

Diabetic Retinopathy: Unraveling the Paradoxical Effects of Intensive Insulin Treatment

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ABSTRACT

Background: Several major clinical trials have demonstrated that the tight glycemic control achieved with intensive insulin treatment may lead to an early worsening of diabetic retinopathy but that intensive long-term insulin treatment slows the progression of diabetic retinopathy.

Objective: The goal of this article was to review developments in understanding both the pathophysiologic pathways involved in diabetic retinopathy and the biologic effects of insulin to provide a possible explanation why insulin treatment may have deleterious short-term effects yet provide long-term benefits.

Methods: The content of this article was based on a search of MEDLINE from 1984 through 2006. English-language articles were chosen using the search terms *diabetic retinopathy*, *insulin effects*, and *clinical trials*.

Results: Early worsening of diabetic retinopathy from acute intensive insulin treatment may result from upregulation of retinal vascular endothelial growth factor (VEGF) expression through increased binding of hypoxia-inducible factor-1 α to the VEGF promoter. This results in increased gene transcription and VEGF levels. Increased VEGF levels also promote activation of protein kinase C; both events contribute to increased vasopermeability and a breakdown of the blood-retinal barrier. However, long-term insulin treatment reduces inflammatory and oxidative stress components that may contribute to progressive diabetic retinopathy. Both inflammatory effects and oxidative stress may play a role in the etiology of diabetic retinopathy. The beneficial biologic effects of insulin may thus supercede the acute deleterious effects of the VEGF-mediated blood-retinal-barrier breakdown. We speculate that the long-term beneficial effects of intensive insulin therapy on the progression of diabetic retinopathy arise from insulin's recently realized potent anti-inflammatory effects and ability to modulate redox-sensitive signaling pathways.

Conclusions: Insulin treatment introduced before irreversible pathologic changes develop in the retina can impart beneficial effects to the vascular cells, may help suppress future deleterious events, and may slow the development or progression of retinal microangiopathic changes. (*Insulin*. 2007;2:4-11) Copyright © 2007 Excerpta Medica, Inc.

Key words: diabetic retinopathy, clinical trials, insulin effects, inflammation, oxidative stress, microvasculature, models of retinopathy.

INTRODUCTION

The prevalence of diabetes mellitus (DM) in the United States is at an epidemic level, and DM is one of the most serious challenges to health care worldwide due to the increase in occurrence and economic burden. It is projected that DM will affect 239 million people by 2010, a doubling in prevalence since 1994.¹ The incidence of diabetic retinopathy continues to grow, causing a serious health problem throughout the world.

Diabetic retinopathy has a significant economic impact on society, both in terms of health care resources and lost productivity in the workplace. Diabetic retinopathy affects 75% of patients after 15 years; it affects up to 98% of patients after 15 years who received a diagnosis before age 30.² The number of people at risk of blindness from diabetic retinopathy in the United States alone is >600,000 annually, and dia-

betic retinopathy is the leading cause of blindness in the industrialized world in adults aged 25 to 74 years.³ Gallup polls⁴ show that blindness is the long-term disability of DM that patients fear most, and the one with the most devastating psychological effect. The goal of this article was to review developments in understanding both the pathophysiologic pathways involved in diabetic retinopathy and the biologic effects of insulin to provide a possible explanation why insulin treatment may have deleterious short-term effects yet provide long-term benefits.

MATERIALS AND METHODS

The content of this article was based on a search of MEDLINE from 1984 through 2006. English-language articles were chosen using the search terms *diabetic retinopathy*, *insulin effects*, and *clinical trials*.

FINDINGS FROM CLINICAL TRIALS

In 1993, the results of the 10-year National Institutes of Health-sponsored Diabetes Control and Complications Trial (DCCT) in patients with type 1 DM demonstrated conclusively that intensive insulin therapy slows the development and progression of diabetic retinopathy and imparts a beneficial effect on microvascular dysfunction.⁵ The benefits include a reduction in the risk of progressive retinopathy, including a reduced incidence of macular edema and requirement for laser therapy. Furthermore, the Epidemiology of Diabetes Interventions and Complications study, a 10-year follow-up study tracking participants of the DCCT, determined that patients who received intensive insulin therapy continued to benefit 7 years later, with a reduction in the proportion of patients who had worsening retinopathy, despite regression of glycemic control.⁶

The 20-year United Kingdom Prospective Diabetes Study (UKPDS) extended the conclusion that intensive insulin therapy has long-term benefits in patients with type 2 DM. Intensive insulin therapy in patients with type 1 DM (DCCT) reduced the risk of retinopathy developing in the primary cohort by 27%; it significantly reduced the risk of retinopathy developing by 34% to 76% in DCCT⁷ and by 25% in patients with type 2 DM (UKPDS).⁸ Both studies also showed that retinopathy responds slowly to glycemic corrections, with the patient's history of glycemia influencing amelioration of retinopathy.

These results suggest that hyperglycemia can induce microvascular damage, that early glycemic control affects DM sequelae, and that the pathophysiology of microvascular complications is similar in type 1 and type 2 DM.

ETIOLOGY AND IMPLICATIONS OF EARLY WORSENING

Although the DCCT study demonstrated that intensive insulin therapy slowed the development and progression of diabetic retinopathy, it also demonstrated an early-worsening effect in 13% of (711) patients. An early-worsening effect, defined as a ≥ 3 -step progression in retinopathy scale, is predominantly transient; after 18 months, 51% of patients in the intensive treatment group recovered.⁹

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Complications from early worsening can lead to proliferative retinopathy and macular edema, with subsequent deposition of hard exudates. It has been reported that 23% of patients with type 2 DM who take oral antidiabetes agents and are switched to intensive insulin therapy developed clinically significant progression of retinopathy. Additionally, this effect is more prevalent if the glycosylated hemoglobin (A1C)

level is lowered by $\geq 3.0\%$.¹⁰ It is also known that initiating intensive insulin treatment in patients with chronically poor glycemic control, as indicated by a high A1C level, can threaten vision. Therefore, close ophthalmologic monitoring, or delaying intensive treatment until photocoagulation treatment can be rendered, may be advisable.

The underlying factors that cause early worsening of diabetic retinopathy during intensive insulin treatment remain unknown, and mechanistic explanations of this effect remain speculative. We now know that many of the clinical signs observed during early worsening, such as edema and exudates, are consistent with a compromised integrity or breakdown of the blood-retinal barrier.

A recent finding on an experimental animal model to demonstrate blood-retinal-barrier breakdown has provided initial insight into the early worsening seen during intensive insulin treatment.¹¹ It is hypothesized that acute intensive insulin treatment induces transcriptional upregulation of retinal vascular endothelial growth factor (VEGF) expression, which is mediated by hypoxia-inducible factor-1 α (HIF-1 α). It is proposed that insulin increases binding of HIF-1 α to the hypoxia-responsive elements in the VEGF promoter, resulting in increased VEGF transcription through mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K), and possibly Jun N-terminal kinase pathways. Increased VEGF levels are associated with breakdown of the blood-retinal barrier in DM. This is corroborated by the observation that a VEGF trap can substantially reduce the extent of extravasation of Evans blue dye in the retina after acute insulin treatment and can markedly reduce breakdown of the blood-retinal barrier in mouse models with a compromised blood-retinal barrier.^{11,12}

Additional studies have demonstrated protein kinase C (PKC) activation with increased VEGF levels, thereby suggesting that PKC mediates VEGF-induced vascular permeability. Other studies using intravitreal or oral administration of a selective PKC- β isoform inhibitor demonstrate suppression of vascular permeability.¹³ Together, these studies suggest a direct, modulatory role for PKC in the pathways mediating vascular leakage and macular edema.

LONG-TERM BENEFICIAL EFFECTS OF INSULIN

The results of several major clinical trials provide compelling evidence that long-term intensive insulin therapy slows the development and progression of diabetic retinopathy. Although intensive insulin treatment does not prevent retinopathy, it delays its progression sufficiently to demonstrate beneficial effects that become evident after 3 years of intensive insulin treatment.⁷

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It has been suggested that in addition to lowering A1C levels, insulin possesses unique physiologic properties that

impart direct beneficial effects on the microvascular complications of DM. How can intensive, prolonged insulin treatment rectify metabolic and retinal vascular abnormalities to provide long-lasting benefit on retinal microvascular function? We speculate that when insulin is introduced before irreversible pathologic changes occur, its newly established anti-inflammatory effect and its modulation of redox-sensitive signaling pathways result in a beneficial "imprint" on the endothelium, possibly slowing the development and progression of retinal microangiopathic changes.

Insulin may confer an enduring benefit at the endothelial cell level both directly and indirectly. In addition to regulating glycemia, insulin's direct anti-inflammatory effects include suppression of adhesion molecules and down-regulation of cytokine-induced transcription factors. Insulin also confers an indirect but favorable effect by lowering polyol levels and the formation of advanced glycation end products (AGEs), thereby reducing the deleterious effects of glycation on biomolecules. Insulin's effect on glycemia and AGEs reduces cellular oxidative stress and maintains an appropriate cellular redox state. The molecular mechanism(s) by which insulin imparts these beneficial effects on the endothelium of the retinal microvasculature remain(s) partly unexplained. However, the effect may involve alterations at the level of gene expression, cellular proteins, and cell-surface expression. For these reasons, insulin has been reported to promote a comprehensive metabolic control on endothelial cells that counteracts the many detrimental changes hyperglycemia induces.¹⁴ If insulin treatment is started early in DM, before overt pathologic changes in target organs, it may slow the development and progression of diabetic retinopathy.

Early changes in metabolic function such as oxygen consumption, blood flow, and rheologic properties precede the development of early irreversible structural damage to the retinal microvasculature, such as loss of pericytes and dilation and tortuosity of capillaries. The metabolic milieu appears to play an early, central role in the development and progression of diabetic retinopathy. Failure to rectify the dysmetabolism has a prolonged impact on the progressive pathologic changes in diabetic retinopathy. Inflammation and hyperglycemia unleash a cascade of events that affect cellular proteins, gene expression, and cell-surface-receptor expression in the endothelium, ultimately resulting in progressive pathologic changes and subsequent microvascular complications.

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Therefore, the implementation of early, comprehensive metabolic control could alter progression of microangiopathies by correcting dysmetabolic changes that function as accumulated "metabolic memories" early in the evolution of diabetic retinopathy.¹⁴ We postulate that insulin has an integral role in preserving normal endothelial function by creating the proper metabolic milieu and anti-inflammatory

effects, and that long-term insulin therapy can slow the progression of metabolic abnormalities in diabetic retinopathy. Emerging data suggest that many of the beneficial effects obtained by proper glycemic management may be attributable not only to the direct effects of insulin on the prevention of hyperglycemia, but also to insulin's indirect biologic effects.¹⁵

ROLE OF INFLAMMATION IN DIABETIC RETINOPATHY

Evidence exists that changes to endothelial cells associated with inflammatory responses play an early, prominent role in the development of retinal diabetic microangiopathy. One of the earliest hemodynamic changes observed in the diabetic retina is an increase in leukostasis, which occurred within days of induced diabetes in experimental animal models.¹⁶ The expression of cell adhesion molecules, such as intracellular adhesion molecule 1 (ICAM-1) and P-selectin, was elevated in the retinal vessels of patients with DM.¹⁷ The increased density or leukocytosis with enhanced endothelial cell adhesion of leukocytes in diabetic retinal microvessels is believed to contribute to capillary occlusion, areas of non-perfusion, and subsequent ischemia.

Inflammatory changes have been reported to be responsible for many of the characteristic microangiopathic changes of diabetic retinopathy.¹⁶ The inflammatory-dependent microangiopathic effect may be assisted by the proinflammatory isoforms of VEGF, which have been shown to induce monocyte chemotaxis and increase expression of ICAM-1.¹⁸ The ability of VEGF-164 to selectively induce inflammation to promote leukocyte cell adhesion has been proposed as a prerequisite for the pathologic neovascularization present in diabetic retinopathy.¹⁹ This proinflammatory effect is central in the early pathogenesis of diabetic retinopathy. Other authors have postulated that although leukostasis indicates endothelial cell dysfunction, insulin resistance, and oxidative stress, these elements per se are unlikely to be responsible for the subsequent development of retinopathy.²⁰

Leukocyte infiltration in the retina has also been linked to endothelial cell apoptosis by Fas protein and Fas ligand system mechanisms that could promote blood-retinal-barrier breakdown²¹ and give rise to acellular capillaries, a microangiopathy characteristic of diabetic retinopathy. AGEs have also been reported to cause an increased regulation of nuclear transcription factor κ B (NF- κ B) in the retinal microvasculature along with increases in leukostasis, ICAM-1, and breakdown of the blood-retinal barrier.²² The nonsteroidal anti-inflammatory drug sulindac has been shown in diabetic dogs to prevent basement-membrane thickening, one of the earliest known ultrastructural changes in diabetic retinopathy.²³ Inhibition of PKC- β isoform (by ruboxistaurin) prevents increases in leukostasis and can abrogate vascular dysfunction thought to contribute to the pathogenesis of diabetic retinopathy.^{20,24} The aggregate findings from these studies suggest a component characteristic of a chronic inflammatory disease in the natural history of diabetic retinopathy.

DIRECT ANTI-INFLAMMATORY EFFECTS OF INSULIN ON THE ENDOTHELIUM

Insulin is emerging as a molecule with potent anti-inflammatory properties that can suppress proinflammatory cytokines. Recent evidence has documented intensive insulin therapy's direct and pronounced anti-inflammatory effect in lowering serum C-reactive protein in long-term critically ill patients.^{15,25}

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Of significance to insulin's general anti-inflammatory effect is the suppression of the proinflammatory transcription factor NF- κ B. Activation of NF- κ B increases the release of cytokines and other chemotactic factors involved in inflammation. Included among the genes whose expression is regulated by NF- κ B are VEGF, tumor necrosis factor- α , interleukin-1 β , the receptor for AGEs (RAGE), adhesion molecules such as ICAM-1 and vascular cell adhesion molecule, and monocyte chemoattractant protein.²⁶ Therefore, suppression of NF- κ B by insulin would have a pronounced effect on the inflammatory cascade, which includes regulation of proinflammatory cytokines, adhesion molecules, and chemokines.

NF- κ B activation depends partially on activation of the Rho kinase-guanosine triphosphatase pathway. Modulation of NF- κ B by insulin appears to be complex, involving multiple pathways as well as direct and indirect effects. Insulin—by increasing the activity of geranylgeranyltransferase I and the availability of geranylgeranylated Rho-A—can augment NF- κ B-dependent transcriptional activity in response to AGEs.²⁷ Insulin, by reducing hyperglycemia, also indirectly reduces AGE formation and the available ligands for RAGE-receptor activation, resulting in downstream induction of NF- κ B. Inflammatory cytokines and reactive oxygen species (ROS) also increase NF- κ B. Insulin's direct anti-inflammatory effect suppresses the induced elevations in NF- κ B expression mediated by cytokines or ROS.

INSULIN ACTION ON VASCULAR SMOOTH MUSCLE: RELEVANCE TO DIABETIC MICROANGIOPATHY

Insulin action is regulated by 2 independent biochemical pathways: PI3K and MAPK. In an *in vitro* system, activation of the PI3K pathway was shown to keep isolated vascular smooth muscle cells quiescent.²⁸ In contrast, insulin's effect on the MAPK pathway promotes vascular smooth muscle migration. In insulin resistance, PI3K pathway signaling is impaired and MAPK pathway signaling remains essentially unaffected. This physiologic state favors the transformation of vascular smooth muscle cells from quiescence to a proliferative phenotype. This component of insulin-signaling could present a feasible mechanism for the formation of microangiopathies in the vasoproliferative stages of diabetic retinopathy because either endogenous hyperinsulinemia, as

encountered in the insulin-resistant state, or protracted exogenous administration of insulin could promote a metabolic milieu that supports mitogenic action on vascular smooth muscle cells and promotes vasoproliferative changes in the retinal microvasculature.

However, results from clinical trials using intensive insulin treatment for diabetic retinopathy have shown insulin to have beneficial rather than detrimental effects. This suggests that in the *in vivo* system, additional, and perhaps competing, mechanisms involving insulin exist. For instance, insulin stimulates increased expression of endothelial nitric oxide synthase, which could promote vasodilation and improve retinal oxygenation, thereby reducing ischemic vasoproliferative responses.

THE ROLE OF OXIDATIVE STRESS IN DIABETIC RETINOPATHY

DM is associated with increased oxidative and nitrosative stress, leading to peroxynitrite formation and activation of poly(ADP-ribose) polymerase.²⁹ Oxidative stress can accelerate apoptosis of retinal endothelial cells through peroxynitrite-mediated nitrosylation of tyrosine PI3K, Akt-1 kinase inhibition, and activation of the p38 MAPK signaling pathway.³⁰ This apoptotic mechanism could contribute to the formation of acellular capillaries in the retinal vasculature. Oxidative stress mechanisms have also been implicated in methylglyoxal-mediated apoptosis of retinal pericytes.³¹ Selective destruction of intramural pericytes leads to the formation of "pericyte ghosts," a hallmark microangiopathic event in early diabetic retinopathy. Increased oxidative stress could also result from increased aldose reductase activity. Antioxidant defense enzymes are decreased in the retina of experimental models of retinopathy, and administration of a mixture of antioxidants prevented early microangiopathic changes in 2 established animal models of diabetic retinopathy.³² Oxidative stress also activates the PKC pathway, promoting increased vasopermeability.¹³

Oxidative stress mechanisms have also been implicated in methylglyoxal-mediated apoptosis of retinal pericytes.

THE ROLE OF INSULIN IN OXIDATIVE STRESS AND STRESS-ACTIVATED SIGNALING PATHWAYS

There is experimental and clinical evidence for elevated levels of free radicals and increased generation of ROS in type 1 and type 2 DM. These biochemical pathways play a role in hyperglycemic damage. Sources of oxidative stress in DM include altered carbohydrate and lipid metabolism and decreased levels of antioxidant defenses such as glutathione, glutathione peroxidase, catalase, CuZnSOD, MnSOD, α -tocopherol, and β -carotene.

The precise mechanism by which oxidative stress accelerates development of complications in DM, including diabetic retinopathy, is only partially understood. However,

associations with PKC, AGEs, and activation of NF-κB have been documented. ROS can signal the degradation of IκB, a regulatory factor critical for NF-κB activation and translocation to the nucleus. Once NF-κB enters the nucleus, it binds to DNA and modulates the expression of several genes controlling the inflammatory process. Since NF-κB appears to be central for cytokine-mediated inflammatory responses and ROS can activate NF-κB, insulin—by directly inhibiting NF-κB—can be considered an effective modulator of both pathways.

Long-term insulin treatment may help maintain a steady-state metabolism in the endothelial cell. This steady-state metabolism involves having sufficient substrate available for both the glutathione-redox cycle, which protects the endothelial cell from peroxide damage via oxidation of H₂O₂, and the nitric oxide pathway, which preserves the retinal vasculature's basal tone (Figure 1). The key factor for maintaining the metabolic steady state is the appropriate availability of nicotinamide adenine dinucleotide phosphate (NADPH) for the various competing pathways. The enzyme aldose reductase is also NADPH dependent, and increased flux through this pathway in hyperglycemia will compete for availability of the NADPH cofactor for the nitric oxide

synthase enzyme to generate NO and maintain the vasculature's resting basal tone. By an indirect mechanism, insulin could suppress formation of ROS and oxidative stress by maintaining sufficient NADPH available as cofactor for the glutathione reductase enzyme, which catalyzes the reduction of oxidized glutathione to cellular glutathione. Glutathione is a substrate for glutathione peroxidase, which catalyzes the oxidation of H₂O₂ and protects the cell from peroxide-induced damage.

Insulin may also provide benefit by reducing AGEs. An increase in endogenous glycated proteins, particularly α-oxoaldehyde, which has been implicated in apoptosis of intramural pericytes in diabetic retinopathy, leads to activation of the cytosolic glutathione-dependent glyoxalase system. The glyoxalase system catalyzes the detoxification of methylglyoxal (oxoaldehydes) to aldinate (D-lactic acid). It is composed of the enzymes glyoxalase I and glyoxalase II and the reduced cofactor glutathione.³³ Glutathione levels necessary for H₂O₂ oxidation are consumed in the glyoxalase I catalyzed reaction. Therefore, high circulating levels of oxoaldehydes in hyperglycemia make the cell more susceptible to peroxide damage.

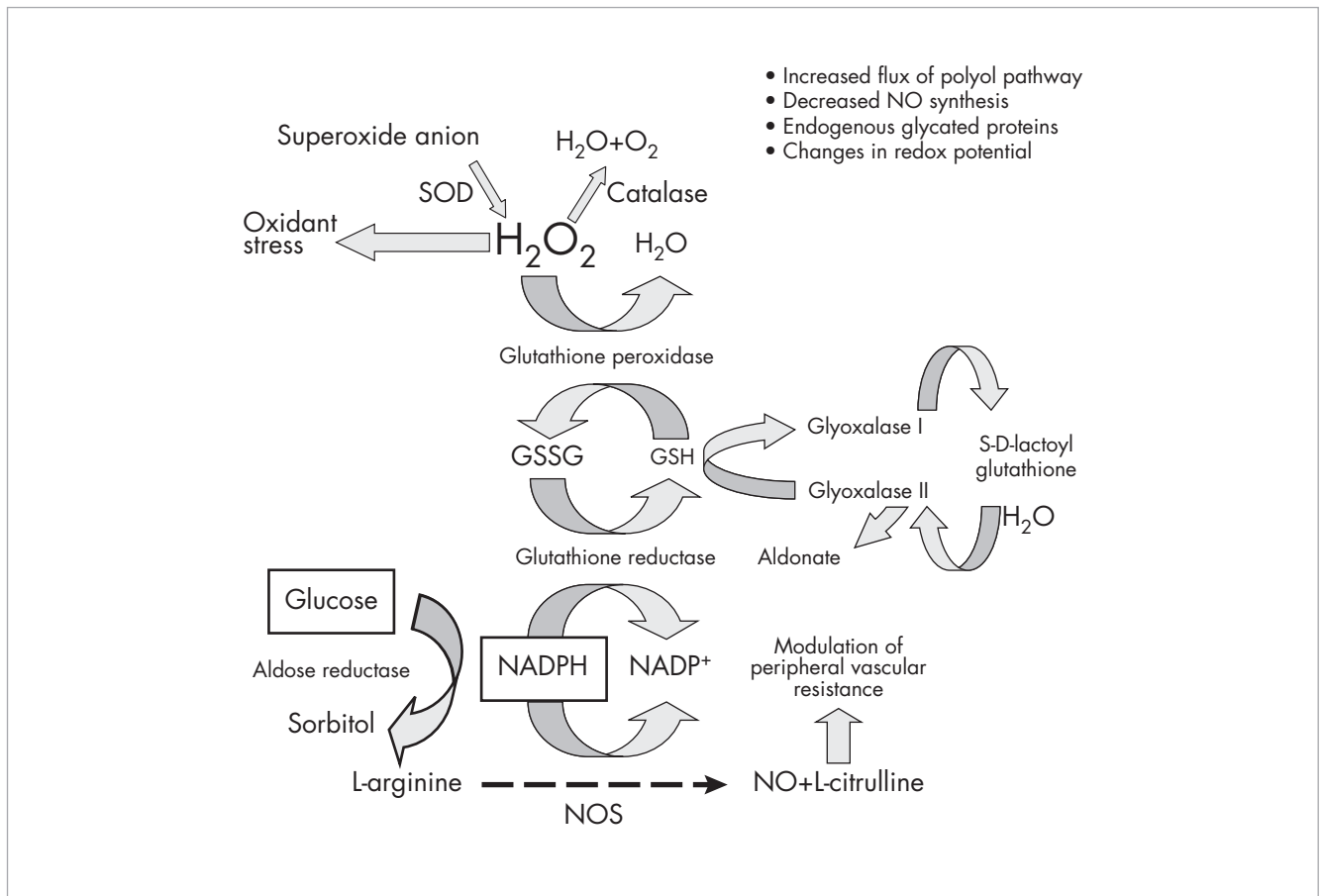


Figure 1. Cellular dysmetabolism in diabetes mellitus and the effect of insulin. NO = nitric oxide; SOD = superoxide dismutase; GSSG = oxidized glutathione; GSH = reduced glutathione; NADPH = nicotinamide adenine dinucleotide phosphate; NADP⁺ = NADPH oxidized form; NOS = NO synthase.

DISCUSSION AND CONCLUSIONS

Growing understanding of the pathogenic mechanisms in diabetic retinopathy, in conjunction with insight into the previously unsuspected biologic effects of insulin, increase knowledge of the pathogenesis of DM in general and of diabetic retinopathy in particular. This knowledge can help researchers explore novel mechanism-based therapeutic modalities.

It is feasible to suppress, by pharmacologic intervention, the particular biochemical pathway(s) believed responsible for early worsening in diabetic retinopathy. Use of a VEGF-trap or PKC- β inhibitor (ruboxistaurin) to suppress vascular leakage might prevent breakdown of the blood-retinal barrier induced by intensive insulin treatment. The concomitant use of VEGF antagonists or PKC- β inhibition in combination with insulin treatment could provide a way to eliminate the early-worsening effect; long-term insulin treatment could then help slow the progression of diabetic retinopathy. Insulin is a particularly attractive long-term treatment modality because of its low cost and proven safety profile.

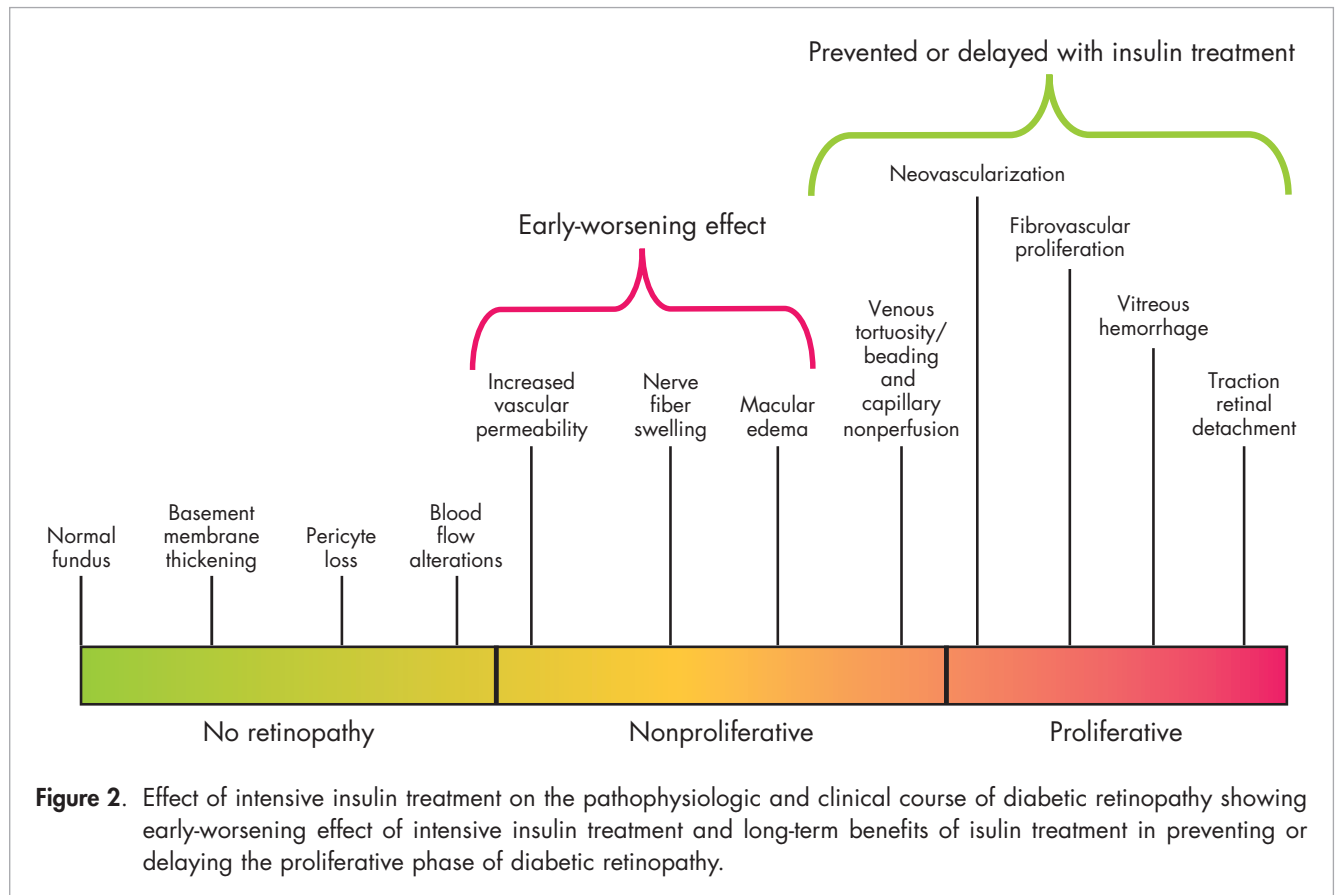
Major breakthroughs in treatment options for patients with diabetic retinopathy may come when researchers fully elucidate the mechanism by which insulin counteracts inflammatory changes, imparts “metabolic memory,” and ameliorates oxidative-stress-mediated signaling pathways.

The use of acute insulin therapy to treat patients with diabetic retinopathy should be initiated in conjunction with a

thorough ophthalmologic evaluation and periodic follow-up examinations for monitoring progression of retinopathy for at least 18 to 24 months. The initial transient early worsening due to tight glycemic control that occurs in a minority of patients with DM might exacerbate retinopathy and lead to development of macular edema and the formation of hard exudates, particularly in patients with a history of poor glycemic control. In high-risk patients, photocoagulation treatment may have to precede initiation of insulin therapy, or the insulin therapy regimen may have to be phased in to avoid lowering A1C $\geq 3.0\%$ between ophthalmic evaluations.

The long-term beneficial biologic effects of insulin supersede its acute deleterious effects associated with blood-retinal-barrier breakdown, such as increased vascular permeability, nerve fiber swelling, and macular edema. Insulin treatment introduced before irreversible pathologic changes occur can impart beneficial effects on vascular cells and help slow the development or progression of retinal microangiopathies in the proliferative stages of diabetic retinopathy (Figure 2).

Clinical trials have shown that tight glycemic control delays the progression of diabetic retinopathy. Recent experimental evidence continues to support these clinical findings and adds to understanding of the mechanistic pathways that involve insulin action. A constitutive insulin-receptor signaling pathway essential for providing an antiapoptotic mecha-



nism functions in the retina and is impaired in DM.³⁴ Loss of the prosurvival pathway in DM may contribute to the early stages of diabetic retinopathy and is reversible with systemic and local insulin. High glucose level has also been shown to accelerate retinal endothelial cell apoptosis due to peroxynitrite formation and disruption of VEGF prosurvival function.³⁵

The long-term use of insulin has both direct and indirect beneficial effects. Maintaining normoglycemia reduces both AGEs and oxidative stress. Oxidative stress from ROS at the mitochondrial level induces PKC activation, formation of AGEs, polyol accumulation, and NF- κ B activation.³⁶ Both experimental and clinical data suggest that insulin may be useful in the long-term treatment of diabetic retinopathy.

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