Diabetes is a serious and growing public health problem that results in reduced life expectancy and increased morbidity as a result of diabetes-specific complications. The hallmark of diabetes is hyperglycaemia, a stressor which can be controlled clinically through the exogenous administration of insulin or through drugs which increase insulin secretion, decrease glucose release from the liver, increase the use of glucose in the skeletal muscle and fat, delay the absorption of glucose from foods and, most recently, act through the incretin system [1]. These advances, together with improved glucose monitoring and better markers of glycaemic control, have led to much tighter control of hyperglycaemia. In spite of such progress in treatment, debilitating vascular complications remain in most diabetic patients.

In the Diabetes Complications and Control Trial (DCCT), Type 1 diabetic patients were either placed on standard or intensive treatment regimens to normalize their glucose levels. Because the progression of microvascular complications was so profoundly reduced in patients with tight glucose control, the DCCT ended after a mean time of 6.5 years and all patients were placed onto intensive therapy [2]. Notably, in the Epidemiology of Diabetes Interventions and Complications (EDIC) trial, a follow-up to the DCCT, compared with their counterparts receiving intensive therapy throughout the trial, patients on the standard treatment regimen during the DCCT still had a higher incidence of complications several years after switching to intensive therapy [3,4]. Furthermore, recent data from EDIC also suggest that the influence of early glycaemic control on the progression to macrovascular events may become more evident with longer follow-up [5,6].

Data from the United Kingdom Prospective Diabetes Study (UKPDS) appear to be consistent with this evidence. Specifically, people with lower fasting plasma glucose values at the time of diagnosis had fewer vascular complications and fewer adverse
clinical outcomes over time as compared with people with higher fasting plasma glucose values, despite similar rates of increasing glycaemia [7], suggesting that early metabolic control has enduring beneficial effects even in Type 2 diabetes.

Collectively, these observations support the concept that early glycaemic environment is remembered, and the authors of the DCCT/EDIC have referred to this phenomenon as ‘metabolic memory’ [6].

Experimental evidence supporting the concept of ‘metabolic memory’ and its possible link with oxidative stress

Several years ago a preliminary report suggested the possibility of a ‘hyperglycaemic memory’ for hyperproduction of fibronectin and collagen in endothelial cells persisting after glucose normalization [8]. Using the same design, 14 days in high glucose followed by 7 days of culture in normal glucose concentration, preliminary data show that in endothelial cells overproduction of free radicals persists after the normalization of the glucose and is accompanied by a prolongation of the induction of PKC-β, NAD(P)H oxidase, Bax, collagen and fibronectin, in addition to 3-nitrotyrosine (3-NY) [9]. This suggests that oxidative stress may be involved in the ‘metabolic memory’ effect.

The effect of re-institution of good glucose control on hyperglycaemia-induced increased oxidative stress and nitrative stress has also previously been determined in the retina of rats maintained in poor glucose control before initiation of good control [10]. In diabetic rats, 2 or 6 months of poor control (glycated haemoglobin >11.0%) was followed by 7 months of good control (glycated haemoglobin < 5.5%). Re-institution of good control after 2 months of poor control reduced elevations in retinal lipid peroxides and nitric oxide (NO) levels by approximately 50%, but failed to have any beneficial effects on nitrotyrosine formation. However, reversal of hyperglycaemia after 6 months of poor control had no significant effect on retinal oxidative stress and NO levels. In the same rats, inducible nitric oxide synthase expression and nitrotyrosine levels remained elevated by > 80% compared with normal rats or rats kept in good control for the duration of the study [10]. In a similar study, caspase-3 activity in diabetic rats kept in poor control for 13 months was 175% that in normal rats [11]. Re-institution of good glycaemic control after 2 months of poor control partially normalized the hyperglycaemia-induced activation of caspase-3 (to 140% of normal values), while re-institution of good control after 6 months of poor control had no significant effect on the activation of caspase-3. In the same study, nuclear factor-kappa B (NF-κB) activity was 2.5-fold higher in diabetic rats kept in poor control than in normal rats. Re-institution of good control after 2 months of poor control partially reversed this increase, but good control after 6 months of poor control had no effect. Initiation of good control soon after induction of diabetes in rats prevented activation of retinal caspase-3 and NF-κB [11].

Similar results are available for the kidney. Diabetic rats were maintained in good glycaemic control (glycated haemoglobin 5.0%) soon after or 6 months after induction of hyperglycaemia, and were killed 13 months after induction of diabetes [12]. For rats in which good control was initiated soon after induction of diabetes, oxidative stress (as measured by the levels of lipid peroxides (LPOs), 8-hydroxy-2′-deoxyguanosine (8-OHdG), and reduced glutathione (GSH)) and NO in urine and renal cortex were not different from that observed in normal control rats, but when re-institution of good control was delayed for 6 months after induction of diabetes, oxidative stress and NO remain elevated in both urine and renal cortex [12].

These data suggest that hyperglycaemia-induced oxidative stress and NO, as well as activation of apoptosis and of NF-κB, can be prevented if good glycaemic control is initiated very early, but are not easily reversed if poor glycaemic control is maintained for longer durations. Therefore, these findings suggest persistence of the hyperglycaemia-induced damage in such organs even after its normalization.

Molecular basis for the ‘metabolic memory’: the possible link between oxidative stress and non-enzymatic glycation

The role of oxidative stress in diabetic complications

Brownlee and co-workers have recently pointed to an excess of superoxide anion (•O₂⁻), a reactive species, in the mitochondria of endothelial cells in response to hyperglycaemia, with the formation of diabetic complications [13]. This new insight further links with four key pathways suggested to be involved in the development of these complications (increased polyol pathway flux, increased advanced glycation end-product (AGE) formation, activation of protein kinase C and increased hexosamine pathway flux), into a unifying hypothesis regarding the effects of hyperglycaemia on the development of diabetic complications [13,14]. This topic is reviewed by Brownlee [15] and Ceriello [16].

However, if excess reactive species are central in the development of hyperglycaemia-related diabetic complications, could this excess explain the persistence of the risk for complications even when the hyperglycaemia is reduced or normalized?

Glycation of mitochondrial proteins, oxidative stress and ‘metabolic memory’

The above reported studies [9–12] suggest that long-lasting effects of hyperglycaemia result in increased oxidative stress, while inhibiting oxidative stress has been shown to reverse these effects in a preliminary study [9]. Mitochondrial over-production of •O₂⁻ in hyperglycaemia has been suggested as the ‘unifying hypothesis’ for the development of diabetic complications [15]. Therefore, it is reasonable to infer that mitochondria are also important players in propagating the ‘metabolic memory’. Chronic hyperglycaemia is thought to alter mitochondrial function through glycation of mitochondrial proteins [17]. Levels of methylglyoxal (MGO), a highly reactive
that collagen AGE formation is an irreversible phenomenon. Therefore, it appears a marker for glycation of extra-cellular proteins and a predictor of end-organ damage [23]. While glycated HbA1c may be partially enzymatically deglycated [27], such a reaction has not yet been found for AGEs incorporated into collagen. Therefore, it appears that collagen AGE formation is an irreversible phenomenon.

The glycation of mitochondrial proteins may be a contributing explanation for the phenomenon of 'the metabolic memory'. Glycated mitochondria overproducing free radicals, independently from the actual glycaemia, can lead to a catastrophic cycle of mitochondrial DNA (mtDNA) damage as well as functional decline, further oxygen radical generation and cellular injury [28], thus maintaining the activation of the pathways involved in the pathogenesis of diabetic complications. Furthermore, mitochondrial proteins become damaged or post-translationally modified as a consequence of a major change in the redox status of a cell [28]. This, however, may also affect mitochondrially destined proteins, which are imported into the mitochondrial outer membrane, inner membrane or matrix space via specific import machinery transport components [28]. Finally, oxidative stress may alter mitochondrial protein expression [29,30] and turnover [31], possibly leading up to a perpetuation of the phenomenon.

In other words, it may be postulated that, in the 'metabolic memory', the cascade of events is the same as that proposed by Brownlee [15] — the source of •O2− is still the mitochondria — but that in addition the production of reactive species is unrelated to the presence of hyperglycaemia and depends on the level of glycation of mitochondrial proteins. This hypothesis is depicted in Fig. 1.

Therapeutic implications and prospects

The emerging evidence that hyperglycaemia leaves a very early imprint on the development of future complications has important therapeutic implications: it seems mandatory to commence early aggressive treatment of hyperglycaemia from diagnosis of diabetes. However, while this strategy can be accepted easily in Type 1 diabetic patients, some concerns may arise in Type 2 patients because this approach may include early insulin use. Moreover, tight control of hyperglycaemia may also have to address 'postprandial' hyperglycaemia [32,33], not only because postprandial hyperglycaemia is accompanied by specific formation of both reactive species [36] and AGEs, not only in the plasma [37], but also intracellularly [38].

Another possible strategy is to reduce AGE formation and oxidative stress generation concomitant with glucose normalization. Several compounds have already been developed which block AGE formation. Metformin and pioglitazone have been shown in vitro to prevent AGE formation [39]. ACE inhibitors and AT-1 blockers are compounds used to control blood pressure, however, they are also able to reduce AGE formation [40]. Interestingly, these drugs also work as antioxidants [16] and, at least for AT-1 blockers, there is evidence of a specific action.
against hyperglycaemia-induced oxidative stress [41]. Finally, statins may potentially also be beneficial in reducing reactive species [41]. Thus, one could envisage a future strategy consisting of compounds active on AGE formation [42], together with another compound capable of specifically targeting mitochondrial reactive species generation [43].

Conclusions

Consistent new emerging evidence suggests that hyperglycaemia can leave an early imprint in cells of the vasculature and of target organs, favouring the future development of complications. Additionally, evidence suggests that this ‘memory’ can appear even when good glycaemic control is achieved. This phenomenon has been named as ‘metabolic memory’ [6]. This evidence raises many questions regarding the therapeutic management of diabetes. In particular, the existence of the metabolic memory suggests that very early aggressive treatment of hyperglycaemia is mandatory.

Competing interests

None to declare.

References

8 Roy S, Sala R, Cagliero E, Lorenzo M. Overexpression of fibronectin induced by diabetes or high glucose: phenomenon with a memory. Proc Natl Acad Sci USA 1990; 87: 404–408.
16 Creillioli A. New insights on oxidative stress and diabetic complications may lead to a ‘causal’ antioxidant therapy. Diabet Care 2003; 26: 1589–1596.


