REVIEW ARTICLE

The role of "metabolic memory" in the natural history of diabetes mellitus

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KEY WORDS

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ABSTRACT

There is growing evidence that early, intensive treatment of new-onset diabetes mellitus aimed at tight glucose control reduces the risk of micro- and macrovascular complications. Metabolic memory is a term used to describe beneficial effects of immediate intensive treatment of hyperglycemia and the observation that they are maintained for many years, regardless of glycemia in the later course of diabetes. This phenomenon was first observed in preclinical studies and was later confirmed in large clinical trials. It has been suggested that early glycemia normalization can halt hyperglycemia-induced pathological processes associated with enhanced oxidative stress and glycation of cellular proteins and lipids. The phenomenon of metabolic memory suggests that antioxidants and agents degrading advanced glycation end products in addition to strict hypoglycemic treatment can be used to prevent chronic diabetic complications.

The results of large-scale clinical trials of key importance to contemporary diabetology, including DCCT (Diabetes Control and Complications Trial), UKPDS (United Kingdom Prospective Diabetes Study) and STENO-2, have provided evidence that intensive treatment of chronic hyperglycemia started in the initial stage of diabetes mellitus yields beneficial outcomes of long-term duration.[1-3] In patients who had been previously intensively treated, a significant decrease in the rate of chronic diabetes complications has been observed. On the other hand, a delay in effective treatment of hyperglycemia in a diabetic patient may cause a wide spectrum of adverse biological reactions in vascular endothelial cells. These processes cannot be stopped at later stages even if normal glucose levels are achieved. They lead to endothelial dysfunction, and to the irreversible morphological alterations typical for diabetic micro- and macroangiopathy.

This particularly negative phenomenon has been defined as metabolic memory.[4,5] Potential existence of such memory was suggested in the 1980s.[6] These reports indicated that hyperglycemia-induced metabolic stress left permanent vascular abnormalities in the target organs (e.g. the retina, kidneys, heart and lower extremities) despite achieving good glycemic control. Since then, the hypothesis of metabolic memory has been supported by the results from numerous experimental and clinical studies involving patients with types 1 and 2 diabetes.

Preclinical evidence One of the first trials which suggested a prolonged negative effect of hyperglycemia on blood vessels despite achieving tight glycemia control was conducted by Engerman and Kern.[6] The aim of this study was to evaluate whether improved metabolic control could inhibit the progression of retinopathy in dogs with alloxan-induced diabetes mellitus. Diabetic dogs were categorized into three groups according to glycemic control: the first with poor control for 5 years, the second with hyperglycemia for 2.5 years and with good control for the next 2.5 years, and the third with good control achieved by insulin administration and maintained during 5 years of observation. The reference group comprised non-diabetic dogs. It was observed that the introduction of intensive glucose level lowering treatment during 2 months since diabetes induction inhibited the development of microaneurysms and other histopathological lesions in the retinal vessels. However, such response was not observed when

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strict metabolic control was introduced after 2.5 years of hyperglycemia. The results indicate that progression of diabetic retinopathy may be inhibited when the treatment of diabetes mellitus is initiated as early as possible.

A study conducted by Kowluru et al. in Wistar rats yielded similar results. The aim of the study was to investigate whether achieving good glycemic control after a relatively short exposure to high glucose levels alters the level of oxidative stress in the retina.[7] Diabetes mellitus was induced by intraperitoneal streptozocin administration and the rodents were protected against ketoacidemia and body mass loss by insulin injections. In two groups of rats the treatment was administered to maintain poor metabolic control (glycated hemoglobin [HbA_{1c}] >11%) for 2–6 months. Subsequently, the insulin therapy was intensified so that satisfactory metabolic control could be achieved (HbA_{1c} <5.5%) and maintained for the next 7 months. The reference groups involved rodents that remained under poor or good metabolic control for 13 months. The experiments demonstrated an increase in lipid peroxide levels and a significant decrease in glutathione levels in the retinas of rodents with poor metabolic control. Additionally, poor metabolic control was accompanied by an increase in nitric oxide (NO) levels, inducible NO synthase (iNOS) expression and peroxynitrite formation. Reinstitution of good metabolic control improved retinal lipid peroxide and NO levels in the rats which were hyperglycemic for 2 months. A further decrease in glutathione levels in the retina and a significant effect on nitrotyrosine levels were not observed. Normalization of glucose metabolism in rats that were hyperglycemic for 6 months did not have a significant effect on the levels of oxidative stress markers and NO in the retina. The iNOS expression and nitrotyrosine accumulation in the retinas of these animals were ≥80% greater than in the groups with good control. The data confirmed that pathological lesions in retinal capillaries, induced by hyperglycemia-related oxidative stress and by nitrogen molecules, might occur after a relatively short exposure to high glucose (after 6 months these changes become irreversible). The data also indicated that hyperglycemia--induced biochemical alterations in the retina were hardly reversible. A chance of achieving such a beneficial effect depends on the duration of poor metabolic control prior to its improvement.

Another trial which might further support the theory of metabolic memory was performed on Lewis rats with streptozocin-induced diabetes.[8] One group of animals was subjected to 12 months of poor glycemic control (HbA_{1c} >12%). In the other group, 6 months of poor glycemic control was followed by 6 months of tight control (HbA_{1c} <6%). The reference group comprised nondiabetic rats. At the end of the study, the nitrotyrosine level, total antioxidant capacity and a number of acellular capillaries in the retina were measured. It was shown that total nitrotyrosine concentrations increased by 30% in the animals after 12 months of poor glycemic control and was 2.5-fold higher in isolated retinal microvessels compared to nondiabetic rats. In the poorly controlled animals almost 4-fold increase in the number of acellular capillaries, 50% decrease in superoxide dismutase activity and 25% decrease in total antioxidant capacity were noted. Achievement and maintenance of normoglycemia after 6 months of poor metabolic control did not reverse pathological changes in retinal vessels induced by prolonged hyperglycemia. These observations suggest that apart from excess of free radicals and oxidative stress, peroxynitrite accumulation, protein nitration in the whole retina and in the capillaries, as well as impaired ability to neutralize free radicals generated in response to high glucose levels in the mitochondria of endothelial cells, play a significant role in the phenomenon of metabolic memory. Normalization of carbohydrate metabolism after a few-month exposure of the retina to high glucose levels did not inhibit the development of hyperglycemia-induced histopathological lesions in the capillaries.

An interesting study by Kowluru et al. confirmed the association between the time needed to achieve normal blood glucose level and the persistence of biochemical changes induced by hyperglycemia in rats.[9] There were no marked differences in the levels of oxidative stress markers in the renal cortex and urine of the rats with good metabolic control achieved shortly after induction of diabetes, compared to nondiabetic rats. Achievement of good control after 6 months did not change the values of evaluated parameters. These results suggest that oxidative stress in tissues and organs of diabetic animals could be prevented by early initiation of tight glycemic control.[9]

In a study on diabetic, sucrose-fed rats Hammes et al. demonstrated that transplantation of isolated Langerhans islets after 6 weeks since the onset of disease inhibited diabetic retinopathy. Islet transplantation after 12 weeks of diabetes did not have equally positive effects. These findings confirm what other researchers have already suggested, namely that the earlier and more effective metabolic control, the lower the risk of negative effects of metabolic memory and of vascular complications.[10]

The hypothesis of metabolic memory has been supported by another experiment involving dogs on a 30% galactose diet. After 24 months, one group returned to a standard diet and the second continued to receive galactose. After 60 months the animals were sacrificed and their retinas examined. Pathological changes typical of diabetic retinopathy occurred despite completion of galactose administration.[11]

A study by Robinson et al. corroborates the above findings showing that thickening of retinal capillary basement membrane in rats on a 4- or 8-month 50% galactose diet was inhibited when a potent aldose reductase inhibitor

TABLE 1 Major clinical trials supporting the hypothesis of metabolic memory – basic trial and extended phase

Trial	Basic trial	Extended phase
	mean duration (years)	mean duration (years)
DCCT/EDIC	6.5 years (DCCT)	11 (EDIC)
UKPDS	10 (10.7)[a]	10
STENO-2	7.8	5.5

a Patients treated with sulphonylurea derivatives or insulin were followed for 10 years, whereas the group treated with metformin was followed for 10.7 years.

Abbreviations: DCCT – Diabetes Control and Complications Trial, EDIC – Epidemiology of Diabetes Interventions and Complications, UKPDS – United Kingdom Prospective Diabetes Study

in combination with galactose (primary prevention) was used. If the administration of aldose reductase inhibitor or withdrawal of the galactose diet occurred after 4 or 8 months (secondary prevention), the effects of such intervention were less pronounced and considerably delayed. These findings support the concept of the beneficial effect of a rapid, preventive reduction of hyperglycemia, and indicate that delayed aggressive intervention is far less effective.[12]

Clinical evidence The results of the extended phases of the landmark clinical trials[13-15], which included patients with types 1 or 2 diabetes mellitus, also indicate that metabolic memory does exist as previously suggested by the results of interventional studies (TABLE 1).

The purpose of DCCT study sought to investigate whether intensive insulin therapy had advantage over conventional treatment in terms of its impact on the chronic complication rate in type 1 diabetes. The researchers decided to preterminate the trial after approximately 6.5 years when they observed that the incidence of retinopathy was significantly lower in the intensive treatment group compared to that on conventional treatment. The risk of retinopathy progression was reduced by 78.5% in patients who underwent primary prevention and by 64.5% after implementation of secondary prevention.[16] A significant percentage of these patients took part in an 11-year follow-up study – EDIC (Epidemiology of Diabetes Interventions and Complications).[13] The study results showed that the incidence of the first composite cardiovascular endpoint and the first non-fatal myocardial infarction, stroke or cardiovascular death was lower by 42% and 57%, respectively, in patients undergoing intensive insulin therapy from the beginning compared to those treated conventionally. Such a substantial difference was observed despite similar HbA_{1c} levels in the two groups.[13]

Evidence for the existence of the metabolic memory was also provided by the extended phases of the UKPDS and STENO-2 trials performed in patients with type 2 diabetes.[14,15] The UKPDS study demonstrated that beneficial effects of 10-year intensive glycemic control on microangiopathy were maintained for the subsequent 10 years of follow-up. In patients intensively treated with sulphonylureas and insulin, the rate of microangiopathic complications was reduced by 24% ($p = 0.001$), macroangiopathic complications by 9% ($p = 0.04$), myocardial infarction by 15% ($p = 0.01$) and all-cause death by 13% ($p = 0.007$). In obese patients treated with metformin, the incidence of any diabetes-related endpoint decreased by 21% ($p = 0.01$), myocardial infarction by 33% ($p = 0.005$) and death from any cause by 27% ($p = 0.002$). It should be emphasized that as early as after 1 year of the extended phase, there were no significant differences in HbA_{1c} levels between intensive and conventional treatment groups. It indicates that the positive effects observed in the extended phase of the UKPDS trial were not closely associated only with HbA_{1c} levels. Most likely, the memory of good metabolic control in the initial stage of type 2 diabetes mellitus was one of the most important factors. Holman et al. defined the phenomenon observed in the extended phase of UKPDS trial as the "legacy effect". It refers to the extended positive effects of good metabolic control achieved in the early stage of type 2 diabetes mellitus.[14] The legacy effect augments the patients' chance for a significant reduction in micro- and macrovascular risk.

The results from the extended phase of the STENO-2 trial provide compelling evidence that an effective management of hyperglycemia, elevated blood pressure and lipid disorders has beneficial effects.[15] The study showed that despite the lack of significant differences in cardiometabolic risk factors, including HbA_{1c}, systolic and diastolic pressure, triglyceride, total cholesterol and LDL-cholesterol levels, a substantial difference in the incidence of defined endpoints was maintained over many years, with much better outcomes in the intensive treatment group.[15]

Another study which indicated the relevance of early good glycemic control was conducted by Chen et al. on patients with new-onset type 2 diabetes. After 10–14 days of intensive insulin therapy, the patients were scheduled for further treatment with insulin or oral antidiabetic drugs. At 6 months, the HbA_{1c} level was significantly lower and β-cell function more efficient in the insulin group compared to the oral treatment group.[17]

A coincidence of results, indicating the beneficial effect of early intensive therapy on the rate of chronic complications in types 1 and 2 diabetes, leads to the concept that the phenomenon of metabolic memory is now not merely a hypothesis but a clinically relevant biological mechanism.

Clinical evidence suggests that it is easier to prevent the unwanted effect of metabolic memory in patients with type 1 diabetes than in those with type 2 diabetes. It results from the fact that type 2 diabetes is preceded by a long period of prediabetes, usually lasting a few years, and then by asymptomatic phase of diabetes mellitus. Over

TABLE 2 Major hypotheses that account for the presence of metabolic memory

poor control of glycemia – an increased risk for tissue and organ damage as a result of enhanced oxidative stress or augmented production of advanced glycation end products
poor control of glycemia – an increased risk for renal damage, which in turn heightens the risk of cardiovascular events
poor control of glycemia – an increased risk for mitochondrial DNA damage by oxygen and nitrogen free radicals and advanced glycation end products

several years, glucose levels exceed normal, which facilitates passive, insulin-independent intracellular transport of higher amounts of glucose, especially in the endothelium, immune system as well as the central and peripheral nervous system cells. Typical clinical symptoms in type 1 diabetes mellitus usually develop rapidly, allowing earlier intervention and normalization of glycemia. Thus, it may be assumed that the risk of "encoding" of hyperglycemic stress exerted on blood vessels in type 1 diabetes mellitus is probably lower than it is in type 2 diabetes mellitus.

Pathogenesis of metabolic memory Many investigators seek an answer to the question of why the effect of good glycemic control at an early stage of diabetes mellitus is maintained over many years after its discontinuation. A number of hypotheses have been developed (TABLE 2).

It is believed that chronic hyperglycemia in diabetic patients creates environment in which closely related pathologies may lead to the development of chronic diabetic complications (FIGURE). One of the key factors responsible for complications in diabetes mellitus is an excessive production of free oxygen radicals in the mitochondria of the endothelial cells and an increase in oxidative stress.[18-20] As it was mentioned above, the higher glucose levels in extracellular space, the higher the levels in the endothelial cells. Higher amount of glucose is metabolized in the mitochondria of these cells, and more free radicals are produced. In these conditions, and with a simultaneous lower activity of endogenous systems that protect cellular structures against damage by these extremely active molecules, especially peroxides, the level of oxidative stress increases.

A considerable amount of data support the fact that excessive production of the reactive oxygen species in the mitochondria is the first stage in a cascade of interconnected events leading to the development of chronic complications in diabetes mellitus. Ceriello et al. defined this phenomenon as the vicious circle of metabolic memory.[21] Free radicals released in excess in the mitochondrial respiratory chain trigger many intracellular pathways, including activation of protein kinase C (PKC), increased polyol and hexamine pathway fluxes and increased advanced glycation end products (AGEs) formation. In addition, free radicals may change expression of a number of genes involved in the pathogenesis of chronic diabetic complications.[22-26] Activated PKC intensifies expression of various adhesive molecules, proinflammatory cytokines and growth factors. It also stimulates nuclear factor κB (NF-κB), which subsequently activates over 150 proinflammatory genes in vascular cells, including tumor necrosis factor β (TNF-β), interleukin (IL)-1, IL-6, IL-8, cyclooxygenase 2 and nitric oxide synthase.[27,28]

With an increased level of oxidative stress, synthesis of the extracellular matrix and production of plasminogen activator inhibitor-1 increases, while production of prostacyclin decreases. Adhesion of leucocytes and platelets to the endothelium is accelerated. Activation and then migration of macrophages and lymphocytes into the subendothelial space is another factor that triggers the release of free radicals as well as hydrolytic enzymes and proinflammatory cytokines, including TNF-α and IL-1. Interaction of reactive oxygen species with NO leads to peroxynitrite production. This compound has strong oxidizing effect on thiol and thioether groups and nitration effect on proteins, such as mitochondrial complexes I and IV, MnSOD (manganese superoxide dismutase), GAPDH (glyceraldehyde 3-phosphate dehydrogenase) and voltage-dependent anion channel. Furthermore, it decreases intracellular glutathione concentration and damages DNA strands.

Hyperglycemia and oxidative stress increase expression and serum concentration of metalloproteinases MMP-2 and MMP-9, which heightens the risk of chronic inflammation. All these processes worsen vascular contraction and relaxation, increase platelet activity and stimulate proliferation of vascular smooth muscles. This in turn contributes to the development and progression of micro- and macroangiopathic lesions.[29-33]

It has been demonstrated that high blood glucose levels inhibit endothelial production of NO, which causes abnormal vascular reactivity and worsens perfusion of tissues and organs.[34] On the other hand, it was established that hyperglycemia and the related oxidative stress increase iNOS activity, accelerating NO synthesis.[35] It creates conditions conducive to the damage of proteins of the mitochondrial electron transfer and reduction of peroxide detoxication and, consequently, glucose metabolism is directed to polyol and hexokinase pathways. At the same time, intensified protein and lipid glycation, endothelial cell apoptosis, and vascular reactivity impairment occur.[36,37] The toxic effect of hyperglycemia on tissues and organs is also associated with enhanced AGE formation, which is often an irreversible process.[38] These molecules are formed as a result of non-enzymatic reaction of reduced glucose with free amine groups of proteins, lipids and nucleic acids.

Intracellular protein glycation and AGE formation modify transcription of numerous genes, which increases mitochondrial production of free radicals as in the case of hyperglycemia.[39] AGEs may create cross bonds with proteins

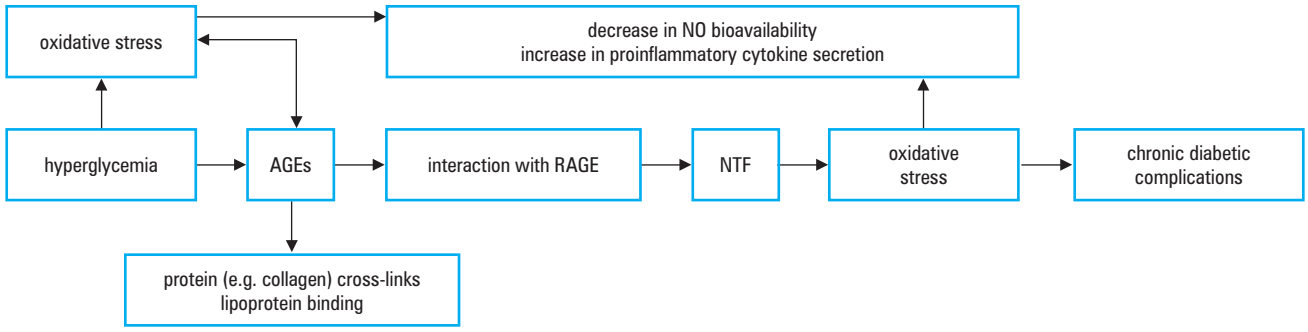

FIGURE The vicious circle of metabolic memory.
Abbreviations: AGE – advanced glycation end product, NO – nitric oxide, NTF – neurotrophic factors, RAGE – advanced glycation end product receptor

of the extracellular matrix, and thus change its function and structure. By binding to a suitable receptor (advanced glycation end product receptor, RAGE) in various cells, they send a signal for NF-κB activation, which influences expression of genes associated with diabetic complications.[23,35] Of note, AGEs may modify LDL cholesterol so that this lipid molecule undergoes oxidation more easily, becoming more atherogenic.[40]

Mitochondrial respiratory chain protein glycation seems to be especially important in the concept of metabolic memory.[41] Excessive inflow of glucose and retention of AGEs in the mitochondria activate and maintain overproduction of reactive oxygen species.

The results of a limited number of studies conducted to date indicate that AGEs accumulated in the skin of diabetic patients are a strong predictor of survival. Elevated serum levels of AGEs correlate with heart failure and major cardiovascular events, total mortality and mortality related to coronary artery disease.[42] The EDIC study showed that incidence of retinopathy and neuropathy positively correlated with the level of AGEs. Based on this finding it has been suggested that AGEs reveal higher prognostic significance than duration of diabetes mellitus and HbA_{1c} levels. It is because the risk associated with the accumulation of AGEs in the human body probably represents cumulative burden of hyperglycemia, dyslipidemia, oxidative stress, and chronic inflammation.[43,44]

Once the role of AGEs in metabolic memory and chronic diabetic complications was demonstrated, researchers were prompted to develop methods of AGE determination in different tissues and their elimination from the body (e.g. fluorescence, biochemical, immunologic, and mass spectrometry techniques). The latter may be achieved by using inhibitors of the cross-links. One such inhibitor, aminoguanidine, has been found to inhibit neuropathy and retinopathy development in a diabetic rat model.[45]

The cycle of events in the vascular cells might suggest that oxidative stress, associated with delayed therapeutic intervention, is essential in the phenomenon of metabolic memory.[5] It was shown that the use of antioxidants, acting at mitochondrial level (e.g. α-lipoic acid) after glycemia normalization, may further reduce the production of free radicals and oxidative stress markers in human endothelial cells and in the retina of diabetic rats. It was also demonstrated that the compounds inhibiting free radical generation outside the mitochondria only partially prevent the effect of metabolic memory.[20]

Considering the phenomenon of metabolic memory it has to be emphasized that not only uncontrolled hyperglycemia but also the abnormal values of other biochemical (lipids) and hemodynamic (blood pressure) parameters play a crucial role in the development of diabetic complications in the later stage of diabetes. However, substantial evidence shows that insufficient metabolic control in the past causes numerous biochemical reactions, which cannot be prevented later despite achieving normoglycemia. Numerous investigators including the Polish diabetologist, Artur Czyżyk, strongly emphasized the necessity of prompt preventive glucose lowering in patients with hyperglycemia.[46]

The most recent data indicate that some oral antidiabetic drugs exert a beneficial effect on the development of good metabolic memory. Glyclazide may serve as an example. A study on the endothelial cell culture collected from the human umbilical vein demonstrated that a few-day exposure to elevated glucose levels (30 mmol/l) leads to increased reactive oxygen species, 8-OHdG, nitrotyrosine, and caspase-3 levels and reduced Bcl-2 expression. Despite normalized glucose level in the environment (to 5 mmol/l), the adverse effects of hyperglycemia were not abolished. However, these effects were counteracted if glyclazide was added to the culture prior to the exposure to a very high glucose concentration. A similar beneficial effect was not achieved by means of glibenclamide. Likely, the difference resulted from specific antioxidant properties of glyclazide.[23] Recent experimental studies have shown that some compounds that have the ability to decrease free radical and AGE production or increase AGE degradation may be even more effective than free radical scavengers.[47] It was reported that these compounds might have retino- and nephroprotective activity.[45]

It is suggested that a number of drugs currently used in diabetic patients may probably prevent

AGE formation. These include pioglitazone, metformin, benfotiamine, vitamin B derivatives, lipoic acid, acetylsalicylic acid and angiotensin II receptor antagonists.[19,48,49] However, clinical trials conducted to date have not provided sufficiently strong evidence for their efficacy and safety.

In summary, the mechanism(s) of metabolic memory have not been fully clarified. However, it has been well documented that poor metabolic control at the onset of diabetes leads to excessive generation of free oxygen radicals in the mitochondria of endothelial cells and overproduction of AGEs. These changes activate a number of cellular pathways, which in turn increase the risk of micro- and macroangiopathic diabetic complications. The metabolic memory might be "programmed" by early, intensive treatment of hyperglycemia. A prompt and aggressive management of glycemia, blood pressure and lipid disturbances may significantly reducte the risk of chronic complications and premature death in diabetic patients.

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