Trying to prevent diabetic cardiovascular autonomic neuropathy: More questions than answers

Robert J. Gropler, MD^a

^a Division of Radiological Sciences, Cardiovascular Imaging Laboratory, Edward Mallinckrodt Institute of Radiology, Washington University School of Medicine, St. Louis, MO

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The prevalence of diabetes mellitus in the United States has increased dramatically over the past 30 years, to where it now affects ~24 million of adults and with that number expected to double by 2050.¹ This greater prevalence includes both type-1 diabetes mellitus (T1DM) and type-2 diabetes mellitus (T2DM), although the latter predominates primarily because of the increase in obesity. Cardiovascular disease is the leading cause of morbidity and mortality in diabetic patients accounting for approximately two-thirds of all deaths.² In addition to its tremendous health burden, there is a substantial economic burden with the cost of diagnosis and treatment of cardiovascular complications in diabetic patients estimated to be ~\$22 billion annually.³ Although coronary atherosclerosis is the major cause of cardiovascular disease, diabetes mellitus can impair cardiovascular function independent of atherosclerosis. For example, the presence of T2DM is an independent risk factor for developing left ventricular hypertrophy and congestive heart failure and worsens the prognosis for those who develop these conditions. Cardiovascular autonomic dysfunction (CAN) is another often overlooked complication of this disease. It reflects abnormalities in the sympathetic and/or parasympathetic neuronal systems with their relative contributions

defining patient symptomatology. The prevalence of CAN ranges from ~1% to 90% in T1DM and from ~20%-75% in patients with T2DM.^{4,5} The wide ranges reflect both inconsistencies in criteria used to define CAN and variable study design. CAN may be present the time of diagnosis of diabetes and the prevalence increases with age, disease duration, and worsening glycemic control. There are multiple clinical manifestations of CAN including resting tachycardia, impaired heart rate variability, exercise intolerance, abnormal regulation of blood pressure, orthostatic hypotension, and silent myocardial ischemia. Moreover, CAN is associated with impaired diastolic function and has been posited to be a potential contributor to the cardiomyopathy of diabetes mellitus, although direct causality has been difficult to prove. These diverse manifestations of CAN are likely the reasons it is a key contributor to the increase in CV morbidity and mortality associated with diabetes mellitus. The pathogenesis of CAN is likely multifactorial and reflects the yet to be defined proportional contributions of numerous factors such as hyperglycemia-induced neuronal ischemia/injury, neurovascular insufficiency, autoimmune damage, and neurohormonal growth factor deficiency.⁶ The importance of hyperglycemia in the pathogenesis of CAN is supported by multi-center trials such as the Diabetes Control and Complications Trial and the Epidemiology of Diabetes Interventions and Complications Trial demonstrating that intensive glycemic control had persistent beneficial effects on the incidence, severity, and progression of CAN in patients with T1DM. The results in patients with T2DM are less clear but appear to suggest that intensive glycemic control in combination with aggressive CV risk factor modification reduces the prevalence of CAN in patients with microalbuminuria. Despite these encouraging results, there is intense interest in developing therapeutic approaches to reduce the incidence and clinical severity of CAN.

Reprint requests: Robert J. Gropler, MD, Division of Radiological Sciences, Cardiovascular Imaging Laboratory, Edward Mallinckrodt Institute of Radiology, Washington University School of Medicine, 510 S. Kingshighway, St. Louis, MO 63110; Groplerr@mir.wustl.edu
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One theory, that was the basis for the study is reported in this issue of the Journal by Thackeray et al⁷, that very early treatment to control hyperglycemia can prevent or attenuate CAN. In the study, the anti-diabetic agents metformin (which decreases liver gluconeogenesis and improves skeletal muscle glucose uptake and oxidation via activation of AMP-activated kinase) and rosiglitazone (a PPAR γ -agonist that reduces peripheral lipolysis resulting in improved insulin sensitivity) were compared to insulin in a rodent model that attempts to recapitulate the T2DM phenotype. The model attempts to exploit the well-known observation that the Sprague-Dawley rat develops insulin resistance and hyperinsulinemia but normoglycemia with high-fat feeding. By administering a small dose (typically 25–50 mg·kg⁻¹) of streptozocin to impair but not eliminate pancreatic beta-cell function, hyperglycemia is induced while still maintaining insulin resistance and relative hyperinsulinemia, and thus, T2DM is exhibited. In this study 6-week-old rats were placed on a high-fat diet for 2 weeks at which time they received streptozocin or placebo. After an additional week, those animals exhibiting hyperglycemia were placed into either no-treatment or one of the three treatment groups for 8 weeks of therapy. Small animal PET imaging using the norepinephrine analog ¹¹C-*meta*-hydroxyephedrine (HED) to assess cardiac adrenergic neuronal function was performed at baseline and at 8 weeks of therapy. Echocardiography was used to assess both systolic and diastolic function. In vitro measurements included plasma insulin, substrates, and norepinephrine levels and immunoblotting of myocardial tissue to measure the expression of various proteins involved with noradrenergic signaling and the transport of glucose and fatty acids to assess metabolic changes. The major findings from the study were that despite improved plasma lipid levels, neither metformin nor rosiglitazone reduced plasma glucose levels and that only insulin-restored lipid and glucose levels to normal. At 8 weeks, both the untreated and treated diabetic rats displayed a significant reduction in myocardial HED uptake (~40%, $P < .05$) that did not differ amongst the groups. This decline was paralleled by an elevation in plasma norepinephrine levels in all diabetic groups (2.3- to 3.3-fold compared with non-diabetic rats, $P < .05$) and in general, a decline in myocardial norepinephrine reuptake transporter expression (28%–35% lower compared with non-diabetic rats, $P < .05$). All of these findings are consistent with CAN. With respect to diastolic function, the presence of diabetes significantly extended mitral valve deceleration time and increased the E/A ratio suggesting diastolic dysfunction. Only insulin therapy demonstrated significant prevention of these functional abnormalities. Based on these results, the investigators concluded that

very early institution of anti-diabetic therapy did not prevent the development of CAN in this pre-clinical model of diabetes.

The authors should be complemented for performing a highly complex study. However, based on the results of the clinical trials mentioned above demonstrating the benefits of tight glycemic control on CAN, it is surprising that very early institution of anti-diabetic therapy did not have at least a partial beneficial effect in preventing CAN. This leaves the reader with a couple of unanswered questions. The most notable is why did the very early institution of various anti-diabetic therapies fail to prevent or reduce the development of CAN? The most likely explanation is the variability in the diabetic phenotype exhibited by the rat model used for the study. Unfortunately, the success rate in achieving T2DM with this model is fairly variable and highly dependent upon the dose of streptozocin used with hypoinsulinemia frequently occurring at the higher streptozocin doses leading to a phenotype consistent with T1DM. This appears to have occurred in this study (45 mg·kg⁻¹ were administered) as the rats exhibited insulin deficiency and inconclusive evidence of insulin resistance given a hyperglycemic-euglycemic clamp was not performed. Thus, it is not surprising that either metformin or rosiglitazone failed to exhibit a beneficial effect on CAN. Moreover, the diabetic animals were non-obese further mitigating potential therapeutic effects of rosiglitazone whose primary site of action is adipose tissue. However, it is less clear why insulin replacement initiated very early after the onset of hyperglycemia failed to reduce or prevent CAN? As with all studies, there may be unexplained technical or procedural anomalies that may account for the lack of expected results. In addition, sex plays an important role in normal cardiac autonomic function and on the impact of diabetes on various cardiac parameters such as myocardial hypertrophy, substrate metabolism, and mechanical function. Moreover, CAN tends to be more common in diabetic men than women.^{4,5} In the current study, only male rats were studied, so whether similar findings would be obtained in female rats is unclear. The authors raise the intriguing possibility that the relatively young age of rats at the time treatment may have led to confounding of the observations by normal postnatal development of the cardiac sympathetic nervous system. However, this appears unlikely given that in rats, adrenergic innervation patterns in cardiac tissues and the vasculature reach adult levels by postnatal week 3, whereas the thickness and density of nerve fibers reach adult levels by postnatal week 5, well before the administration of streptozocin in this study.⁸ Given these numerous variables, the author's conclusion that the diabetic rat model used for this study displayed insulin resistance and that

the early institution of anti-diabetic therapies in it does not prevent CAN, is probably a bit too firm. As a consequence, the potential utility of early therapy to prevent CAN, particularly in T2DM is still an open question.

However like many good research studies, the results do provide some unexpected insights, in this case into the relationship between CAN and myocardial diastolic dysfunction. As mentioned previously, it is still unclear as to whether CAN contributes to the development of the diabetic cardiomyopathy or is merely associated with it. A recent observational clinical study would suggest causality but without an intervention component the link cannot be proven definitively.⁹ In an earlier study by Thackeray et al, it was shown that 8 weeks of insulin therapy instituted at 8 weeks of diabetes in the same rat model resulted in an improvement in CAN (e.g., recovery of myocardial HED retention and a decrease in plasma NE levels). However, no improvement in diastolic function was observed.¹⁰ This is in contrast to the present study where the reverse findings were observed (e.g., no effect on CAN but preservation of diastolic function). Taken in sum, these observations suggest that CAN is associated with diabetic cardiomyopathy but is not a prerequisite for it, at least in a pre-clinical model consistent with T1DM. This would suggest other causes of the diabetic cardiomyopathy such altered myocardial metabolism, enhanced fibrosis, and increased oxidative stress/inflammation may be more contributory. Clearly, more detailed in

studies in other pre-clinical diabetic models are needed to help confirm this observation.

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