Review Article

Metabolic memory and diabetic retinopathy: Legacy of glycemia and possible steps into future

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The response of retinal pathology to interventions in diabetic retinopathy (DR) is often independent of the glycated hemoglobin (HbA1c) values at the point of care. This is despite glucose control being one of the strongest risk factors for the development and progression of DR. Previous preclinical and clinical research has indicated metabolic memory, whereby past cumulative glucose exposure may continue to impact DR for a prolonged period. Preclinical studies have evaluated punitive metabolic memory through poor initial control of DM, whereas clinical studies have evaluated protective metabolic memory through good initial control of DM. In this narrative review, we evaluate the preclinical and clinical evidence regarding metabolic memory and discuss how this may form the basis of preventive care for DR by inducing "metabolic amnesia" in people with a history of uncontrolled diabetes in the past. While our review suggested mitochondrial biology may be one such target, research is still far from a possible clinical trial. We discuss the challenges in such research.

Key words: Diabetes mellitus, diabetic retinopathy, metabolic memory, prevention

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Diabetic retinopathy (DR) is a microvascular complication of diabetes mellitus (DM) with neurodegenerative retinal changes preceding or coexisting with vascular insult.[1] It is a slowly progressive disease leading to significant visual impairment. It impacts socioeconomic, functional, and psychological well-being. DR is classified as nonproliferative DR (NPDR), proliferative DR (PDR), and diabetic macular edema (DME).[2] Vision-threatening DR (VTDR) involves severe NPDR, PDR, and DME.[3] Currently, the worldwide prevalence of DR is 22.27% (103.12 million) and VTDR is 6.17% (28.54 million).[4] The management strategies for VTDR include systemic control of DM, periodic eye examination, retinal laser photocoagulation, intravitreal anti-vascular endothelial growth factor (anti-VEGF) or steroid injection, and vitreoretinal surgery, depending on the extent of VTDR.[5] However, despite maintaining "reasonable" systemic control, DR progresses from early (NPDR) to advanced (PDR) stages; aggressive treatment is not always successful.[6]

Continued worsening of DR, despite stabilized blood glycemia, has been linked to the effect of metabolic memory (MM).[7,8] This is a clinical phenomenon driven by biochemical, structural, and genomic changes at the cellular level, which make the progress of DR refractory to long-sustained glycemic alterations. The concept of MM was initiated from observations on the long-term beneficial effects of early intensive treatment of hyperglycemia on DR in clinical studies, regardless of the glycemic status in the later course of DM for people with poor diabetes control initially.[9] Later, MM was also proven in various short-term preclinical models evaluating changes following enforced poor to good glycemic control [Table 1]. There is a considerable time lag between the onset of DM and the onset of VTDR; this period is an opportunity where newer therapeutics may be evaluated to retard DR. MM offers one such target.[10]

The current knowledge on MM is largely from the *post hoc* analyses of intervention trials in DM and preclinical research. There are strategic differences between the preclinical and clinical research. This narrative review presents these differences and summarizes the pathophysiology and the cellular and clinical effects of MM. In addition, we also explored the possibility of therapies that can counteract the adverse effects of MM on the retina.

Search strategy and selection criteria

We searched PubMed and Google Scholar for articles on MM in English published from January 1988 to December 2022,

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Table 1: Preclinical studies explaining metabolic memory utilizing strategy of alternate poor and good control of glucose

Site of metabolic memory	Author	Experimental model	Pathway/process affected due to hyperglycemia	Duration of PC	Minimum time taken by the pathway to normalize after GC initiation
Mitochondria	Madsen-Bouterse *et al.*[11]	Rats	Mitochondrial oxidative stress and its DNA damage and repair system	6 months	6 months of GC did not revert the changes
	Zhong and Kowluru[12]	Rats	Degeneration of mitochondrial cristae, alteration in gene expression	6 months	6 months of GC did not revert the changes
	Santos and Kowluru[13]	Rats	Mitochondrial membrane transport systems	4 months	4 months of GC did not revert the changes
	Santos and Kowluru[14]	Rats	Mitochondrial biogenesis (nuclear and transcriptional factors)	6 months	6 months of GC did not revert the changes
	Mishra and Kowluru[15]	Cultured retinal endothelial cells and rats	Mitochondrial DNA mismatch repair genes	Endothelial cells – 4 days Rats – 3 or 6 months	4 days or 6 months of GC did not revert the changes
	Zhong and Kowluru[16]	Rats	Histone acetylation of manganese sodium dismutase gene (antioxidant machinery)	6 months	6 months of GC did not revert the changes
	Tewari *et al.*[17]	Rats	Retinal mtDNA replication	6 months	6 months of GC did not revert the changes
Epigenetic changes	Mishra *et al.*[18]	Rats	Antioxidant machinery (Keap1, Nrf2)	3 months	3 months of GC did not revert the changes
	Mishra *et al.*[19]	Rats	Nrf2-mediated retinal glutathione biosynthesis	3 months	3 months of GC did not revert the changes
	Kowluru and Chan[20]	Rats	Activation of retinal capillary cell apoptosis	6 months	6 months of GC did not revert the changes
	Kowluru *et al.*[21]	Rats	Apoptotic machinery	6 months	Partial recovery if GC initiated after 2 months, but no change if GC started after 6 months
	Zhong and Kowluru[22]	Rats	Regulation of the manganese superoxide dismutase gene	4 months	4 months of GC did not revert the changes
	Mishra and Kowluru[23]	Rats	DNA methylation	3 months	3 months of GC did not revert the changes
	Olsen *et al.*[24]	Zebra fish	MM is transmissible to the offspring and correlated with DNA hypomethylation and aberrant gene expression	-	-
microRNAs	Zhao *et al.*[25]	Human retinal endothelial cells	Effect of microRNA miR-23b-3p on sirutin 1	High glucose for 1 week or transient high glucose for 2 days	5 days of GC failed to revert the changes

GC=Good control, MM=Metabolic memory, mtDNA=Mitochondrial DNA, PC=Poor control

using the keywords "metabolic memory" or "legacy effect" in combination with the term "diabetic retinopathy"/"diabetes." Relevant references cited in those articles were also reviewed. The search yielded 125 articles containing both preclinical and clinical data. Three authors (AS, KB, BT) reviewed all studies for relevance, and after removing duplicates, 24 preclinical and 26 clinical articles were reviewed.

Preclinical evidence of MM

Use of preclinical models in diabetes research: Studies described later in this review[16-43] have used animal models with either induced autoimmune diabetes, cellular exposure to high glucose, or caused hyperglycemia by damaging the pancreatic beta cells (most common).[26] Streptozotocin (STZ), a glucosamine-nitrosourea compound, is one such drug which is toxic to pancreatic beta cells and their DNA with permanent effects.[27] It can affect other vital organs, too, by changing P450 isozymes.[28] This is a limitation, where drug-induced diabetes in an animal model completely abolishes insulin production and can affect other organs too, whereas even severe human DM can have variable insulin production as the glycemic control also depends upon the metabolic profile and lifestyle factors of the subject.

Preclinical studies show the independence of DR from the glycemic status following prolonged hyperglycemia.

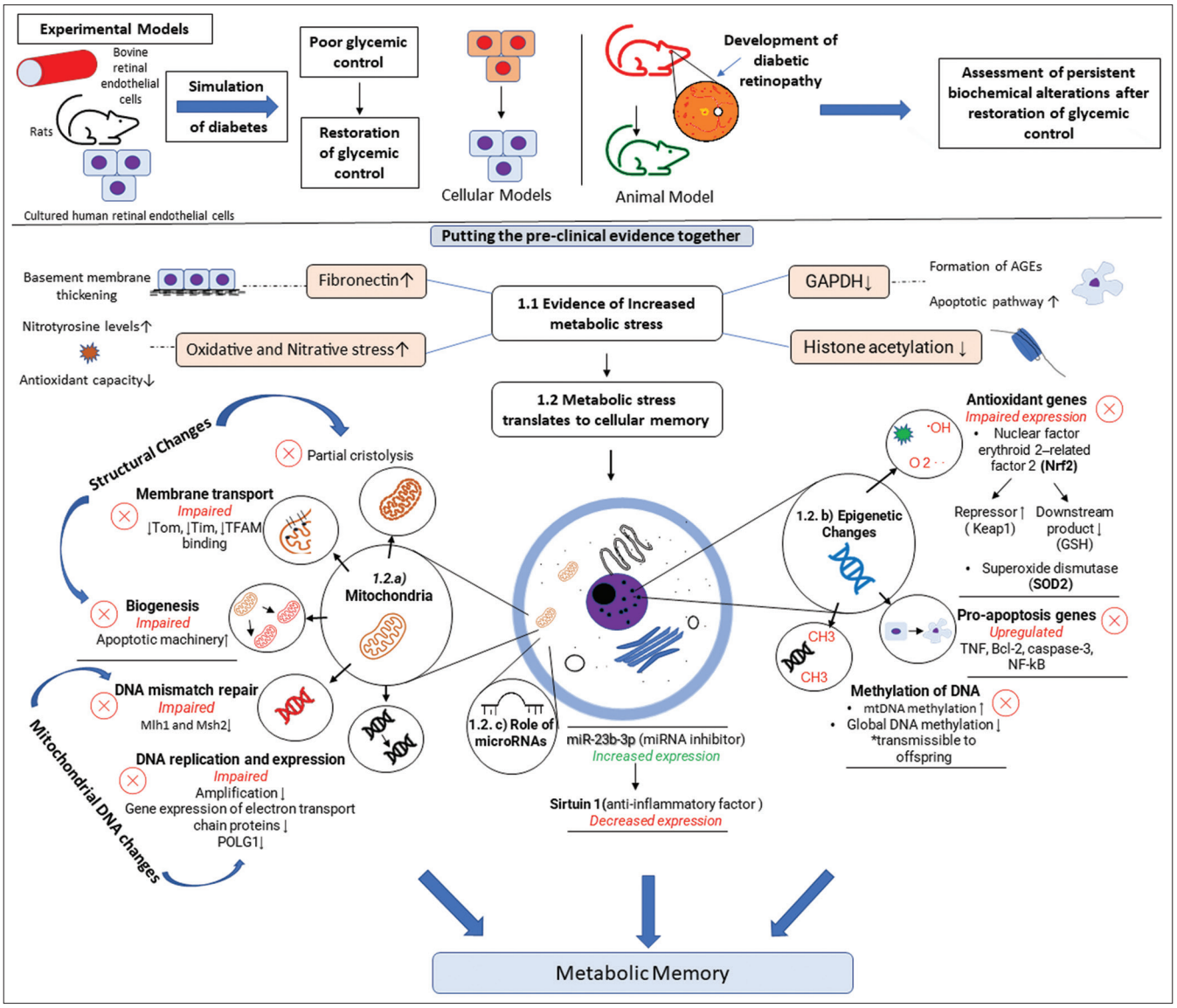

Figure 1: Illustration showing the source and cellular origins of metabolic memory collated as findings of the preclinical studies. The figure represents the phased studies with varying glycemic control discussed in Section 1 and indicates mitochondrial changes, epigenetics, and miRNA to be the cellular site of the memory. miRNA = microRNA

These studies have used the model of poor glycemic control followed later by good glycemic control to demonstrate MM [Table 1 and Fig. 1]. For example, Kowluru and Chan[20] studied the effect of poor control (PC) for 6 months, followed by good control (GC) for 6 months on apoptosis of retinal capillary cells in diabetic rats. Reinstitution of GC following PC did not reduce capillary cell apoptosis in the retinal vessels. Retinal genes, mainly from tumor necrosis factor (TNF) ligand and receptor, caspase, B-cell lymphoma 2 (Bcl-2), and death domain subfamilies, were upregulated around two-fold in PC rats and remained upregulated even after the reversal of hyperglycemia. The authors also studied the effect of hyperglycemia on apoptotic machinery in diabetic rats, which showed upregulated caspase-3 and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) activity.[21] Animal models suggest that MM arises from core injury induced by reactive oxygen species (ROS) primarily at the mitochondrial level, leading to sustained deleterious effects refractory to improved glycemic control. Advanced glycation end products (AGEs) and inflammation are the key initiators of this metabolic and oxidative stress.[29,30]

Chronic metabolic stress – sources and stimuli

Roy *et al.*[31] measured fibronectin in animal (diabetic rats) and experimental models (human umbilical vein endothelial cells [HUVEC]). Fibronectin causes basement membrane thickening. The authors found elevated fibronectin associated with hyperglycemia, which persisted even after restoring GC for 2 months. Furthermore, elevated fibronectin levels were noted in daughter cells despite multiple cell divisions of the parent cell in the absence of high glucose. This indicated the persistence of metabolic changes despite reversion to GC, suggesting MM.

Kowluru[32] studied the effect of varying duration of PC (2 or 6 months) followed by GC (7 months) on oxidative and

nitrative stress levels in the retina of diabetic rats. In the 2-month PC group, GC reduced retinal lipid peroxides and nitric oxide (NO) levels by 50% without affecting nitrotyrosine formation. However, compared to normal rats with GC, inducible NO synthase expression and nitrotyrosine levels were continuously >80% in these rats with 6 months of PC. In a similar rat model, with 6 months of PC followed by GC, the nitrotyrosine concentration in the retina, nitrotyrosine-positive retinal capillary cells, and the number of acellular capillaries were similar in PC to GC group and PC group. The retinal manganese superoxide dismutase (MnSOD) activity remained subdued, and the antioxidant capacity remained low 6 months after the reversal of PC, indicating this mechanism to be a source of MM.[33]

Madsen-Bouterse *et al*.[34] studied the effect of high glucose concentration on bovine retinal endothelial cell glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and the changes taking place after reversal to normal glucose. It was found that high glucose exposure decreases the activity of GADPH and leads to its translocation from cytosol to the nucleus. In the nucleus, it activates apoptotic pathways and is covalently modified. However, this modification is not immediately inhibited by reversal to normal glycemia.

Zhong and Kowluru[35] studied the role of histone acetylation in the development of the MM phenomenon in diabetic rat retina. Histone deacetylase (HDAC) was activated with increased gene expression, histone acetyltransferase (HAT) activity was reduced, and the acetylation of histone H3 was decreased in PC for 6 months. Such changes were independent of acute hyperglycemia, persisted after the restoration of normoglycemia, and formed the basis of chronic metabolic stress [Fig. 1].

Engerman and Kern[36] studied MM in DR by evaluating the effect of reversal of PC in dogs. There was development of retinal capillary aneurysms, loss of pericytes, intraretinal microvascular abnormalities (IRMA), and other lesions during 60 months in PC, and these changes were inhibited if GC was initiated early within 2 months. In PC followed by GC, the retinopathy was absent or similar at 2.5 years of PC and developed subsequently despite GC. The retinopathy in this study was greater at autopsy than at 2.5 years and was greater than in the group with GC, indicating sustained MM.

Ihnat *et al*.[37] evaluated MM in HUVEC, retinal pigment epithelial (ARPE-19) cells, and the retina of diabetic rats. In cell culture line and diabetic rats with PC for 3 weeks or 1 week, the levels of protein kinase C-beta, NAD (P) H oxidase subunit p47phox, Bcl-2-associated X protein, 3-nitrotyrosine, fibronectin, and poly (ADP-ribose) – the markers of metabolic stress – remained high for 1 week after the levels of glucose had normalized. This indicated a memory at the level of metabolic stress.

Metabolic stress to cellular memory

The generated chronic metabolic stress causes changes at the cellular level that are resistant to glycemic reversions. These changes have been broadly detected at the level of mitochondria, epigenetics, and microRNA (miRNA) [Fig. 1].

a. Mitochondria

***Structural and functional changes*:** Hyperglycemia-related metabolic stress induces mitochondrial dysfunction, leading to MM and DR.[11,38,39] Zhong and Kowluru[12] studied Wistar rats maintained in PC for 6 months, followed by GC for 6 months. Retinal vessels from rats with PC showed enlarged mitochondria with partial cristolysis (degeneration of mitochondrial cristae). Out of 84 genes, six retinal genes were upregulated and 12 were downregulated. There were alternations in the gene expressions of fusion, carrier, and fission proteins. Normalization of glycemic levels for 6 months did not reverse these changes, suggesting a long-lasting effect.

Santos and Kowluru[13] studied the role of mitochondrial membrane transport systems in the MM phenomenon in diabetic rats. Mitochondrial transcription factor-A (TFAM) is an essential factor in the activation of mitochondrial transcription and replication. The study found that the binding of TFAM with mitochondrial DNA (mtDNA) was impaired after 4 months of PC. The amelioration of hyperglycemia for 4 months had no beneficial effect on the decreased function of TFAM. Santos and Kowluru[14] also studied the role of mitochondrial biogenesis in MM. Six months of PC reduced mtDNA copy number, increased gene transcripts of peroxisome proliferator-activated receptor-γ coactivator-1α (PGC1), nuclear respiratory factor 1 (NRF1), TFAM, and decreased mitochondrial TFAM. The binding of TFAM with the chaperones remained lower, and these failed to improve with 6 months of GC. However, supplementation with lipoic acid (reduces oxidative stress) during 6 months of GC significantly reduced impaired mitochondrial biogenesis and also reduced the progression of retinopathy. Madsen-Bouterse *et al*.[11] too found significantly increased superoxide, DNA glycolases, and reduced mtDNA amplification, glutathione, and oxidoreductases of the electron transport chain during PC of DM. These four studies indicate a change in mitochondrial structure and function following uncontrolled metabolic stress, refractory to immediate forced euglycemia.

***Mitochondrial DNA repair defects*:** Mishra and Kowluru[15] studied the role of mtDNA mismatch repair genes in the pathogenesis of MM in retinal endothelial cells and the retina of diabetic rats. The sequence variants in mtDNA's displacement loop (D-loop) region were elevated, and repair proteins (Mlh1 and Msh2) were reduced. Overexpression of Mlh1 in the endothelial cells mitigated a glucose-induced increase in D-loop sequence variants, a decrease in respiration rate, and an increase in apoptosis, whereas overexpression of Msh2 did not protect the mitochondrial damage. Return to normoglycemia did not reverse the decrease in repair enzymes or increase in D-loop sequence variants.

Zhong and Kowluru[16] evaluated the role of histone methylation of *Sod2* (gene encoding MnSOD) in developing DR and the MM phenomenon in diabetic rats. Three or six months of PC reduced histone methylation and increased the binding of lysine-specific demethylase-1 (LSD1) and specificity protein 1 (Sp1) at SOD. Reversal of PC did not reverse these changes. Similarly, Tewari *et al*.[17] studied mtDNA replication and showed three consecutive months of PC, and then GC failed to reverse the downregulation of the mtDNA replication enzyme. These studies showed changes in mtDNA due to hyperglycemia, which did not revert following the normalization of glucose levels.

b. Epigenetic modifications

Epigenetic modifications are heritable altered chromatin structures without an actual alteration of the sequence of DNA.

Mishra *et al*.[18] evaluated the role of epigenetic modifications of the Kelch-like ECH-associated protein 1 (Keap1) promoter in retinal endothelial cells of diabetic rats kept on PC for 3 months, followed by GC for 3 months. Hyperglycemia increased the binding of transcriptional factor (Sp1) at Keap1, and the promoter continued to be methylated with increased expression of Keap1-regulated genes and decreased expression of NF-E2-related factor 2 (Nrf2)-regulated genes. Reversal of hyperglycemia did not reverse these changes.

In another unrelated study, Mishra *et al*.[19] evaluated the role of epigenetic modifications in Nrf2-mediated retinal glutathione biosynthesis and its implications in MM in retinal endothelial cells of diabetic rats. In diabetes, the binding of Nrf2 at antioxidant response element region 4 (ARE4) is decreased. Diabetic rats were kept on PC for 3 months, followed by GC for 3 months. Histone methylation was altered in diabetes, which led to impaired binding of Nrf2 at catalytic subunit of enzyme glutamate–cysteine ligase (Gclc)-ARE4, thus reducing Glutathione (GSH) and increasing oxidative stress. Reversal to GC for 3 months did not ameliorate these changes.

Zhong and Kowluru[22] studied the role of epigenetic regulation of SOD2 in the MM phenomenon. Diabetic rats were kept on PC or GC for 4 months or on PC for 2 months, followed by GC for 4 months. PC caused alterations at the promoter and enhancer of retinal SOD2. Return to normoglycemia did not prevent these changes, and SOD2 continued to be abnormal, hinting at a mechanism for MM.

Mishra and Kowluru[23] studied the role of DNA methylation in MM in diabetic rats kept on PC for 3 months, followed by GC for 3 months. They also evaluated the effect of the presence/absence of inhibitors of DNA methylation in human retinal endothelial cells (HREC). Four days of GC after 4 days of PC did not decrease the activity of DNA methyltransferase 1 (Dnmt 1), hypermethylation of mtDNA with impaired transcription, upregulation of hydroxymethylation enzyme, and matrix metalloproteinase-9. Similarly, 3 months of euglycemia following hyperglycemia of 3 months could not reverse these changes.

Olsen *et al*.[24] showed in a zebrafish model that MM is transmissible to the offspring and correlated this with DNA hypomethylation and aberrant gene expression. STZ-induced DM was followed by recovery to normoglycemic levels, which took 2 weeks. However, caudal fin regeneration and skin wound healing remained impaired as in diabetic zebrafish, and this impairment was transmissible to the daughter zebrafish, though it lacked any AGE accumulation or increased oxidative stress. The study showed that hyperglycemia-induced global DNA hypomethylation correlated with aberrant gene expression for a subset of loci in the daughter's tissue.

These preclinical studies by Mishra *et al*.,[23] Zhong *et al*.,[22] and Olsen *et al*.[24] have thus suggested epigenetic changes to be induced due to hyperglycemia that persist through a later period of euglycemia, with the latter group's study even showing heritability across generations.

c. RNAs as a mediator of MM

Bixler *et al*.[40] studied changes in the retinal transcriptome of diabetic rats in comparison to euglycemic rats. The rats were evaluated for 3 months, and one group received insulin after 1.5 months. The gene expression changes were analyzed by quantitative polymerase chain reaction (PCR). About 57% of diabetes-induced mRNA changes (789 probes) observed at 3 months were fully normalized to control levels with insulin therapy, whereas 37% of probes (514) were only partially normalized. Few genes (5%, 65 probes) remained significantly dysregulated even in the insulin-treated diabetic rats. The genetic changes not rescued or prevented by insulin treatment may suggest MM.

The miRNAs are small noncoding RNAs that regulate the gene expression of the coding genes. Zhao *et al*.[25] demonstrated MM in human Retinal Endothelial cell (REC), where high glucose increased miRNA, which downregulated SIRT1. The, iRNA did not decrease even after return to normal glucose. Reduced miR-23b-3p expression inhibited proinflammatory expression by inhibition of SIRT1, negating the effect of MM. Similar results were also replicated in the diabetic rat model of MM. There is less evidence suggesting the role of mRNA and miRNA compared to that of mitochondrial changes and epigenetics changes for the development of MM [Fig. 1].

Clinical evidence of MM

Surprisingly, almost all human evidence on MM and DR comes from data investigated for the management of DM and not that of DR. These are either a *post hoc* analysis of clinical trials or long-term observational studies evaluating cumulative exposure to hyperglycemia in people with DM [Table 2 and Fig. 2].

2.1. Diabetes Control and Complications Trial and Epidemiology of Diabetes Interventions and Complications

The Diabetes Control and Complications Trial (DCCT) studied intensive blood sugar control in type 1 DM (T1DM).[54] At 6.5 years, all patients in the DCCT study were enrolled in Epidemiology of Diabetes Interventions and Complications (EDIC), a long-term observational study.[55] After 10 years of follow-up, no significant difference was seen in the glycated hemoglobin (HbA1c) level between intensive and conventional groups (8.07% vs. 7.98%), but there was a significant difference of ≥3-step retinopathy

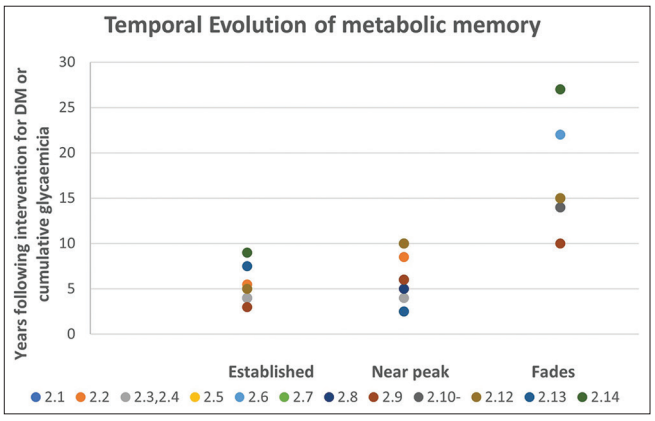

Figure 2: Scatter graph showing the temporal evolution of metabolic memory. Study 2.11 (VADT) did not account for the development of metabolic memory, study 2.9 is an analytic dataset, while 44-year results of study 2.14 (UKPDS) are not fully published yet. The evolution presented is authors' understanding from the papers quoted, and not the numeric provided in these individual studies. UKPDS = United Kingdom Prospective Diabetes Study, VADT = Veteran Affairs Diabetes Trial

Table 2: Clinical studies explaining metabolic memory

	Author and Study type	DCCT/EDIC[41]/prospective randomized clinical trial
1	DM type/duration	T1/18 years/*n*=1214
	Objective/method	To evaluate the effect of intensive blood sugar control on progression of DR
	Results	No significant difference in HbA1c level between intensive and conventional groups (8%) Significant difference of ≥3-step retinopathy progression between the two groups (41.1% vs. 58.7%) Persistence of risk reduction, though lowered compared to 10 years of follow-up (41% vs. 60%)
	Conclusion	Lower risk of incidence of retinal complications is maintained in the intensive group, though gap between the two groups decreases with time, while HbA1c is comparable
2	Author and Study type	Lind *et al.*[42]/retrospective analysis of DCCT/retrospective analysis
	DM type/duration	T1/12.3 years
	Objective/method	To evaluate the relative risk contribution of HbA1c Used values at different points in time before the event, indicating progression of retinopathy occurred
	Results	HbA1c of last 2–3 years had the greatest relative risk contribution to the current progression of retinopathy HbA1c of last 5 years made a greater contribution than the current values, while values from last 8 years still had a significant impact Effects take 2–3 years to appear after lowering of HbA1c The numbers needed to treat when reducing HbA1c from 8.3% to 8% from diagnosis were estimated to be 1688 for the first 3 years and 13 for the period 9–12 years
	Conclusion	The present HbA1c value is not the most important; rather, 2–3 years earlier HbA1c had the highest impact The shape of metabolic memory of HbA1c influences the present-day retinopathy changes even if current glycemic control is good
3	Author and study type	Giordano *et al.*[43]/retrospective cohort
	DM type/duration	T1/15 years/*n*=376
	Objective/method	To evaluate the predictive role of the main clinical and biochemical parameters in determining microvascular complications
	Results	A higher probability of developing DR was found in patients with higher mean HbA1c levels during follow-up (HR 2.35, 95% CI 1.34–4.12; *P*=0.003), as well as at onset (HR 1.85, 95% CI 1.06–3.24; *P*=0.030)
	Conclusion	Among various clinical, metabolic, immunologic, and biochemical factors evaluated at onset, only HbA1c is predictive for the microangiopathy development in T1DM Only the metabolic memory exerts a role in DR development
4	Author and study type	Hirose *et al.*[44]/retrospective cohort
	DM type/duration	T1/20 years/*n*=15
	Objective/method	To evaluate if mean "metabolic memory-free" HbA1c values covering total diabetes duration could predict retinopathy in younger-onset T1DM
	Results	Significant difference of mean HbA1c between retinopathy-positive and retinopathy-negative patients (*P*=0.0048) No significant difference between the two groups in mean HbA1c (10^{th}–20^{th} year)
	Conclusion	Mean HbA1c values had a substantial capacity to predict retinopathy at year 20 after onset HbA1c monitoring for the entire duration of DM is more important than the partial initial period linked to metabolic memory
5	Author and study type	Bolotskaya *et al.*[45]
	DM type/duration	T1/20 years/*n*=155
	Objective/method	To assess the time course of changes in the level of HbA1c for 20 years after the onset of T1DM and to compare its correlation with the development of microvascular complications, DR, and DN
	Results	Level of HbA1c at the onset of the disease in patients developing the complications was higher than in those without complications (10.2%±0.6% and 8.5%±0.2%, *P*=0.003) Significant differences in HbA1c levels between the groups persisted during 15 years of follow-up, averaging 9.2%±1.5%, 9.7%±0.9%, and 8.1%±0.7% after 5, 10, and 15 years, respectively, in the complication group and 7.1%±0.3%, 8.1%±0.4%, and 7.2%±0.2% in the no complication group (*P*<0.01) Mean duration of T1DM was 9.6±6.2, 11.0±2.0, and 13.6±4.6 years for the nonproliferative, preproliferative, and proliferative stages
	Conclusion	Microvascular complications in patients with poor glycemic control at onset suggest metabolic memory phenomenon

Contd...

Table 2: Contd...

	Author and Study type	DCCT/EDIC[41]/prospective randomized clinical trial
6	Author and study type	Ducos *et al*.[46]/retrospective cohort
	DM type/duration	T2/13±10 years/*n*=334
	Objective/method	To evaluate whether DR in T2DM is related to metabolic memory
	Results	The association between DR and having an HbA1c higher than the median was significant only for the oldest previous HbA1c values (OR=6.75, 95% CI 1.90–23.90)
	Conclusion	25.1% of well-controlled T2DM patients had DR, which was related to their HbA1c levels from 5 years before study admission
7	Author and study type	Gaede *et al*.[47]/prospective clinical trial
	DM type/duration	T2/21 years/*n*=160
	Objective/method	To study the potential long-term impact of a 7.8-year intensified, multifactorial intervention in patients with T2DM and microalbuminuria in terms of gained years of life and years free from incident cardiovascular disease
	Results	Progression of retinopathy reduced by 33% in the intensive therapy group Blindness in at least one eye was reduced in the intensive-therapy group with an HR of 0.47 (95% CI 0.23–0.98, *P*=0.044)
	Conclusion	Significant beneficial effect of early intensive treatment seen
8	Author and study type	Azad *et al*. (VADT)[48]/prospective randomized controlled study
	DM type/duration	T2/17 years/*n*=133
	Objective/method	To assess the long-term role of INT compared to standard glycemic control in accumulated eye procedures in patients with advanced diabetes
	Results	A mild nonsignificant increase in number of procedures in the INT group
	Conclusion	INT played no protective role, probably due to the level of advancement of DR
9	Author and study type	ACCORD[49]/prospective randomized controlled study
	DM type/duration	T2/4 years/*n*=10,251
	Objective/method	To study whether intensive blood glucose control, combination therapy for dyslipidemia (simvastatin + fenofibrate), and intensive blood pressure control would help to limit the progression of DR
	Results	7.3% of had progression of retinopathy in the intensive therapy group compared to 10.4% in the standard therapy group (OR=0.67; 95% CI -0.51 to 0.87; *P*=0.003) The rate of moderate vision loss was 16.3% and 16.7% among patients receiving intensive and standard glycemic therapy, respectively (adjusted HR 0.95; 95% CI 0.80–1.13; *P*=0.56) There was significant difference in progression of DR in the fenofibrate group (6.5%) when compared to the placebo group (10.2%) (adjusted OR=0.60, 95% CI 0.42–0.87; *P*=0.006)
	Conclusion	The study concluded that INT and intensive combination treatment with anti-lipid agents reduced the rate of progression of DR, but not intensive blood pressure control
10	Author and study type	ACCORDION[50]/prospective observational study, follow-up of ACCORD
	DM type/duration	T2/4 years/*n*=1310
	Objective/method	Observational follow-up study of ACCORD
	Results	5.8% of participants in the intensive group and 12.7% in the standard group showed progression of DR at the end of eighth year (95% CI 0.28–0.63, *P*=0.0001), concluding significant benefit of intensive blood glucose control in reducing the risk of progression of DR Rates of moderate vision loss were 29.6% in the intensive group and 31.7% in the standard group at the end of 8 years (95% CI 0.90–1.07, *P*=0.67)
	Conclusion	Significant benefit of intensive blood glucose control in reducing the risk of progression of DR in long term
11	Author and study type	ADVANCE[51]/prospective randomized controlled study
	DM type/duration	T2/5 years/*n*=11,140
	Objective/method	To study the effects of intensive glucose control on major microvascular and macrovascular events in T2DM
	Results	Intensive control resulted in reduction in the incidence of major microvascular events (HR 0.86, 95% CI 0.77–0.97; *P*=0.01), but not in the major macrovascular events (HR 0.94, 95% CI 0.84–1.06; *P*=0.32) 10% reduction in the incidence of nephropathy, but not DR in the intensive glucose control group
	Conclusion	No direct evidence of reduction in the risk of progression of retinopathy, but metabolic memory cannot be completely excluded Participants needed to be followed for longer duration than 5 years to assess for progression of DR

Contd...

Table 2: Contd...

	Author and Study type	DCCT/EDIC[41]/prospective randomized clinical trial
12	Author and study type	VISS[52]/prospective randomized controlled study
	DM type/duration	T1/32 years/*n*=451
	Objective/method	To evaluate HbA1c as a predictor for severe microvascular complications and to formulate target HbA1c levels for treatment of T1DM
	Results	After 32 years, 9% had no retinopathy, 64% had NPDR, and 27% had PDR; HbA1c values with the least risk of development of PDR and nephropathy were 7.3% (56 mmol/mol) and 8.1%, respectively
	Conclusion	Long-term weighted mean HbA1c is a very strong biomarker of PDR and nephropathy, suggestive of long-term effect of glycemic control
13	Author and study type	SDIS[53]/prospective randomized controlled study
	DM type/duration	T1/10 years/*n*=102
	Objective/method	To evaluate the effects of ICT and RT in T1DM individuals with NPDR and unsatisfactory glucose. At 7.5 years, the RT group was given intensified treatment
	Results	The mean HbA1c was significantly lower in the ICT group and HbA1c was reduced from 9.5%±1.4% in the ICT group and 9.4%±1.2% in the RT group to a mean (for 10 years) of 7.2%±0.6% and 8.3%±1.0% (P<0.001), respectively The life table analysis of risk of developing serious retinopathy showed that about 60% of ICT patients did not develop serious retinopathy compared to about 30% in the ST group
	Conclusion	The intensive treatment reduced the DR complications significantly at year 10, suggestive of a metabolic memory effect
14	Author and study type	UKPDS (58)/prospective randomized controlled study
	DM type/duration	T2/44 years/*n*=4209
	Objective/method	To compare the effect of intensive and conventional glucose control therapy on microvascular complications in T2DM
	Results	2%–6% fewer microvascular complications such as DR and DN following early intensive blood glucose control with sulfonylureas or insulin Early intensive blood glucose control with metformin also showed reduction in heart attacks by 31% and in deaths by 25%
	Conclusion	The 44-year follow-up results are suggestive of a positive legacy effect of intensive treatment

ACCORD=Action to Control Cardiovascular Risk in Diabetes, ACCORDION=Action to Control Cardiovascular Risk in Diabetes Follow-On, ADVANCE=Action in Diabetes and Vascular Disease, CI=confidence interval, DCCT=Diabetes Control and Complications Trial, DM=diabetes mellitus, DN=diabetic nephropathy, DR=diabetic retinopathy, EDIC=Epidemiology of Diabetes Interventions and Complications, HbA1c=glycated hemoglobin, HR=hazard ratio, ICT=intensified conventional treatment, INT=intensive glycemic control, NPDR=nonproliferative DR, OR=odds ratio, PDR=proliferative DR, RT=regular treatment, SDIS=Stockholm Diabetes Intervention Study, T1DM=type 1 diabetes mellitus, T2DM=type 2 diabetes mellitus, UKPDS=United Kingdom Prospective Diabetes Study, VADT=Veteran Affairs Diabetes Trial, VISS=Vascular diabetic complications in south-east Sweden

progression between the two groups (35.8% vs. 60.6%). The risk reduction was greater in the first 4 years compared to 10 years (74% vs. 57%).[56,57] At 18 years of follow-up, there was no significant difference in HbA1c level between the intensive and conventional groups (8%).[41] The two groups significantly differed in ≥3-step retinopathy progression (41.1% vs. 58.7%, P < 0.0001). The risk reduction effect of intensive treatment persisted, though it was lower than 10-year follow-up (41% vs. 60%, P < 0.0001). In summary, the study results showed the effect of MM on DR to persist for a long duration, though it faded with a lack of intensive control.

2.2. Steno diabetes studies

The Steno trial compared the effect of targeted, intensified, multifactorial intervention with conventional treatment on the risk factors for cardiovascular disease and microvascular complications in DM.[47,58] The Steno-2 trial studied the long-term effect of intensive treatment in people with type 2 DM (T2DM) and microalbuminuria. At the end of the main trial, 19 (23.8%) patients in the intensive group and 33 (41.3%) in the conventional group showed DR progression (odds ratio [OR] 0.45 [95% confidence interval {CI} 021–0.95], P = 0.04). After 13.3 years (7.8 years of trial and 5.5 years of follow-up), there was a significant reduction in the need for retinal photocoagulation in the intensive group than the conventional group (OR = 0.45 [95% CI 0.23–0.86], P = 0.02), with concurrent reduced DR progression in the intensive group (OR = 0.57 [95% CI 0.37–0.88], P = 5 0.01). At the end of the 21.2-year follow-up, DR progression reduced by 33% in the intensive group, with a significant reduction in blindness in at least one eye (hazard ratio [HR]: 0.47, 95% CI 0.23–0.98; P = 0.044).[59] In summary, the lowering risk of diabetic complications was attributed directly to the early intensive treatment, thus validating the continuing effects of MM during the observation phase.

2.3,4. Action to Control Cardiovascular Risk in Diabetes, Action to Control Cardiovascular Risk in Diabetes Follow-On

Action to Control Cardiovascular Risk in Diabetes (ACCORD) studied the impact of intensive blood glucose control, combination therapy for dyslipidemia (simvastatin + fenofibrate), and intensive blood pressure control in people with T2DM.[49,60] The Action to Control Cardiovascular Risk in Diabetes Follow-On (ACCORDION) study was an observational follow-up study of ACCORD.[50] The difference in HbA1c levels between the groups did not sustain at the end of

the ACCORDION study. The mean HbA1c (mA1c) values were 7.3% and 7.7% at the beginning and 7.8% and 7.9% at the end in the intensive and standard groups, respectively. Thirty-eight of 658 (5.8%) participants in the intensive group and 83 of 652 (12.7%) in the standard group showed progression of DR at the end of the eighth year (95% CI 0.28–0.63, $P = 0.0001$). In summary, the study documented significant benefit of early intensive blood glucose control in reducing the risk of DR progression. Intensive glycemic control conferred protection from the progression of retinopathy, even though the difference between the glycated A1c values diminished. This was consistent with the phenomenon of MM seen in T1DM as reported earlier.[61]

2.5 Action in Diabetes and Vascular Disease

The Action in Diabetes and Vascular Disease (ADVANCE) trial studied the effects of intensive glucose control on major microvascular and macrovascular events in T2DM.[51] The study documented a reduced incidence of major microvascular events (HR 0.86, 95% CI 0.77–0.97; $P = 0.01$) with intensive control of DM. There was no direct evidence of a reduction in the risk of retinopathy progression. However, the possibility of a positive MM effect in the intensive control group could not be entirely excluded as the follow-up was limited to only 5 years.

2.6 Vascular diabetic complications in south-east Sweden

Vascular diabetic complications in south-east Sweden (VISS) evaluated HbA1c as a predictor for severe microvascular complications and determined the target HbA1c level for T1DM.[62] It was a longitudinal observational study that recruited 451 people with early T1DM diagnosed before age 35 and followed up for a mean of 22 years. Patients were grouped by HbA1c values (<6.7%, 6.8%–7.6%, 7.7%–8.6%, 8.7%–9.5%, >9.5%), and the prevalence of different grades of retinopathy was studied. DR was detected in each group, except for those with HbA1c <6.7%. The prevalence of laser-treated retinopathy increased with an increase in HbA1c values. After 32 years of follow-up, HbA1c 7.3% was the lowest value associated with developing PDR, which manifested at lower HbA1c, indicating the need for long-term weighted mA1c as a biomarker of PDR.[52]

2.7. Giordano *et al*.[43] studied the impact of clinical and biochemical parameters in determining microvascular complications (microalbuminuria and retinopathy) in T1DM and found only HbA1c to be predictive. The study documented a higher risk of retinopathy with higher mA1c (HR 2.35; 95% CI 1.34–4.12; $P = 0.003$), including at diagnosis (HR 1.85, 95% CI 1.06–3.24; $P = 0.030$). It was asserted that higher HbA1c at diagnosis predicts retinopathy.

2.8. Ducos *et al*.[46] studied the role of "glycemic memory" in DR. They analyzed the HbA1c values of previous years in well-controlled T2DM. The HbA1c values from previous years were collected for .-4±3 months ($n = 255$), -16 ± 4 months ($n = 152$), -30 ± 4 months ($n = 93$), and -62 ± 26 months ($n = 105$). The study found a significant association between DR and the oldest previous HbA1c higher than the median (OR = 6.75, 95% CI 1.90–23.90).

2.9. Lind *et al*.[42] analyzed the DCCT data to determine the impact of HbA1c values at different points in time on retinopathy progression and how it shapes up MM. The median duration of DM at entry was 5.8 years, the observation time for retinopathy progression (under DCCT) was 6.5 years, and the mean total exposure to HbA1c was 12.3 years. HbA1c values from the past 2–3 years had the maximum relative risk contribution to the current progression of retinopathy (about 2.8× greater contribution from the present value), and the contribution to the relative risk of DR progression was 50% and 25% for the HbA1c values from the past 6.5 and 8 years, respectively. The HR for HbA1c 8% versus HbA1c 7% was 1.05 at 1 year and 1.63 at 5 years; similarly, on lowering of HbA1c from 8% to 7% after a prolonged period, the hazard function did not meet the HbA1c 7% level until 2–3 years later. In summary, past glycemic exposure apparently impacts the status of present retinopathy, and it takes 5 years or longer to negate the impact of past hyperglycemic exposure.

2.10. Hirose *et al*.[44] retrospectively analyzed people with T1DM evaluated shortly after the diagnosis, so that the impact of past unknown glycemia on retinopathy can be negated and only the mean MM-free HbA1c values can be correlated with retinopathy. Fifteen people enrolled within 1 year of T1DM diagnosis were followed for 20 years with serial monitoring of HbA1c. The mA1c values were calculated for the entire period. The study showed that the mA1c at every level, 1^{st}–20^{th} year, 4^{th}–20^{th} year, and 7^{th}–20^{th} year, significantly predicted retinopathy ($P = 0.0048$, 0.0101, and 0.0275, respectively). The predictive significance decreased for those under 20 years of diabetes, and there was no significant difference between the two groups for mA1c (10^{th}–20^{th} year, $P = 0.0662$).

Veteran Affairs Diabetes Trial

Analysis of the Veteran Affairs Diabetes Trial (VADT) by Azad *et al*.[48] is a prospective longitudinal trial that compared the effect of intensive and standard treatment in people with advanced T2DM. Accumulated eye procedures (eye events) during the 9-year intervention period, 2000–2008; the interim VADT follow-up study, 2000–2013; and the entire 17 years of VADT follow-up, 2000–2017, were analyzed. The analysis did not show a significant difference in total eye events between the intensive and standard treatment groups during the 17-year follow-up, and the study did not identify any apparent long-term MM effect on DR.

2.12 Bolotskaya *et al*.[45] studied the course of HbA1c values over 20 years in people with T1DM, from its onset to its correlation with microvascular complications. In the study, 155 people completed the measurement of HbA1c and checked it 2–4 times a year. There was no significant difference in the mA1c values between the two groups in the last 5 years of the follow-up (HbA1c 7.8% ±0.3% and 7.4% ±0.6%; $P > 0.05$ in the complication and no complication groups, respectively). The mA1c in people without microangiopathy remained <7.5% at 5, 10, and 15 years; it was >7.5% throughout the study in people who developed microangiopathy. In summary, glycemia levels at the onset or early in the disease shape the future course of retinopathy, thus confirming the concept of MM.

The Stockholm Diabetes Intervention Study

The Stockholm Diabetes Intervention Study (SDIS, 1982) evaluated the effects of intensified conventional treatment (ICT; $n = 48$) and regular treatment (RT; $n = 54$) in people with T1DM with NPDR and suboptimal serum glucose. The initial analysis

was done at 18 months, and then subjects were followed up for 10 years.[53,63] At 7.5 years, the treatment was intensified in the RT group, and patients in the ICT group were allowed to self-monitor their glucose without close tutoring, aiming at an HbA1c of 7.0%. At the 10-year follow-up, analysis was done for 43 (89.6%) patients in ICT and 48 (88.9%) patients in the RT group. The 10-year mA1c was significantly lower in the ICT group (from 9.5% ±1.4% to 7.2% ±0.6%, $P < 0.001$) and in the RT group (from 9.4% ±1.2% to 8.3% ±1.0%, $P < 0.001$). Advanced retinopathy (PDR or DME requiring treatment) was significantly lower in the ICT group than in the RT group ($n = 33$ vs. $n = 63$, $P = 0.003$). In addition, life table analysis showed a lesser risk of developing advanced retinopathy at 10 years in the ICT group (60% vs. 30%).

The United Kingdom Prospective Diabetes Study

The United Kingdom Prospective Diabetes Study (UKPDS; 1977–1991) was a randomized, prospective, multicenter trial that compared the effects of intensive and conventional glucose control on microvascular complications in T2DM.[64] The study randomized 4209 people to receive either conventional therapy (dietary restriction) or intensive therapy (sulfonylurea, insulin, or metformin) in overweight patients for glucose control. The trial closed in 1997, but the patients continued to be in follow-up at regular intervals after the first analysis. The study showed that the benefits of good glycemic control persisted despite the early loss of within-trial differences in HbA1c levels between the intensive therapy and the conventional therapy groups. After 12 years, there was a 21% reduction in DR progression and a 29% reduction in the need for laser photocoagulation in the intensive versus conventional treatment group. The recent results of 44-year follow-up showed 26% fewer microvascular complications such as DR and diabetic nephropathy following early intensive blood glucose control with sulfonylureas or insulin.[65-67]

Hypothetical ophthalmic evidence suggesting the role of MM in DR

There are no major intervention studies on DR vis-à-vis sequential HbA1c variations with outcomes, but assumptive evidence exists. Epidemiologic studies Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR) have reported the rate of DR progression to decline with each 6-year consecutive interval of DM control, but the reduction was <2% in the first three intervals, suggesting the possible rigidity of immediate response to glycemic control.[68] For PDR, long-term therapies are known to be needed for acceptable control of retinopathy. Protocol S (DRCR.net) showed that despite a comparable baseline HbA1c of 8.5%, about 47% required four or more anti-VEGF injections, while 12% required PRP in the fifth year of the study.[69] The Diabetic Retinopathy Study (DRS) also reported persistent new vessels despite absent initial signs of fibrosis following adequate laser at the end of the study (274 in the argon laser group, 284 in the xenon laser group).[70] Similarly, the RISE and RIDE studies compared anti-VEGF with sham injections for DME over 36 months and found apparent recently controlled glycemia not to reduce the number of treatment sessions significantly in the third year.[71,72] Such patterns have also been noted in real-world studies (VISION trial), where >20% of patients continued to require anti-VEGF injections till 5 years of therapy. The Early treatment of diabetic retinopathy study (ETDRS), having a very high baseline HbA1c, could document spontaneous improvement in DME in only 11% of eyes without treatment.[73,74]

In the absence of analysis on cumulative glycemic control in such ophthalmic studies on NPDR, DME, or PDR, it may be incorrect to attribute the long-term requirement of therapies or spontaneous improvements to MM alone. These changes could have also resulted from permanent and irreversible vascular ocular changes. But this certainly makes a case for evaluation of the effect of MM on therapy for DR.

Possible use of glycemic MM in containing DR

A legacy effect of good systemic control can be protective, while PC can be punitive, as shown in Sections 1 and 2. The divide between preclinical and clinical methodology is noticeable. The preclinical studies have broadly used the strategy of PC of DM, followed by GC of DM to demonstrate the punitive nature of MM. Meanwhile, the clinical studies have used the strategy of maintaining or relaxing GC and comparing findings to the initially poorly DM-controlled group that later converted to GC, demonstrating the protective nature of MM. It is unethical to cross over the GC of DM human groups to PC today, but based on the study models discussed before, we summarized possible methods of preventing MM in Table 3. Mitochondrial biology (especially the one that alleviates chronic oxidative stress) could be an ideal target for enforcing metabolic amnesia to counter the punitive effects of past poor systemic control [Fig. 2]. In support, a diet supplemented with antioxidants has been shown to reduce the incidence of DR in a large clinical study.[75] However, such therapy will likely act best in the early stages of DR and in people with the least cumulative HbA1c, as shown in Table 1 and Fig. 2.

Summary and way forward

MM has been largely evaluated for its protective effect in clinical studies and its punitive effect in preclinical studies. Our review indicates that a positive impact of MM on DR was observed in 12 of 14 clinical studies evaluating good glycemic control. The temporal evolution of memory occurs by 3–5 years of exposure to high glucose. The positive effect of tight control of hyperglycemia was seen even after about 5 years of stopping antiglycemic interventions in most clinical trials [Fig. 2]. While many preclinical studies explain this memory, few also suggest possible therapeutic targets, especially to alleviate oxidative stress at the mitochondrial level. Executing such therapies during the initial period of retinal memory may prevent DR or retard its progression.

It may be unethical for clinical studies to evaluate the punitive effects of MM today, as this would need subjecting individuals with good glycemic control to PC. However, future preclinical studies can focus on crossover good and PC designs to attain similarities with human studies. This will allow us to evaluate the protective effects of MM, which is largely lacking from the preclinical literature on MM till now. This would also reinforce the therapeutic potential of targeting MM for good DR-related outcomes in the long term. However, a major challenge in reversing MM or implementing metabolic amnesia research is our inability to spatially localize the memory in humans; it could be the retinal neurons or even an extraocular focus. Additional work is required in this area. In addition, early phase studies in humans are required to establish the

Table 3: Probable pharmacological targets for metabolic memory

Author	Study objective	Result	Remarks
Madsen-Bouterse *et al*.[11]	Effect of high glucose concentration on bovine retinal endothelial cell GAPDH	High glucose exposure decreases the activity of GADPH and leads to its translocation, which is not reversed by normal glycemia. Overexpression of GADPH in a high-glucose environment inhibited processes related to DR	Regulating GADPH activity or increasing its expression despite high glycemia may limit the pathways responsible for DR
Mishra and Kowluru[15]	Role of mtDNA MMR genes in the pathogenesis of metabolic memory	MMR proteins Mlh1 and Msh2 were reduced after hyperglycemia, causing elevated sequence variants in the D-loop region of mtDNA. The overexpression of Mlh1 in endothelial cells mitigated glucose-induced increase in D-loop sequence variants	Preventing damage to mtDNA can be a focus for obviating metabolic memory-related effects
Santos and Kowluru[14]	Role of mitochondrial biogenesis in MM	Poor control led to decreased mtDNA copy number due to dysregulation of mitochondrial biogenesis Supplementation with lipoic acid (reduces oxidative stress) during 6 months of good control significantly reduced impaired mitochondria biogenesis and reduced the progression of retinopathy too	Reduction of oxidative stress at the level of mitochondria may contain MM
Zhao *et al*.[25]	Effect of miRNAs on MM in DR	High glucose has been shown to activate NF-κB (proinflammatory) and stimulate miR-23b-3p expression and reduce SIRT1 expression (anti-inflammatory) Reduced miR-23b-3p expression ameliorated inhibition of SIRT1 expression and also negated the effect of MM	miRNAs can be a target of therapy as also gene therapy for sirutin

D-loop=displacement loop, DR=diabetic retinopathy, GAPDH=glyceraldehyde-3-phosphate dehydrogenase, miRNA=microRNA, MM=metabolic memory, MMR=mismatch repair, mtDNA=mitochondrial DNA

safety profile of interventions targeted at MM. Although we attempted to draw a chronology of the development of MM, it is noteworthy that newer hypoglycemic agents/strategies and better control of hyperglycemia in recent times may have a different impact on MM compared to the results of historic trials. Moreover, the clinical trials included in this review were conducted in diverse populations at various time points, utilizing varying methodologies and data analysis. At this point, it can be envisioned that a meticulous longitudinal evaluation of prospective cohorts observed through various phases of MM and DR is required to understand MM better and identify ideal time points and novel target agents for interventions.

Abbreviations

AGE advanced glycation end product
ARE4 antioxidant response element region 4
ARPE-19 retinal pigment epithelium
Bcl-2 B-cell lymphoma 2
DR diabetic retinopathy
DM diabetes mellitus
NPDR nonproliferative DR
PDR proliferative DR
D-loop displacement loop
Dnmt 1 DNA methyltransferase 1
DME diabetic macular edema
GAPDH glyceraldehyde-3-phosphate dehydrogenase
GC good control
HAT histone acetyltransferase
HDAC histone deacetylase
HREC human retinal endothelial cells
HUVEC human umbilical vein endothelial cells
IRMA intraretinal microvascular abnormalities
LSD1 lysine-specific demethylase-1
Micro RNA (miRNA) miR-23b-3p expression
MM metabolic memory
MnSOD manganese superoxide dismutase
SOD2 manganese superoxide dismutase gene
mtDNA mitochondrial DNA
NO nitric oxide
PC poor control
PKC protein kinase C
PGC1 peroxisome proliferator-activated receptor-γ coactivator-1α
ROS reactive oxygen species
VTDR vision-threatening DR
VEGF vascular endothelial growth factor
STZ streptozotocin
Sod2 superoxide dismutase 2
Sp1 specificity protein 1
TFAM mitochondrial transcription factor-A
TNF tumor necrosis factor
Tim translocases of the inner mitochondrial membrane
Tom translocase of the outer mitochondrial membrane
TFAM mitochondrial transcriptional factor
NRF1 nuclear respiratory factor 1
Nrf2 NF-E2-related factor 2
Keap1 Kelch-like ECH-associated protein 1
Gclc catalytic subunit of enzyme Glutamate–cysteine ligase
PCR polymerase chain reaction.

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