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REVIEW

Glucose toxicity: The leading actor in the pathogenesis and clinical history of type 2 diabetes — mechanisms and potentials for treatment

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Oxidative stress

Abstract *Aim:* Although it is now well established that the deleterious effects of chronic hyperglycaemia (i.e., glucose toxicity) play an important role in the progressive impairment of insulin secretion and sensitivity, the two major actors of the pathogenesis of type 2 diabetes mellitus, the precise biochemical and molecular mechanisms responsible for the defects induced by glucose toxicity still remain to be defined.
Data synthesis: here we will briefly report on convincing evidence that glucose toxicity acts through oxidative stress, modifications in the exosamine pathway, protein kinase C and others. After inducing or contributing to the genesis of type 2 diabetes, these same mechanisms are considered responsible for the appearance and worsening of diabetic specific microvascular complications, while its role in increasing the risk of cardiovascular diseases is less clear. Recent intervention studies (ADVANCE, ACCORD, VADT), conducted to evaluate the effects of strict glycaemic control, apparently failed to demonstrate an effect of glucose toxicity on cardiovascular diseases, at least in secondary prevention or when diabetes is present for a prolonged time. The re-examination, 20 years later, of the population studied in the UKPDS study, however, clearly demonstrated that the earliest is the strict glycaemic control reached, the lowest is the incidence of cardiovascular diseases observed, including myocardial infarction.
Conclusion: The acquaintance of the role of glucose toxicity should strongly influence the usual therapeutic choices and glycaemic targets where the reduced or absent risk of hypoglycaemia, durability of action, and data on prolonged safety should be the preferred characteristics of the drug of choice in the treatment of type 2 diabetes mellitus.

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In 1940 Charles Best proposed the idea that hyperglycaemia could alter the β cell function with pioneering descriptive alterations in glucose transporter and in glucose response in pre-diabetic and type 2 diabetic patients [1–3]. The first experimental evidence in humans was based on the description of the amelioration of abnormalities related to high glucose levels including insulin secretion and sensitivity, after normoglycaemia was achieved [4–6]. Nevertheless, the most influential studies used phloridzin as a tool to reverse the hyperglycaemia.

Dihydrochalcone phloridzin is a natural product and dietary constituent found in a number of fruit trees. Phloridzin's principal pharmacological action is to produce renal glycosuria through inhibition of the sodium-glucose symporters located in the proximal renal tubule. Thus, its main effect is the reduction of glycaemia with no direct amelioration of insulin secretion or sensitivity. Interestingly, this mechanism of action is presently used in a new class of hypoglycaemic agents: the sodium-glucose transporter 2 (SGLT-2) inhibitors.

In partially pancreatectomised rats, a model of the hyperglycaemia condition, Rossetti et al. noted that normalisation of blood glucose levels after phloridzin treatment restored insulin secretion and insulin sensitivity [7,8]. The same glycaemia-normalising treatment improved insulin-mediated glucose transport in adipocytes without restoring glucose transporter gene expression [9]. This relationship between hyperglycaemia and β cell dysfunction was further supported by other in vivo and in vitro studies in which the restored physiological glucose levels (however achieved) determined a recovery of β cell function and glucose-mediated insulin secretion [4–6,10]. Further, Kim et al. showed a significant reduction in whole body glucose uptake in mice with a specific inactivation of GLUT 4 in muscle. This change involves muscle, liver and adipose tissue. After phloridzin treatment, the impairment in muscle glucose utilisation was still decreased, but the uptake in the other tissues was restored [11], thus confirming the role of glucose toxicity in impairing glucose uptake.

In vitro studies used islets and perfused-pancreas preparations from obese diabetic rats [12] and rats rendered diabetic by streptozotocin injection [10–14]. They showed a marked impairment in insulin secretion and response to glucose. The restored normal glucose levels, reached by using insulin infusion or lowering the perfused glucose concentration, normalised the impaired insulin response.

In order to further define glucotoxic mechanisms in vivo, investigators have used both multiple models of empirical hyperglycaemia and animal models with spontaneous hyperglycaemia. The former includes the administration of streptozotocin in the early stages of life [13,15], partial pancreatectomy [16,17], acute or chronic glucose infusion [18], inhibition of the sodium-dependent glucose transporter [19], the latter one involves GK rats [20], Zucker diabetic fatty (ZDF) rats [21] and *Psammomys obesus* fed on a high-energy diet [22]. Rossetti et al. performed a meal-tolerance test and used hyperglycaemic clamps on partially pancreatectomised rats with the result that glucose metabolism was strongly impaired and was associated with a decreased ability of beta cells after an acute glucose challenge. The first phase of insulin secretion was totally blunted and the second markedly impaired. Insulin responsiveness came back to normal after treatment with phloridzin [23]. These abnormalities have been further demonstrated in non-diabetic rats in a chronic hyperglycaemic state (glucose infusion for 72 h) [18,24], with the same reversibility after normoglycaemic intervention.

From these first research steps, hyperglycaemia, however obtained, has been described as a condition responsible for reduced responsiveness to the glucose stimulus of β cells, with impaired response and altered secretion. This hypothesis is further supported by the finding that restored normoglycaemic status, reached through different ways, restores the defects of the insulin-releasing cells related to chronic exposure to high glucose levels.

It is clear from the above mentioned experiments that different variables are involved in the metabolic effects of hyperglycaemia; considering the metabolic machinery as a single system including muscle, adipose tissue and liver, it should be reasonable that they interact in an inter-tissue circuitry resulting in adaptations to glucose overload (''metabolic flexibility''). Thus, the term glucose toxicity also represents the whole metabolic effect of prolonged exposure to pathological glucose levels: pancreatic function deterioration is only one of the deleterious effects exerted by prolonged hyperglycaemia, but we should consider these all together as a unique alteration of the effect on insulin sensitivity and all the tissues altered by hyperglycaemia.

Nevertheless, even as several studies have been carried out in the last 40 years, there is no unifying theory explaining the mechanism of the deleterious effects of glucose toxicity. The cellular and biochemical proposed mechanisms include increased flux through the hexosamine pathway, elevation of the inflammatory state, oxidative stress, toxic effect of increased levels of malonyl-CoA, diacylglycerol, enhanced activity of protein kinase C, disruption in the insulin-secreting machinery and reduced activity of voltage-dependent calcium channels.

Hexosamine pathway

Almost 20 years ago, Steve Marshall first investigated the role of hexosamines, after the observation that the presence of glutamine was required for glucose-induced desensitisation of glucose transport in adipocytes. Insulin resistance appeared only if glutamine was in the pre-incubation media with high glucose; the glucose toxicity-induced desensitisation of the glucose transport machinery could be prevented by molecules able to inhibit the hexosamine pathway [25]. Further experiments revealed that down-regulation depended upon formation of hexosamines, although this pathway is of quantitatively minor importance (2–3%) for glucose metabolism of these cells [26]. The physiological hexosamine pathway is shown in Fig. 1.

In order to elucidate the relationship between the hexosamine biosynthetic pathway and glucose toxicity-insulin resistance, the majority of authors frequently used three experimental approaches: exposure to elevated and prolonged high glucose levels; exposure to glucosamine, which enters the hexosamine biosynthetic pathway bypassing GFAT (Fig. 1); increasing GFAT or OGT enzymes activities or

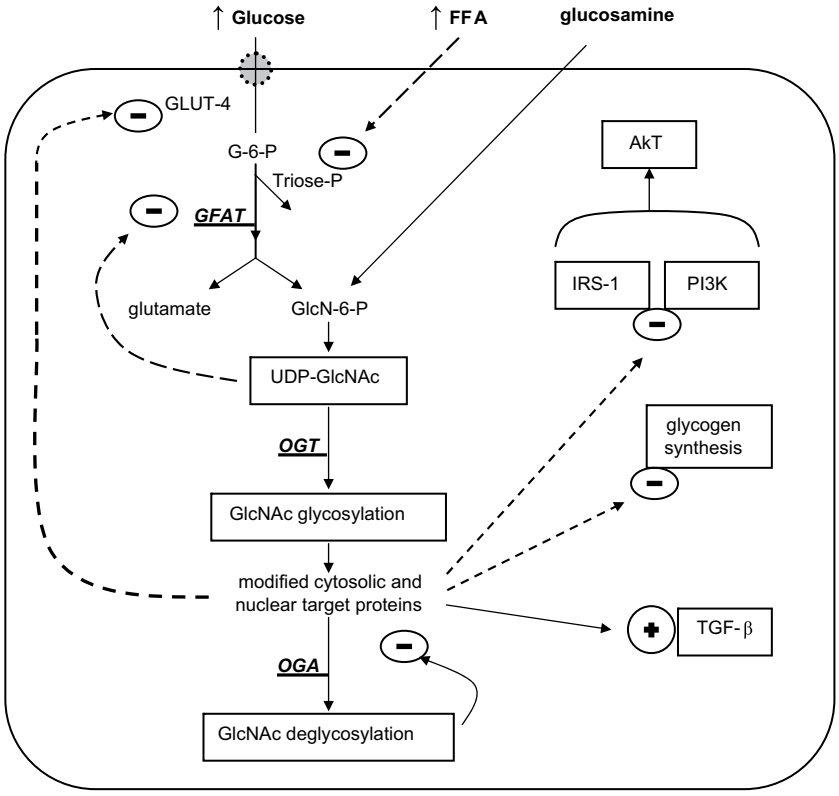

Figure 1 Physiologically, glucose flux through the hexosamine biosynthetic pathway (HBP) explains only 1—3% of the total incoming glucose in the cells. After the transport and the phosphorylation of glucose to glucose-6-phosphate, fructose-6-phosphate and glutamine are substrate of the rate-limiting enzyme of the pathway, glutamine:fructose-6-phosphate amidotransferase (GFAT), with the resultant glutamate and glucosamine-6-phosphate. After a few intermediate steps, the ultimate substrate of the hexosamine biosynthetic pathway is uridine diphosphate-*N*-acetylglucosamine (UDP-GlcNac). This compound is an allosteric feedback inhibitor of GFAT and the obligatory substrate of *O*-linked β-*N*-acetylglucosamine transferase (OGT). The latter enzyme catalyses a reversible (by the action of β-D-*N*-acetylglucosaminidase, OGA) inducible and dynamic posttranslational modification, the GlcNAcylation, i.e. the transfer of a single *N*-acetylglucosamine moiety to specific hydroxyl groups of serine and or threonine residues of target proteins. The *O*-GlcNAc modification is strictly dependent on the amount of UDP-GlcNAc produced, which is dependant on the total income of glucose into the cell. This dynamic modification may represent an important mechanism able to regulate the localisation, the stability and the function of proteins, considering that the target residues for the *O*-GlcNAc glycosylation/deglycosylation are often the same regulatory residues of the phosphorylation/dephosphorylation, one of the most important mechanisms of regulation in cell biology. For example, some proteins relevant to insulin resistance, which have been shown to undergo *O*-GlcNAc modification, include phoshatidylinositol 3 kinase (PI3K), insulin receptor substrate-1 (IRS-1), glycogen synthesis enzyme, glucose transporter 4 (GLUT-4), thus inducing, respectively, a reduced insulin signaling and action as well as a decrease of glucose income. In addition, the *O*-GlcNAc modification may stimulate the synthesis of TGB-β, worsening the hyperglycaemia.

inhibition of OGA's. In rat adipocytes, glucosamine was able to completely shut down the insulin-stimulated glucose transport [25] and this effect was absent in cells not expressing GLUT-4, even with GLUT-1 [27]. This suggests that the more involved tissues in hexosamine biosynthetic pathway-induced glucose toxicity are those that are insulin sensitive. In vivo experiments showed that the continued infusion of different glucosamine concentrations for 2 or 4 h into conscious rats could decrease the efflux through the glycolytic pathway, alter the effectiveness of glucose transport machinery and reduce the glycogen synthesis [26,28]. In transgenic mice overexpressing GFAT in fibroblasts [29], adipose tissue and skeletal muscle [30,31], there is a remarkable reduction in peripheral insulin sensitivity (glycogen synthesis and glucose uptake) and altered translocation of GLUT-4. Furthermore, an increased flux in the hexosamine biosynthetic pathway is responsible for obesity, impaired glucose tolerance and insulin resistance, when GFAT is overexpressed in the liver [32], and hyperinsulinaemia (when the overexpression is limited to β cells) [33]. Also, a moderate overexpression of OGT (∼20%) in muscle-adipose tissues of transgenic mice can lead to sustained insulin resistance [13]. Over-physiological function levels of OGT in the liver, instead, cause an impairment of insulin-responsive genes [34]. Insulin resistance was also reached by the inhibition of OGA in adipocytes treated with *O*-(2-acetamido-2deoxy-D-glucopyranosylidene)-amino-*N*-phenylcarbamate (PUGNAc): the mechanism seems to be related to a modified activation of Akt/PKB, resulting in reduced insulin signalling [35].

Regarding the molecular mechanism through which GlcNAc-modification may lead to insulin resistance, there is no single unique theory. Nonetheless, the major evidence

suggests that the acceleration of the hexosamine pathway, however obtained, may lead to an impaired translocation, docking or fusion to the plasma membrane, accelerated degradation and decreased intrinsic activity of GLUT-4 [36–38].

The insulin signalling system is also involved in a deleterious effect of the increased hexosamine biosynthetic pathway. IRS seems to be modified by the GlcNAc-glycation on the same serine/threonine residues usually regulated by phosphorylation. This reduces the downstream through the insulin cascade, as the block of activation of PI-3-kinase and its reduced association with IRS-1 and -2, as well as a decreased activation of Akt/PKB, resulting in reduced insulin signalling [38]. Recently, it has been demonstrated that GlcNAc-modified protein levels are increased in the pancreatic islets of diabetic rats and this may cause disruption of the glucose-sensing and insulin-secreting function of the β cells. In fact, under high glucose concentrations, the GlcNAcylation of two transcription factors which regulate glucose-dependent insulin gene expression and secretion, NeuroD1 and PDX-1 (pancreatic duodenal box-1), is elevated, increasing the insulin synthesis. On the other hand, the GlcNAcylation of forkhead box other-1 (FOX-1), a transcriptional factor that modulates several cellular functions as cell cycle, growth and differentiation, may cause an alteration on β-pancreatic function and survival [39].

Unfortunately, clinical data regarding the role of the hexosamine biosynthetic pathway in glucose toxicity are few and in contrast with each other. In a recent study, levels of stable hexosamine pathway end-products were found not to be increased in the adipose and muscle tissue of patients with insulin resistance, and there was no correlation between hexosamine levels and parameters associated with insulin resistance. These findings seem to argue against involvement of the hexosamine pathway in insulin resistance in humans; however, hexosamine levels were correlated with circulating FFA and leptin, thus strongly suggesting an intriguing role as a trigger fuel sensor [40]. A negative relationship between GFAT activity and glucose utilisation was demonstrated in type 2 diabetic subjects [41]. Other studies reported increased GFAT activity in the skeletal muscle of insulin resistant patients affected by type 2 diabetes [42]. Recently, a cross-sectional study showed a strong correlation between increased flux in the hexosamine biosynthetic pathway and insulin resistance (even if not measured by the clamp), postprandial hyperglycaemia and oxidative stress in Asian Indian type 2 diabetic patients [43].

Therefore, several investigators [44] have proposed that the hexosamine pathway acts as a fuel sensor in insulin sensitive cells, so that when the cell is inundated with substrates (i.e., glucose and/or FFAs), insulin resistance develops and prevents cell engorgement by oxidizable compounds. Acceleration of the hexosamine pathway also causes leptin expression in skeletal muscle [45] which, by regulating appetite and the route of fat disposal, could potentially close a negative feedback loop and inhibit the self-maintenance of a vicious cycle between lipid/glucose excess and insulin resistance [46]. Alterations in this feedback system could eventually lead to a break in glucose homeostasis [43].

In light of these observations, both in vitro and in vivo experiments in animal models as well as some evidence in humans show the hexosamine biosynthetic pathway as one of the mechanisms through which hyperglycaemia can worsen β cell function and insulin resistance (Table 1).

Clearly, any intervention designed to act on the altered hexosamine pathway may slow the vicious cycle of glucose-toxicity. Further, it is well established that hexosamines are also involved in the pathogenesis of microvascular diabetic complications [42]. It can therefore be speculated that any intervention able to act on this pathway (including restoration of euglycaemia) can reduce the burden of both diabetic hyperglycaemia and microvascular complications.

Oxidative stress

Several studies show that experimental conditions of oxidative stress in in vitro models cause a decrease in glucose-stimulated insulin response [47] and defects in the insulin-stimulated pathway [48]. In agreement with this, chronic hyperglycaemia is an example of an increased nutrient availability in which the normal and physiologic pathway may be disrupted. The increased oxidative stress is a potentially unifying mechanism in the nutrient-activated pathway. So, for many years it has been suggested that oxidative stress may be related with the progression of diabetic disease. In fact, elevated oxidants and markers of oxidative tissue damage, as damage of DNA bases, and high levels of hydroperoxides, have been demonstrated in type 2 diabetic patients and treatment with hypoglycaemic drugs ameliorates the glucose metabolism as well as the antioxidant defences [49].

Physiologically, reactive incomplete reduced forms of oxygen (ROS) are produced in the mitochondria ATP-production chain, and only 0.1% of totally consumed oxygen generates ROS. In conditions in which electrochemical changes occur in the respiratory chain (such as in increased nutrient availability, e.g., high glucose state), there is an increment of partial reduction of O_2, leading to the generation of free radical anion superoxides [50]. Moreover, hyperglycaemia determines a decrease in NADPH and glutathione levels, two antioxidant reducing equivalents, with a consequent enhanced sensitivity to ROS-related oxidative stress [51].

The treatment with *N*-acetyl-cysteine, aminoguanidine or carnosine (and its isoforms resistant to the degradation), models of antioxidant agents, reduce the degree of hyperglycaemia and glycosuria in Zucker rats [52] and improves the whole insulin-producing system (improvement of insulin secretion, reduced rate of apoptosis, increased β cells mass) [53,54]. As inferred from the above, the toxic effects of oxidative stress are exerted, above all, in the β cells, since their antioxidant enzyme levels are lower than the ones in other tissues [55].

Several mechanisms have been reported for increased β cell susceptibility to ROS thereby determining a number of deleterious effects as shown in Table 1.

In addition, Maassen [56] suggests that the increase in oxidative stress may cause mutations in mtDNA because of its proximity to the ROS-producing systems. It is well known

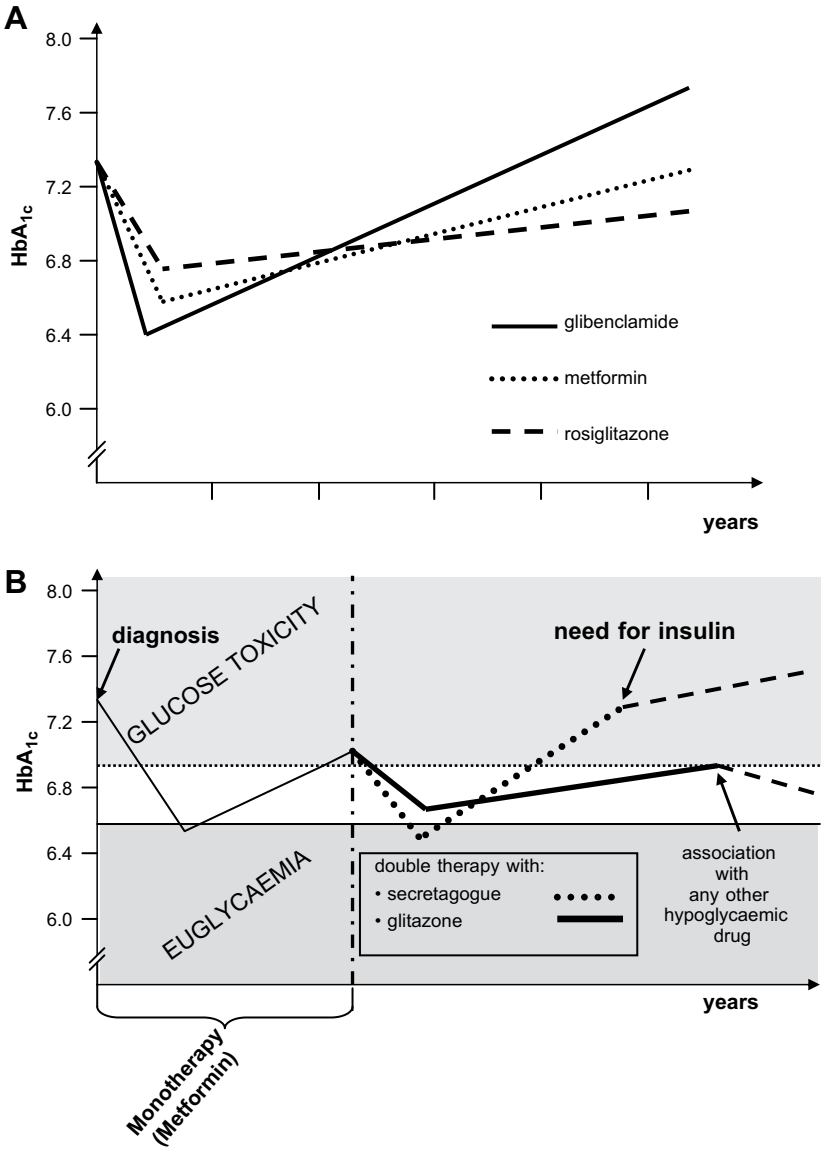

Figure 2 Panel A: Redrawn from the ADOPT study [105]: newly diagnosed type 2 diabetic patients were allocated to three treatment groups (glibenclamide, rosiglitazone and metformin). Glibenclamide was faster in reaching the target HbA_{1c} but caused an early failure of beta cells. Rosiglitazone showed a delayed effect on HbA_{1c} but demonstrated a significantly prolonged durability of the beta cell. Panel B: Hypothetic application of data from the ADOPT study in current clinical practice. At the onset of diabetes metformin, if tolerated, is usually the first drug option. However, the glycaemic control is lost over the years and it is necessary to add other diabetic drugs to the initial therapy. The addition of sulphonylureas could appear more powerful in again reaching a satisfactory control, but complete beta cell failure would probably be reached soon. The addition of a glitazone should prolong the efficacy of the therapy (durability), possibly preserving the beta cell function for further secretagogues therapy, thus delaying the need for insulin.

that mitochondria are the engine when glucose fuel is burnt, and some defects in mtDNA are listed as a causative role of diabetes [57], as shown by in vitro experiments [58] and in vivo observations [59]. Accumulating in vitro evidence has shown that increased mitochondrial reactive species lead to the activation of stress kinases such as c-Jun N-terminal kinase, p38, I kappaB kinase, and extracellular receptor kinase 1/2, resulting in a down-regulation of the cellular response to insulin, reduced ability of insulin to promote glucose uptake, and glycogen and protein synthesis. The mechanisms leading to this down-regulation involves increased serine/threonine phosphorylation of insulin receptor substrate-1 (IRS1), impaired insulin-stimulated redistribution of IRS1, reduced protein kinase-B phosphorylation and GLUT-4 synthesis and translocation to the plasma membrane.

Thus, it is largely accepted that oxidative stress plays a key role in the development of insulin resistance. In vivo observations clarified that this association is not restricted to insulin resistance in type 2 diabetes, but is also evident in obese, non-diabetic individuals, and in patients with the metabolic syndrome [60,61].

Table 1 Impact of oxidative stress on β cells and possible mechanisms of action.

Mechanisms of action	→ Effects
Posttranscriptional loss of PDX-1 mRNA	→ Reduced PDX-1 protein and binding to insulin promoter
Posttranslational changes in MafA activity	→ Down-regulation of insulin promoter activity
Pro-apoptotic effect, with over-expression of Bid, Bad, Bix, and opposite influence on anti-apoptotic Bcl-xl	→ Reduced β cell mass
Activation of UCP-2 (member of a group of nuclear-encoded uncoupling protein, the proton carrier proteins in mitochondria)	→ Decrease in ATP/ADP ratio, closure of ATP-sensitive $K+$ channels and impaired exocytose of insulin stores

Hyperglycaemia, the hallmark of diabetic disease, leads to chronic oxidative stress which in turn contributes to the pathogenesis of diabetic microvascular complications [51] as well as being responsible for decreases in insulin promoter activity, insulin gene expression, insulin secretion, death rate of β cells and impairment in energy production, contributing to the development and progression of diabetes.

The acute glucose fluctuation of post-meal hyperglycaemia, which is a frequent phenomenon in people with type 1 and 2 diabetes, exhibits a more powerful trigger on oxidative stress than chronic hyperglycaemia per se [62—64], even though the metabolic control appears adequate as evaluated by HbA1c [65,66]. As reported, a small intervention trial actually demonstrated that postprandial hyperglycaemia, but not fasting hyperglycaemia, could predict cardiovascular disease [67]. However, most of the effects of high glucose concentrations have been studied in cell cultures or animal models, and only a few studies investigated the actual role of the effect of postprandial hyperglycaemia in humans. A significant increase in nitrotyrosine plasma levels, a marker of oxidative stress, was observed during a 2 h hyperglycaemic clamp in healthy subjects [68]. Recent data demonstrated that 3 h of hyperglycaemia, while maintaining basal fasting insulinaemia in healthy subjects, induced the expression of a number of genes involved in detoxification and free radical scavenging [69]. Although these experiments are extremely suggestive, it has to be mentioned that the study of the effects of postprandial hyperglycaemia/glucose toxicity per se is limited by the difficulty of reproducing these fluctuations in an experimental model.

PKC

It is well known that protein kinase C is involved in the pathogenesis of diabetic microvascular complications. When the blood glucose level rises, the elevated glucose income into the cells leads to an increment of DAG synthesis in a glucose-dependent manner. Since PKC is activated by intracellular DAG [70], there is no doubt that it may represent a toxic effect of hyperglycaemia. The PKC pathway mediates different cellular signals and is involved in the activation of NF-κB, with a significant pro-inflammatory cytokine secretion [71].

Several studies (as reviewed by Avogaro et al.) [72] clearly demonstrate that, in type 2 diabetic patients, the insulin receptor activity is markedly decreased both in adipocytes [73,74] and skeletal muscle [75,76]. The defect is reversible in relation to the improvement of glycaemic control and, as shown by an in vitro experiment, PKC inhibitors blocked insulin desensitisation caused by high glucose levels [77] and prevented glucose-induced decreases in insulin receptor kinase activity and insulin resistance [78,79]. Therefore, while the role of PKC is well established in diabetic microvascular complications, it still remains to be defined whether the glucose-induced activation of protein kinase C (PKC) [78] may act directly on the insulin receptors or via stimulation of an inflammatory state.

Inflammation

Another major mechanism involved in glucose toxicity is inflammation and the diabetic state has been proposed as an inflammatory state [80]. Hyperglycaemia, in fact, promotes elevation of plasma C-reactive protein [81], chemokines and adhesion molecules and the induction of cytokine secretion by different cell types, such as monocytes, adipocytes and β cells [82,83]. A paradigm of the hyperglycaemia-induced pro-inflammatory state is represented by IL-1β. This cytokine is normally involved in autoimmune processes as the destruction of β cells in type 1 diabetes mellitus [84]. Meadler et al. have shown that human islets from non-diabetic organ donors, exposed to a prolonged high glucose concentration, release 2.2 fold amounts of IL-1β compared with islets under normoglycaemic stimulation [85].

Since IL-1β expression is not detectable in non-diabetic human pancreatic islets [86] and the overexpression of this cytokine is prevented in *Psammomys obesus* treated with phloridzin, it can be supposed that islet cells are the source of glucose-dependent IL-1β production. The release of IL-1β is followed by NF-κB activation and up-regulation of FAS, resulting in impaired β cell function and an increased islet apoptosis rate.

In light of these observations, the role of IL-1β in mediating glucose-induced deleterious effects on β cells may be the linking feature between type 1 and 2 diabetes, although both conditions have different aetiological causes [85]. A recent study performed in monocytic cells has shown that Toll-like receptors are involved in high-glucose-induced deleterious effects [83]. Physiologically, Toll-like receptors, expressed in different tissues and cells, play a role in inflammation, atherosclerotic processes and innate immune responses [87].

In vitro hyperglycaemic stimulation causes, in a dose- and time-dependent manner, a strong increase in TLRs expression and activity, by activation of PKC and NADPH oxidase activation. The resulting inflammation response

may modulate and worsen hyperglycaemia-induced effects such as peripheral insulin resistance and atherosclerosis [83].

In conclusion, glucose toxicity is able to generate or exacerbate the typical chronic inflammatory state of type 2 diabetes mellitus thus playing, through this and the above reported mechanisms, the leading role in its pathogenesis and clinical history, becoming the crucial target for treatment.

Clinical evidence

From all the above studies in animal and cellular models we can now strongly confirm that glucose toxicity plays the pivotal role (i.e., is the ''best actor'' of the scene) in inducing insulin resistance and beta cell failure in type 2 diabetes mellitus. Presently, we have few data regarding the actual role of glucose toxicity in humans, but we believe that the evidence described below will supply proof of the involvement of chronic hyperglycaemia per se in the natural history of human type 2 diabetes.

In patients with type 1 diabetes, insulin resistance is an acquired and reversible phenomenon since insulin sensitivity is normal during remission of the disease [88]. The degree of fasting hyperglycaemia and HbA1c are, in both patients with type 1 and 2 diabetes, inversely related to the magnitude of peripheral insulin resistance. Even in populations such as the Pima Indians [89], in which insulin resistance is severe and partly genetically determined [90], glycaemia is the most important determinant of insulin sensitivity. In patients with type 1 diabetes, if glycaemic control is normalised, insulin sensitivity returns to normal [91]. Twenty-four hours of experimental hyperglycaemia in patients with type 1 diabetes [92] again induces insulin resistance, particularly non-oxidative glucose metabolism (which mainly consists of glycogen synthesis in skeletal muscle) thus closely resembling the most common defect of type 2 diabetes. Therefore, glucose toxicity can induce insulin resistance in humans, and in patients with only secondary insulin-resistance (type 1 diabetic patients) removal of glucose toxicity normalises insulin resistance.

A simplistic mechanism of glucose toxicity at the level of pancreatic beta cells can be hypothesised as a continuous over-stimulation of insulin secretion with depletion of insulin stores, worsened hyperglycaemia and glucose toxicity closing the loop again up to beta cell exhaustion. The consequent loss of β cell mass was reported to be an important contributor to the onset of diabetes and evolution of the diabetic state [93,94]. Studies performed on pancreatic tissues from patients with type 2 diabetes, and control subjects obtained from 124 autopsies, showed a low frequency of β cell replication in all cases (diabetic patients and control groups) [95]. However, the frequency of β cell apoptosis was increased 10 fold in the lean, and 3 fold in the obese, cases of type 2 diabetes. These evidences clarify the presence of the deleterious effects exerted by chronic hyperglycaemia on human β cells, and compel the use of hypoglycaemic drugs in order to avoid/slow the onset of β cell loss and diabetic complications.

An early pharmacological intervention, preferably at the onset of diabetes, should slow the decline in insulin secretion in the natural history of diabetes. In fact, in people newly diagnosed with type 2 diabetes who failed 3–6 weeks of diet therapy, a 2–3 week course of intense insulin therapy, subsequently discontinued, showed a good long term response in glycaemic control [96], even with diet alone. Recent data confirmed that at the onset of type 2 diabetes in drug-naïve patients the early intensive therapy had a better outcome with regard to the recovery and maintenance of β cell function than treatment with oral hypoglycaemic agents [97].

All hypoglycaemic agents remove glucose toxicity with different mechanisms of action and may lay a basis for long term glycaemic control. Metformin and/or PPAR gamma agonists mostly ameliorate insulin resistance, while secretagogues such as sulphonylureas and glinides have a beneficial effect on the improvement of insulin secretion. Among these, gliclazide seems to possess antioxidant properties that might protect the beta cell, at least in vitro, when beta cells are exposed to high glucose concentrations [98]; it also prevents apoptosis.

The biguanide compound metformin increases whole body glucose disposal by stimulating insulin independent glucose transport through AMPK activation [99], but also protects the beta cell from the glucose toxicity oxidative effects directly reducing intra-cellular oxidative stress [100].

Thiazolidinediones (TZDs) improve glycaemic control in people with type 2 diabetes mostly by remodelling adipose tissue. They promote the differentiation of subcutaneous adipose tissue (and therefore its volume, ensuing augmented body weight) resulting in a reduction of visceral fat and of circulating free fatty acids. TZDs also reduce inflammatory cytokines, usually invoked in the occurrence of insulin resistance and beta cell apoptosis [101]; TZDs might therefore exert protective effects against the progression of type 2 diabetes that are beyond and over the removal of glucose toxicity.

Acarbose is an oral agent that acts through the inhibition of the gut enzyme a-glycosidase, slowing or abolishing the absorption of poly-saccharides. The compound is not absorbed, and its simple mechanism (lowering of postprandial glucose excursion) suggests its use for the study of selective postprandial glucose-toxicity. Data on diabetes prevention and cardiovascular risk reduction have been published [102], although largely debated. Unfortunately the significant side effects of the drug limit its use on a large scale.

More recently drug companies introduced injectable GLP 1 analogues and oral inhibitors of DPP IV, the key enzyme in GLP 1 catabolism, which are able to induce beta cell secretion with an incretin mimetic mechanism [103], while inhibiting glucagon secretion and having a trophic effect on beta cell mass [104], at least in vitro. As any other hypoglycaemic agent, these new drugs certainly reduce glycaemia and therefore glucose-toxicity; the demonstration of their durability, however, still needs to be revealed.

Encouraging results from the PPAR gamma agonist rosiglitazone [105] suggest that the more durable effect of this drug on glucose control, as compared with glibenclamide and metformin, may be due to its protective effect on the beta cell over the years. Nevertheless, the absence of hypoglycaemia with the PPAR gamma agonists, and

therefore the possibility to reach the highest therapeutic doses without severe side effects (i.e., without reducing patients' compliance), can be considered another basis for the success of the durability of rosiglitazone.

Glucose toxicity and cardiovascular diseases

Several epidemiological studies have indicated that chronic hyperglycaemia, as measured by fasting plasma glucose concentrations or glycosylated haemoglobin HbA1c [106,107], is an independent risk factor for cardiovascular disease in patients with T2DM [108,109]. This raises the obvious question of whether the targeting of near normal levels of glucose may reduce the risk of cardiovascular events in diabetic patients.

In 1996, a progressive positive correlation was found by Hanefeld between the degree of postprandial glycaemia and the risk of myocardial infarction and cardiovascular mortality in people with diabetes. Interestingly, such a link was not found with fasting plasma glucose [67]. The larger UKPDS, however, failed to demonstrate a reduction in cardiovascular events and myocardial infarction with intensive treatment, excluding metformin only in overweight diabetic patients [110].

Reports from the Diabetes Control and Complications Trial (DCCT)/Epidemiology of Diabetes Interventions and Complications (EDIC) [111] has provided evidence to support hyperglycaemia as an independent risk factor for cardiovascular disease. The DCCT study was designed to evaluate the effect of intensive insulin treatment on diabetic complications in type 1 diabetic patients. After demonstrating these effects, the study was interrupted and the patients entered a subsequent observational study (EDIC), without any difference in treatment. Intensive treatment during the first (DCCT) study reduced the risk of any cardiovascular disease event only in the following observational (EDIC) study, including the risk of non-fatal myocardial infarction, stroke, or death from cardiovascular disease.

Recently, two completed multicentre clinical trials, the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial [112] and the Action in Diabetes and Vascular Disease (ADVANCE) [113] were designed to evaluate the effects of intensive treatment for glycaemic control on vascular outcomes in patients with type 2 diabetes who were considered to be at high risk. In the ADVANCE trial there was a relative reduction of 10% in the primary composite outcome of major microvascular events, primarily as a consequence of a reduction in the progression of microalbuminuria in the intensive treatment group. No effects, however, were observed on macrovascular disease or mortality. In the ACCORD trial the primary outcome (the first occurrence of non-fatal myocardial infarction or non-fatal stroke or death from cardiovascular diseases) failed to be significantly reduced in the intensively treated group. In the same trial, however, patients intensively treated suffered from a significantly increased risk for all cause mortality and this, for obvious ethical reasons, caused the interruption of the study. Unfortunately, the trial was not designed to discern if one drug or the other was responsible for the increased deaths. Nevertheless, in the intensively treated patients the episodes of hypoglycaemia requiring any assistance or requiring medical assistance increased almost 3 fold; this strongly suggests that the higher rate of severe hypoglycaemia could be the major cause of death. Further, more than 90% of patients in the intensively treated patients used rosiglitazone, as compared with ''only'' 57.5% in the control group, inferring a role for rosiglitazone as a cause for the adverse events. Nevertheless, the observation of a comparable rate of fatal or non-fatal heart failure between the two groups seems to exclude a causative role for glitazones in the increased death rate. Trying to confirm earlier preliminary data obtained by the Veterans Administration researchers, the VADT [114] was again designed to evaluate whether intensification of glucose control can reduce major CV events in patients with long standing type 2 diabetes. The results of the VADT study did not show that a target of HbA1c below 7% had a statistically significant effect on reducing major CV events, death, or microvascular complications, excluding the progression of microalbuminuria. The compelling message from these three major studies seems to be that near normal glycaemic control (i.e., the abolishment of glucose toxicity) does not reduce CV events but actually causes (at least in the ACCORD trial) an increased risk of death. The earlier reported UKPDS [115] was designed with a similar aim to these trials, but involved patients with newly diagnosed type 2 diabetes and without any evidence of previous CV disease. As reported above, even after 10 years of intensive treatment for glucose control, no reduction of CV events was observed. After the end of the trial all the patients were treated intensively and, 1 year after the end of the trial, no significant difference in glycated haemoglobin levels was present between groups. Despite this finding, in the original intensive-therapy group, re-examined in the post-trial UKPDS monitoring conducted 10 years later, there was a reduction in the risk of myocardial infarction and of death from any cause, as well as of microvascular complications and of any diabetes-related outcome. This delayed effect clearly demonstrates the cumulative effects of glucose toxicity on cardiovascular diseases. Therefore, treating intensively, early and avoiding hypoglycaemic events should be considered as mandatory in the treatment of type 2 diabetes. But, with what drugs?

Glucose toxicity and diabetic microvascular complications

It is well-known that hyperglycaemia is the major (if not the only) cause of microvascular diabetic complications, including nephropathy, retinopathy and neuropathy [116]. Actually, the glycaemic threshold for the diagnosis of diabetes is solely based on the increased risk of the appearance of diabetic retinopathy with a fasting glucose concentration above 126 mg/dl (7 mmol/l) [117]. As fully clarified by several, even recent, clinical trials, the control of hyperglycaemia and glucose toxicity exerts a preventative role in microvascular complications which is more powerful and evident than in macrovascular ones [110–114,118,119]. Even if the molecular explanation of their onset is not our aim in this paper, it is interesting

to report that several different mechanisms that are involved in the pathogenesis of these complications, such as the altered hexosamine pathway, increased oxidative stress and enhanced polyol and PKC-DAG pathway (determining changes in vascular function and endothelial permeability), are shared with the mechanisms of glucose toxicity in the beta cell and skeletal muscle [120–123]. More importantly, the tight control of glycaemia may eliminate or, at least, reduce the deleterious effects of glucose toxicity on these tissues, preventing or slowing their appearance and/or progression. Approaches to prevent glucose toxicity will be discussed below.

Therapy

Any anti diabetic treatment which lowers the fasting glucose concentration (the main determinant of mean glucose concentration throughout the day) ameliorates glucose toxicity and leads to an improvement in beta cell function and, as seen above, if hypoglycaemia is not provoked, a reduced risk of cardiovascular events can be expected.

At the earliest stages, when the impaired beta cell function appear to be reversible, the mechanism of beta cell dysfunction should be targeted by lifestyle changes and pharmacological intervention; lifestyle and rosiglitazone being the two most effective strategies to prevent or delay diabetes [124].

Sulphonylureas act mostly by increasing insulin secretion. The increased insulin levels in the portal vein reduce the hepatic glucose production while peripheral insulin enhances the uptake of glucose in the muscle [125]. Sulphonylureas reach the therapeutic target faster than other oral drugs, but cause hypoglycaemia [112–114] thus reducing compliance. More importantly, the durability of sulphonylureas is much shorter than metformin and TZDs [105]. This means a shorter time to the addition of another drug, or a shorter time to the addition of insulin and, again, increasing the risk of hypoglycaemia and reducing compliance (Fig. 2).

The incretin hormones (as GLP 1 and GIP [126]) are physiologically secreted in response to an oral glucose load and may act through the inhibition of glucose release, delay in gastric emptying and the reduction in appetite. Their main mechanisms are glucose-dependent insulin secretion and avoiding hypoglycaemia, the most common side effect of diabetes treatment [127]. However, there are no data regarding the durability and the decrease of cardiovascular events due to missing clinical trials testing long term therapy with incretins.

A promising approach to the treatment of glucose toxicity in type 2 diabetes may be represented by SGLT2 inhibitors. In animal models remogliflozin [128] exhibited efficacy; this data was confirmed by short-term studies with dapagliflozin [129] (12 weeks) and sergliflozin [130] (2 weeks) in patients. If these drugs are available they represent a useful option since they improve glycaemic control thereby inducing glycosuria without any direct effect on insulin secretion, but will improve it just like phloridzin in animal models; these drugs might in fact be considered as phloridzin analogues.

As mentioned above, metformin may act through the AMPK pathway [99] by increasing insulin sensitivity with no hypoglycaemic event. In addition, it has a protective effect on beta cells, attenuating the reactive oxygen species production [100]. Consequently, it represents the first weapon in the treatment of patients with insulin resistance and type 2 diabetes.

As seen above (Fig. 2), the beneficial effects of the oral agents are usually lost over the years, mainly due to progressive beta cell failure. In all patients a partial failure of the beta cell is usually seen, with moderate but significant hyperglycaemia, i.e., glucose-toxicity which, in turn, contributes to the progression of the β cell failure. This vicious cycle of glucose toxicity-induced continuous deterioration of insulin secretion is the pathogenic force that causes the final need for insulin treatment in type 2 diabetic patients. If glucose toxicity is the cause of beta cell failure, driving patients to insulin dependence, glucose toxicity is the major characteristic that should be aggressively treated in all patients, seeking durability and compliance but avoiding life-threatening hypoglycaemias.

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