

# The Role of Inflammatory Cytokines in Diabetes and Its Complications

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The prevalence of diabetes worldwide is increasing rapidly in association with the increase in obesity. Complications are a major fear of patients with diabetes. Complications of diabetes affect many tissues and organs, causing retinopathy, nephropathy, neuropathy, cardiovascular diseases, peripheral vascular diseases, stroke, and periodontal pathologies. Immunologic abnormalities are associated with type 1 and type 2 diabetes and diabetic complications. T cell abnormalities are believed to be the major cause of autoimmune disease in type 1 diabetes, leading to the destruction of pancreatic islets. In type 2 diabetes, inflammation and activation of monocytes are postulated to be important for enhancing insulin resistance and may contribute to the loss of insulin secretory function by islet cells. Many factors can enhance insulin resistance, including genetics, a sedentary lifestyle, obesity, and other conditions, such as chronic inflammation or infection. Increases in inflammation, such as activation of monocytes and increased levels of inflammatory markers, e.g., C-reactive protein, plasminogen activator inhibitor-1, and other cytokines, were reported in insulin-resistant states without diabetes. One possible mechanism is that abnormal levels of metabolites, such as lipids, fatty acids, and various cytokines from the adipose tissue, activate monocytes and increase the secretion of inflammatory cytokines, enhancing insulin resistance. According to this model, obesity activates monocytes and enhances insulin resistance, increasing the risk for type 2 diabetes. Abnormalities in innate immunity might also participate in the development of diabetic complications. In general, hyperglycemia is the main initiator of diabetic retinopathy, nephropathy, and neuropathy, and it participates in the development of diabetic cardiovascular diseases. Although the precise role of inflammation in the development of diabetic microvascular diseases is still unclear, it is likely that inflammation induced by diabetes and insulin resistance can accelerate atherosclerosis in patients with diabetes. Also, it was shown that conditions with an inflammatory basis, such as obesity and type 2 diabetes, can contribute to periodontal disease, suggesting that periodontal abnormalities may be partly influenced by inflammatory changes. Further research is required to confirm the role of inflammation and the onset of diabetes, microvascular diseases, and periodontal pathologies. *J Periodontol* 2008;79:1527-1534.

## KEY WORDS

**Complications; diabetes; hyperglycemia; inflammation; insulin resistance; periodontal disease.**

**D** diabetes is a growing concern. Its incidence is increasing rapidly and is predicted to increase further, in parallel with the trends observed for obesity.<sup>1-3</sup> The increasing prevalence of diabetes represents a significant burden to human health because of its numerous and often serious complications. These include nephropathy, retinopathy, neuropathy, cardiovascular disease, and periodontitis.<sup>4</sup> Diabetes also has an economic cost, with total direct and indirect medical costs already >\$132 billion in the United States alone.<sup>5</sup>

Many factors are known to contribute to the development of diabetes and its complications. These include genetics, diet, sedentary lifestyle, perinatal factors, age, and obesity.<sup>6</sup> Nevertheless, an inflammatory basis for diabetes and its complications has been gaining interest. Inflammatory processes are associated with type 1 and type 2 diabetes; however, the distinct etiology of the two types of diabetes suggests that different causal mechanisms are involved. Type 1 diabetes is frequently found in childhood or young adulthood and arises from the autoimmune destruction of the pancreatic islet cells leading to loss of insulin production.<sup>5</sup> Type 2 diabetes is the more common form and occurs mainly in

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adults, although the prevalence in younger people is beginning to increase in conjunction with childhood obesity.<sup>5</sup> Type 2 diabetes is characterized by an increase in insulin resistance in conjunction with the inability of pancreatic beta cells to secrete sufficient insulin to compensate.<sup>5</sup>

Investigations into the role of inflammatory mechanisms in diabetes and its complications are expected to provide insight into the processes underlying the onset and progression of the disease. Such improved understanding of the inflammatory basis for diabetes may prove valuable for introducing novel approaches to treatment, alongside currently used non-pharmacologic and pharmacologic interventions.

### **INFLAMMATORY PROCESSES ARE ASSOCIATED WITH PROGRESSION OF TYPE 1 DIABETES**

Although there is some controversy surrounding the precise role of inflammatory processes in type 1 diabetes, intriguing findings have emerged from studies of C-reactive protein (CRP) levels, a measure of circulating inflammatory biomarkers.<sup>7</sup> Although CRP concentrations in individuals with the new onset (within days of diagnosis) of type 1 diabetes were similar to those observed in healthy controls, levels in individuals with long-term diabetes were significantly higher ( $P=0.04$ ).<sup>7</sup> These findings suggest that the inflammatory process may play a greater role in the long-term progression of type 1 diabetes than in its onset. The strongest factor to support inflammation as being important in type 1 diabetes is in the area of complications. Increases in inflammatory and oxidative stress markers are also found in conjunction with the development of complications of diabetes, with increases in plasma levels of CRP and in concentrations of soluble vascular cell adhesion molecule-1 and nitrotyrosine in patients with microvascular disease compared to those without.<sup>8</sup> Increases in monocyte release of interleukin (IL)-1 $\beta$  and superoxide anions were also reported in patients with type 1 diabetes, suggesting the elevations in inflammatory marker activity in microvascular and cardiovascular diseases.<sup>8</sup>

### **INFLAMMATORY PROCESSES ARE ASSOCIATED WITH DEVELOPMENT OF TYPE 2 DIABETES**

Research has focused more on the role of inflammatory processes in the development and progression of type 2 diabetes than type 1 diabetes. Increases in inflammatory markers are detected in apparently healthy individuals who later go on to develop type 2 diabetes,<sup>9-11</sup> suggesting that inflammation occurs early during the period of impaired glucose tolerance, prior to the diagnosis of type 2 diabetes. In adult Pima Indians, a population characterized by a high prevalence of type 2 diabetes, individuals with white blood cell counts within the highest tertile were more

likely to develop type 2 diabetes over the 20-year period studied compared to those in the lowest tertile.<sup>9</sup> Similarly, in a prospective, nested case-control study<sup>10</sup> of apparently healthy, middle-aged women in the United States, inflammatory markers IL-6 and CRP within the highest quartiles were associated with an increased risk for developing type 2 diabetes over a 4-year period compared to those in the lowest quartile (unadjusted relative risk [RR], 7.5; [95% confidence interval (CI): 3.7 to 15.4] for IL-6 and 15.7 [95% CI: 6.5 to 37.9] for CRP;  $P<0.001$  for both). These findings were mirrored in the Monitoring of Trends and Determinants in Cardiovascular Diseases study of healthy, middle-aged men, in which CRP concentrations in the highest quartile were associated with an increased risk for developing type 2 diabetes over the 7-year period studied (unadjusted RR, 2.84 [95% CI 1.5 to 5.36];  $P=0.003$  for trend).<sup>11</sup>

### **INFLAMMATORY PROCESSES CONTRIBUTE TO INSULIN RESISTANCE IN TYPE 2 DIABETES**

Insulin resistance begins prior to the onset of type 2 diabetes, at which time impaired glucose tolerance occurs as a result of beta cell decomposition and relative insulin deficiency. Several factors are linked to the development of insulin resistance in individuals with impaired glucose tolerance and type 2 diabetes, including genetics and environmental influences, obesity, and other conditions associated with chronic inflammation or infection. The possibility that obesity, and the activation of adipose tissue in particular, may enhance the release of inflammatory factors that underlie the development of insulin resistance has generated intense interest in the field of diabetes for a number of reasons. First, a significant proportion of individuals with type 2 diabetes are overweight or obese, and obesity is a risk factor for the development of type 2 diabetes.<sup>2,12</sup> Second, the increased release of adipocyte-derived metabolites, such as lipids, fatty acids, and various inflammatory cytokines, in obese individuals has been linked to the development of insulin resistance.<sup>13</sup> Third, chronic inflammation is associated with obesity, insulin resistance, and type 2 diabetes, all of which are features of the clustering of metabolic pathologies known as “metabolic syndrome.”<sup>14</sup> It should be emphasized, however, that despite interest in obesity as a predisposing factor to insulin resistance in type 2 diabetes, other equally important mechanisms for the loss of insulin sensitivity have been proposed. One study<sup>15</sup> of young, lean offspring of patients with type 2 diabetes demonstrated similar body mass index measurements and plasma concentrations of inflammatory markers tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), IL-6, and adiponectin in insulin-resistant and insulin-sensitive individuals. This suggested that obesity and systemic

inflammatory factors do not play a significant role in the development of insulin resistance in this population; the loss of insulin sensitivity was ascribed to a dysregulation of intramyocellular fatty acid metabolism. Other studies<sup>5,16</sup> demonstrated a high risk for type 2 diabetes in Asian subjects, despite low concentrations of CRP and other inflammatory markers compared to other ethnic groups. Taken together, such findings suggest that mechanisms in addition to obesity and systemic inflammation are involved in predisposing individuals to insulin resistance and type 2 diabetes.

In the obesity-related model for the development of insulin resistance, adipocytes, once activated, release abnormal levels of bioactive molecules, such as lipids, fatty acids, monocyte chemoattractant protein-1 (MCP-1), and various inflammatory cytokines, e.g., CRP, plasminogen activator inhibitor-1, and TNF- $\alpha$ .<sup>13</sup> The release of these cytokines and other mediators results in the local recruitment of monocytes within adipose tissues. With differentiation of the monocytes into macrophages comes an increased release of inflammatory factors and chemokines locally within adipose tissue and systemically, such that the inflammatory response is propagated to various tissues.<sup>13</sup>

One of the earliest studies<sup>17</sup> to link the release of inflammatory substances from adipose tissues to insulin resistance in type 2 diabetes involved rodent models of obesity and diabetes. In these mouse and rat models, expression of TNF- $\alpha$  mRNA and protein was induced locally within adipose tissue, as well as systemically in the plasma. By inhibiting TNF- $\alpha$  expression in one of the rodent models (fa/fa) using a recombinant TNF- $\alpha$  receptor-immunoglobulin G chimeric protein, insulin sensitivity improved, suggesting a direct role for TNF- $\alpha$  in the development of insulin resistance.<sup>17</sup> Such increases in insulin sensitivity with the use of a specific TNF- $\alpha$  inhibitor have not been replicated in human clinical trials,<sup>18</sup> although larger studies and large trial periods may be needed to confirm the findings.<sup>14</sup>

A mechanism has been proposed linking the expression of TNF- $\alpha$  and other inflammatory mediators to the development of insulin resistance in obesity and type 2 diabetes.<sup>13</sup> In this model, inflammatory cytokines and/or bacterial lipopolysaccharide stimulate I-kappa-B (I $\kappa$ B) kinase- $\beta$  (IKK $\beta$ ), and possibly IKK $\alpha$ , to induce activation of nuclear factor-kappa B (NF- $\kappa$ B) (Fig. 1). Other non-inflammatory cell/cytokine-mediated agents also contribute to NF- $\kappa$ B activation, including free fatty acids, obesity, hyperglycemia, protein kinase C (PKC) activators, and oxidants. Following activation, NF- $\kappa$ B translocates to the nucleus, resulting in the subsequent transcription of genes that promotes the development of insulin resistance.<sup>13</sup> Induction of

target genes by NF- $\kappa$ B leads to the increased expression of inflammatory markers and mediators associated with insulin resistance and is associated with the production of other factors, including receptors, inflammatory mediators, chemokines, and transcription factors; among other functions, these stimulate recruitment of monocytes and their differentiation into macrophages (Fig. 1).<sup>13</sup>

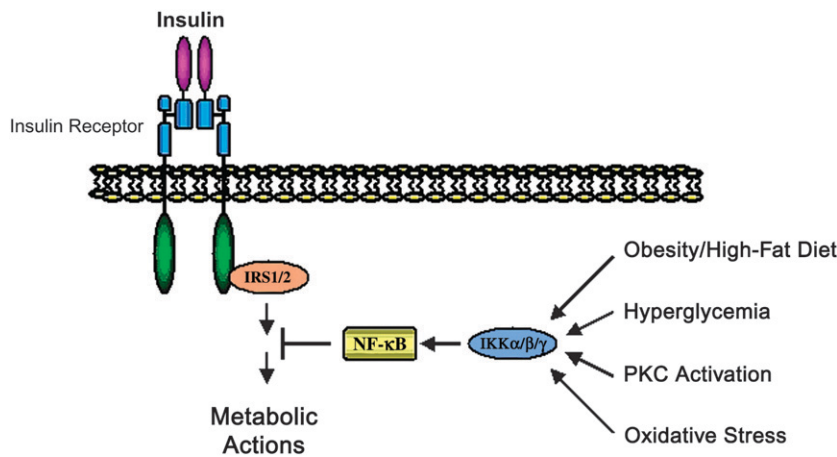
### **INHIBITION OF IKK $\beta$ /NF- $\kappa$ B PATHWAY IMPROVES OBESITY-RELATED INSULIN SENSITIVITY**

Several lines of evidence support the critical role of the IKK $\beta$ /NF- $\kappa$ B pathway in the development of insulin resistance, including a study in an animal model<sup>19</sup> and a human clinical trial.<sup>20</sup> The heterozygous deletion of IKK $\beta$  in an obese mouse model fed a high-fat diet over 23 weeks postnatally conferred protection from the development of insulin resistance, as measured by lower fasting insulin and glucose concentrations compared to normal/homozygous IKK $\beta$  controls.<sup>19</sup> Additionally, in a clinical study<sup>20</sup> of young, obese, non-diabetic adults, treatment with salsalate, a non-acetylated salicylate and known disruptor of the IKK $\beta$ /NF- $\kappa$ B pathway, improved insulin sensitivity compared to placebo control. Salsalate treatment also improved inflammatory markers in these patients, resulting in a reduction in free fatty acids ( $P < 0.05$ ) and increases in levels of the anti-inflammatory cytokine adiponectin ( $P < 0.01$ ) and circulating concentrations of CRP ( $P < 0.05$ ).<sup>20</sup>

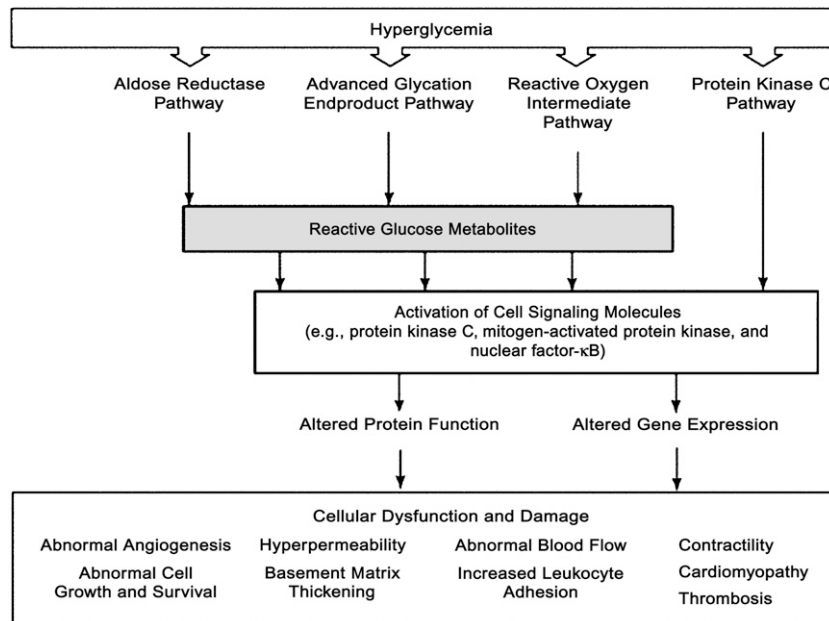
### **ASSOCIATION BETWEEN INFLAMMATORY PROCESSES AND COMPLICATIONS OF DIABETES**

Insulin resistance and insulin deficiency give rise to a hyperglycemic state that is a major risk factor for the development of diabetic complications. For example, primary pulmonary disorder is rare in diabetes, although the pulmonary tree is heavily vascularized and exposed to all the systematic factors of diabetes. Hyperglycemia is considered the key contributor to microvascular complications, including retinopathy, neuropathy, and nephropathy. It is also one of several major risk factors associated with cardiovascular disease along with insulin resistance or deficiency, free fatty acidemia, hypertension, hyperlipidemia, and inflammation. Periodontitis is commonly associated with diabetes, as demonstrated by a 2.9-fold increase in risk in individuals with poorly controlled glycemia;<sup>21</sup> however, the major risk factors contributing to its development are unknown.

Hyperglycemia was shown to act deleteriously through a number of pathways, including the aldose reduction pathway, advanced glycation end product



**Figure 1.** Activators of IKK/NF-κB pathway inhibit insulin signaling. IKK = IκB kinase; IRS = insulin receptor substrate.



**Figure 2.** Major pathways initiated by hyperglycemia that contribute to complications of diabetes. Reprinted with permission from Blackwell Publishing.<sup>22</sup>

contribute to cellular dysfunction and damage associated with micro- and macrovascular complications.

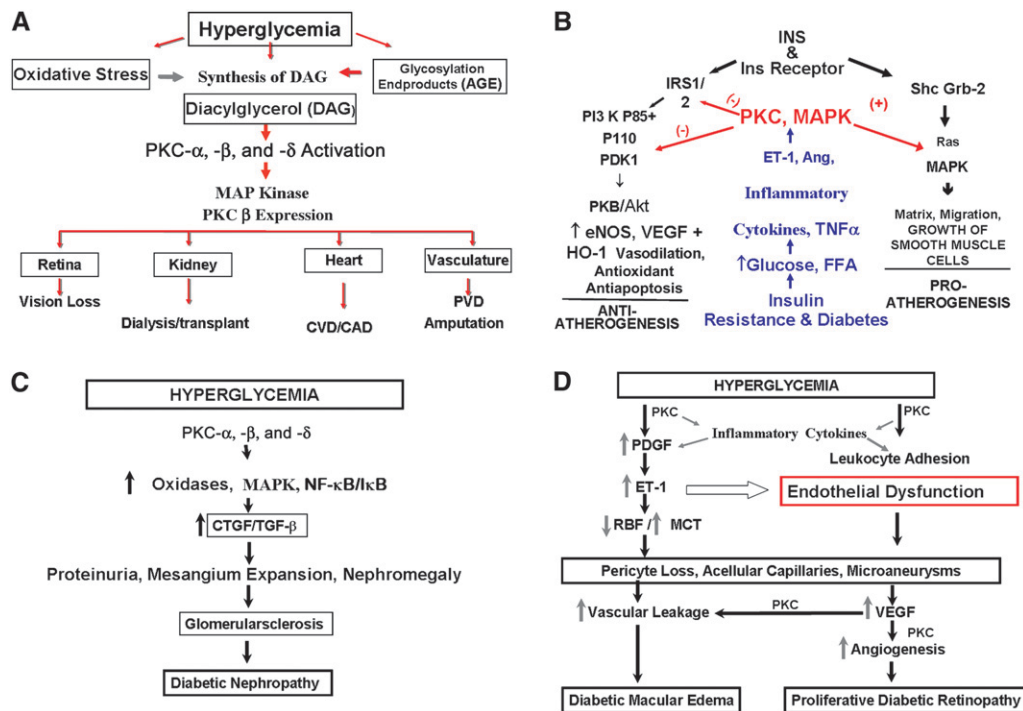
The actions of inflammatory and oxidant pathways at the local tissue level are key to understanding their contribution to the pathogenesis of diabetic complications.<sup>23</sup> Evidence suggests that increases in systemic markers of inflammation, such as CRP and IL-6, are associated with complications such as diabetic nephropathy<sup>24</sup> in type 2 diabetes. However, systemic inflammatory factors are only weakly associated with the development of diabetic retinopathy,<sup>25,26</sup> and the relationship is uncertain in other complications, such as periodontal disease. Such studies underscore the importance of investigating local, downstream pathways and mediators for resolving processes underlying vascular pathologies in diabetes.<sup>23</sup>

**LOCAL MECHANISMS FOR DIABETIC COMPLICATIONS**

Hyperglycemia induces the expression of the PKC pathway.<sup>23</sup> This results in the development of complications through altered gene expression and/or protein function, thus contributing to cellular dysfunction and damage.<sup>23</sup> A well-described pathway for the development of diabetic vascular complications involves activation of the diacylglycerol (DAG)-PKC pathway.<sup>23,27</sup> Increases in DAG levels and PKC activity are found in a wide variety of tissues and cultured cells isolated from diabetic animals and humans exposed to high glucose levels.<sup>27</sup> Several processes may underlie the increased formation of DAG, including the induction of oxidants, such as H<sub>2</sub>O<sub>2</sub>, and the accumulation in AGEs (Fig. 3A). Stimulation of DAG synthesis activates PKC

(AGE) pathway, reactive oxygen intermediate pathway, and PKC pathway (Fig. 2).<sup>22</sup> All four pathways give rise to oxidant and inflammatory mediators that result in deleterious effects, both systemically and locally in the tissues. Activation of the pathways leads to enhanced production of gluco-oxidants and AGEs, as well as increased flux through the aldose reduction pathway and activation of signaling cascades by mediators such as PKC, mitogen-activated protein kinase (MAPK), and NF-κB.<sup>22</sup> The effects include altered gene expression and/or protein function that

isoforms, with PKC-β considered to play a central role in the development of complications in tissues such as retina, kidney, heart, and the vasculature.<sup>23,27</sup> Activation of PKC in the vasculature has been associated with processes including increases in basement matrix protein synthesis, activation of leukocytes, endothelial cell activation and proliferation, smooth muscle cell contraction, endothelial permeability, activation of cytokines, transforming growth factor-β (TGF-β), vascular endothelial growth factor (VEGF), endothelin, and angiogenesis.<sup>27-29</sup>



**Figure 3.**

**A)** PKC- $\beta$  pathway to diabetic vascular complications. **B)** Selective local insulin resistance in cardiovascular tissues in combination with the activation of PKC (especially the  $\beta$  isoform) and MAPK inhibits the anti-atherogenic pathway while enhancing proatherogenesis. **C** and **D)** Diabetic nephropathy and retinopathy progress through a series of hyperglycemia-dependent pathways in which activation of PKC isoforms enhances activation and/or production of intermediaries, resulting in pathologic changes. The precise role of inflammatory cytokines in the progression of retinopathy remains to be determined. Ang = angiotensin; CAD = coronary artery disease; CTGF = connective tissue growth factor; CVD = cardiovascular disease; ET-1 = endothelin-1; eNOS = endothelial nitric oxide synthase; FFA = free fatty acid; Grb-2 = growth factor receptor-bound protein 2; HO-1 = heme-oxygenase-1; I $\kappa$ B = I $\kappa$ B kinase; INS/Ins = Insulin; IRS = insulin receptor substrate; PI3 K P85 = phosphoinositide-3 kinase p85 expression; PKB = protein kinase B; PVD = peripheral vascular disease; RBF = retinal blood flow; Shc = src homology domain c-terminal adaptor homolog.

Vascular complications arise from increased PKC and MAPK activity in cardiovascular tissues in the presence of selective insulin resistance.<sup>23,27</sup> Under normal physiologic conditions, insulin interacts with insulin receptors to stimulate two main pathways in cardiovascular tissues: a phosphoinositide-3 kinase (PI3K) pathway that inhibits atherogenesis and has antiatherogenic effects and a MAPK-activated pathway that promotes cellular growth and enhances atherogenesis (Fig. 3B).<sup>23,27</sup> In the presence of insulin resistance and diabetes, the increase in glucose and free fatty acids leads to an increased release of inflammatory cytokines and altered regulation of PKC and MAPK activity.<sup>23,27</sup> PKC inhibits the PI3K pathway and results in enhanced atherogenesis through such processes as a reduction in antiatherogenic nitric oxide production and impaired endothelium-dependent vasodilation. In addition, processes initiated by insulin resistance and diabetes stimulate the MAPK pathway to exert proatherogenic actions.

Several lines of evidence suggest that the selective activation of PKC- $\beta$  is also a likely mechanism for the

pathogenesis of microvascular complications in diabetes, including diabetic retinopathy and nephropathy (Figs. 3C and 3D). In diabetic nephropathy, hyperglycemia is believed to induce damage by activation of the PKC pathway, resulting in proteinuria, mesangium expansion, and nephromegaly followed by glomerular sclerosis. In retinopathy, PKC activation leads to endothelial dysfunction, followed by pericyte loss, formation of acellular capillaries, and microaneurysms. Such pathologic changes in the retina lead to increased vascular leakage and diabetic macular edema (DME) or to increased VEGF and angiogenesis associated with proliferative diabetic disease<sup>30</sup> and may contribute to DME through the stimulation of PKC.

**SELECTIVE INHIBITION OF PKC- $\beta$  AMELIORATES THE VASCULAR COMPLICATIONS OF DIABETES**

With findings that PKC- $\beta$  may play a critical role in the development of pathologies associated with insulin

resistance and diabetes, there is increased interest in the benefits of inhibiting PKC- $\beta$  to reduce harmful sequelae in micro- and macrovascular complications. Studies<sup>23,31</sup> have focused on the orally administered selective inhibitor of PKC- $\beta$ , ruboxistaurin mesylate (RBX; LY333531). RBX is currently in Phase II/III clinical trials for the treatment of diabetic retinopathy, neuropathy, and nephropathy.<sup>23</sup>

Preliminary evidence demonstrated that RBX may be effective in preventing some of the vascular abnormalities associated with cardiovascular pathologies in animal models. In an early study,<sup>32</sup> RBX decreased the mitogenic activity of VEGF by selectively inhibiting PKC- $\beta$  in endothelial cells from bovine aorta.

Evidence suggests that the inhibition of PKC by RBX prevents the progression of diabetic nephropathy, including the inhibition of mesangial expansion in a diabetic (db/db) rodent model of type 2 diabetes.<sup>33</sup> Although the administration of RBX (10 mg/kg body weight/day) for 16 weeks to db/db mice had no effect on blood glucose levels, body weight, or kidney weight compared to untreated controls, the treatment did result in inhibition of glomerular PKC- $\beta$  activation and mesangial expansion and a reduction in urinary albumin excretion rates.<sup>33</sup> More recently, RBX treatment (32 mg/day) in adults with type 2 diabetes for 1 year maintained estimated glomerular filtration rate ( $-2.5 \pm 1.9$  ml/minute per  $1.73$  m<sup>2</sup>;  $P = 0.185$ ), whereas it was significantly reduced in the placebo group ( $-4.8 \pm 1.89$  ml/minute per  $1.73$  m<sup>2</sup>;  $P = 0.009$ ).<sup>34</sup> Because these subjects were already treated with stable doses of an angiotensin-converting enzyme inhibitor and/or angiotensin receptor blocker, the findings suggested that inhibition of PKC through RBX treatment acts through a distinct pathway offering additional benefits to traditional therapies for diabetic nephropathy.

In diabetic retinopathy, treatment with RBX inhibited the progression of DME as well as reduced visual loss in clinical studies.<sup>35,36</sup> In a 3-year, randomized controlled study<sup>35</sup> of 685 subjects with type 1 or type 2 diabetes attending 70 clinics, oral treatment with RBX (32 mg/day) was investigated in patients with moderately severe to very severe non-proliferative retinopathy. Administration of RBX was associated with a 40% reduction in the prevalence of sustained moderate visual loss (sustained from months 30 to 36 of study; 9.1% versus 5.5% in placebo- and RBX-treated subjects, respectively;  $P = 0.034$ ). There was also a two-fold increase in the baseline-to-endpoint visual improvement of  $\geq 15$  letters ( $P = 0.005$ ) and inhibition of the progression of clinically significant macular edema ( $P = 0.003$ ) compared to placebo control.<sup>35</sup> A 30-month follow-up study of subjects with DME demonstrated that daily oral administration of RBX, 32 mg, reduced the pro-

gression of DME to a sight-threatening stage compared to placebo ( $P = 0.054$ ).<sup>36</sup>

### ARE PERIODONTAL PATHOLOGIES OF DIABETES MICRO- OR MACROVASCULAR COMPLICATIONS?

Diabetic complications are usually separated into micro- or macrovascular diseases because they differ in some of the risk factors. Some of the metabolic abnormalities, such as lipids, fatty acids, and insulin resistance, may be present in obesity and diabetes, whereas hyperglycemia is only present in diabetes. Therefore, the development of the diabetic complications involving cardiovascular or macrovascular diseases are associated with all of the risk factors stated above. However, hyperglycemia is essential for the development of diabetic microvascular diseases but not for cardiovascular complications. This conclusion is derived from the clinical observations that, without hyperglycemia, such as in obese or insulin-resistant people without diabetes, classic lesions of diabetic retinopathy or nephropathy are generally not found. Multiple studies,<sup>37-39</sup> including those of Dr. Robert Genco, suggested that the increased risk for periodontal disease exists in patients with obesity without diabetes. These findings suggested that periodontal disease associated with diabetes may be more related to macrovascular complications of diabetes. This separation could be potentially important for understanding the mechanism of diabetic periodontal disease. Several mechanisms related to diabetic complications could be applicable to micro- and macrovascular diseases of diabetes. These mechanisms include the following: increases in oxidative stress, inflammation, AGE formation, and activation of several signaling pathways involving PKC, MAPK, and others.

In this issue, the role of inflammation and oxidative stress in causing periodontal disease has been reviewed by other authors and, therefore, will not be done here. Increases in oxidative stress and inflammatory factors have been clearly identified and shown in subjects with obesity and insulin resistance with or without diabetes. Therefore, it is very likely that similar factors, which cause increases in inflammatory molecules and oxidative stress in obese and insulin-resistant states, such as increased fatty acids, could also be causing problems in the periodontal tissue. Furthermore, when diabetes occurs, hyperglycemia would be an additional risk factor that induces oxidative stress and inflammation to accelerate dysfunctions in periodontal tissue, which are already present because of obesity or insulin resistance. One example of the specific adverse effects of hyperglycemia, which is not present in insulin-resistant and obese subjects, is its inhibitory effect on neutrophils to resolve infection. Increases in susceptibility

to infection could accelerate the destruction of periodontal tissues. Clearly, studies are needed to clarify the role of hyperglycemia and other metabolic factors, such as lipids, fatty acids, and the role of insulin resistance, in periodontal tissue metabolism and function. It is very likely that those metabolic changes and insulin actions play an important role in the function and survival of gingiva. In addition, it is also possible that periodontal disease could be a manifestation of an important syndrome, the metabolic syndrome, which manifests multiple metabolic abnormalities that may or may not include diabetes but is strongly associated with insulin resistance. Therefore, it is possible that periodontal disease could be a marker for metabolic syndrome. Reciprocally, it is also possible that people with metabolic syndrome may have an increased risk for the development of periodontal disease. In the screening of patients with metabolic syndrome, data need to be gathered to determine whether the evaluation of periodontal disease should be included.

## CONCLUSIONS

Diabetes and its complications are a growing concern, given the increases in its worldwide prevalence and its association with obesity. Inflammatory processes are implicated in the onset of diabetes and the progression of complications. Adiposity, and in particular the release of inflammatory cytokines and other mediators from adipose cells, is associated with the development of insulin resistance in type 2 diabetes, partially through activation of the NF- $\kappa$ B pathway. Other factors that activate the NF- $\kappa$ B pathway and are associated with the development of insulin resistance include obesity and a high-fat diet, hyperglycemia, PKC activation, and oxidative stress. Hyperglycemia is the major risk factor for the development of microvascular diabetic complications, such as retinopathy, neuropathy, and nephropathy. Diabetic cardiovascular pathology has a combination of risk factors, including insulin resistance, free fatty acidemia, hypertension, hyperlipidemia, and inflammation. Although evidence suggests that periodontal disease is associated with increases in inflammatory markers, the major risk factors for its development in diabetes are still unclear. It is important to clarify whether the causes of periodontal disease associated with diabetes are similar to micro- or macrovascular diseases, because the treatment may be different. A well-described pathway underlying the development of diabetic vascular complications is via activation of PKC isoforms, PKC- $\beta$  in particular. At the level of individual tissues, activation of PKC- $\beta$  is associated with the development and/or progression of microvascular and macrovascular complications. Administration of the oral agent RBX to selectively inhibit PKC- $\beta$  activation ameliorated the vascular complications of diabetes. It is a novel

therapy that potentially could be used in conjunction with established agents for diabetic complications.

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## REFERENCES

1. (CDC) Center for Disease Control and Prevention. Diabetes data & trends. Number (in millions) of persons with diagnosed diabetes, United States, 1980-2005. Available at: <http://www.cdc.gov/diabetes/statistics/prev/national/figpersons.htm>. Accessed February 9, 2008.
2. (CDC) Center for Disease Control and Prevention. Diabetes data & trends. Overweight and obesity. U.S. Obesity Trends 1985-2006. Available at: <http://www.cdc.gov/nccdphp/dnpa/obesity/trend/maps/>. Accessed February 9, 2008.
3. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030. *Diabetes Care* 2004;27:1047-1053.
4. Soskolne WA, Klinger A. The relationship between periodontal diseases and diabetes: An overview. *Ann Periodontol* 2001;6:91-98.
5. (CDC) Center for Disease Control and Prevention. National diabetes fact sheet. United States, 2005 General information. Available at: [http://apps.nccd.cdc.gov/DDTSTRS/template/ndfs\\_2005.pdf](http://apps.nccd.cdc.gov/DDTSTRS/template/ndfs_2005.pdf). Accessed February 9, 2008.
6. Singh R, Shaw J, Zimmet P. Epidemiology of childhood type 2 diabetes in the developing world. *Pediatr Diabetes* 2004;5:154-168.
7. Treszl A, Szereday L, Doria A, King GL, Orban T. Elevated C-reactive protein levels do not correspond to autoimmunity in type 1 diabetes. *Diabetes Care* 2004;27:2769-2770.
8. Devaraj S, Cheung AT, Jialal I, et al. Evidence of increased inflammation and microcirculatory abnormalities in patients with type 1 diabetes and their role in microvascular complications. *Diabetes* 2007;56:2790-2796.
9. Vozarova B, Weyer C, Lindsay RS, Pratley RE, Bogardus C, Tataranni PA. High white blood cell count is associated with a worsening of insulin sensitivity and predicts the development of type 2 diabetes. *Diabetes* 2002;51:455-461.
10. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *JAMA* 2001;286:327-334.

11. Thorand B, Löwel H, Schneider A, et al. C-reactive protein as a predictor for incident diabetes mellitus among middle-aged men: Results from the MONICA Augsburg cohort study, 1984-1998. *Arch Intern Med* 2003;163:93-99.
12. Mooradian AD. Obesity: A rational target for managing diabetes mellitus. *Growth Horm IGF Res* 2001; 11(Suppl. A):S79-S83.
13. Shoelson SE, Lee J, Goldfine AB. Inflammation and insulin resistance. *J Clin Invest* 2006;116:1793-1801.
14. Hotamisligil GS. Inflammation and metabolic disorders. *Nature* 2006;444:860-867.
15. Petersen KF, Dufour S, Befroy D, Garcia R, Shulman GI. Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes. *N Engl J Med* 2004;350:664-671.
16. Albert MA, Ridker PM. C-reactive protein as a risk predictor: Do race/ethnicity and gender make a difference? *Circulation* 2006;114:e67-e74.
17. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor- $\alpha$ : Direct role in obesity-linked insulin resistance. *Science* 1993;259: 87-91.
18. Ofei F, Hurel S, Newkirk J, Sopwith M, Taylor R. Effects of an engineered human anti-TNF- $\alpha$  antibody (CDP571) on insulin sensitivity and glycemic control in patients with NIDDM. *Diabetes* 1996;45: 881-885.
19. Yuan M, Konstantopoulos N, Lee J, et al. Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of I $\kappa$ B $\beta$ . *Science* 2001;293:1673-1677.
20. Fleischman A, Shoelson SE, Bernier R, Goldfine AB. Salsalate improves glycemia and inflammatory parameters in obese young adults. *Diabetes Care* 2008; 31:289-294.
21. Tsai C, Hayes C, Taylor GW. Glycemic control of type 2 diabetes and severe periodontal disease in the US adult population. *Community Dent Oral Epidemiol* 2002;30:182-192.
22. Scott JA, King GL. Oxidative stress and antioxidant treatment in diabetes. *Ann N Y Acad Sci* 2004;1031: 204-213.
23. He Z, King GL. Protein kinase C beta isoform inhibitors: A new treatment for diabetic cardiovascular diseases. *Circulation* 2004;110:7-9.
24. Dalla Vestra M, Mussap M, Gallina P, et al. Acute-phase markers of inflammation and glomerular structure in patients with type 2 diabetes. *J Am Soc Nephrol* 2005;16(Suppl. 1):S78-S82.
25. Izuora KE, Chase HP, Jackson WE, et al. Inflammatory markers and diabetic retinopathy in type 1 diabetes. *Diabetes Care* 2005;28:714-715.
26. Meleth AD, Agron E, Chan CC, et al. Serum inflammatory markers in diabetic retinopathy. *Invest Ophthalmol Vis Sci* 2005;46:4295-4301.
27. Das Evcimen N, King GL. The role of protein kinase C activation and the vascular complications of diabetes. *Pharmacol Res* 2007;55:498-510.
28. He Z, King GL. Can protein kinase C beta-selective inhibitor, ruboxistaurin, stop vascular complications in diabetic patients? *Diabetes Care* 2005;28:2803-2805.
29. Ohshiro Y, Ma RC, Yasuda Y, et al. Reduction of diabetes-induced oxidative stress, fibrotic cytokine expression, and renal dysfunction in protein kinase C beta-null mice. *Diabetes* 2006;55:3112-3120.
30. Aiello LP, Avery RL, Arrigg PG, et al. Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. *N Engl J Med* 1994;331:1480-1487.
31. Jirousek MR, Gillig JR, Gonzalez CM, et al. (S)-13-[(dimethylamino)methyl]-10,11,14,15-tetrahydro-4,9:16, 21-dimetheno-1H, 13H-dibenzo[e,k]pyrrolo[3,4-h][1,4,13]oxadiazacyclohexadecene-1,3(2H)-dione (LY333531) and related analogues: Isozyme selective inhibitors of protein kinase C beta. *J Med Chem* 1996; 39:2664-2671.
32. Xia P, Aiello LP, Ishii H, et al. Characterization of vascular endothelial growth factor's effect on the activation of protein kinase C, its isoforms, and endothelial cell growth. *J Clin Invest* 1996;98:2018-2026.
33. Koya D, Haneda M, Nakagawa H, et al. Amelioration of accelerated diabetic mesangial expansion by treatment with a PKC beta inhibitor in diabetic db/db mice, a rodent model for type 2 diabetes. *FASEB J* 2000; 14:439-447.
34. Tuttle KR, Bakris GL, Toto RD, McGill JB, Hu K, Anderson PW. The effect of ruboxistaurin on nephropathy in type 2 diabetes. *Diabetes Care* 2005;28:2686-2690.
35. Aiello LP, Davis MD, Girach A, et al. Effect of ruboxistaurin on visual loss in patients with diabetic retinopathy. *Ophthalmology* 2006;113:2221-2230.
36. PKC-DMES Study Group. Effect of ruboxistaurin in patients with diabetic macular edema: Thirty-month results of the randomized PKC-DMES clinical trial. *Arch Ophthalmol* 2007;125:318-324.
37. Al-Zahrani MS, Bissada NF, Borawski EA. Obesity and periodontal disease in young, middle-aged, and older adults. *Journal of Periodontology* 2003;74:610-615.
38. Genco R, Grossi S, Ho A, Nishimura F, Murayama Y. A proposed model linking inflammation to obesity, diabetes, and periodontal infections. *Journal of Periodontology* 2005;76:2075-2084.
39. Ritchie C. Obesity and periodontal disease. *Periodontology* 2000 2007;44:154-163.

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