 63). A significant observation was that correction of hyperglycemia with phloridzin (an inhibitor of renal tubular glucose reabsorption) which does not alter insulin levels, normalized insulin action in 90% pancreatectomized rats (Fig. 4) (7). In addition, human subjects with poorly controlled T1DM were also found to be insulin resistant, and institution of intensive insulin therapy to normalize glucose concentrations restored insulin sensitivity (64). Together, these data demonstrated that peripheral insulin target tissues in vivo could be desensitized to insulin by high glucose. These studies utilized the hyperinsulinemic-euglycemic clamp technique, a highly sensitive measure of in vivo insulin-

stimulated muscle glucose uptake (65). This idea has been supported by other in vivo reports and in vitro studies in muscle tissue and adipocytes. Significantly, incubation of skeletal muscle tissue (66-68) and adipocytes (8) in the presence of increasing medium glucose concentrations leads to insulin resistance of glucose uptake. This has been associated with a decrease in both the total number of glucose transporters and the ability of insulin to stimulate their translocation (68,69). Since the ability of glucose to enhance its own uptake independent of insulin, i.e. by mass action, has also been found to be downregulated, and the fact that this phenomenon has been observed in non-insulin target tissues such as brain (70,71), implies that basal glucose transporters such as GLUT1, as well as GLUT4 (the insulin-sensitive glucose transporter) are downregulated. Recent data support these concepts.

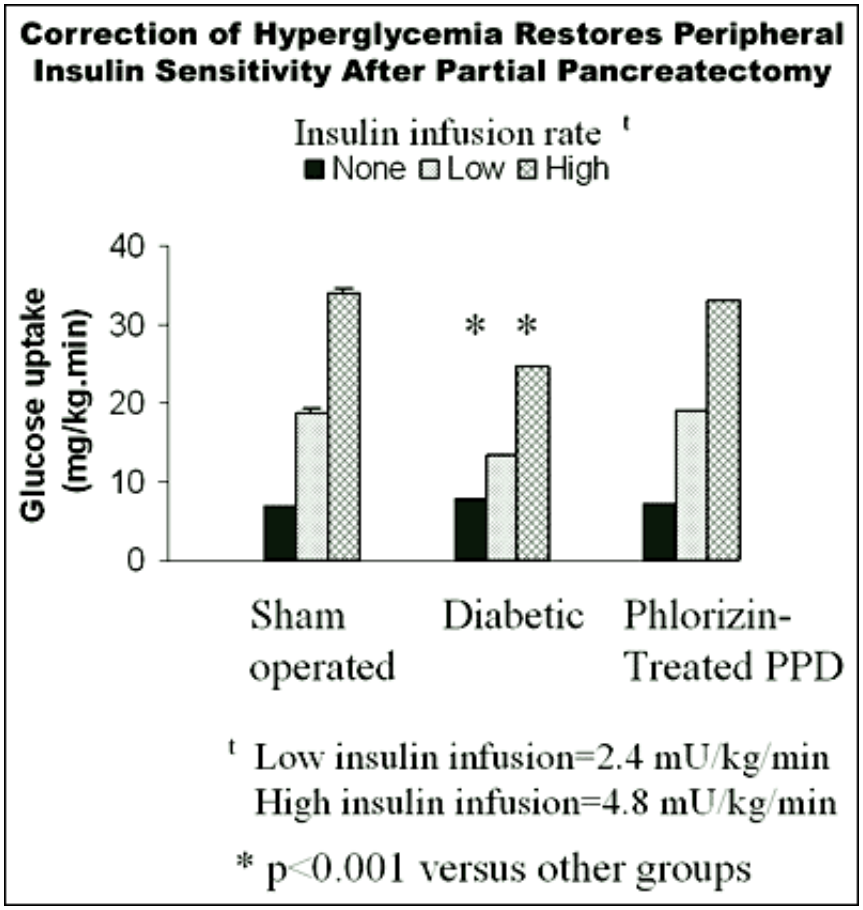

Figure 4.The effects of hyperglycemia on insulin sensitivity was demonstrated in the pancreatectomized rat model which is described in Figure 1. Euglycemic clamp studies were performed at 2 different rates of insulin infusion to measure whole body insulin sensitivity 6 weeks post-pancreatectomy. The insulin levels achieved during the infusions were similar in all groups, 77 â€“ 82 ÂµU/ml at the low dose, and 154-174ÂµU/ml at the high dose. Whole body glucose uptake was significantly decreased in the diabetic group by about 30%, while phlorizin administration, which maintained normal glucose concentrations, prevented this impairment of insulin action. Furthermore, when phlorizin was stopped, and hyperglycemia allowed to occur, the defect in insulin action was documented within 2 weeks (not shown). Phlorizin itself had no effect on insulin sensitivity. (Reproduced with permission from ref. 7).

It is important to note that high glucose-induced insulin resistance requires increased glucose uptake and metabolism, and therefore, in the peripheral insulin target tissues muscle and fat, is accentuated by increasing amounts of insulin (72, 81). In the in vivo studies of pancreatectomized rats, liver insulin responsiveness was unaffected (7). Under the clamp conditions employed hepatic glucose production was nearly completely suppressed by insulin. Thus, whether the hepatic insulin resistance found in T2DM can be exacerbated by hyperglycemia was not adequately addressed. In more recent studies, evidence has been provided that insulin signaling is impaired by hyperglycemia in the liver (73). So, although clearly not all hepatic metabolic abnormalities observed in T2DM are caused by high glucose, there is likely a substantial contribution of hyperglycemia to insulin resistance, as observed in muscle and adipose tissue.

CELLULAR AND MOLECULAR MECHANISMS OF GLUCOSE TOXICITY

A number of cellular abnormalities resulting from exposure to high glucose have been observed in both pancreatic Î²-cells and peripheral insulin target tissues: These include: 1) enhanced oxidative stress, 2) increased flux of glucose through the hexosamine biosynthesis pathway (HBP), 3) activation of ser/thr kinases such as PKC (protein kinase C) and 4) altered gene expression (Table 3). It is noteworthy that many of these same signaling pathways and/or pathophysiological changes have been documented to occur in non-insulin target tissues which are subject to the long term complications of diabetes, namely vascular endothelial cells, and cells of the kidney, retina and peripheral nerves (74-76). Thus, the data suggest that there is overlap among the mechanisms of â€œglucose toxicityâ€ of Î²-cells, insulin metabolic target tissues, and that of other organs and tissues which manifest the chronic complications of diabetes. In addition, these mechanisms have been reported in tissues subject to pathological responses to high glucose such as the developing embryo (77,78).

Table 3: General Mechanisms of Glucose Toxicity	
1. Oxidative Stress 2. Activation of Protein Kinase C (PKC) 3. Increase flux through the Hexosamine Biosynthesis Pathway (HBP) 4. Formation of Advanced Glycation Endproducts (AGEs) 5. Altered Polyol Pathway Flux 6. Changes in gene expression (directly or	

as consequence of any of the above)	

Pancreatic Î²-cells

In discussing the Î²-cell, it should be noted that there are differences among authors in the definition of â€œglucose toxicityâ€. In most reports, although it is recognized that changes in insulin secretory responses, pancreatic insulin content, gene expression and irreversible Î²-cell injury occur in a continuum reflective of the duration of high glucose exposure, all of these processes are encompassed by the term â€œglucotoxicityâ€. However, Poitout and Robertson (79) have emphasized that early Î²-cell secretory refractoriness after short term exposure to high glucose may be viewed as a physiological adaptation and should be termed â€œglucose desensitizationâ€. Medium term exposure results in â€œÎ²-cell exhaustionâ€ in which releasable insulin is depleted. The term â€œglucose toxicityâ€ was reserved for progressive and irreversible Î²-cell failure resulting from long term exposure. In this chapter the term is used to describe the widespread effects of exposure to high glucose, recognizing the varied mechanisms, tissue responses and time courses of these effects.

As alluded to above, one mechanism for which substantial evidence has been provided is â€œoxidative stressâ€. Oxidative stress refers to the presence of an excess amount of reactive oxygen intermediates (ROIs), due to an imbalance between their formation and degradation. The presence of oxidative stress caused by high glucose is well documented in many tissues subject to complications, e.g. endothelial cells (80), nerve cells (81), proximal renal epthelial cells (82), as well as Î²-cells (83). Recent studies support the important role of oxidative stress in Î²-cell failure. First, treatment of ZDF (Zucker Diabetic Fatty) rats with the antioxidant, NAC (N-acetylcysteine) between 6 and 12 weeks of age, prevented Î²-cell failure and ameliorated hyperglycemia (84). In vitro, similar treatment of HIT-T15 cells or isolated islets exposed to high glucose for several months preserved insulin gene expression and glucose stimulated insulin secretion (84,85). These data were reproduced in another rodent model of type 2 diabetes, the db/db mouse (86). Other in vitro studies demonstrated that while inhibition of GSH (glutathione) synthesis in islets augmented ribose-induced oxidative stress and decreased insulin mRNA and secretion, overexpression of the antioxidant enzyme, glutathione peroxidase, using adenovirus-mediated gene transfer, protected human islets from ribose toxicity (87). Notably, part of the Î²-cell response to hyperglycemia is an increase in gene expression of various antioxidant enzymes, namely heme oxygenase-1, glutathione peroxidase, Cu/Zn SOD (superoxide dismutase) and Mn-SOD (Table 2) (22). This observation in the rat partial pancreatectomy model suggests that a compensatory protective response to the increased oxidative stress may occur and contribute to the prolonged time course observed for â€œglucose toxicityâ€ to become irreversible (59).

There have been few studies investigating of the role of increased flux via the HBP in the induction of Î²-cell glucose toxicity. Overexpression of GFA (glutamine fructose-6-phosphate amidotransferase), the rate-limiting enzyme of this pathway (Fig.5) in a Î²-cell specific manner in mice resulted in hyperinsulinemia, insulin resistance and (in male mice) the eventual development of mild type 2 diabetes (88). Furthermore, GFA overexpression in rat islets using adenovirus, or exposure of islets to glucosamine which bypasses GFA and greatly augments

flux through the HBP, impaired glucose-stimulated insulin secretion and reduced Î²-cell gene expression (e.g. insulin, GLUT 2) (89). Interestingly, it was found that these maneuvers increased oxidative stress and that the antioxidant, NAC, was able to block these effects (89). Thus, although enhanced protein O-GlcNAcylation (the covalent and reversible modification of proteins on Ser and/or Thr residues by addition of single moiety of N-Acetylglucosamine has been suggested to mediate the effects of increased HBP flux (90, 91), its exact role remains to be defined.

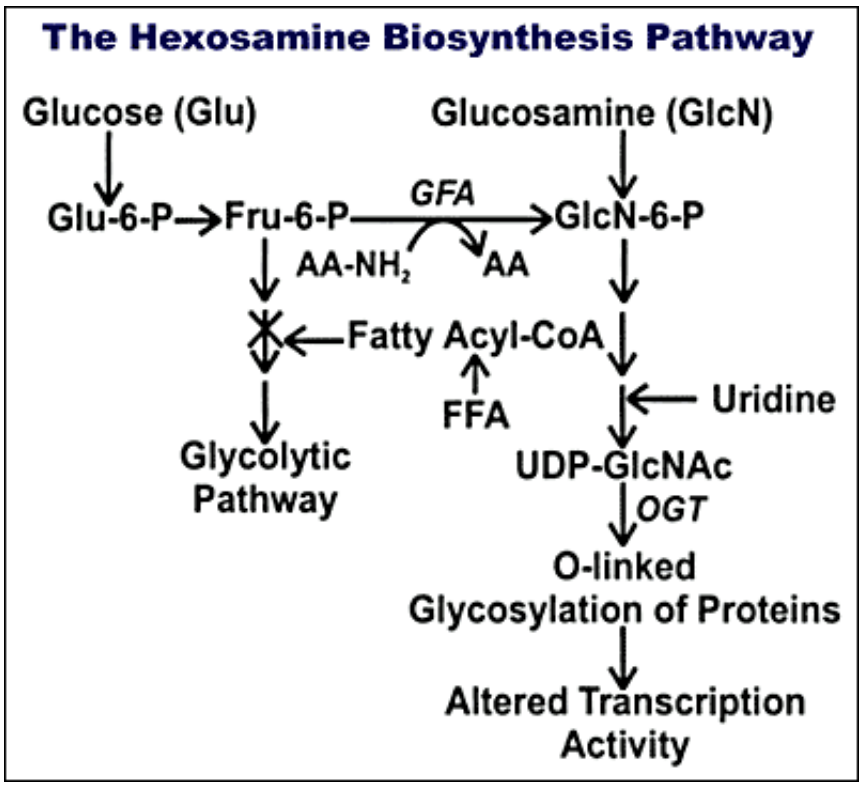

Figure 5.The hexosamine biosynthesis pathway (HBP) splits from the glycolytic pathway with the reaction of fructose-6-phosphate (F6P) with the NH2 donor amino acid, glutamine, being converted to glucosamine-6-phosphate (GlcN6P) by the rate-limiting enzyme, glutamine fructose-6-phosphate amidotransferase (GFA). Subsequent reactions result in the formation of UDP-GlcNAc (uridine diphosphate N-acteylglucosamine) which is utilized as a substrate for N- and O-linked glycosylation. It is intracellular protein O-glycosylation mediated by OGT (O-linked GlcNAc transferase) which has been postulated to contribute to glucose toxicity by altering gene expression. Many studies use glucosamine as an agent to drive this pathway to investigate the consequences of increased flux through the HBP. Glucosamine bypasses the rate limiting GFA.

In the pathogenesis of diabetes complications, the activation of the b isoform of PKC appears to be particularly important (92), and currently, clinical trials in humans of a PKC-b inhibitor are underway (93). It has now been documented that the PKC-b isoform is also activated by high glucose in Î²-cells, and that expression of a PKC b2-isoform specific dominant-negative mutant

in islets blocked the induction of c-myc (94). The transcription factor c-myc is known to be upregulated by high glucose in various diabetic models and appears to mediate a number of the changes in Î²-cell gene expression seen in high glucose (22, 79, 95). A recent study demonstrated that enhanced HBP flux activates PKC-b in glomerular mesangial cells (96). While this has not yet been demonstrated in Î²-cells, these data suggest a close interaction amongst the 3 mechanisms discussed above, namely oxidative stress, flux through the HBP and activation of PKC.

Peripheral Insulin Target Tissues

Similar to the Î²-cell, in vitro and in vivo studies of muscle and adipose tissue have implicated oxidative stress, the HBP, and PKC. Incubation of rat adipocytes in the presence of high glucose and insulin induced insulin resistance which could be blocked by GFA inhibitors (97). Furthermore, exposure to glucosamine in the presence of low concentrations of insulin reproduced the insulin resistance (97). There has been some controversy about the mechanism and relevance of the glucosamine effect (98), however more recent data demonstrate that insulin resistance can be induced by PUGNAc [O-(2-acetamido-2-deoxy-D-glucopyranosylidene) amino-N-phenylcarbamate], an inhibitor of N-acetylglucosaminidase, which blocks deglycosylation and thus turnover of the O-GlcNAc modified proteins (99). In vivo, overexpression of GFA in transgenic mice causes insulin resistance (100). Infusion of glucosamine into rats increases flux through the HBP, induces insulin resistance and has been proposed to increase modification of IRS-1 by O-GlcNAc (101,102). As described in detail in Chapter 2, insulin signals its metabolic effects by stimulating autophosphorylation of its cell surface receptor and its subsequent activation as a tyrosine protein kinase. The IRK (insulin receptor kinase) phosphorylates IRS (insulin receptor substrate) proteins 1 and 2 on tyrosine residues which results in the recruitment of signaling proteins with SH2 (Src homology 2) domains. One of the most important of these is the lipid kinase, phosphatidylinositol-3-kinase (PI3K), which phosphorylates the 3 position of phosphatidylinositols such as PI(4,5) P2 to generate PI (3,4,5) P3. The latter phospholipid recruits other proteins to membranes via their PH (pleckstrin homology) domains. Ultimately serine kinases such as akt/PKB (protein kinase B) and PKC-z are activated. This pathway, responsible for glucose transport and glycogen synthesis, may be inhibited at multiple steps in various states of insulin resistance. The effects of hyperglycemia have been reported to involve IRS-1 and/or akt/PKB (67,69,102-105). It is not clear whether O-GlcNAc on IRS-1 is a direct cause of either its decreased tyrosine phosphorylation or decreased binding of the p85 regulatory subunit of PI3-kinase.

There are several studies in cultured cells as well as in muscle and adipose tissue implicating activation of the Ser kinase, PKC, by high glucose as a contributor to insulin resistance (68,103, 106-108). The resistance appears to be mediated at the level of the insulin receptor (106,107,109) and/or IRS-1 (110). Ser phosphylation of IRS-1 has been demonstrated to impair its tyrosine phosphorylation and, in some cases, convert IRS-1 from an effective substrate to an inhibitor of the IRK (111). Cotransfection of various conventional PKC (cPKC) and novel PKC (nPKC) iosforms with the insulin receptor and IRS-1 demonstrated that most isoforms, particularly the novel PKC-d and -e, inhibit the IRK only in the presence of IRS-1 (112).

The activation of the cPKCs and nPKCs is dependent on DAG (diacylglycerol) (113). It has been found that DAG levels are elevated in muscle tissue in insulin-resistant hyperglycemic rodent models (114). It has been proposed that excess glucose flux to glyceraldehyde-3-phosphate is shunted towards DHAP (dihydroxyacetone phosphate) and DAG synthesis. At the same time, it has been found that high glucose also increases malonyl-CoA, thus inhibiting CPT-1 (carnitine palmitoyl transferase-1) and fatty acid oxidation. The resultant increase in LCFA (long chain fatty acyl)-CoA will also promote the generation of DAG. Exposure of rat adipocytes to high glucose (30 mM) has been associated with an impairment of IRK activity which was blocked by PKC inhibitors (107).

While IRS-1 is a key signaling intermediate subject to multiple forms of regulation, e.g. phosphorylation, O-glycosylation and enhanced degradation (99,102,109-112, 115), it should be noted that in some studies exposure of target tissues to high glucose has been found to be associated with insulin resistance at a step distal to IRS-1 and PI3K, namely akt/PKB (67,73,105,115). The mechanism of inhibiton of akt/PKB in the context of normal PI3 kinase activation is not clear (68), but in the case of exposure to the fatty acid palmitate, an increase in akt/PKB dephosphorylation has been reported (116). This has been attributed to increased ceramide synthesis in response to excess palmitate (117). Exposure to high glucose alone has not been associated with elevated ceramide (68). However, a recent report suggests that in human subjects with diabetes, elevated ceramide concentrations may exist in peripheral insulin target tissues (118). Further work is necessary to sort out the varied signaling defects contributing to the insulin resistance induced by hyperglycemia.

Several studies suggest that oxidative stress may play a role in the pathogenesis of insulin resistance (119-122). First, exposure of cells directly to H2O2 causes insulin resistance (121,122). Second, exposure to high glucose has been associated with oxidative stress in many tissues and cell types (123,124) and recently, in adipocytes (125). Third, antioxidant treatment of rats exposed to short term hyperglycemia was able to prevent insulin resistance (126). Finally, several small studies in human subjects with T2DM showed improvement in insulin resistance with oral vitamin E (127) or intravenous vitamin C treatment (128). ROIs have been associated with activation of multiple signaling pathways (129-133), including activation of PKCs (132), and altered gene expression (133). Thus, it is plausible that oxidative stress contributes to â€œglucose toxicityâ€ in insulin target tissues but its precise role remains to be defined.

Microvascular and other complications

It has been well documented that the chronic microvascular complications of diabetes along with fetal malformations are caused by hyperglycemia. The clinical and pathological features of diabetic retinopathy, nephropathy and neuropathy are described in chapters 33, 34 and 35, respectively. The current views of the molecular mechanisms of these complications share similarities with those of â€œglucose toxicityâ€ discussed above. Thus the major contributors appear to be: 1) increased flux of glucose through the aldose reductase (polyol) pathway, 2) the DAG-PKC activation pathway, 3) the AGE (advanced glycation endproduct) formation pathway and 4) the hexosamine biosynthesis pathway (reviewed in 76). In addition, the importance of high glucose-induced oxidative stress has been emphasized. Various sources of increased

ROS include, 1) glucose autoxidation in the presence of metal ions (134), 2) a byproduct of formation of AGEs via 2-deoxyglucosone and/or methylglyoxal (135), 3) increased glucose oxidation in mitochondria (136), and 4) depletion of NADPH via the polyol pathway (137) and by inhibition of glucose-6 phosphate dehydroxygenase (G6PDH) by HBP flux, which increases the concentration of the G6PDH inhibitor, glucosamine – 6- P (138). Decreased NADPH limits the regeneration of the intracellular antioxidant reduced glutathione (GSH) by glutathione (GSSG) reductase (139). In some subjects additional susceptibility to oxidative stress may result from a genetically determined decrease in endogenous antioxidant enzymes, e.g. catalase, SOD (superoxide dismutase) (140).

Recently, the role of oxidative stress has been proposed to be primary. Thus increased mitochondrial glucose metabolism leads to increased ROS which inhibit glyceraldehyde phosphate dehydrogenase (GAPDH) blocking the glycolytic pathway (136). The increase in upstream metabolites redirects metabolic flux through the pathways mentioned above (Fig.6), which in turn lead to alterations in cell signaling and gene expression associated with complications. Evidence supporting this concept includes the finding that normalization of increased flux and reversal of several outcomes is achieved by inhibition of mitochondrial ROS generation (78,91,136), as well as by shunting of metabolites to the pentose phosphate pathway by treatment with benfotiamine (lipid soluble thiamine) (141) (Fig.6). Furthermore, antioxidant treatment has been demonstrated to have salutory effects on high glucose induced Î²-cell dysfunction (84-87), insulin resistance (126-128), neuropathy (142,143), retinopathy (143,144), and congenital malformations (145,146). Whether these novel observations will lead to a better treatment and improved outcomes remains to be determined

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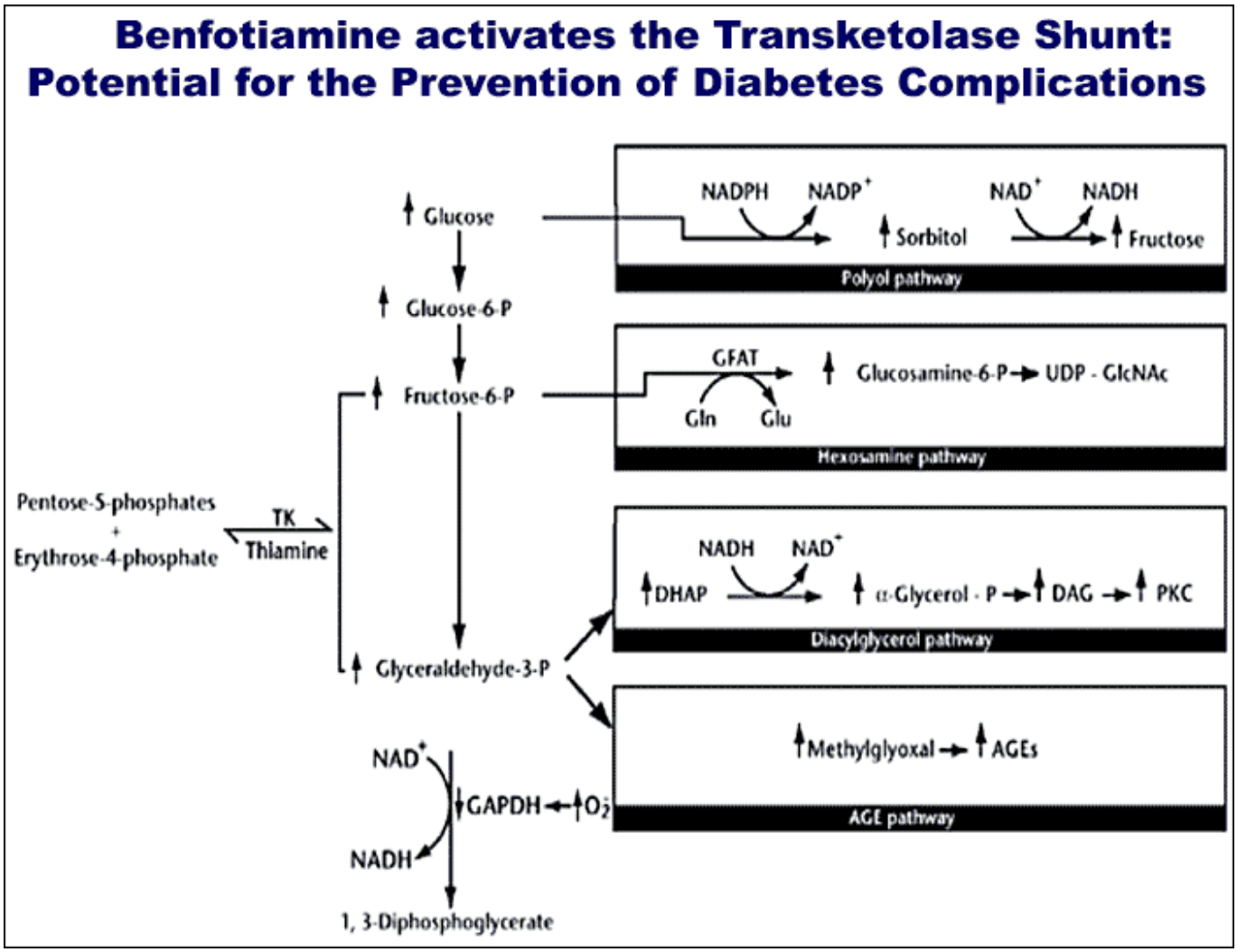

Figure 6.A unifying hypothesis has been postulated to explain the pathogenesis of the microvascular complications of diabetes. The contribution of these pathways to glucose toxicity in the Î²-cell and peripheral insulin target tissues is likely, but remains to be well defined. Briefly, in this model increased mitochondrial glucose metabolism results in the increased generation of ROS (reactive oxygen species). These in turn inhibit the key glycolytic enzyme GAPDH (glyceraldehyde 3-phosphate dehydrogenase), both directly (it is redox sensitive) as well as by decreasing intracellular [NAD+]. A block at this site results in increased glucose flux through various pathways, namely the polyol pathway, the HBP, the diacylglycerol synthesis-PKC activation pathway and the AGE (advanced glycation endproduct) pathway. The consequences of the products of these pathways are cell and tissue toxicity, i.e. dysfunction and damage. A recently proposed mechanism to reverse these fluxes is to enhance transketolase activity with large doses of thiamine, a cofactor. This would convert the 6- and 3-carbon sugars to products metabolized by the pentose phosphate pathway. (Reproduced with permission from ref. 141).

CLINICAL IMPLICATIONS

The clinical implications of â€œglucose toxicityâ€ are clear. Since hyperglycemia leads to insulin resistance, and in subjects with pre-existing insulin resistance, is an exacerbating factor, and because high glucose also leads to impaired insulin secretion and contributes to Î²-cell failure, it is evident that meticulous control of glycemia should be an early goal of therapy in diabetes. Whether better glucose control is able to preserve endogenous insulin secretion in type 1 or type 2 diabetes in humans is not proved. However, insulin resistance, which presents a stress on the Î²-cell, has been associated with more rapid deterioration of Î²-cell function in type 1 diabetes (147). On the other hand, in the UKPDS study, apparent Î²-cell function deteriorated at similar rates in conventional and more intensively controlled groups in type 2 diabetes. Since the Î²-cell is sensitive to even small elevations in circulating glucose concentrations, it is possible that the glycemic control achieved in the intensively treated group in the UKPDS was not adequate to prevent glucose toxicity.

In the case of microvascular complications, it has been well documented that intensive therapy to lower glucose in both types of diabetes and importantly, by any manoeuver in T2DM, i.e. diet/exercise/oral hypoglycemic agents/insulin, will lead to improved outcomes. In summary, glucose toxicity may be viewed as a general pathophysiological process in diabetes, encompassing adverse effects on multiple tissues, with at least overlapping, if not identical molecular mechanisms. Current and future treatment strategies (Table 4) are based on these concepts.

Table 4: Prevention of the Consequences of Glucose Toxicity		
Current	Potential	
ACE Inhibitors Angiotensin Receptor Blockers Statins ASA Aldose Reductase Inhibitors* (only available in some countries) Metformin (?)**	Antioxidants PKC Inhibitors AGE formation inhibitors Benfotiamine (Thiamine)	
* Most studies to date show limited benefit. ** Metformin was found to have a protective effect against microvascular complications which was out of proportion to its blood glucose lowering effect in the UKPDS.		

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