Increased morbidity due to 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. These advances, together with improved glucose monitoring and better markers of glycaemic control, have led to

**Key words:** Metabolic memory. Non-enzymatic glycation. Oxidative stress. Mitochondria. Diabetic complications.

**INTRODUCTION**

Diabetes is a serious and growing public health problem that results in reduced life expectancy and increased morbidity due to 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. These advances, together with improved glucose monitoring and better markers of glycaemic control, have led to
much tighter control of hyperglycaemia. In spite of these progresses 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. Notably, in the Epidemiology of Diabetes Interventions and Complications (EDIC) trial, a follow-up to the DCCT, patients on the standard treatment regimen during the DCCT still had a higher incidence of complications as compared to their counterparts receiving intensive therapy. Notably, in the Epidemiology of Diabetes Interventions and Complications (EDIC) trial, a follow-up to the DCCT, patients on the standard treatment regimen during the DCCT still had a higher incidence of complications as compared to their counterparts receiving intensive therapy. 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.

Data from the United Kingdom Prospective Diabetes Study (UKPDS) appear to be consistent with this evidence. Specifically, people with lower fasting plasma glucose (FPG) values at the time of diagnosis had fewer vascular complications and fewer adverse clinical outcomes over time as compared to people with higher FPG values, despite similar rates of increasing glycaemia, 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.”

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 has 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. Even if increased •O2- generation in hyperglycaemia is a key event in activating the other pathways involved in the pathogenesis of diabetic complications, it represents only a first step in the production of global cellular oxidative stress and the subsequent vascular damage that ensues. Hyperglycaemia also favors, through the activation of NFKB, an increase in the expression of both NAPDH and of iNOS, which would be expected to result in an excess of both NO and •O2-. NO is thought to contribute to endothelial dysfunction in two different ways. First, •O2- may also directly react with and quench NO, thereby reducing the efficacy of a potent endothelium-derived vasodilator system that participates in the general homeostasis of the vasculature, and evidence suggests that during hyperglycaemia reduced NO availability exists. Second, as mentioned above, •O2- overproduction when accompanied by increased NO generation favors the formation of the strong oxidant ONOO- and an overproduction of both •O2- and NO has been reported in response to hyperglycaemia. It has been shown that a stable protein adduct, 3-nitrotyrosine (3-NY), is a marker of ONOO- and •NO2- and can be readily measured using ELISA or western blot. The possibility that diabetes is associated with increased ONOO formation is supported by the recent detection of increased nitrotyrosine plasma levels in type 2 diabetic patients. Several pieces of evidence support a direct role of hyperglycaemia in favoring this phenomenon. 3-NY formation is detected in the artery wall of monkeys during hyperglycaemia, in the plasma of healthy subjects during hyperglycaemic clamp and in diabetic patients during an increase of postprandial hyperglycaemia. Hyperglycaemia is also accompanied by 3-NY deposition in a perfused working heart from rats, and it is reasonably related to unbalanced production of NO and •O2-, through iNOS over-expression and through the many •O2- sources described above. 3-NY formation is also associated with the development of an endothelial dysfunction in both healthy subjects and in coronaries of perfused hearts of rats. Interestingly, the clinic 3-NY has been found to be an independent predictor of vascular disease. All the above described pathways are summarized in Figure 1.
From the above reported findings it seems clear that both oxidative and nitrosative stress play a central role in the development of diabetic complications, both micro and macrovascular. However, if excess reactive species are central in 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?

Several years ago the possibility that a “hyperglycaemic memory” for a hyperproduction of fibronectin and collagen in endothelial cells persisting after glucose normalization, was preliminarily reported. Using the same design, 14 days in high glucose followed by 7 days of culture in normal glucose, preliminary data show that in endothelial cells an 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-NY, suggesting that oxidative stress may contribute to explain the appearance of the so called “metabolic memory”.

Fig. 1. Intracellular hyperglycaemia induces overproduction of superoxide at the mitochondrial level. Overproduction of superoxide is the first and key event in the activation of all other pathways involved in the pathogenesis of diabetic complications, such as polyol pathway flux, increased AGE formation, activation of protein kinase C and Nrf4, increased hexosamine pathway flux. Mitochondrial proteins are glycated in hyperglycaemia and this effect induces mitochondria to overproduce superoxide anions. In this case, even when glycemia is reduced or normalized, glycated mitochondria continue to overproduce superoxide, therefore activating the same pathways involved in the generation of diabetic complications. This hypothesis may contribute to explain the appearance of the so-called “metabolic memory”.

Mitochondrial overproduction of •O2– in hyperglycaemia has been suggested as the “unifying hypothesis” for the development of diabetic complications. Therefore, it is reasonable that mitochondria are also important players in propagating the “metabolic memory.”

Chronic hyperglycaemia is thought to alter mitochondrial function through glycation of mitochondrial proteins. Levels of methylglyoxal (MGO), a highly reactive α-dicarbonyl by-product of glycolysis, are increased in diabetes. MGO readily reacts with arginine, lysine and sulfhydryl groups of proteins in addition to nucleic acids, inducing the formation of a variety of structurally identified AGEs, both in target cells and in the plasma. MGO has an inhibitory effect on mitochondrial respiration and MGO-induced modifications are targeted to specific mitochondrial proteins. These premises are important because a recent study, for the first time, has described a direct relationship between the formation of intracellular AGEs on mitochondrial proteins, the decline in mitochondrial function and the excess formation of reactive species. Therefore, mitochondrial respiratory chain proteins which underwent glycation were prone to produce more •O2–, independently from the level of hyperglycaemia.

AGE formation is a prolonged phenomenon. In the DCCT, AGE formation was examined in 215 patients who underwent a skin biopsy 1 year before the close of the trial. Compared with conventional treatment, intensive treatment was associated with significantly lower levels of AGEs. Retinopathy, nephropathy and neuropathy outcomes were significantly associated with the levels of AGEs and increased levels of AGEs.
in the skin has been found to be significantly associated with the outcomes for microvascular complications in the EDIC. Furthermore, it is reasonable that AGEs may also explain the results regarding increased incidence of cardiovascular complications in the EDIC, considering that AGEs have been found associated with CVD even in non diabetic women.

What is really important is the clinical evidence that inclination of proteins, particularly collagen to be glycated is independent of the actual ambient glucose level. It has also been proposed that glycation of extracellular collagen may be a marker for glycation of intracellular proteins and a predictor of end-organ damage. While glycated HbA1c may be partially enzymatically deglycated, such a reaction has been not yet found for AGEs incorporated into collagen. Therefore, it appears that collagen AGEs formation is an irreversible phenomenon.

In conclusion, the glycation of mitochondrial proteins may be a contributing explanation for the phenomenon of the “metabolic memory.” Glycated mitochondria overproduce free radicals, independently from the actual glycaemia, maintaining the activation of the pathways involved in the pathogenesis of diabetic complications. In other words, it may be postulated that in the “metabolic memory” the cascade of the events is the same as that proposed by Brownlee—the source of $\text{O}_2^-$ is still the mitochondria—but that in addition the production of reactive species is unrelated to the presence of hyperglycaemia, depending by the level of glycation of mitochondrial proteins. This hypothesis is reported in the Figure 1.

THE “METABOLIC MEMORY” AND ENDOTHELIAL DYSFUNCTION: RELEVANCE TO CARDIOVASCULAR RISK IN DIABETES

Diabetes mellitus is associated with an increased incidence of macrovascular diseases. The accelerated macrovascular disease is due partly to the increased incidence of classical risk factors, such as hypertension and dyslipidemia. However, recent evidence suggests that hyperglycaemia also plays a significant role.

The endothelium is a major organ involved in the development of cardiovascular disease even in diabetes. All risk factors involved in the pathogenesis of cardiovascular disease, such as dyslipidemia and hypertension, can induce endothelial dysfunction, which has been largely shown to be predictive of a future cardiovascular event.

The presence of endothelial dysfunction has often been reported in diabetes. However, while several studies have shown that hyperglycaemia induces an endothelial dysfunction in both diabetic and non-diabetic subjects, a clear demonstration that controlling hyperglycaemia can restore/normalize endothelial dysfunction is still lacking. Particularly, in type 1 diabetic patients endothelial dysfunction has been reported to present even when normoglycaemia was achieved. Furthermore, several studies indicate that hyperglycaemia induces endothelial dysfunction through the generation of oxidative stress, which has been suggested to be the key player in the generation of the diabetic complications, both micro and macrovascular.

In a recent study, 36 type 1 diabetic patients and 12 controls were enrolled. The diabetic patients were divided in three groups. The first group was treated for 24h with insulin, achieving a near-normalization of glycaemia. At the 12 h of this treatment vitamin C was added for the remaining 12 hours. The second group was treated for 24 hours with vitamin C. At the 12 hours of this treatment insulin was started, achieving a near-normalization of glycaemia for the remaining 12 hours. The third group was treated for 24 hours with both vitamin C and insulin, achieving near normalization of glycaemia. Neither normalization of glycaemia or vitamin C treatment alone was able to normalize endothelial dysfunction or oxidative stress. Combining insulin and vitamin C normalized endothelial dysfunction and decreased oxidative stress to normal level. This study suggests that long-lasting hyperglycaemia in type 1 diabetic patients induces permanent alterations in endothelial cells, which may contribute to endothelial dysfunction by increased oxidative stress even when hyperglycaemia is normalized. The results of this study are explained in the figure 2.

The finding that only the simultaneous control of glycaemia and oxidative stress can normalize endothelial function in type 1 diabetic patients is clearly relevant. This evidence seems to suggest the existence of 2 different pathways working in the generation of endothelial dysfunction in type 1 diabetes: one directly related to hyperglycaemia and one not. A possible explanation for this evidence is that 2 pathways are simultaneously working: one due to the actual level of glycaemia generating free radicals during glucose utilization at the mitochondrial level, and another one to the long lasting damage induced in the endothelial cells by chronic hyperglycaemia, possibly through non-enzymatic glycation of mitochondria.

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 start in diabetic patients on an
early aggressive treatment of their hyperglycaemia. However, while this strategy can be easier accepted in type 1 diabetic patients, some concerns may arise in type 2 patients because this approach may include early insulin use. Moreover, a tight control of hyperglycaemia may also have to include the treatment of “postprandial” hyperglycaemia, not only because postprandial hyperglycaemia is a strong contributor to HbA1c in both type 1 and type 2 diabetic patients, but because postprandial hyperglycaemia is accompanied by a specific formation of both reactive species and AGEs not only in the plasma, but also intracellularly.

Another possible strategy is to reduce AGEs formation and oxidative stress generation concomitant with glucose normalizations. Several compounds have already shown the capacity of blocking AGEs formation. Metformin and pioglitazone have shown in vitro to prevent AGE formation. ACE inhibitors and AT-1 blockers are compounds used to control blood pressure, however, they are also able of reducing AGEs formation. Interestingly, these drugs also work as antioxidants, and at least for AT-1 blockers, there is evidence a specific action against hyperglycaemia-induced oxidative stress. Finally statins may be also potentially beneficial in reducing reactive species.

Putting this together, one could envision a future strategy consisting of compounds active on AGE formation, together with another compound capable of specifically targeting mitochondrial reactive species generation.

CONCLUSIONS
Consistent new emerging evidence suggests that hyperglycaemia can leave an early imprint in cells of the vasculature and of target organs, favoring the future development of complications. Additionally, evidence suggests that this “memory” can appear even when a good control of glycaemia is achieved. This phenomenon has been named as “metabolic memory.” The metabolic memory seems to be, however, a more common phenomenon and not only related to hyperglycaemia. Taken together, this evidence raises many questions regarding the therapeutic management of diabetes. In particular, because aggressive multifactorial intervention has already been demonstrated to reduce the risk of both micro and macroangiopatichic complications of diabetes, the existence of the metabolic memory suggests that a very early aggressive treatment of the various risk factors seems to be mandatory.  

Fig. 2. Glycemia, flow mediated dilation and nitrotyrosine plasma levels in the type 1 diabetic patients treated with:
- Insulin and Vit. C 24 h;
- Vit. C 24 h + Insulin 12 h;
- Insulin 24 h + Vit. C 12 h

From reference 40
REFERENCES


