

The “Metabolic Memory”: Is More Than Just Tight Glucose Control Necessary to Prevent Diabetic Complications?

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Context: The concept of a “metabolic memory,” that is of diabetic vascular stresses persisting after glucose normalization, has been supported both in the laboratory and in the clinic and in both type 1 and type 2 diabetes.

Evidence Acquisition: Using PubMed, we searched for publications on diabetic micro- and macrovascular complications using terms such as persistence, prolongation, sustained, and “memory” and focusing on the mechanistic basis behind this metabolic memory.

Evidence Synthesis: We found that as early as the mid-1980s this memory phenomenon was described in diabetic animals and isolated cells exposed to high glucose followed by normalized glucose and then, beginning around 2002, in results from large clinical trials such as the Diabetes Complications and Control Trial–Epidemiology of Diabetes Interventions and Complications and the United Kingdom Prospective Diabetes Study. Furthermore, mechanisms for propagating this memory appear focused on the nonenzymatic glycation of cellular proteins and lipids and on an excess of cellular reactive oxygen and nitrogen species, in particular originating at the level of glycated mitochondrial proteins and perhaps acting in concert with one another to maintain stress signaling independent of glucose levels.

Conclusions: The emergence of this metabolic memory suggests the need for early aggressive treatment aiming to “normalize” metabolic control together perhaps with the addition of agents which reduce cellular reactive species and glycation in order to minimize long-term diabetic complications. (*J Clin Endocrinol Metab* 94: 410–415, 2009)

The epidemic of diabetes is a serious and growing public health problem that results in reduced life expectancy and increased morbidity due to disease-specific vascular complications. Despite significant recent advances in hyperglycemia treatment, blood glucose monitoring, and markers of glycemic control, debilitating vascular complications remain in most diabetic patients. In particular, whereas the role of glycemic control in preventing the development of microvascular complications of diabetes is clear for both type 1 and type 2 diabetes (1, 2), its role for prevention of cardiovascular disease seems to be established in type 1 diabetes but is still a matter of debate in type 2 diabetes.

Most recently, the results of the ADVANCE and the ACCORD trials raise the debate about whether extremely tight glu-

cose control is beneficial at all in diabetes (3, 4). Specifically, the ADVANCE trial found that tight glucose control in type 2 diabetic patients involving gliclazide only resulted in decreased nephropathy with no change in the incidence of retinopathy or macrovascular complications (3). The results of the ACCORD trial found that tight glucose control resulted in increased mortality in high-risk type 2 diabetic patients (4). Conversely, whereas the results of the United Kingdom Prospective Diabetes Study (UKPDS) were unable to show a significant effect of strict glycemic control on myocardial infarction (2), a recent follow-up to the same study seems to confirm the utility of long-term hyperglycemic control in type 2 diabetes in preventing cardiovascular disease (5).

ISSN Print 0021-972X ISSN Online 1945-7197

Printed in U.S.A.

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doi: 10.1210/jc.2008-1824 Received August 19, 2008. Accepted November 25, 2008.

Abbreviations: AGE, Advanced glycation end-product; ARB, angiotensin receptor-1 blocker; ETC, electron transfer chain; MGO, methylglyoxal; mtDNA, mitochondrial DNA; NF- κ B, nuclear factor κ B; 3-NT, 3-nitrotyrosine; RAGE, receptor for AGEs; ROS, reactive oxygen species; STZ, streptozotocin.

However, the question seems to be more complex and is not simply related to the control of hyperglycemia. What is emerging is that hyperglycemia has long-lasting deleterious effects both in type 1 and type 2 diabetes and that glycemic control, if not started at a very early stage of the disease, is not enough to completely reduce complications.

In this review we seek to begin to address this question, first with clinical and experimental evidence, then with a potential mechanistic explanation, and finishing with a list of possible agents that may be useful for therapeutic intervention.

Evidence for a Metabolic Memory of Vascular Stress after Glucose Normalization

One way to explain this apparent discrepancy between glycemia and diabetic complication incidence and severity is what has been termed the “metabolic memory” (6), the idea that early glycemic environment is remembered in the target organs (*i.e.* eye, kidney, heart, extremities). The first investigation of such a memory was reported over 20 yr ago by the laboratory of Kern (7) in the retina of diabetic dogs who were switched to good glucose control after either 2 months or 2.5 yr of poor control and then analyzed at 5 yr after beginning the study. The animals switched to good glucose control at 2 months had little evidence of retinopathy, as did their control counterparts receiving good glucose control throughout the study. In contrast, the animals switched to good control at 2.5 yr had a similar incidence of retinopathy as their control counterparts who received poor glucose control throughout the 5-yr study. Just after this first study was published, the laboratory of Lorenzi (8) showed that there was a memory of basement membrane (collagen IV, fibronectin) mRNA induction in isolated endothelial cells and in the kidneys of streptozotocin (STZ)-induced diabetic rats 1 wk after glucose normalization after 2 wk of high glucose. Hammes *et al.* (9) next found that in a sucrose-fed diabetic rat model, islet transplantation at 6 wk, but not 12 wk, after the onset of diabetes could rescue aspects of diabetic retinopathy.

This metabolic memory phenomenon emerged, clinically, when the results of a large type 1 diabetes clinical trial, the Diabetes Complications and Control Trial (DCCT) and its follow-up Epidemiology of Diabetes Interventions and Complications (EDIC) trial, came to light. In the DCCT, type 1 diabetic patients were placed on either standard or intensive treatment regimens to normalize their glucose levels. Because the progression of microvascular complications was so profoundly reduced in patients with intensive glucose control, the DCCT ended early after a mean time of 6.5 yr and all patients were placed onto intensive therapy (1). Then in the EDIC follow-up trial with the same patient population, it was found that patients on the standard treatment regimen during the DCCT still had a higher incidence of microvascular diabetic complications such as nephropathy and retinopathy compared with their counterparts receiving intensive therapy throughout the trial several years after switching to intensive therapy, despite the fact that the mean glycated hemoglobin A1c levels of the groups were nearly equivalent (10, 11). Furthermore, recent data from the EDIC also

suggest a persistent influence of early glycemic control on the progression of macrovascular complications such as carotid intima-media thickness and cardiovascular disease (6, 12).

The metabolic memory has also been shown to be present in type 2 diabetes. Data from the follow-up of the UKPDS have shown that type 2 diabetic patients, like type 1 diabetic patients in the DCCT-EDIC, who were on the standard treatment regimen during the study still have a higher incidence of microvascular and cardiovascular complications compared with their counterparts receiving intensive therapy throughout the trial and the follow-up period (5). This suggests that early metabolic control has enduring beneficial effects also in type 2 diabetes.

A Potential Link between Oxidative Stress and the Metabolic Memory

The laboratory of Brownlee (13) has recently pointed to a relationship between an excess of superoxide anion (O_2^-), a reactive species associated with oxidative stress, in the mitochondria of endothelial cells in response to hyperglycemia with the formation of diabetic complications. This new insight further links this hyperglycemia-driven superoxide excess with four key pathways suggested to be involved in the development of diabetic complications—increased polyol pathway flux, increased advanced glycation end-product (AGE) formation, activation of protein kinase C, and increased hexosamine pathway flux—forming a unifying hypothesis for the formation of diabetic complications (reviewed in Ref. 14).

An apparent discrepancy between the hypothesis of superoxide excess and the metabolic memory phenomenon is that of time. Superoxide, like most reactive species, is a fleeting molecule with a half-life of at most minutes, whereas the metabolic memory can last years. A potential answer to this discrepancy is that there are a number of cellular biochemical targets for superoxide and similar reactive species including nucleic acids, proteins, and lipids/lipoproteins with a long half-life. Once modified by reactive species, these molecules can exert an altered cellular function over a prolonged time.

As mentioned above, Brownlee (13) found that superoxides emanating from the mitochondria were responsible for the deleterious effects of hyperglycemia on the vasculature. It is well known that the mitochondria are the “powerhouses” of the cell, and they have through their production of energy as ATP allowed for the existence of large multicellular organisms such as humans and have arguably allowed for us to exist in the oxygen-rich environment of the earth’s atmosphere. The electron transfer chain (ETC) or respiratory chain is responsible for this production of ATP, and as its name implies, electrons are transferred between the protein complexes of the ETC as part of energy production. When the ETC becomes uncoupled or dysfunctional, as in response to high glucose, electrons can be transferred to molecules like oxygen (O_2) forming superoxide (O_2^-) and other reactive species. These reactive species can interact with mitochondrial proteins such as those of the ETC, and it has been shown that certain reactive species such as peroxide (H_2O_2) and peroxynitrite ($ONOO^-$) can cross membranes and damage mac-

romolecules in other parts of the cell (reviewed in Ref. 15). Indeed, increased levels of 3-nitrotyrosine (3-NT), a reactive species protein adduct, have been shown to be a marker of oxidative stress in diabetic complications (16). Another unique property of the mitochondria is mitochondrial DNA (mtDNA), which unlike nuclear DNA is not tightly wound and compacted by chromatin, but exists instead in a rather open conformation, making it especially susceptible to damage from reactive species (reviewed in Ref. 17).

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

Indeed, there is a growing mass of evidence supporting the role of oxidative stress in the metabolic memory phenomenon. In STZ-induced type 1 diabetic rats, the laboratory of Kowluru (18) has shown that reinstitution of good glucose control for 7 months after 2 months of poor control inhibited elevations in retinal oxidized lipids but failed to have any beneficial effect on 3-NT induction. However, institution of good glucose control for 7 months after 6 months of poor control had no significant effect on retinal oxidative stress and 3-NT levels. In a similar study, the same group (19) showed that caspase-3 activity, a measure of apoptotic cell death, and nuclear factor κ B (NF- κ B), a key oxidative stress inducible inflammatory marker, were induced in the retina of diabetic rats kept in poor control for 13 months. Furthermore, reinstitution of good glycemic control after 2 months of poor control partially normalized the hyperglycemia-induced activation of caspase-3 and NF- κ B, whereas the reinstitution of good control after 6 months of poor control had no significant effect on the activation of either caspase-3 or NF- κ B (19). More recently, Kowluru *et al.* (20) have further supported and extended these findings by showing that 6 months of poor glycemic control followed by 6 months of good glycemic control showed no significant reduction in 3-NT levels in the retina of STZ-induced diabetic rats compared with counterparts receiving poor glycemic control throughout the study.

Furthermore, the functional role of oxidative stress in maintaining the metabolic memory was recently confirmed by our laboratories (21). Using the same design of the Roy *et al.* (8) memory studies almost 20 yr earlier, namely 14 d of high glucose followed by 7 d of normal glucose, data in endothelial and retinal cells and in the retinas of STZ-induced diabetic rats show that an overproduction of oxidative stress persists after the normalization of glucose levels. Furthermore, this oxidative stress excess is accompanied by a prolongation of the induction of the diabetic complication markers protein kinase C- β , reduced nicotinamide adenine dinucleotide phosphate oxidase, Bax, collagen and fibronectin, in addition to 3-NT (21). For the first time, it was definitively shown in endothelial cells that reducing intracellular production of free radicals, particularly at the mitochondrial level, was capable of switching off the metabolic memory (21). Similar results have also been reported in diabetic rats, where the antioxidant α -lipoic acid was given during the glucose normalization period (21). Interestingly, a recent paper has described how transient hyperglycemia (6 h) induces long-lasting activation of epigenetic changes in the promoter of the NF- κ B subunit

p65 in aortic endothelial cells both *in vitro* and in nondiabetic mice, leading to increased p65 gene expression (22). Both the epigenetic changes and the gene expression changes persist for at least 6 d of subsequent normal glycemia, as do NF- κ B-mediated increases in monocyte chemoattractant protein 1 and vascular cell adhesion molecule 1 expression (22). Furthermore, hyperglycemia-induced epigenetic changes and increased p65 expression were prevented by reducing mitochondrial superoxide production or superoxide-induced α -oxoaldehydes (22). These data are consistent with a preliminary report showing that oscillating periods of 12 h of high glucose induce extensive levels of oxidative stress mediated through NRF2, NQO1, BAX and TIGAR in endothelial cells, and this stress results in a cellular memory effect (23).

A recent study from our laboratory (24) has confirmed in type 1 diabetic patients that endothelial dysfunction, a causative marker of diabetic complications (reviewed in Ref. 25), persists even after normalizing glycemia. For the first time, this study has also been able to show that combining antioxidant therapy (vitamin C) with the normalization of glycemia can be used to almost interrupt endothelial dysfunction (24). The role of oxidative stress in this phenomenon appeared crucial: after 12 h of normalization of glycemia alone or after 12 h of vitamin C treatment alone, 3-NT levels remained increased and endothelial function was still altered, whereas combining glycemic control with vitamin C treatment normalized 3-NT levels and endothelial function (24). A similar memory effect has also been reported in a human study looking at the impact of glucose fluctuation on endothelial function and oxidative stress generation (26). Two periods of high glucose (10 and 15 mmol/liter) were able to induce a memory in endothelial dysfunction in normal subjects (26).

Role of AGEs in the Metabolic Memory

As mentioned above, it is reasonable to begin with the mitochondria as important players in propagating the metabolic memory. Along with oxidative stress, chronic hyperglycemia is thought to alter mitochondrial function through the glucose modification (glycation) of mitochondrial proteins. Specifically, levels of methylglyoxal (MGO), a highly reactive α -dicarbonyl by-product of glycolysis, are increased in diabetes (27). MGO readily attacks cellular proteins (28) and nucleic acids (29), inducing the formation of a variety of what are termed "advanced glycation end-products" (AGEs), known to play a causative role in diabetic complications (reviewed in Ref. 30). Furthermore, MGO has been shown to inhibit mitochondrial respiration, and MGO-induced modifications have been shown to target specific mitochondrial proteins (31). These premises are important because a recent study for the first time has described a direct relationship between the AGE modification of mitochondrial proteins, the decline in mitochondrial function, and the excess formation of mitochondrial reactive species (32).

Specifically, mitochondrial respiratory chain proteins that underwent glycation were prone to produce more superoxide, independent of the level of hyperglycemia (32). Finally, oxidative stress may alter mitochondrial protein expression (33, 34) and

The vicious circle of the “Metabolic Memory”

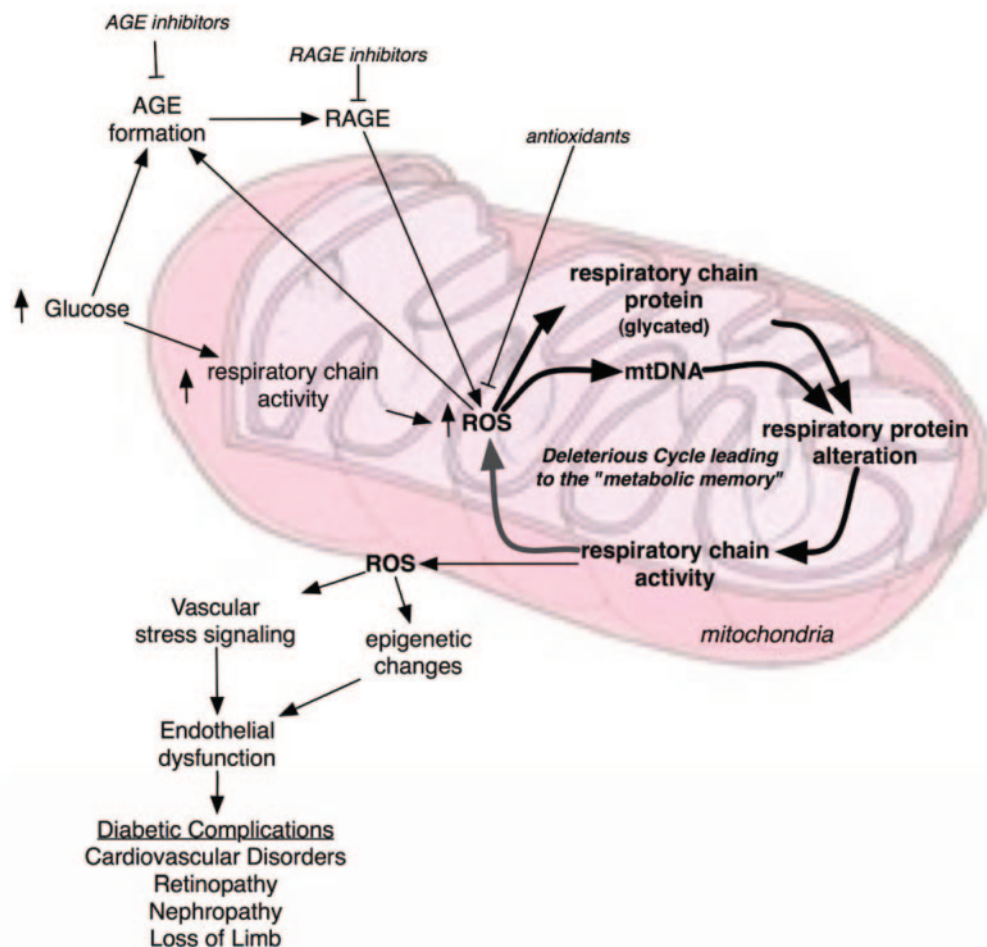


FIG. 1. Intracellular hyperglycemia induces overproduction of superoxide, a ROS, at the mitochondrial level as a possible cause of the metabolic memory of hyperglycemic stress after glucose normalization. Overproduction of ROS is the first and key event in the activation of all other pathways involved in the pathogenesis of diabetic complications, such as polyol pathway flux, increased AGE formation, activation of protein kinase C, and increased hexosamine pathway flux. Mitochondrial proteins are glycosylated in hyperglycemia, and this effect induces mitochondria to overproduce superoxide anion, a condition that does not depend on glycemic levels. Binding of AGEs to RAGE results in generation of intracellular ROS generation, which promotes the expression of RAGE themselves. mtDNA may influence gene expression and at the same time may contribute to an overgeneration of free radicals at the mitochondrial level. These self-maintaining conditions, leading to a persistent oxidative stress generation independent of the actual glycemic levels, may contribute to the appearance of the metabolic memory.

turnover (35), and AGEs are capable of inducing reduced nicotinamide adenine dinucleotide phosphate, which in turn can generate more free radicals (36).

Turning back to the clinical scenario, in the DCCT trial, AGE formation was examined in type 1 diabetic patients who underwent a skin biopsy 1 yr before the close of the trial (37). Compared with conventional treatment, intensive treatment was associated with significantly lower levels of AGEs. Furthermore, the 10-yr incidence of retinopathy and nephropathy were significantly associated with the levels of AGEs in the EDIC (37). Another important point is the clinical evidence that the inclination of proteins, particularly collagen, to be glycosylated was found to be independent of the actual ambient glucose level (38). Although glycosylated hemoglobin A1c may be partially enzymatically deglycosylated, such a reaction has not yet been found for other AGEs (38). Therefore, it is possible that mitochondrial AGE formation is an essentially irreversible phenomenon and could be responsible for the long-term nature of the metabolic memory.

Finally, evidence suggests that AGEs and the receptor for AGE called “RAGE” may be involved in the metabolic memory. There is a growing body of evidence that engagement of RAGE with AGEs elicits oxidative stress (39). Binding of AGEs to RAGE results in generation of intracellular reactive oxygen species (ROS) generation and subsequent activation of the redox-sensitive transcription factor NF- κ B in vascular wall cells, which promotes the expression of a variety of vascular damage genes including RAGE itself (40). Taken together, AGE modification of mitochondrial respiratory chain proteins can lead to excess reactive species. As mentioned in the oxidative stress section above, this can lead to a catastrophic cycle of mtDNA damage as well as functional respiratory chain decline, which further triggers reactive species generation and cellular injury. This aids in maintaining the activation of the pathways involved in the pathogenesis of diabetic complications via a metabolic memory now independent of glucose levels. This hypothesis is represented in Fig. 1.

Therapeutic Implications and Future Prospects

The possibility of "switching off" the metabolic memory could be, in the near future, an important strategy for the prevention of diabetic complications. Because oxidative stress seems to be a key player in the phenomenon, the use of antioxidants would be theoretically useful. However, it is well established that clinically available antioxidants do not have any beneficial effects in diabetes, at least at doses used in the available trials (41). However, it has already been suggested that a new therapeutic strategy would be the prevention of the overproduction of free radicals rather than scavenging those already produced (42). In this scenario, a possible strategy might be to reduce AGE formation, RAGE expression, and oxidative stress generation concomitant with glucose normalization. Several clinically used compounds have already shown the capacity of blocking AGE formation. The antidiabetic agents metformin and pioglitazone have been shown *in vitro* to prevent AGE formation (43). Furthermore, the antihypertensive drugs angiotensin-converting enzyme inhibitors and angiotensin receptor-1 blockers (ARBs) are also capable of reducing AGE formation (44). Indeed, telmisartan, an ARB, has been shown to down-regulate RAGE mRNA levels and subsequently inhibit generation of superoxide (45). Interestingly, these drugs also work as antioxidants, and at least for the ARBs, there is evidence that a specific action exists against hyperglycemia-induced oxidative stress and the metabolic memory (46, 47). Additionally, an oral antidiabetic compound, gliclazide, the main drug used in the ADVANCE study (3), may also be potentially beneficial in abolishing the memory, as has been found in cultured endothelial cells (48). Finally, an interesting option would be the use of benfotiamine, a B vitamin (thiamine) derivative, which has been capable both *in vitro* and *in vivo* of inhibiting the activation of the pathways induced by superoxide in response to high glucose (49, 50).

Putting this together, one could envision a future strategy consisting not only in an early aggressive treatment of hyperglycemia, but with the simultaneous use of compounds active on AGE formation, together with compounds capable of specifically targeting mitochondrial reactive species. This strategy, which clearly must be validated with further studies, has the potential to reduce the deleterious metabolic memory effect of hyperglycemia on diabetic complications.

Acknowledgments

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Disclosure Statement: The authors have nothing to disclose.

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