Regression and Progression of Cardiac Sympathetic Dysinnervation Complicating Diabetes: An Assessment by C-11 Hydroxyephedrine and Positron EmissionTomography

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Cardiovascular denervation complicating diabetes has been implicated in sudden cardiac death potentially by altering myocardial electrical stability and impairing myocardial blood flow. Scintigraphic evaluation of cardiac sympathetic integrity has frequently demonstrated deficits in distal left ventricular (LV) sympathetic innervation in asymptomatic diabetic subjects without abnormalities on cardiovascular reflex testing. However, the clinical significance and subsequent fate of these small regional defects is unknown. This study reports the results of a prospective observational study in which positron emission tomography (PET) with (—)-[^{11}C]-*meta*-hydroxyephedrine ([^{11}C]-HED) was used to evaluate the effects of glycemic control on the progression of small regional LV [^{11}C]-HED retention deficits in 11 insulin-dependent diabetic subjects over a period of 3 years. The subjects were divided into two groups based on attained glycemic control during this period: group A contained six subjects with good glycemic control (individual mean HbA1c $< 8\%$), and group B contained five subjects with poor glycemic control (individual mean HbA1c $\geq 8\%$). Changes in regional [^{11}C]-HED retention were compared with reference values obtained from 10 healthy aged-matched nondiabetic subjects. At baseline, abnormalities of [^{11}C]-HED retention affected 7.3% ± 1.4% and 9.9% ± 6.6% of the LV in group A and B subjects, respectively, with maximal deficits of LV [^{11}C]-HED retention involving the distal myocardial segments. At the final assessment in group A, the extent of the deficits in [^{11}C]-HED retention decreased to involve only 1.7% ± 0.7% of LV ($P < .05$ *v* baseline scan), with significant increases in [^{11}C]-HED retention occurring in both the distal and proximal myocardial segments. In contrast, in group B with poor glycemic control, the extent of [^{11}C]-HED deficits increased to involve 34% ± 3.5% of the LV ($P < .01$ *v* baseline), with retention of [^{11}C]-HED significantly decreasing in the distal segments ([^{11}C]-HED retention index, 0.066 ± 0.003 *v* 0.057 ± 0.002, $P < .05$, at baseline and final assessment, respectively). Poor glycemic control was associated with increased heterogeneity of LV [^{11}C]-HED retention, since three of five group B subjects developed abnormally increased [^{11}C]-HED retention in the proximal myocardial segments. In conclusion, defects in LV sympathetic innervation can regress or progress in diabetic subjects achieving good or poor glycemic control, respectively. In diabetic subjects with early cardiovascular denervation, institution of good glycemic control may prevent the development of myocardial sympathetic dysinnervation and enhanced cardiac risk.

DIABETIC AUTONOMIC NEUROPATHY (DAN) has been implicated in the excess sudden cardiac death observed in diabetic patients[1-6] potentially by altering myocardial electrical stability and/or by impairing myocardial blood flow.[7-9] Absent respiratory sinus arrhythmia secondary to DAN is predictive of left ventricular (LV) functional failure and is associated with increased mortality.[5] Indeed, cardiovascular DAN has been reported to confer a 5-year mortality rate of 16% to 53%,[1-3] with the highest mortality observed in advanced cases with symptomatic sympathetic denervation and orthostatic hypotension.[3] A clear beneficial effect of metabolic control on the development or progression of cardiac DAN should therefore significantly improve the overall prognosis for diabetes; however, such an effect has been difficult to demonstrate clinically.

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Improved metabolic control has been reported to slow the progression of heart rate variability (HRV) deficits in insulin-dependent diabetic patients in some studies[10-14] but not others,[15-17] leading to speculation that autonomic nerve function may be less responsive to improved glycemic control than somatic nerve function.[17] However, in the Diabetes Control and Complications Trial (DCCT), intensive insulin therapy, which resulted in a decrease of mean HbA1c values of approximately 2%, decreased the risk of developing subclinical neuropathy (defined by abnormal nerve conduction or autonomic function testing) by up to 71%,[12,13] suggesting that autonomic dysfunction can be prevented by achieving near-euglycemia. However, the DCCT was not designed to test the efficacy of intensive therapy in the treatment and potential reversal of diabetic neuropathy, and patients with overt diabetic neuropathy were excluded from the study.

Uncertainty as to the effects of improved metabolic control on cardiovascular DAN may, in part, reflect the techniques used to assess DAN. Conventional measures of autonomic function use indirect methods relying on cardiovascular reflexes, which are unable to quantify regional cardiac denervation. Recently, direct assessment of cardiac sympathetic integrity has become possible with the introduction of radiolabeled analogs of norepinephrine, which are actively taken up by the sympathetic nerve terminals of the heart.[9,18-25] In cross-sectional studies, deficits of LV [^{123}I]-metaiodobenzylguanidine (MIBG) retention have commonly been identified in diabetic subjects even without abnormalities on cardiovascular reflex testing[19,22] and have been reported even in newly diagnosed diabetes.[23] The radiotracer [^{11}C]-HED has recently been developed as a norepi-

nephrine analog for positron emission tomography (PET).[21,25,26] This has been shown to undergo highly specific uptake and retention in the sympathetic nerve terminals,[21,26] which thus facilitates the quantitative regional characterization of sympathetic neuronal dysfunction and loss.[21,26,27] In the rat heart, [^{11}C]-HED retention is abolished by chemical sympathetic denervation with 6-hydroxydopamine[28] or desimpramine.[25] In the transplanted human heart, studies using [^{11}C]-HED have demonstrated increased tracer retention in the proximal anterior wall that correlated with the presence of axons on histological assessment[29] and with increased coronary blood flow in response to sympathetic activation,[30] thus confirming the neuronal specificity of HED-PET and its ability to quantify regional sympathetic reinnervation.

Small (<10%) deficits of LV [^{11}C]-HED retention have been reported in 40% of diabetic subjects without DAN on reflex testing,[27] with defects observed selectively in the distal inferolateral wall of the LV.[21,26,27] In more severe DAN, the retention of [^{11}C]-HED in the LV is remarkably heterogeneous, since as the extent of distal deficits increase,[21,26,27] [^{11}C]-HED retention becomes paradoxically increased in the proximal myocardial segments.[27] However, the etiology and clinical importance of these scintigraphically demonstrated deficits, the pattern of progression of LV sympathetic denervation, and its relationship to glycemic control are unknown. This communication reports the results of a 3-year follow-up study of previously characterized subjects[21] in which PET with [^{11}C]-HED was used to evaluate the effects of glycemic control on the progression of small distal deficits of LV [^{11}C]-HED. The findings presented herein demonstrate that regression of [^{11}C]-HED retention deficits or progression to a markedly heterogeneous pattern consistent with a sympathetic "dysinnervation"[27] is closely related to the degree of antecedent glycemic control.

SUBJECTS AND METHODS

To minimize the possibility of including subjects with occult coronary artery disease, only young subjects without clinical (or ECG) evidence of macrovascular disease were studied. All subjects had undergone SPECT myocardial perfusion scintigraphy using thallium 201 at rest and [Tc-99m] hexakis-2-methoxy-2-isobutyl-isonitrile (Sesta-MIBI) during maximal adenosine stimulation (140 μg/kg/min) within 1 year of the baseline study that excluded the presence of significant coronary artery disease. Moreover, both at baseline and at the final assessment, the presence of regional discrete perfusion defects consistent with occult coronary artery disease was evaluated using PET by visual and semiquantitative analysis of myocardial blood flow at rest. All subjects had homogeneous N-13 ammonia retention on visual inspection of standardized color-coded blood flow images taken from the vertical and horizontal long axis and the distal and proximal short axis of the LV. In addition, all diabetic patients had z scores of less than 2 for N-13 ammonia retention in their individual LV polar map sectors when compared with the corresponding sector in the whole nondiabetic control group, consistent with the absence of clinical attributes of coronary artery disease.

Eleven previously studied[21] type I (insulin-dependent) diabetic subjects who were known to have distal LV [^{11}C]-HED retention deficits underwent repeat HED-PET assessment after 3 years. Results in these diabetic subjects were compared with reference values obtained at baseline in 10 healthy age-matched nondiabetic subjects (these reference values were consistent with values previously reported in other healthy subjects,[21] and retention of sympathetic neurotransmitter analogs in the heart remains constant over a 3-year period[31]). Clinical details are listed in Table 1. In response to the results of the DCCT,[12,13] it was considered unethical to randomize subjects into intensive or conventional therapy groups, and thus attempts were made to intensify glycemic control in all diabetic subjects. However, to attempt to explore the influence of antecedent glycemic control on the progression of LV [^{11}C]-HED deficits, it was decided prospectively to use a HbA1c value of 8% (consistent with the DCCT "action-required" level[32]) as the cutoff to separate subjects achieving good versus poor glycemic control over the 3-year period. HbA1c values were determined at 6-month intervals. Group A subjects were defined as those who achieved a significant improvement in glycemic control (mean HbA1c, <8%), whereas in group B subjects, there was no overall change in glycemic control from baseline, with mean HbA1c values of 8% or higher. At baseline, nine subjects (five in group A and four in group B) had decreased HRV to deep breathing and two subjects in each group had DAN on standardized cardiovascular reflex testing, since these subjects had at least two abnormal reflex tests and reported symptoms attributable to DAN. These symptoms included early satiety, nausea, persistent constipation with periodic episodes of diarrhea, and dizziness on standing. Written informed consent was obtained from all patients, and the study protocol was approved by the institutional review board of the University of Michigan.

Autonomic Function Testing

A battery of autonomic function tests were used to assess cardiovascular autonomic neuropathy. Subjects were fasted and abstained from both prescription and nonprescription medications on the day of assessment. All subjects were instructed to delay their morning insulin injection until after testing, and were excluded if they experienced a hypoglycemic episode within 24 hours of testing. Blood glucose values were within the range of 120 to 250 mg/dL during the assessment period.

HRV with deep breathing was assessed[33-36] by measuring the maximum and minimum R-R intervals and calculating a mean value for the six cycles. A 5-minute postural study was performed in which the systolic and diastolic blood pressure was recorded three times at 1-minute intervals while supine and the change in pressure was recorded at 1-minute intervals for 5 minutes after standing. The lowest standing systolic pressure was used for calculation of postural change in blood pressure. The Valsalva maneuver[33] was performed, and the ratio of the longest R-R interval after the maneuver to the shortest R-R interval during the maneuver was calculated. This test was repeated twice with a 5-minute period of rest between Valsalva maneuvers, and the mean value was calculated. Abnormality on these standardized tests was defined as results less than the 5th percentile for age.[33-36]

PET Studies and Image Construction

Patients on medication (including caffeine) known to interfere with neuronal uptake of norepinephrine analogs had the medication discontinued at least 2 weeks prior to study. Cardiac PET imaging was

Table 1. Clinical Details of the Subjects

	Nondiabetic Controls (n = 10)	Group A (n = 6)	Group B (n = 5)
Male:female ratio	3:7	1:5	3:2
Age (yr)			
Mean ± SD	37 ± 11	36 ± 6	34 ± 13
Range	26-56	26-45	20-50
Diabetes duration (yr)	—	20 ± 10	19 ± 15
Baseline autonomic symptoms	—	2	2
Proliferative retinopathy	—	2	2
Albuminuria (>30 mg/24 h)	—	1	2

performed in a whole-body PET scanner (model CTI 931 and Siemens/ECAT 931, Knoxville, TN) with 20 mCi [^{11}C]-HED and 20 mCi [^{13}N]-ammonia.[21] The scanner has eight circular detector rings, thereby facilitating the simultaneous acquisition of 15 contiguous transaxial images (oriented perpendicular to the sagittal and coronal planes of the body) with a slice thickness of 6.75 mm. Following placement of an intravenous cannula in the antecubital vein and positioning of the tomograph with the aid of a scout image, a 15-minute transmission study using a retractable germanium 68 ring source was performed to subsequently correct emission data for tissue attenuation. [^{11}C]-HED was made using a modified version of the synthesis previously reported by Rosenspire et al.[26] In brief, [^{11}C]-HED was produced by direct methylation of (—)-metaraminol using [^{11}C]methyl triflate in dimethylformamide and purified using a cation-exchange semi-prep high-performance liquid chromatography column (Phenomenex SCX, Torrance, CA) using 100 mmol/L sodium phosphate monobasic as vehicle. Specific activities were greater than 400 mCi/mmol, with radiochemical purities greater than 98%.

Dynamic scan acquisition was initiated simultaneously with injection of [^{11}C]-HED, and the protocol comprised 15 images with varying frame duration (six × 30 seconds, two × 60 seconds, two × 150 seconds, two × 300 seconds, two × 600 seconds, and one × 1,200 seconds). The emission data were attenuation-corrected and reconstructed using filtered backprojection using a Hanning filter with a cutoff frequency of 1.12 cycles/cm. A SUN workstation (SUN Microsystems, Mountain View, CA) was used to realign the images perpendicular to the long axis of the LV yielding eight contiguous short-axis views (slice thickness, 0.8 cm) of myocardial tracer distribution extending from the apex to the base of the LV. After waiting 1 hour for the C-11 decay after the end of data acquisition, resting myocardial perfusion was quantified using N-13 ammonia as previously reported.[21,37,38] [^{13}N]-ammonia was produced using a procedure based on the method of Berridge and Landmeier.[39] In brief, [^{13}N]-ammonia was produced in-target using distilled water containing 5 mmol/L EtOH and H_2 over pressure at 680 kPa. The target was irradiated with 15 MeV protons at 15 μA for 5 minutes. After irradiation, the target volume (~2 mL) was driven through an anion-exchange column using He gas to remove nitrates and nitrites and subsequently filtered using a 0.22-μm filter to ensure sterility. A 10-minute delay was imposed before delivery to the PET Clinic to allow for decay of [^{15}O] water that is simultaneously produced within the target. Twenty millicuries of N-13 ammonia was administered into a peripheral arm vein over 30 seconds. Dynamic scan acquisition was then initiated with varying frame duration (12 × 10 seconds, six × 30 seconds, two × 300 seconds).

Polar Map Generation to Assess Homogeneity of [^{11}C]-HED Retention

The homogeneity of LV [^{11}C]-HED retention was assessed as previously reported.[21] Circumferential count-profile analysis was performed on each of the eight short-axis images. Each short-axis slice was divided into 36 angular regions of interest ("sectors"), and the myocardial concentration of [^{11}C]-HED in each sector, as reflected by the mean PET counts in the sector, was determined. Regional variation of myocardial retention of [^{11}C]-HED was assessed by dividing the mean PET counts in each of 288 sectors (eight images × 36 sectors per image) by the value found in the sector containing the maximum mean PET counts. These normalized [^{11}C]-HED retention data were then displayed as polar coordinate maps of relative tracer activity (C-11/N-13) from the short-axis blood flow and the 40- to 60-minute postinjection [^{11}C]-HED images. The map was divided into nine regions. In this map, the LV myocardium is depicted with the apex at the center, the distal LV segments (anterior, septal, inferior, and lateral) as the inner ring, and the corresponding proximal segments as the outer ring. Apical values were obtained by averaging together all sectors in the two most apical short-axis slices. Distal values for the other segments were obtained by averaging together the appropriate sectors in the three planes adjacent to the two apical planes. Similarly, the corresponding proximal values were obtained using the final three short-axis slices toward the base of the heart.

Studies performed in the normal subjects were similarly processed and averaged together to determine reference values for the homogeneity of [^{11}C]-HED retention in the healthy LV for comparison to the homogeneity of tracer retention in the diabetic subject. In normal subjects, regional differences in relative [^{11}C]-HED retention do not achieve statistical significance[21] and are consistent with homogeneous uptake of [^{11}C]-HED within the normal LV. The heterogeneity of regional LV [^{11}C]-HED retention in each diabetic patient was compared with this normal homogeneous distribution by calculating a z score, $z_i = (q_i - \mu_i)/\sigma_i$, where q_i is the relative [^{11}C]-HED retention value in the ith sector value of the diabetic polar map, and μ_i and σ_i are the mean and standard deviation of the relative [^{11}C]-HED retention in the ith sector of the control polar map. In diabetic subjects, sectors that had a z score greater than 2.5 (ie, relative [^{11}C]-HED retention in the patient's sector was less than the corresponding sector mean relative [^{11}C]-HED retention in normal subjects by >2.5 SD) were defined as abnormal. Thus, the calculated z scores represent a validated[21] measure of the individual subject's myocardial tracer retention heterogeneity, with an increase of heterogeneity being consistent with distal LV denervation.[21] The "extent" of the heterogeneity was expressed as the percentage of sectors in the polar map that were abnormal, ie, z_i more than 2.5.

Retention Index Calculation to Quantify Regional Changes in Absolute [^{11}C]-HED Retention

A "retention index" approach[21] was additionally used to quantify absolute changes in retention of [^{11}C]-HED in the proximal and distal LV myocardium. This technique normalizes [^{11}C]-HED retention to myocardial tracer delivery. All subjects had homogeneous myocardial perfusion on quantitative blood flow analysis, and thus it was assumed that the delivery of [^{11}C]-HED to the LV would also be homogeneous. Absolute tissue retention of [^{11}C]-HED between 40 and 60 minutes after injection was measured for distal and proximal anterior, septal, inferior, and lateral myocardial segments by averaging the appropriate sectors from the last frame of the dynamic data set. Mean tracer counts per pixel within the myocardial regions were determined. To correct this measurement for the amount of tracer delivered to the myocardium, retention was divided by the total counts in the blood over the period from injection to 60 minutes later. Total blood counts were determined from the area under the time-activity curve for a small region of interest (five × five pixels) placed at the center of the LV blood pool on a basal plane. This yielded a [^{11}C]-HED retention index as follows:

$$\text{retention index (mL blood/min/mL tissue)} = \frac{\text{tissue counts between 40 and 60 minutes}}{\int \text{blood counts from time 0 to 60 minutes}}.$$

Statistics

Statistical analysis was performed using Super ANOVA (Abacus Concepts, Berkeley, CA). The equality of means of the experimental groups was tested by a one-way ANOVA, and if significant, the differences were assessed by the Student-Newman-Keuls multiple-range test. If the variances for the variables were found to differ significantly, a logarithmic transformation was performed to correct the unequal variances. All analyses were then performed on the transformed data. A Mann-Whitney U test was used for two-group comparisons. Significance was defined at the .05 level.

RESULTS

A comparison of the 6-month HbA1c values in the two groups of diabetic subjects is shown in Fig 1. At baseline, the difference in mean HbA1c values in group A and group B subjects was not statistically significant. However, over the following 36 months, the mean 6-month HbA1c was significantly higher in group B versus group A ($P < .05$), except at 24 months ($P = .08$). The mean HbA1c over the entire 36-month study period for group A was 7.3% ± 0.7%, compared with 9.7% ± 0.3% in group B ($P < .05$).

Change in Extent of Regional LV [^{11}C]-HED Retention Deficits

The extent of deficits in LV [^{11}C]-HED retention were comparable at baseline in both groups of diabetic subjects (Fig 2), with observed deficits restricted to the distal inferior and lateral myocardial segments. In group A, good glycemic control resulted in a 77% ($P < .05$) reduction in the extent of these deficits after 3 years, which contrasted markedly with group B, in which [^{11}C]-HED retention deficits increased 3.4-fold ($P < .05$) (Fig 2). In group B, abnormalities of [^{11}C]-HED retention were spread circumferentially and proximally involving the anterior, inferior, and lateral LV walls in an ascending neuroanatomical pattern. However, the proximal anterior and septal walls of the LV retained islands of sympathetic innervation even in severe DAN.

Quantitative Assessment of Regional Changes in LV [^{11}C]-HED Retention

To quantify regional changes in LV [^{11}C]-HED retention, retention index values were determined in the distal and proximal myocardial segments of diabetic subjects and compared against the reference values measured in 10 healthy nondiabetic subjects (in the nondiabetic control group, no significant regional differences in LV retention index values were observed). In group A at the baseline assessment, the distal retention index was decreased by 30% ($P < .05$) (Fig 3). Three years of good glycemic control was found to increase the distal

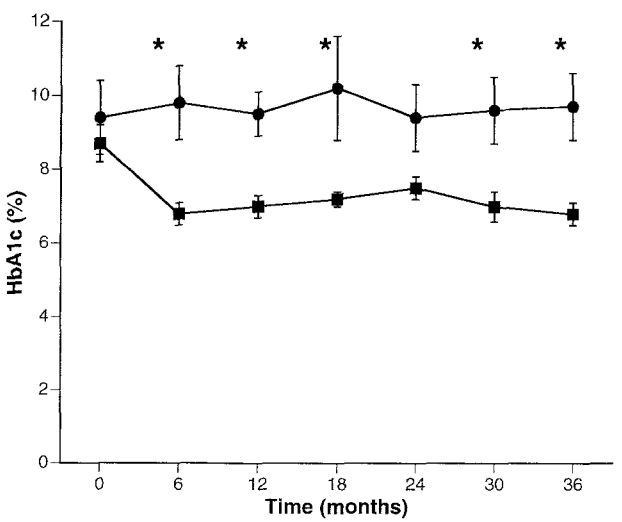

Fig 1. Comparison of 6-month HbA1c in diabetic subjects with mean HbA1c <8% (group A; ■) versus mean HbA1c ≥8% (group B; ●). Data are the mean ± 1 SEM. *$P < .05$ *v* group A.

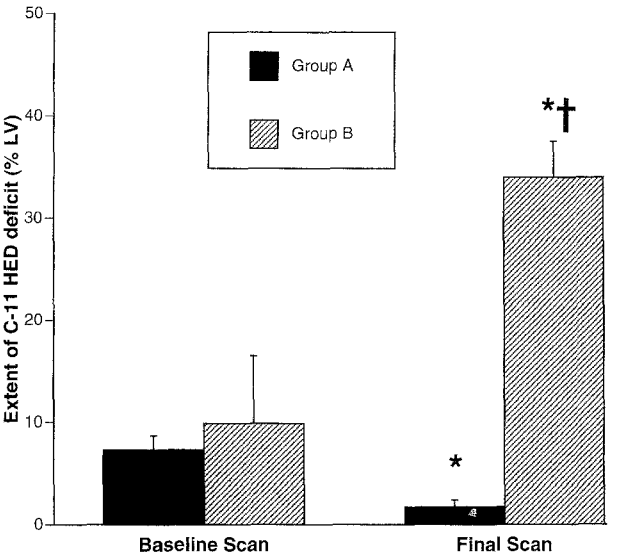

Fig 2. Extent of regional HED retention abnormalities detected at baseline and after 3 years in diabetic subjects with good glycemic control (group A) versus poor glycemic control (group B). Data are the mean ± 1 SEM. *$P < .05$ *v* baseline study. †$P < .01$ *v* group A.

retention index by 25% ($P < .05$) to levels that were not significantly different from normal control values. In a similar fashion, [^{11}C]-HED retention was decreased by 22% ($P < .05$ *v* reference values) in the proximal myocardial segments and increased by 24% ($P < .05$) after 3 years to levels that were also not significantly different from control values.

The corresponding regional changes in [^{11}C]-HED retention in group B are shown in Fig 4. At baseline, the distal [^{11}C]-HED retention index was decreased by 21% ($P < .05$) compared with the reference values and decreased another 14% ($P < .05$ *v* baseline and corresponding change in distal retention index in group A) after 3 years of poor glycemic control. However, in group B, [^{11}C]-HED retention was not significantly different from reference values at baseline in the proximal myocardial segments and paradoxically increased by 21% after 3 years. A similar heterogeneous pattern of [^{11}C]-HED retention was observed in three of five group B subjects with the greatest deficits (>20% of the LV) in LV sympathetic innervation.

Effects of Glycemic Control on the Progression of Cardiac Sympathetic Denervation

The effect of poor glycemic control (mean HbA1c, 9.2% ± 1.4%) on the progression of cardiac sympathetic denervation in a young diabetic subject from group B is shown in Fig 5. At the baseline study, abnormalities of [^{11}C]-HED retention affected only 23% of the LV and were restricted to the distal inferior and lateral myocardial segments. At the final visit, abnormalities extended proximally to involve approximately 52% of the LV with a paradoxical increase in HED retention observed in 40% of the proximal segments, which contrasted with decreased tracer retention distally. Corresponding assessments of cardiovascular reflex testing initially demonstrated a single abnormality of HRV to deep breathing alone at the baseline assessment, whereas at the final visit, abnormalities were also detected in the Valsalva ratio and systolic blood

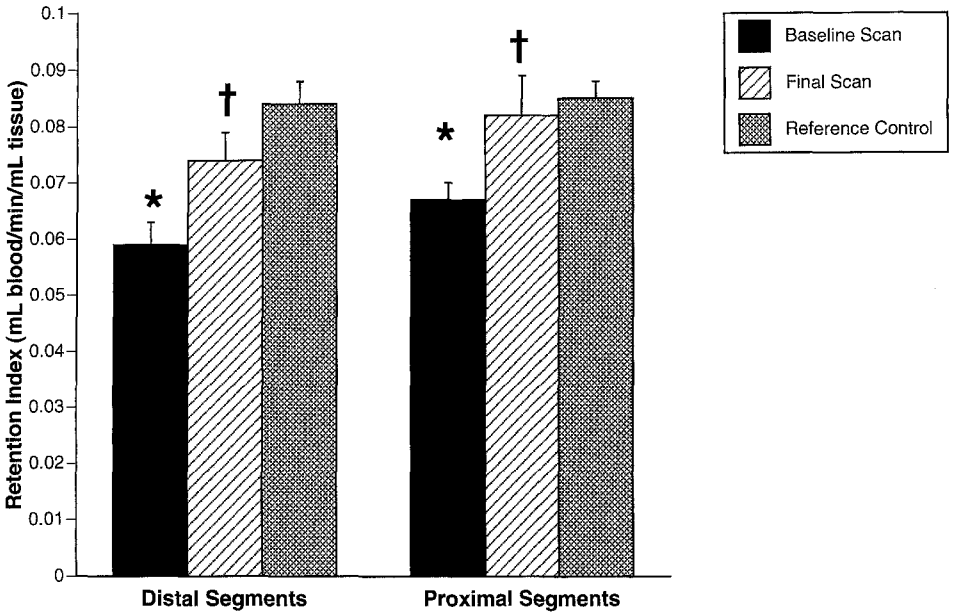

Fig 3. Effect of good glycemic control on regional LV retention of [^{11}C]-HED in group A compared with reference nondiabetic controls. Data are the mean ± 1 SEM. *$P < .05$ *v* reference control. †$P < .05$ *v* baseline.

pressure decrease, thus meeting the criteria for DAN. Clinically, the patient developed gastroparesis, nocturnal diarrhea, urinary retention, and postural hypotension. Thus, progression of cardiac sympathetic dysinnervation in this subject was characterized by increased heterogeneity of LV [^{11}C]-HED retention.

Reproducibility of HED-PET in Diabetic Subjects

To assess the reproducibility of LV HED-PET assessment, three diabetic subjects with small (<10%) LV [^{11}C]-HED retention deficits underwent repeat evaluations performed 7 to 8 days after the first scan. Mean LV retention index values were then generated for each subject's first and second studies, and the difference between the two values was calculated. The mean ± 1 SD of the differences for these three studies was 0.008 ± 0.008.

Changes in Reflex Cardiovascular Autonomic Function Testing

The changes in reflex autonomic function testing in diabetic subjects at baseline compared with the final visit are shown in Table 2. Overall, HRV was decreased[28,30] in both subject groups at the baseline assessment and was unchanged at the final visit. The Valsalva ratio remained unchanged in group A, but declined by 16% in group B, which did not achieve statistical significance ($P = .3$). However, at the final visit, three of five group B subjects had DAN by reflex testing, compared with two of five at the baseline visit.

DISCUSSION

Cardiovascular denervation is associated with increased cardiac risk, particularly in diabetic patients.[1-8] Good metabolic

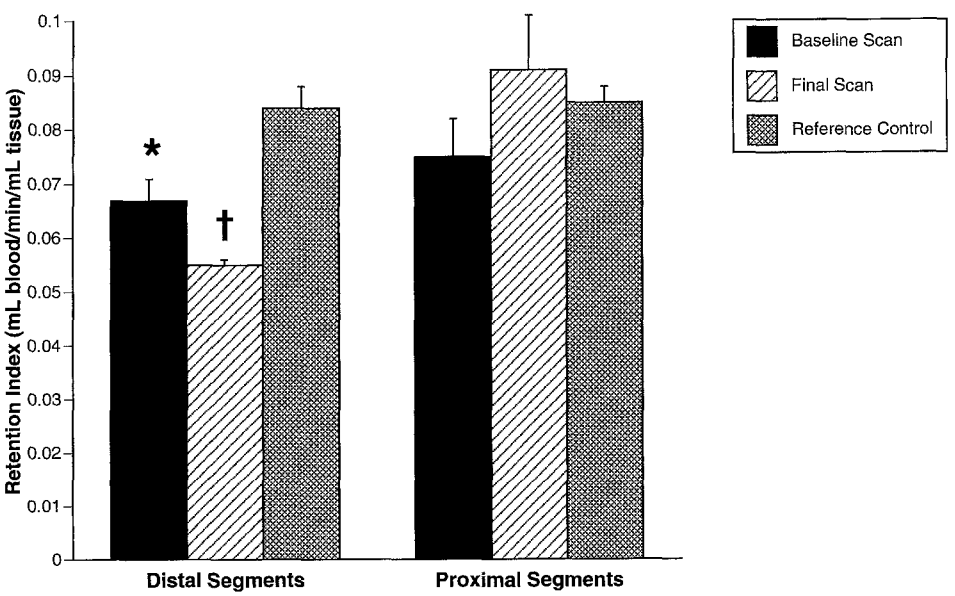

Fig 4. Effect of poor glycemic control on regional LV retention of [^{11}C]-HED in group B. Data are the mean ± 1 SEM. *$P < .05$ *v* reference control. †$P < .05$ *v* baseline study.

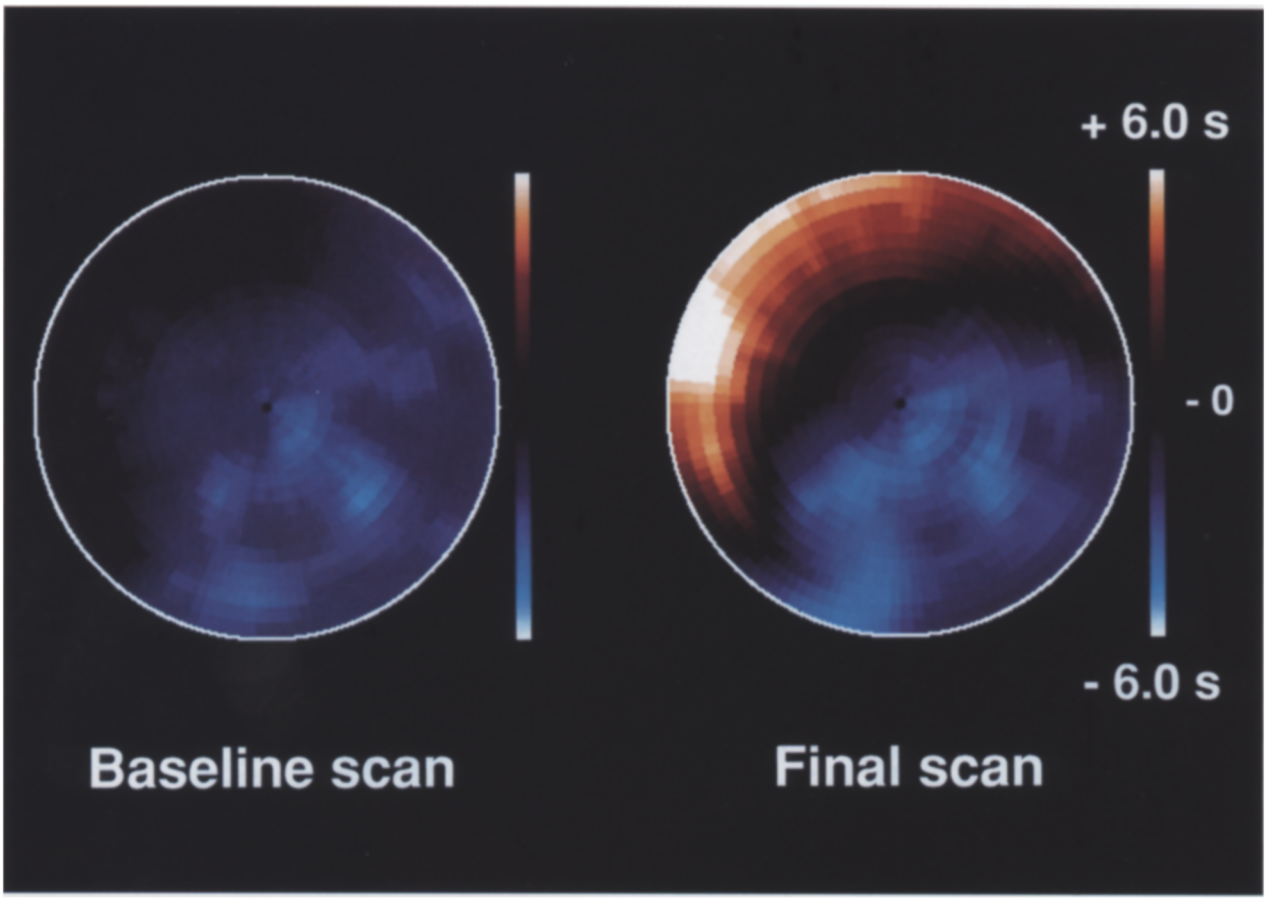

Fig 5. Rapid progression of cardiac sympathetic denervation in a subject with poor glycemic control. Changes in LV tracer activity are displayed as color-coded "polar maps." The LV myocardium is depicted with the apex at the center and the base peripherally, and within each LV region (divided into apical, anterior, septal, inferior, and lateral myocardial segments) the mean relative tracer activity values are determined. Regions of decreased [^{11}C]-HED retention are shown as blue/green and depict denervated myocardium. Regions of increased [^{11}C]-HED retention are shown as white/red and depict increased innervation. At baseline (left), only decreased [^{11}C]-HED retention was observed in the distal myocardial segments. However, 3 years later (right), the extent of the distal deficits was increased, and paradoxically increased [^{11}C]-HED retention was observed in the proximal myocardial segments.

control can slow the development or progression of DAN,[10-14] but it is uncertain whether established cardiovascular denervation can be reversed. The recent development of sympathetic neurotransmitter analogs permits direct quantitation of early regional deficits in cardiac sympathetic innervation.[9,18-31] This study reports the results of a follow-up study in which PET with [^{11}C]-HED was used to evaluate the effects of glycemic control on progression of previously reported[21] regional deficits of LV [^{11}C]-HED retention. In subjects with small [^{11}C]-HED deficits, the instigation of good metabolic control (mean HbA1c, 7.3% ± 0.7%) over 3 years was associated with a reduction in the extent of LV [^{11}C]-HED deficits and normalization of [^{11}C]-HED retention within both the distal and proximal myocardial segments. In contrast, subjects with continuing poor glycemic control (HbA1c, 9.7% ± 0.3%) demonstrated an increase in the extent and heterogeneity of LV [^{11}C]-HED retention defects, with a further decrease in the retention of tracer in the distal LV segments contrasting with an increase in tracer retention proximally. The findings are consistent with the hypothesis that scintigraphically detected deficits in distal LV [^{11}C]-HED retention reflect early LV sympathetic denervation that can regress or progress to a marked heterogeneous pattern in diabetic subjects achieving good or poor glycemic control, respectively. It is tempting to speculate that in diabetes, the development of LV sympathetic dysinnervation may contribute to enhanced cardiac risk.

Table 2. Neuropathy Details of the Diabetic Subjects

	Baseline Visit		Final Visit	
Parameter	Group A	Group B	Group A	Group B
HRV (beats/min)	6 ± 5	9 ± 7	7 ± 6	8 ± 11
Valsalva ratio	1.43 ± 0.4	1.52 ± 0.5	1.40 ± 0.4	1.28 ± 0.2
Systolic BP decrease (mm Hg)	19 ± 15	19 ± 25	18 ± 16	20 ± 17

NOTE. Data are the mean ± 1 SD.

The data presented in this study indicate that early deficits of cardiovascular sympathetic innervation are reversible by improved metabolic control. Many studies have confirmed the beneficial effects of improved glycemic control on the progression of peripheral somatic nerve deficits including neuropathic

symptoms,[40-43] nerve conduction velocity slowing,[10,12,13,15,17,44,45] and vibration perception thresholds.[10,14] However, the relationship of metabolic control to the progression of abnormalities of autonomic function has been less clear. The inconsistent effects of improved metabolic control on the progression of DAN could reflect the inability of some studies to normalize HbA1c, insufficient study duration, advanced severity of DAN at initiation of the study, or, potentially, insensitivity of the reflex cardiovascular autonomic function tests used. Inadequacy of attained glycemic control, in general, appears not to be the principal factor responsible for the reported resistance of the autonomic nervous system to improvement. In this study, for example, in contrast to the findings using HED-PET, improved glycemic control was without discernible effect on the reflex tests of autonomic function. This potentially may have reflected the small number of patients enrolled in this study. However, in other larger studies that achieved levels of metabolic control equivalent to those reported herein[15-17] or in which euglycemia was achieved after pancreas transplantation,[46-48] a similar lack of efficacy was found on cardiovascular reflex testing. Thus, indirect cardiovascular reflex testing may be less sensitive than quantitative scintigraphic imaging techniques such as HED-PET[21,26,27] or MIBG-SPECT[18-20,22-24] in detecting small changes in myocardial sympathetic nerve fiber density or function in response to therapeutic intervention.

The responsiveness of neuropathy to improved glycemic control may be critically dependent on its severity. In the DCCT, for example, the likelihood of abnormal nerve conduction was significantly reduced in patients with mild neuropathy on recruitment to the study (defined as "possible or definite neuropathy") treated with intensive compared with conventional therapy for 5 years.[13] Additionally, studies reporting beneficial effects of therapeutic interventions such as aldose reductase inhibitors on somatic nerve conduction slowing and nerve fiber regeneration were conducted in diabetic patients with milder degrees of nerve involvement.[49,50] The patients included in this report were selected on the basis of very early cardiac sympathetic nerve dysfunction. Indeed, in both groups of subjects, only two subjects had abnormalities of two or more reflex autonomic function tests and all had small deficits of LV HED retention. However, the possibility exists for the reversal of more advanced cardiac sympathetic dysinnervation by therapeutic approaches that either maintain near-euglycemia or block the detrimental effects of hyperglycemia. For example, studies in the transplanted human heart in nondiabetic subjects using [^{11}C]-HED have demonstrated sympathetic reinnervation of the proximal anterior wall,[29] and healthy human cardiac transplant recipients develop significant myocardial MIBG uptake that is associated with the release of myocardial norepinephrine.[51] Future studies using scintigraphic techniques are therefore required to assess the reversibility of more extensive cardiac denervation in diabetic subjects.

Although scintigraphic imaging techniques appear able to detect early deficits in sympathetic cardiovascular innervation, the etiology of these defects is unknown. HED (and MIBG) is taken up into the neuron by energy-dependent uptake,[25] and unlike norepinephrine, it is not metabolized within the cytosol by monoamine oxidase (MAO).[52,53] However, the retention of [^{11}C]-HED is dependent on both continuous recycling into and out of the neuron[25,54] and intact vesicular storage,[52] since its washout is greatly potentiated by both the amine uptake-1 inhibitor desipramine[25,53] and the potent inhibitor of vesicular uptake reserpine.[54] Therefore, the small metabolically correctable deficits of [^{11}C]-HED retention observed in the distal inferolateral wall of diabetic subjects in this study could reflect different types of neuronal dysfunction including impaired neurotransmitter uptake or defective vesicular storage and/or complete neuronal loss. These localized deficits appear to differ from the widespread abnormalities of myocardial MIBG uptake reported in metabolically compromised newly diagnosed IDDM subjects that are partially correctable by intensive insulin therapy,[24] which are more indicative of a hyperglycemia- or insulin deficiency–induced acute neuronal dysfunction. The clinical significance of the distal defects identified in the metabolically stable longer-duration diabetic patients presented in this study is strengthened by the observation that with continuing poor glycemic control, they can progress to a pattern of sympathetic dysinnervation, which we have previously observed in subjects with advanced DAN.[21,27] Indeed, the development of such advanced deficits in HED-PET is associated with the development of autonomic symptoms including postural hypotension.[21] Further characterization of these early deficits will be facilitated by the use of other neuronal tracers such as C-11 epinephrine, which is a substrate for MAO,[52] and [^{11}C]phenylephrine,[53] which is particularly suited to the evaluation of vesicular integrity.

Progression of cardiac sympathetic denervation in diabetic subjects with poor glycemic control resulted in increased heterogeneity of LV [^{11}C]-HED retention and sympathetic dysinnervation.[21,27] In the three diabetic subjects who demonstrated the greatest progression of sympathetic denervation, retention of [^{11}C]-HED was found to decrease in the distal myocardial segments, which contrasted with a paradoxical increase in [^{11}C]-HED retention proximally. This pattern of heterogeneous [^{11}C]-HED retention in severe DAN[27] appears to represent an advanced stage in the development of cardiac sympathetic denervation. While the progressive impairment in distal [^{11}C]-HED retention is consistent with neuronal dysfunction or loss, this process cannot be easily invoked to explain the finding in the proximal segments. Dense innervation of the heart base compared with the distal myocardium may contribute to its resistance to denervation and serve as the foundation for ventricular reinnervation, which in nondiabetic animals[55] and man[29,30] proceeds from the base of the heart to the apex. In nondiabetic animals, reinnervation of organs after partial denervation requires axonal sprouting and hyperinnervation within the islands of retained innervation[56,57] that extends into the denervated regions and then becomes downregulated as reinnervation is completed. Thus, the increase in proximal [^{11}C]-HED retention despite the progression of neuropathy may reflect a futile localized increase in sympathetic axonal regeneration and sprouting stimulated in response to the distal denervation that nevertheless fails to result in distal reinnervation. Alternatively, since [^{11}C]-HED is in competition with endogenous norepinephrine for neuronal uptake and storage, a reduction in sympathetic tone leading to decreased norepinephrine release may facilitate

[^{11}C]-HED retention by decreasing its washout. However, since little or no [^{11}C]-HED is washed out of the neuronal terminals during the imaging period used in this study,[52] this explanation does not readily account for our findings. More detailed tracer kinetic studies together with histological examination of the myocardium are required to address these possibilities.

In diabetes, regional myocardial imbalance of sympathetic innervation may contribute to the reported excess of cardiac deaths,[1-5,58-61] although direct evidence of a proarrhythmogenic effect of DAN is currently lacking. Diabetic patients have increased mortality post–myocardial infarction,[58-62] which may reflect the extent of coronary artery disease or increased susceptibility to other triggering factors[62-64] including autonomic imbalance.[1-5] Impaired retention of sympathetic neurotransmitter analogs consistent with neuronal dysfunction or loss has been reported in patients with malignant ventricular dysrhythmias in both the absence[65-67] and the presence[68] of ischemic heart disease. In diabetic subjects, impaired retention of MIBG in the LV has been shown to be predictive of sudden death.[69] Conversely, the presence of abnormally increased retention of [^{11}C]-HED in the proximal myocardial segments of advanced-DAN subjects consistent with the presence of regional myocardial sympathetic hyperinnervation may also predispose to ventricular fibrillation by decreasing the arrhythmogenic threshold.[64] Indeed, regional cardiac sympathetic hyperactivity increases the risk of arrhythmias during myocardial ischemia,[8,58] and selective cardiac sympathetic denervation or β-blockers[8,58-61,70] reduce the risk of cardiac arrhythmias and sudden death in these patients, implicating regional sympathetic imbalance as a factor promoting arrhythmogenesis. Therefore, in cardiovascular DAN, it is tempting to speculate that myocardial sympathetic dysinnervation may contribute to electrical, chemical, and vascular instability particularly if denervation hypersensitivity is also present.

In conclusion, the studies presented herein demonstrate that small deficits in distal LV sympathetic innervation can regress or progress in diabetic subjects achieving good or poor glycemic control, respectively. Progression of cardiac sympathetic neuropathy can result in a marked heterogeneity of LV sympathetic innervation with an apparent increase in proximal innervation contrasting with denervation distally. In diabetic subjects with early cardiovascular denervation, it is tempting to speculate that the institution of good glycemic control may prevent the development of such myocardial sympathetic dysinnervation and thereby reduce cardiac risk.

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