Effect of Diabetes Mellitus on Sympathetic Neuronal Regeneration Studied in the Model of Transplant Reinnervation

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The transplanted heart is initially denervated but undergoes subsequent sympathetic reinnervation. It thus provides a unique model for studying regeneration as a specific component of autonomic nerve biology. The aim of this study was to determine the effect of diabetes mellitus on the regenerational capacity of sympathetic neurons using molecule-targeted PET. **Methods:** Twenty-two nonrejecting, otherwise healthy cardiac transplant recipients underwent PET with the ^{11}C-labeled physiologic neurotransmitter epinephrine at 4.0 ± 3.3 y after surgery. Sympathetic reinnervation was defined as regional restoration of epinephrine retention to values within normal limits. **Results:** Reinnervation was observed in 8 of 12 patients with no evidence of diabetes and in 6 of 10 patients with a long-term history of diabetes mellitus. The regional extent of reinnervation (4.7% ± 5.3% of left ventricle vs. 19.1% ± 20.6% for nondiabetic recipients, $P = 0.04$) and the regeneration rate (0.8% ± 1.0% of left ventricle per year vs. 8.0% ± 10.1% for nondiabetic recipients, $P = 0.04$) were significantly reduced in diabetic subjects. In a multivariate model, diabetes mellitus was an independent determinant of allograft reinnervation. Finally, the reappearance of innervation was found to correlate with an improved chronotropic and inotropic response to stress in a standardized, symptom-limited exercise test including radionuclide angiography. **Conclusion:** The regenerational capacity of the sympathetic nervous system of the heart is reduced, but not abolished, by diabetes mellitus. This study on cardiac transplant recipients further supports a general link between impaired glucose handling and cardiac autonomic nerve function.

Key Words: sympathetic nervous system; autonomic neuropathy; diabetes mellitus; heart transplantation; PET

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The sympathetic nervous system of the heart plays a key role in regulating its performance by modulating rhythm, conduction, and contractility. Diabetes mellitus can cause autonomic neuropathy, which often involves the cardiac autonomic nervous system and is then associated with an adverse outcome, possibly through induction of electromechanic instability (*1,2*). A detailed understanding of the effects of diabetes mellitus on cardiac autonomic innervation is thus important for refining diagnostic algorithms and improving preventive strategies.

Nuclear imaging with radiolabeled catecholamine analogs allows for early detection of impaired cardiac sympathetic nerves. In diabetes mellitus, abnormalities on neuronal imaging seem to precede alterations on standard cardiovascular reflex tests (*3–5*), but the exact mechanisms by which diabetes mellitus contributes to these alterations of innervation are not yet clear. In addition to stimulated regional nerve sprouting resulting in hyperinnervation, other factors such as destruction of nerve terminals, malfunction, and reduced regenerational capacity may contribute to dysinnervation.

In the present study, we sought to specifically evaluate the effect of diabetes on growth and regeneration of cardiac sympathetic nerves. A unique model to provide insight into this component of sympathetic nerve biology is the transplanted heart, because it is completely denervated early after transplantation (*6*). Observations on nondiabetic transplant recipients have shown that sympathetic reinnervation occurs at later stages after surgery, remains regionally limited, but exerts functional and physiologic effects (*7,8*). We sought to investigate the relationship between nerve integrity and cardiac function by measuring the presence and extent of sympathetic reinnervation in diabetic transplant recipients and then comparing the findings to those in nondiabetic patients and determining the effects on cardiac performance.

MATERIALS AND METHODS

Patients and Study Design

Twenty-two symptom-free cardiac transplant recipients (3 women and 19 men; mean age ± SD, 59 ± 7 y) were studied at 4.0 ± 3.3 y after surgery. Before the patients were included in the study, the presence of acute rejection, significant transplant vasculopathy, diabetic vasculopathy, or allograft dysfunction

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was ruled out by clinical follow-up, echocardiography, coronary angiography, and endomyocardial biopsy. No patient received medication known to interfere with the presynaptic (antidepressants, clonidine, reserpine) or postsynaptic (β- or α-adrenergic blockers or agonists) sympathetic nervous system. All other cardioactive drugs were discontinued 24 h before inclusion. Immunosuppressive therapy was not interrupted.

The patients constituted 2 groups: Group 1 consisted of 12 otherwise healthy recipients with no history or evidence of diabetes mellitus, as documented by repeatedly normal fasting blood glucose levels at routine posttransplantation examinations. Group 2 consisted of 10 transplant recipients with a history of diabetes mellitus. In 8 of these patients, type 2 diabetes was diagnosed early after transplantation, whereas 2 patients had a history of type 1 diabetes with onset before transplantation. The median duration of diabetes was 5.8 y. At the time of the study, none of the patients had clinical evidence of diabetic complications or other concomitant diseases. The available characteristics of the patients are summarized in Table 1.

The presence and regional extent of sympathetic allograft reinnervation were quantified noninvasively using PET and ^{11}C-epinephrine. Additionally, using electrocardiographically gated equilibrium radionuclide angiography, we determined cardiac performance at rest and in response to standardized, symptom-limited exercise on the same day. Heart rate, blood pressure, and a 12-lead electrocardiogram were recorded continuously throughout all procedures. Before patients were included in the study, they signed informed consent forms approved by the ethical committee of the medical faculty of the Technischen Universität München.

PET

^{11}C-Epinephrine was synthesized as previously described (*9*). PET was performed using an ECAT EXACT scanner (CTI/Siemens). After adequate positioning, a transmission scan of 15 min was acquired for correction of photon attenuation. For measurement of perfusion, a 15-min static scan was obtained 5 min after injection of 250–300 MBq of ^{13}N-ammonia. After a break of 20 min to allow for radioactivity decay, total events in the field of view of the scanner were monitored to rule out significant residual activity. Then, 200–450 MBq of ^{11}C-epinephrine were injected, and a dynamic imaging sequence (14 frames: 6 × 30 s, 2 × 60 s, 2 × 150 s, 2 × 300 s, and 2 × 600 s) was acquired. To determine the contribution of ^{11}C-labeled metabolites to blood activity, we drew venous blood samples at 1, 5, 10, 20, and 40 min after injection.

Assessment of Left Ventricular Function and Hemodynamics

Autologous erythrocytes were labeled with 800–1,000 MBq of ^{99m}Tc by combined in vivo/in vitro technique and reinjected after purification. After 5 min to allow for equilibrium, patients were positioned semiupright on a bicycle table. Planar gated blood-pool images at rest (frame mode, 24 time bins, 3-min acquisition) were acquired in "best septal" left anterior oblique view using an open-gantry γ-camera (SkyLight; Philips). Then, symptom-limited bicycle exercise was started, using a standardized protocol with a 50-W initial workload. Imaging (parameters similar to rest) began after 1 min in each exercise stage to allow for hemodynamic stabilization and continued for the remaining 3 min of the stage. Every 4 min, the next stage was started and workload was increased by 50 W. Exercise was stopped when the patient reached exhaustion. Similar to the first stage of 50 W, imaging was performed at each subsequent stage (100 W, 150 W, etc.) after a stabilization phase of 1 min. During poststress recovery, 3 min after exercise cessation, a final image was acquired.

Data Analysis

PET. Attenuation-corrected transaxial PET images were reconstructed by filtered backprojection. Using volumetric sampling of static ^{13}N-ammonia perfusion images, we defined myocardial radioactivity in 460 left ventricular segments, depicted in a polar map (*10*). Polar maps were normalized to their maximum and used for qualitative assessment of regional perfusion. Myocardial segments were transferred to the dynamic imaging sequence for ^{11}C-epinephrine, and time–activity curves were obtained. The initial frame of the sequence was checked to rule out residual activity from the previous ^{13}N-ammonia injection. Arterial input function was derived from a small circular region of interest in the left ventricular cavity and was corrected for the presence of ^{11}C-labeled epinephrine metabolites, which were assayed from serial blood samples using Sep-Pak cartridges (Waters) as previously described (*11*). An epinephrine retention index, measured as percentage per minute, was calculated by normalizing myocardial activity at 40 min to the integral under the metabolite-corrected arterial blood time–activity curve. The global extent of reinnervation was quantified as the percentage of the polar map showing a retention index within 2.5 SDs of the average segmental values of a previously described normal database (*11*). Finally, a regeneration rate assuming initial complete denervation after transplantation surgery was calculated by normalizing the extent of reinnervation to the time between surgery and PET measurement

TABLE 1
Patient Characteristics

Characteristic	Overall (n = 22)	No diabetes (n = 12)	Diabetes (n = 10)
Age (y)	59 ± 7	58 ± 9	59 ± 5
Body weight (kg)	84 ± 13	78 ± 12	91 ± 11*
Body mass index (kg/m^2)	27.3 ± 3.9	25.6 ± 3.5	29.3 ± 3.6*
Age at transplantation (y)	54 ± 6	56 ± 7	53 ± 4
Age of donor (y)	38 ± 13	38 ± 12	38 ± 15
Time after surgery (y)	4.0 ± 3.4	2.7 ± 2.7	5.5 ± 3.5†
Exposure of heart to diabetes (mo)	—	—	49 ± 28

*$P < 0.05$ vs. no diabetes.
†$P = 0.05$ vs. no diabetes.

and was expressed as the percentage of left ventricular reinnervation per year.

Left Ventricular Function. Radionuclide angiography was analyzed according to international standards using commercial software (*12*). Based on semiautomatic definition of regions of interest for the left ventricle and background in end-systolic and end-diastolic frames, ventricular counting rates throughout the cardiac cycle were obtained and left ventricular ejection fraction was calculated.

Statistical Analysis

Values are expressed as mean ± SD. Results in groups were compared by the unpaired Student *t* test. The relationship between pairs of continuous variables was described by the Pearson correlation coefficient and was tested for significance by the Fisher *r*-to-*z* transformation. Stepwise regression analysis was performed to identify independent determinants of the neuronal growth rate. *P* values of less than 0.05 were defined as significant.

RESULTS

Restoration of Catecholamine Storage Capacity in the Heart

Myocardial viability was confirmed in all individuals by the absence of perfusion defects (defined as ^{13}N-ammonia uptake below 50% of maximum), ruling out a contribution of structural damage to the impairment of sympathetic innervation.

The results of ^{11}C-epinephrine PET are summarized in Table 2. Presynaptic myocardial catecholamine storage capacity was regionally restored to values within normal limits in 8 of 12 transplant recipients without diabetes mellitus, but also in 6 of 10 with diabetes mellitus. Reinnervation remained regionally limited and primarily occurred in the territory of the left anterior descending coronary artery, as indicated by the highest regional epinephrine retention indices. Plasma metabolite analysis did not reveal differences in systemic metabolic degradation of epinephrine between transplant recipient groups. Figure 1 shows examples of PET investigations in representative diabetic and nondiabetic individuals.

Despite the presence of reinnervation, both the actual extent and the rate of regeneration were significantly reduced in diabetic patients (Table 2). Additionally, regeneration rate significantly correlated inversely with patient age ($r = -0.58$; $P = 0.004$) and age at transplantation ($r = 0.49$; $P = 0.02$) but did not correlate with age of donor, body mass index, body weight, or systolic blood pressure. When these available variables were entered into a stepwise regression model, the presence of diabetes remained an independent determinant of reduced neuronal regeneration. The only other independent determinant in the model was higher patient age.

Relationship Between Sympathetic Neuronal Regeneration and Cardiac Performance

The results for hemodynamics and left ventricular function at rest, during exercise, and in the early recovery phase are summarized in Table 3. No significant difference was found between nondiabetic and diabetic transplant recipients, except for the observation that diabetic subjects had a mildly but significantly lower global ejection fraction, which remained within normal limits at all stages.

The maximal myocardial epinephrine retention index did not correlate with any hemodynamic parameter at rest or during recovery but correlated significantly with increases in heart rate and ejection fraction during exercise ($r = 0.63$ and $P = 0.001$ for heart rate; $r = 0.57$ and $P = 0.006$ for ejection fraction). If groups were analyzed separately (Fig. 2), the correlation between epinephrine retention and chronotropic response to exercise remained significant for nondiabetic recipients ($r = 0.66$; $P = 0.01$) but not for diabetic recipients ($r = 0.26$; $P = 0.5$), whereas the correlation of epinephrine retention index and inotropic response to exercise tended to remain in both groups ($r = 0.54$ and $P = 0.06$ for nondiabetic recipients; $r = 0.65$ and $P = 0.05$ for diabetic recipients).

DISCUSSION

Using reinnervation of cardiac allografts as a model, our study showed that diabetes mellitus influences sympathetic neuronal recovery. The regenerational capacity of myocardial innervation is inhibited but not abolished. Although occurring at a slower rate, restoration of sympathetic neurons remains physiologically relevant, as indicated by

TABLE 2
Left Ventricular Catecholamine Storage Capacity

Parameter	Overall (*n* = 22)	No diabetes (*n* = 12)	Diabetes (*n* = 10)
Extent of restored innervation (% of left ventricle)	12.6 ± 17.0	19.1 ± 20.6	4.7 ± 5.3*
Restoration rate (% of left ventricle/y)	4.7 ± 8.2	8.0 ± 10.1	0.8 ± 1.0*
Global ^{11}C-epinephrine retention index (%/min)	6.8 ± 2.6	7.6 ± 3.3	5.8 ± 1.0
^{11}C-Epinephrine retention index, LAD territory (%/min)	8.5 ± 4.2	9.7 ± 5.2	7.0 ± 1.9
^{11}C-Epinephrine retention index, LCX territory (%/min)	5.6 ± 1.9	6.2 ± 2.4	4.9 ± 0.6
^{11}C-Epinephrine retention index, RCA territory (%/min)	5.4 ± 1.5	5.8 ± 1.9	5.0 ± 0.7
Maximal ^{11}C-epinephrine retention index (%/min)	13.7 ± 6.7	15.4 ± 8.2	11.7 ± 3.7
Totally denervated patients	8 (36%)	4 (33%)	4 (40%)

*$P = 0.04$ vs. no diabetes.
LAD = left anterior descending coronary artery; LCX = left circumflex coronary artery; RCA = right coronary artery.

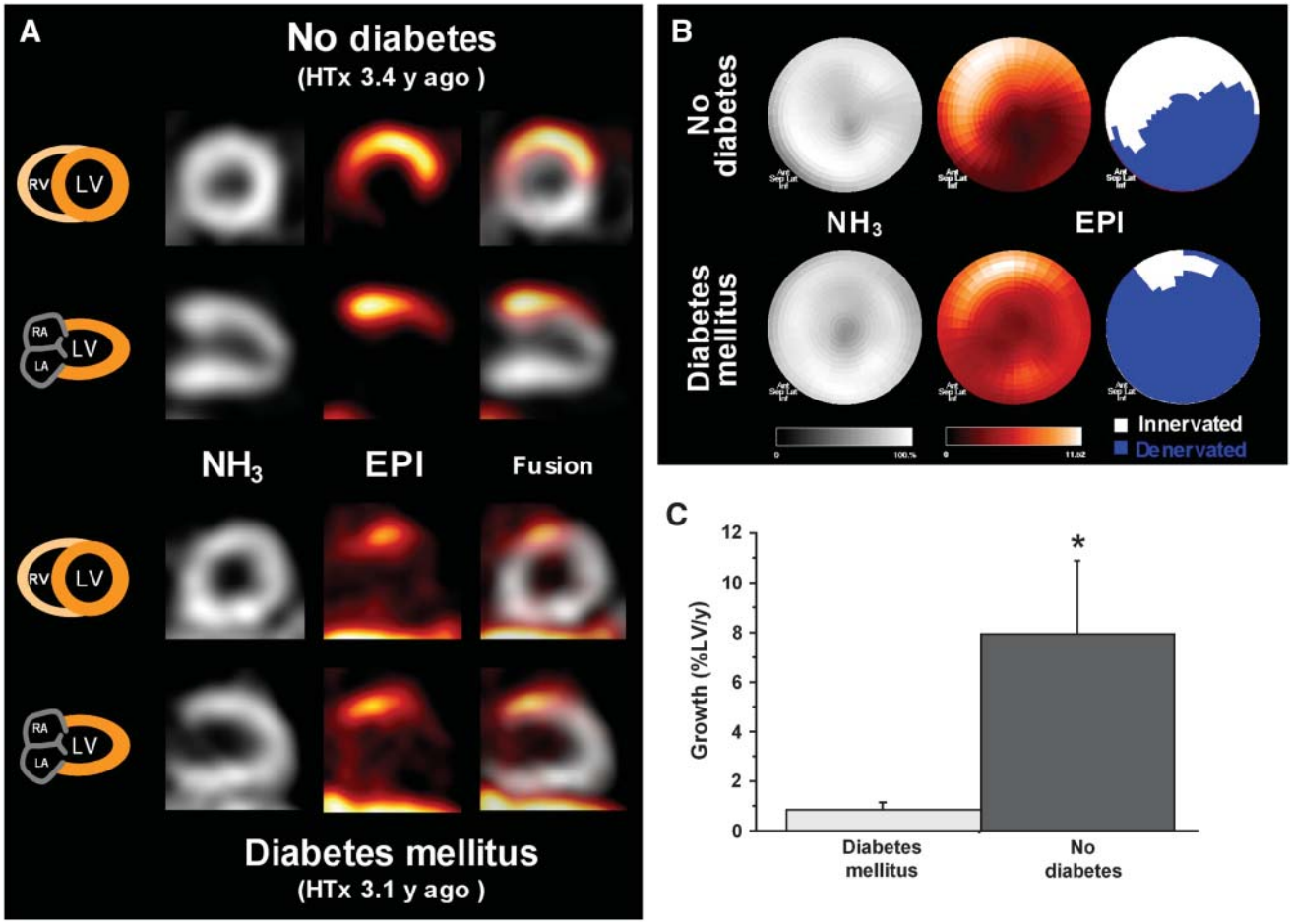

FIGURE 1. (A and B) Assessment of neuronal regeneration by PET. Shown are representative left ventricular short- and long-axis tomographic images (A) and polar maps (2-dimensional display of 3-dimensional tracer distribution throughout myocardium, with apex displayed at center, base at periphery, septum on left, lateral wall on right, anterior wall on top, and inferior wall on bottom) (B) of cardiac transplant recipient without evidence of diabetes mellitus (top) and another recipient with history of diabetes (bottom). Gray-scale images show homogeneous myocardial perfusion, determined by ^{13}N-ammonia. Color-scale images show regional uptake of ^{11}C-epinephrine, indicating reinnervation in basal anterior wall. Extent of reinnervation was 42% in nondiabetic recipient and 13% in diabetic recipient. (C) Group results (mean ± SE) for neuronal regeneration rate. EPI = ^{11}C-epinephrine; HTx = heart transplantation; LA = left atrium; LV = left ventricle; RA = right atrium; RV = right ventricle. *$P < 0.05$.

improved contractile response to exercise in reinnervated transplant recipients despite the presence of diabetes.

After initial complete denervation, reinnervation of the transplanted heart occurs in a regionally heterogeneous pattern, with evidence of restoration occurring first in the basal anterior wall and extending later toward the apex, septum, and lateral wall, whereas the inferior wall remains denervated. Regrowth of nerve fibers along arterial structures is the most likely explanation for this pattern of reappearance (*8,13*). Our results showed inhibited nerve regeneration in diabetic individuals in the setting of transplant reinnervation.

We used ^{11}C-labeled epinephrine as a tracer for molecule-targeted PET neuronal imaging. Epinephrine is a truly physiologic neurotransmitter that is thought to reflect not only presynaptic catecholamine uptake but also vesicular storage capacity. This belief is supported by studies on isolated perfused rat hearts in which the addition of the norepinephrine uptake blocker desipramine to the medium after ^{11}C-epinephrine delivery did not reduce its myocardial retention, supporting the probability that vesicular storage protects intraneuronal tracer from metabolic degradation (*14*). This is in contrast to other, previously used, tracers such as ^{11}C-hydroxyephedrine, which is a catecholamine analog that predominantly reflects uptake but not vesicular storage in nerve terminals (hydroxyephedrine is washed out from isolated perfused rat hearts when desipramine is added after tracer delivery (*15*)), and ^{123}I-metaiodobenzylguanidine, which is an analog used for conventional scintigraphic imaging at lower resolution and without absolute quantification and whose in vivo biokinetics are not defined in comparable detail. Although epinephrine can be relied on as a physiologic biomolecule, differences in the biologic behavior of analogs may explain the results of previous imaging studies. The previously observed pattern of reduced uptake of ^{123}I-metaiodobenzylguanidine in the

TABLE 3
Hemodynamics and Exercise Performance

Parameter	Overall (n = 22)	No diabetes (n = 12)	Diabetes (n = 10)
Rest			
Heart rate (min^{-1})	91 ± 11	91 ± 13	91 ± 9
Systolic blood pressure (mm Hg)	142 ± 19	138 ± 18	148 ± 19
Diastolic blood pressure (mm Hg)	93 ± 14	95 ± 15	90 ± 12
Rate–pressure product (mm Hg/min)	12,991 ± 2,551	12,530 ± 2,521	13,544 ± 2,627
Left ventricular ejection fraction (%)	65 ± 10	70 ± 7	58 ± 10*
Exercise			
Maximal workload (W)	114 ± 28	121 ± 33	106 ± 17
Exercise duration (min)	7.0 ± 2.2	7.2 ± 2.3	6.7 ± 2.2
Peak heart rate (min^{-1})	129 ± 19	135 ± 23	121 ± 10
Heart rate increase (min^{-1})	38 ± 20	44 ± 22	31 ± 12
Peak systolic blood pressure (mm Hg)	194 ± 26	190 ± 29	198 ± 23
Peak diastolic blood pressure (mm Hg)	97 ± 14	98 ± 16	95 ± 12
Peak rate–pressure product (mm Hg/min)	25,267 ± 6,124	24,137 ± 3,745	26,115 ± 7,495
Peak left ventricular ejection fraction (%)	70 ± 12	78 ± 7	61 ± 12*
Increase of ejection fraction (%)	5 ± 7	7 ± 6	2 ± 7
Recovery			
Heart rate (min^{-1})	112 ± 15	114 ± 15	108 ± 15
Systolic blood pressure (mm Hg)	163 ± 27	162 ± 28	165 ± 27
Diastolic blood pressure (mm Hg)	91 ± 9	93 ± 10	88 ± 8
Rate–pressure product (mm Hg/min)	18,409 ± 4,484	18,697 ± 4,670	18,024 ± 4,472
Left ventricular ejection fraction (%)	72 ± 11	77 ± 6	63 ± 10*

*P < 0.01 vs. no diabetes.

inferior wall of diabetic subjects, for example, is a nonspecific finding that has also been reported in arrhythmia, hypertension, cardiomyopathies, and other diseases (*16*).

We investigated a model of cardiac allograft reinnervation to study the interfering effects of diabetes. Transplant reinnervation is a clean model for studying sympathetic neuronal regenerative capacity. Complete denervation early after transplantation is the starting point for all patients, and a close follow-up along with exclusion of other diseases yields comparable conditions for all subjects. Also, the model focuses on neuronal regeneration itself, whereas the results of previous studies of innervation in nontransplantation subjects may have reflected a mixture of degeneration, regeneration, dysfunction, and physiologic heterogeneity of nerve terminals.

But on the other hand, diabetic transplant recipients are a very specific patient group, and the results cannot be generalized to the global population of diabetic patients without caution. The specificity of the patient group was a substantial challenge for patient recruitment and explains the small number of subjects included in the study, which results in some potential limitations. One is the borderline level of significance, attributable to the small sample sizes, for the correlation analysis between innervation and exercise parameters. Nevertheless, r values are within the range reported previously for a larger group of nondiabetic transplant recipients (*7*). Another is the heterogeneity of the diabetic group, which was composed of some type 1 and some type 2 diabetics, with some of the latter having posttransplantation diabetes linked with steroids and insulin

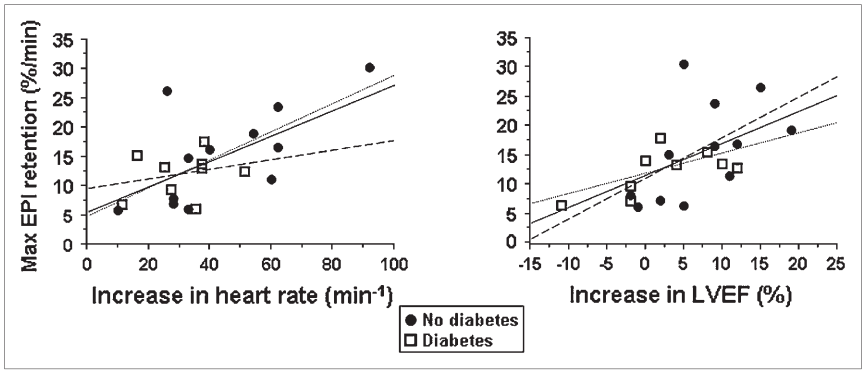

FIGURE 2. Functional importance of neuronal regeneration. Regression plots show relationship between left ventricular maximal ^{11}C-epinephrine retention index and inotropic (increase of heart rate, left) and chronotropic (increase of left ventricular ejection fraction, right) response to exercise. Regression lines are shown for subgroups of diabetic (dashed line) and nondiabetic (dotted line) transplant recipients and for all individuals (solid line). LVEF = left ventricular ejection fraction; max EPI = maximal ^{11}C-epinephrine.

resistance. Specific subsets of these diabetic transplant recipients may show different neuronal regeneration capacities, but the limited patient availability did not allow for a more homogeneous group. Finally, some potentially valuable clinical information about our patients was not retrievable. Information on aortic cross-clamping time during transplantation surgery and complete documentation of rejection episodes were not available. Both are known determinants of reinnervation (*13*) and are possible confounding variables that could not be included in multivariate analysis. Also, because of the difficult recruitment, our diabetic group was studied later after transplantation than was the nondiabetic group. The extent of reinnervation increases with time after transplantation, and differences may thus influence results, but diabetic individuals in our study showed less reinnervation despite being studied later after surgery. Additionally, we calculated an annual regeneration index to normalize for time after transplantation. We did not study patients longitudinally, but the assumption of complete denervation directly after transplantation surgery is justified by previous studies and allows for estimation of regeneration rates without subjecting patients to a second exposure to radioactive tracers.

The exact molecular mechanisms by which diabetes impairs neuronal regrowth to the myocardium cannot be elucidated from this clinical study. Myocardial ischemia, allograft rejection, and diabetic angiopathy were ruled out by functional testing and endomyocardial biopsy during close follow-up after transplantation. Additionally, the finding of normal myocardial perfusion on PET excludes structural damage. Speculatively, nerve growth factors, which are secreted by target organs and are important for maintenance and recovery of neuronal structures, may serve as a potential explanation. Experimental studies have indicated that these proteins are reduced in the diabetic heart, likely because of the cardiomyopathic effects of altered metabolic conditions (*17*). In line with this speculation, the mild reduction of global ventricular function in our diabetic subjects may be interpreted as another indicator of diabetic cardiomyopathic effects. We did not measure glycosylated hemoglobin, because reinnervation is a process taking longer than the time covered by this marker of midterm glycemic control. A correlation with long-term parameters of glycemic control, however, might be of interest and a subject for future investigations.

The results of our study have potential clinical implications. First, regarding the interrelationship between diabetes and autonomic nerve biology, the results provide general mechanistic insights that may be transferred to other neuropathologic settings. Second, the results further support the usefulness of imaging in identifying the involvement of cardiac autonomic innervation in disease. Previous studies have already suggested that nuclear imaging allows for identification of cardiac autonomic nerve alterations at earlier stages than does conventional autonomic reflex testing (*3–5*). Although further studies will be required to establish a clear clinical role, adrenergic neuronal imaging may thus have the potential to be a useful tool for identifying patients who are at risk and for guiding therapy in the future. For the transplant recipient, we expect benefit from reinnervation through improved exercise capacity (*7*). The present data suggest this expectation also for diabetic transplant recipients. It is of note that heterogeneous innervation in the posttransplantation setting is not associated with arrhythmia, because arrhythmia was observed in none of our patients. This finding is not in line with other studies suggesting a link between autonomic innervation heterogeneity and arrhythmogenicity in nontransplantation patient groups (*18*), although transferring results from one setting to the other may be difficult.

CONCLUSION

Our study identified an impairment of the regenerational capacity of sympathetic nerves of the heart in diabetes mellitus. This finding confirms the notion of a link between impaired glucose handling and autonomic nerve function that may adversely influence the clinical course of diabetic patients through functional and electrophysiologic instability. Despite this adverse effect, however, restoration of fully functional nerve terminals, with beneficial physiologic effects, was still observed to some degree, suggesting that preventive or therapeutic strategies aimed at improving autonomic balance may benefit the management of diabetes mellitus.

REFERENCES

1. Valensi P, Sachs RN, Harfouche B, et al. Predictive value of cardiac autonomic neuropathy in diabetic patients with or without silent myocardial ischemia. *Diabetes Care.* 2001;24:339–343.
2. Lee KH, Jang HJ, Kim YH, et al. Prognostic value of cardiac autonomic neuropathy independent and incremental to perfusion defects in patients with diabetes and suspected coronary artery disease. *Am J Cardiol.* 2003;92:1458–1461.
3. Hattori N, Tamaki N, Hayashi T, et al. Regional abnormality of iodine-123-MIBG in diabetic hearts. *J Nucl Med.* 1996;37:1985–1990.
4. Turpeinen AK, Vanninen E, Kuikka JT, et al. Demonstration of regional sympathetic denervation of the heart in diabetes: comparison between patients with NIDDM and IDDM. *Diabetes Care.* 1996;19:1083–1090.
5. Stevens MJ, Raffel DM, Allman KC, et al. Cardiac sympathetic dysinnervation in diabetes: implications for enhanced cardiovascular risk. *Circulation.* 1998; 98:961–968.
6. Schwaiger M, Kalff V, Rosenspire K, et al. Noninvasive evaluation of sympathetic nervous system in human heart by positron emission tomography. *Circulation.* 1990;82:457–464.
7. Bengel FM, Ueberfuhr P, Schiepel N, et al. Effect of sympathetic reinnervation on cardiac performance after heart transplantation. *N Engl J Med.* 2001; 345:731–738.
8. Bengel FM, Ueberfuhr P, Ziegler SI, et al. Serial assessment of sympathetic reinnervation after orthotopic heart transplantation: a longitudinal study using positron emission tomography and C-11 hydroxyephedrine. *Circulation.* 1999; 99:1866–1871.
9. Chakraborty PK, Gildersleeve DL, Jewett DM, et al. High yield synthesis of high specific activity R-(−)-[^{11}C]epinephrine for routine PET studies in humans. *Nucl Med Biol.* 1993;20:939–944.
10. Nekolla SG, Miethaner C, Nguyen N, et al. Reproducibility of polar map generation and assessment of defect severity and extent assessment in myocardial perfusion imaging using positron emission tomography. *Eur J Nucl Med.* 1998;25:1313–1321.

11. Munch G, Nguyen NT, Nekolla S, et al. Evaluation of sympathetic nerve terminals with [^{11}C]epinephrine and [^{11}C]hydroxyephedrine and positron emission tomography. *Circulation.* 2000;101:516–523.
12. Imaging guidelines for nuclear cardiology procedures. American Society of Nuclear Cardiology. Equilibrium gated blood pool imaging protocols. *J Nucl Cardiol.* 1996;3:G26–G29.
13. Bengel FM, Ueberfuhr P, Hesse T, et al. Clinical determinants of ventricular sympathetic reinnervation after orthotopic heart transplantation. *Circulation.* 2002;106:831–835.
14. Nguyen NT, DeGrado TR, Chakraborty P, et al. Myocardial kinetics of carbon-11-epinephrine in the isolated working rat heart. *J Nucl Med.* 1997;38: 780–785.
15. DeGrado TR, Hutchins GD, Toorongian SA, et al. Myocardial kinetics of carbon-11-meta-hydroxyephedrine: retention mechanisms and effects of norepinephrine. *J Nucl Med.* 1993;34:1287–1293.
16. Flotats A, Carrio I. Cardiac neurotransmission SPECT imaging. *J Nucl Cardiol.* 2004;11:587–602.
17. Schmid H, Forman LA, Cao X, et al. Heterogeneous cardiac sympathetic denervation and decreased myocardial nerve growth factor in streptozotocin-induced diabetic rats: implications for cardiac sympathetic dysinnervation complicating diabetes. *Diabetes.* 1999;48:603–608.
18. Kies P, Wichter T, Schafers M, et al. Abnormal myocardial presynaptic norepinephrine recycling in patients with Brugada syndrome. *Circulation.* 2004; 110:3017–3022.