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Diabetes and Dyslipidemia
A New Model for Transplant Coronary Artery Disease

Khanh Hoang, MD; Y.-D. Ida Chen, PhD; Gerald Reaven, MD; Lunan Zhang, MD; Heather Ross, MD; Margaret Billingham, MD; Hannah Valantine, MD

Background—Clinical observations suggest that transplant coronary artery disease (TxCAD) is immunologically mediated but may be accelerated by metabolic derangements. We developed a rat model of heterotopic heart transplantation in the presence of diabetes and dyslipidemia to further study their role in TxCAD development.

Methods and Results—Major histocompatibility complex–mismatched strains of inbred rats underwent heterotopic heart transplantation (ACI-to-Lewis allografts). Diabetes (DM) was induced by streptozotocin injection (80 mg/kg) after transplantation; dyslipidemia was worsened by feeding of a 60% high-fructose diet (+F). Allograft transplants were divided into four groups: (1) +DM/+F; (2) +DM/−F; (3) −DM/+F; and (4) −DM/−F. Isograft transplants (Lewis to Lewis, +DM/±F) were controls. All animals received daily cyclosporine (5 mg/kg). Grafts surviving >30 days were evaluated for TxCAD on histological sections and graded 0 to 5 for intimal thickness. All streptozotocin-treated animals were diabetic within 2 weeks, with fourfold increases in plasma glucose concentrations versus nondiabetics. Severe TxCAD was observed in diabetic allografts only. The mean grade of TxCAD in diabetic allografts was 3.2±0.5 versus 1.1±0.4 in diabetic isografts (P<0.03) and zero TxCAD in nondiabetic allografts (P≤0.0001). Fructose feeding resulted in a 1.5-fold higher triglyceride and a 1.3-fold higher cholesterol level versus the regular diet (−F) but showed no independent contribution to the development of TxCAD.

Conclusions—These findings suggest that metabolic derangements associated with diabetes play an important role in TxCAD development in heterotopic ACI-to-Lewis rat heart transplantation. In this model of TxCAD in major histocompatibility complex–mismatched, diabetic, and dyslipidemic rats, immunologic and metabolic mechanisms that contribute to TxCAD can be further delineated and approaches to its prevention assessed. (Circulation. 1998;97:2160-2168.)

Key Words: transplantation ■ atherosclerosis ■ lipoproteins ■ diabetes mellitus ■ animal model

Transplant coronary artery disease (TxCAD) is the major limitation to long-term survival in cardiac transplant patients. At least 50% of patients have angiographic evidence of TxCAD by 5 years after transplantation, and this condition is associated with a mortality rate >40% during the 2 years after the diagnosis has been made. Despite detailed characterization of the graft morphology by pathological examination, coronary angiography, and intracoronary ultrasound imaging, the pathophysiological mechanisms leading to TxCAD remain unclear. It has been proposed that TxCAD is a chronic alloimmune response on the basis of the diffuse morphology of the disease affecting the entire coronary artery tree and its predilection for the allograft while sparing the native vessels of the recipient. However, few clinical studies have confirmed acute rejection severity or duration as an independent predictor of TxCAD.

We have observed in posttransplant patients that hypertriglyceridemia is an independent predictor of severity in early- and late-phase TxCAD. This finding is consistent with numerous other observations that suggest a correlation between TxCAD and both hyperglycemia and hypertriglyceridemia. More recently, we have observed that glucose intolerance and its characteristic lipoprotein abnormalities, severely elevated plasma triglyceride and VLDL cholesterol levels and low levels of HDL cholesterol, are strongly correlated with the development of TxCAD. This result was intriguing in view of the observations made in nontransplant patients, in whom the clustering of diabetes, dyslipidemia, and hypertension is associated with an exponential increase in the risk for atherosclerosis. A detailed assessment of the metabolic abnormalities in our heart transplant patients revealed that >50% of patients had glucose intolerance, hyperinsulinemia, and dyslipidemia. Thus, we hypothesized that after an initial vascular injury to the heart, either by ischemia at the time of transplantation or as a consequence of activation of the alloimmune response, hyperglycemia and dyslipidemia are important contributors to the accelerated...
tempo of atherosclerosis in the transplanted heart. We propose that consistent with the “injury-response” model of nontransplant atherosclerosis initially proposed by Ross and Agius,11 the metabolic abnormalities of diabetes augment the inflammatory response to injury, leading to the accelerated course of TxCAD.

To test our hypothesis, we have developed a clinically relevant model of heart transplantation that recapitulates the hyperglycemia and dyslipidemia observed in patients after heart transplantation. The background genetics for a number of animal models had been sufficiently characterized to allow for transplantation across major histocompatibility complex (MHC) barriers, a situation similar to that in patients. Existing animal models include rabbit and rat allografts in which TxCAD has been reported in long-term survivors. The disadvantage of the rabbit model12 is that the primary metabolic defect is hypercholesterolemia, specifically LDL cholesterol, a pattern of dyslipidemia that does not appear to be an independent predictor of TxCAD in heart transplant patients.7 Of the two rat heart transplant models previously reported for study of TxCAD, the Lewis–Brown Norway donor heart transplanted into the F344 recipient13 involves transplantation across minor histocompatibility barriers and does not require administration of immunosuppressants, such as cyclosporine (Cs), to prevent acute graft rejection. Although this model of minor histocompatibility mismatch is a reasonable one in which to begin to study TxCAD, a model that involves transplantation across MHC barriers and requires immunosuppressive therapy may be more relevant to human heart transplantation. The second rat model, the ACI rats served as donors and Lewis rats as recipients, and for isografts, Lewis rats served as both donors and recipients. Recipient animals were treated with a single intraperitoneal injection of penicillin G (150 000 U) at the time of abdominal surgery. CsA animals were treated with a single intraperitoneal injection of penicillin G (150 000 U) at the time of abdominal surgery. CsA (Sandoz, Sandimmune oil suspension, 100 mg/dL, diluted with distilled water) was administered daily by gavage, beginning with 10 mg · kg⁻¹ · d⁻¹ on day 14. All recipient animals were weighed weekly, and graft function was assessed by daily palpation. Grafts in which beating was not palpable for 2 consecutive days were deemed to have failed, and these animals were killed within 12 hours. On rare occasions, the time to termination was protracted for up to 36 hours.

Control animals included nontransplanted animals, nondiabetic allografts, and diabetic isografts, in which hearts were transplanted to recipients of identical genetic strain (Lewis to Lewis). Immunosuppression was standardized across all groups by treatment with CsA to prevent acute rejection, which normally occurs within 7 days after transplantation of the allograft. In this report, we present the preliminary results of this new model in which TxCAD develops with an accelerated course in the presence of diabetes and its associated hyperlipidemic state.

**Methods**

**Animals and Transplantation Techniques**

Adult male Lewis and ACI rats weighing 300 to 400 g (Simonsen Laboratories, Gilroy, Calif) were housed individually under conventional conditions and fed a standard Purina rodent chow diet for a minimum of 2 weeks before transplant surgery. After surgery, the rats were either maintained on the standard diet or started on a modified, high-fructose-content diet (60% fructose diet, Teklad Laboratories).

Heterotopic abdominal cardiac allografts were performed by standard microsurgical techniques.17 For the allograft transplants, ACI rats served as donors and Lewis rats as recipients, and for isografts, Lewis rats served as both donors and recipients. Recipient animals were treated with a single intraperitoneal injection of penicillin G (150 000 U) at the time of abdominal surgery. CsA (Sandoz, Sandimmune oil suspension, 100 mg/dL, diluted with distilled water) was administered daily by gavage, beginning with 10 mg · kg⁻¹ · d⁻¹ on the day of transplantation and reduced to 5 mg · kg⁻¹ · d⁻¹ on day 14. All recipient animals were weighed weekly, and graft function was assessed by daily palpation. Grafts in which beating was not palpable for 2 consecutive days were deemed to have failed, and these animals were killed within 12 hours. On rare occasions, the time to termination was protracted for up to 36 hours.

**Metabolic Derangements and Animal Study Groups**

Diabetes was induced in a subset of animals with allografts and isografts by penile vein injection of streptozotocin. The optimal dose of streptozotocin was determined in a prior set of experiments to be 80 mg/kg, given in two divided doses of 40 mg/kg each, on days 3 and 10 postoperatively. This protocol allows for recovery of the animal from the trauma of surgery before rendering it severely diabetic. A subset of diabetic animals also were fed diets high in fructose beginning on the first postoperative day. Animals that died within the initial 10 days after transplant of surgery-related causes, wound infection, or early graft failure (presumed to be due, in part, to surgery-related ischemia) were excluded from the analysis. Table 1 summarizes the control and treatment groups used in this study.

<table>
<thead>
<tr>
<th>Table 1. Animal Study Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of Transplant</td>
</tr>
<tr>
<td>----------------------</td>
</tr>
<tr>
<td>Nontransplant (Lewis)</td>
</tr>
<tr>
<td>Allograft (ACI-Lewis)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Isograft (Lewis-Lewis)</td>
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</table>

*Diabetes was induced by streptozotocin.

Tx indicates transplant; D, diabetes; F, fructose feeding; and CsA, cyclosporin A.
Several control groups were established to note any independent effects of surgery (ie, transplantation), CsA treatment, and allografting, separate from the effects of diabetes. Control groups included the following: (1) nontransplanted Lewis rats without CsA treatment, no diabetes, and no fructose feeding (non-Tx); (2) allograft-transplanted ACI-to-Lewis rats with CsA treatment, no diabetes, and no fructose feeding (allo −D/−F); and (3) isograft-transplanted Lewis rats with diabetes, with or without fructose feeding (iso +D+/F and iso +D+/−F). The remaining treatment groups are shown in Table 1.

**Plasma Glucose and Lipoprotein Measurements**

Blood samples (0.5 to 1.0 mL) were obtained from each animal at baseline and at 2-week intervals for measurement of plasma concentrations of glucose, insulin, triglycerides, and total cholesterol by previously described methods. In addition, the lipid subfractions LDL, HDL, and VLDL were also measured. All animals were fasted for 6 to 7 hours before blood sampling. Diabetes was defined by two measurements of glucose >200 mg/dL. Animals treated with streptozotocin met the criteria for diabetes within 2 weeks of receiving the full dose of the drug (80 mg/kg).

**Processing of Cardiac Tissues and Assessment of TxCAD and Rejection**

Cardiac grafts surviving ≥30 days of the targeted 60-day end point were excised for histological and immunohistochemical analysis. Each excised specimen was immediately cut in cross section (along the short axis of the heart) to form two equivalent halves. The basal half of each cardiac specimen was fixed in 10% formalin and embedded in paraffin. For histological examination, four complete sagittal sections, each 5.0 mm thick, were prepared on glass slides. One of the sections was processed with hematoxylin and eosin for qualitative analysis of mononuclear cell infiltrate and myocyte morphology related to rejection. The remaining sections from adjacent sites were processed with elastin–van Gieson’s stain for detailed assessment of vessels within each section. All vessels in each section that contained an identifiable elastin layer were evaluated for TxCAD. The total numbers of vessels evaluated for each specimen were roughly equivalent (30 to 40 vessels). Each section was graded for the severity of TxCAD on a scale previously published by Adams et al. Tissue inflammation and myocyte damage were assessed qualitatively and graded for the presence of mononuclear cell infiltrate and myocyte damage by the Billingham criteria for histological rejection: grade 0 = normal; grade 1 = mild mononuclear cell infiltrate and no myocyte necrosis; grade 2 = moderate mononuclear cell infiltrate and myocyte necrosis; grade 3 = severe mononuclear cell infiltrate, myocyte necrosis, and interstitial edema; and grade 4 = very severe mononuclear cell infiltrate, myocyte necrosis, interstitial edema, and hemorrhage.

**Statistical Analysis**

The primary end point evaluated for all animals was the presence of TxCAD at the time the rats were killed (60 days after transplantation or at graft failure). Secondary end points were average plasma concentrations of glucose, triglyceride, insulin, total cholesterol, LDL, VLDL, and HDL. To compare the metabolic parameters between groups over the entire study period, mean values for each group were calculated and expressed as mean±SEM. Differences between each study group’s mean values were compared by ANOVA. Differences in the frequency of TxCAD in the experimental groups were compared by χ² analysis. An unpaired t test was used to compare the mean percentages of vessels with TxCAD between groups. Correlation of TxCAD with degree of tissue inflammation and myocyte damage (ie, histological rejection grade) was analyzed by simple regression analysis. In all analyses, a value of P≤0.05 was considered statistically significant.

**Results**

**Graft Ischemic Time**

Graft ischemic time, defined as the interval between cross-clamping of the donor aorta before excision of the heart and the completion of anastomosis of the allograft to the recipient inferior vena cava, was 30±10 minutes. This time did not differ significantly between treatment groups (data not shown).

**Body Weight**

Figure 1 plots the time course of body weight in the experimental groups. Weight gain was less in all transplant groups than in nontransplant controls. After an initial period of weight loss (50 to 75 g) during the first 2 weeks after transplant surgery, nondiabetic groups had a growth rate parallel to that of nontransplanted control animals. Diabetic groups, however, showed blunted growth, which persisted for the duration of the experimental period. The difference in mean body weight between diabetic and nondiabetic animals became statistically significant at 4 weeks (306±9 versus 349±8 g. P=0.004).

**TABLE 2. Metabolic Data: Measurements of Glucose, Insulin, and Lipids After Transplantation (Mean±SEM)**

<table>
<thead>
<tr>
<th>Transplant Group</th>
<th>Glucose</th>
<th>Insulin</th>
<th>Triglycerides</th>
<th>Total</th>
<th>VLDL</th>
<th>LDL</th>
<th>HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Tx</td>
<td>138±4</td>
<td>28±4</td>
<td>64±1</td>
<td>80±4</td>
<td>13±2</td>
<td>14±2</td>
<td>50±12</td>
</tr>
<tr>
<td>−D/−F/−CsA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>−D/−F</td>
<td>131±11</td>
<td>21±3</td>
<td>71±13</td>
<td>64±15</td>
<td>11±3</td>
<td>17±1</td>
<td>33±4</td>
</tr>
<tr>
<td>+D+/−F</td>
<td>419±7†</td>
<td>6±1*</td>
<td>340±63*</td>
<td>114±19</td>
<td>29±2†</td>
<td>25±1</td>
<td>60±25</td>
</tr>
<tr>
<td>−D/+F</td>
<td>148±8</td>
<td>42±2</td>
<td>110±21</td>
<td>69±14</td>
<td>14±8</td>
<td>30±8</td>
<td>23±1</td>
</tr>
<tr>
<td>+D+/+F</td>
<td>447±20*</td>
<td>7±2*</td>
<td>411±87*</td>
<td>146±17</td>
<td>25±3†</td>
<td>68±15</td>
<td>53±16</td>
</tr>
<tr>
<td>Allograft</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>−D/−F</td>
<td>415±10</td>
<td>5±1</td>
<td>352±54</td>
<td>111±7</td>
<td>32±4</td>
<td>21±4</td>
<td>58±12</td>
</tr>
<tr>
<td>+D+/−F</td>
<td>435±36</td>
<td>8±3</td>
<td>460±107</td>
<td>164±7</td>
<td>44±12</td>
<td>60±9</td>
<td>66±4</td>
</tr>
<tr>
<td>Isograft</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>−D/−F</td>
<td>415±10</td>
<td>5±1</td>
<td>352±54</td>
<td>111±7</td>
<td>32±4</td>
<td>21±4</td>
<td>58±12</td>
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<td>+D+/−F</td>
<td>435±36</td>
<td>8±3</td>
<td>460±107</td>
<td>164±7</td>
<td>44±12</td>
<td>60±9</td>
<td>66±4</td>
</tr>
</tbody>
</table>

Tx indicates transplant; D, diabetic; −D, nondiabetic; F, high-fructose (60%) diet; −F, nonfructose diet; CsA, cyclosporin A.

†P<0.001 vs Allo−D/−F, Allo−D+/+F, and Non-Tx−D/−F/−CsA.

‡P<0.004 vs Allo−D/−F and Non-Tx−D/−F/−CsA.
Metabolic Measurements in Animal Study Groups

Mean plasma concentrations of glucose, insulin, triglycerides, total cholesterol, LDL, VLDL, and HDL for each experimental group are shown in Table 2. Figure 2A through 2D plots the time course of glucose, insulin, triglyceride, and total cholesterol.

Control Animals

Transplantation surgery plus CsA treatment did not significantly alter mean glucose, insulin, triglyceride, total cholesterol, or lipid subfraction levels (compare non-Tx and allo−D−F animals, Table 2; P=NS for all values). Metabolic values were also similar for all diabetic animals, whether allografts or isografts (compare groups allo−1D/F and iso−1D/F, or allo−1D/F and iso +D/F; P=NS).

Effects of Streptozotocin on Glucose, Insulin, and Lipid Concentrations

All transplant recipient animals were diabetic by 2 weeks after treatment with streptozotocin, as reflected by a fourfold increase in plasma glucose concentration compared with nondiabetic controls (P<0.0005) and a threefold to fivefold decrease in insulin (P<0.001) (Table 2). These changes persisted throughout the experimental period (Figure 2A and 2B). Compared with nondiabetic allograft controls (allo−D−F), streptozotocin administration (allo−1D/F) resulted in a fourfold to fivefold increase in plasma triglyceride concentration (P<0.001) and a moderate increase in VLDL concentration (P<0.004) but had no significant effect on plasma total cholesterol concentration, LDL, or HDL.

Effects of Fructose in the Absence of Streptozotocin

In nondiabetic animals, fructose feeding alone had no significant effect on plasma glucose concentrations; however, plasma concentrations of insulin were elevated twofold (P<0.001) compared with those of animals fed a standard diet. There was also a slight trend towards increased lipid concentrations in these fructose-fed, non-diabetic animals (not statistically significant).

Combined Effects of Streptozotocin and Fructose

Fructose feeding in the presence of streptozotocin treatment did not significantly change plasma insulin or glucose concentrations compared with nonfructose-fed diabetic animals.
The major effects of fructose in the presence of diabetes were exaggerated increases in plasma concentrations of triglyceride, total cholesterol, and LDL compared with streptozotocin treatment alone. Fructose feeding did not significantly alter levels of VLDL, or HDL in diabetic animals.

**Assessment of TxCAD Frequency and Severity**

The frequency of TxCAD for all groups was evaluated by use of two end points. First, the percentage of animals with “significant TxCAD,” defined as intimal thickening affecting >5% of vessels within a section, was determined for each group. Second, the average percentage of vessels with any TxCAD for all groups was evaluated by use of two end points. First, the percentage of animals with significant TxCAD was calculated for each group of animals (Table 3). Second, the average percentage of vessels with any TxCAD within a section, was determined for each group. The frequency of TxCAD at the time when the animals were killed. No graft was identified by immunohistochemistry and oil red O stains to be lipid-filled macrophages, which are characteristic of foam cells seen in native atherosclerosis (Figure 5). Of note, TxCAD was not detected in native hearts of transplant recipients or in nontransplanted control animals (data not shown).

**Assessment of Myocardial Inflammation and Histological Rejection**

Most histological sections (75%, n=17) of allografts from diabetic recipients showed mononuclear cell inflammatory infiltrates and various degrees of myocyte damage. In contrast, only 11% of nondiabetic allografts and 13% of diabetic isografts had mononuclear infiltrate or myocyte damage. The average histological rejection grade (defined by inflammatory infiltrate and myocyte damage) was significantly higher in diabetic allografts than in nondiabetic allografts (2.2±0.5 versus 0.1±0.2, P≤0.04).

**Correlation of Metabolic Abnormalities With TxCAD**

Elevated plasma concentration of glucose, triglyceride, and VLDL were correlated with the severity of TxCAD in all groups by simple and multiple regression analyses (P<0.03, Table 4). A low insulin level was also correlated with the severity of TxCAD (P<0.04). Plasma concentrations of total cholesterol and LDL were not correlated with the severity of TxCAD.

**Graft and Animal Survival and Correlation With TxCAD**

Table 5 shows the relation between graft failure and the presence of TxCAD at the time when the animals were killed. No graft

**TABLE 3. Frequency and Severity of Transplant Coronary Artery Disease (TxCAD)**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Animals</th>
<th>Percent of Animals With Significant TxCAD*</th>
<th>Mean Percent of Vessels With Any TxCAD†</th>
<th>Mean Grade of TxCAD‡</th>
<th>Mean Tissue Inflammation Grade§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Tx</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>−CsA</td>
<td>6</td>
<td>0</td>
<td>2</td>
<td>...</td>
<td>0.25±0.25</td>
</tr>
<tr>
<td>Allograft</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>−D/−F</td>
<td>8</td>
<td>0</td>
<td>5</td>
<td>...</td>
<td>0.25±0.25</td>
</tr>
<tr>
<td>+D/−F</td>
<td>9</td>
<td>89</td>
<td>73</td>
<td>3.12±0.41</td>
<td>2.67±0.52</td>
</tr>
<tr>
<td>−D/+F</td>
<td>6</td>
<td>0</td>
<td>4</td>
<td>...</td>
<td>0</td>
</tr>
<tr>
<td>+D/+F</td>
<td>8</td>
<td>75</td>
<td>60</td>
<td>3.19±0.48</td>
<td>1.71±0.52</td>
</tr>
<tr>
<td>Isograft</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+D/−F</td>
<td>6</td>
<td>33</td>
<td>28</td>
<td>1.08±0.45</td>
<td>0.50±0.25</td>
</tr>
<tr>
<td>+D/+F</td>
<td>8</td>
<td>25</td>
<td>19</td>
<td>1.10±0.31</td>
<td>0.25±0.25</td>
</tr>
</tbody>
</table>

CsA indicates cyclosporin A; D, diabetic; F, fructose feeding; Allo, allograft; and Iso, isograft.

*Significant TxCAD defined as the presence of any intimal thickening affecting >5% of vessels within a given section (grade >1):
P<0.05 for (Allo +D/−F) vs (Iso +D/−F), and (Allo +D/+F) vs (Iso +D/−F).
P<0.0001 for (Allo +D/−F) vs (Allo −D/−F), and (Allo +D/+F) vs (Allo −D/−F).

†Percent of vessels with TxCAD is the number of vessels with TxCAD divided by the total number of vessels evaluated:
P<0.005 for (Allo +D/+F) vs (Iso +D/+F), and (Allo +D/−F) vs (Iso +D/−F).
P<0.0001 for (Allo −D/−F) vs (Allo +D/+F), and (Allo −D/−F) vs (Allo +D/−F).

‡Animals without significant TxCAD were not graded.

§P≤0.04 for (Allo +D/−F) vs (Allo −D/−F), (Allo −D/−F), and (Iso +D/−F).
failure occurred in nondiabetic allograft groups or diabetic isografts, in contrast to a mean graft survival rate of only 41% (7/17) in diabetic allograft animals ($P$, 0.02; Figure 6). Among diabetic allografts, the graft failure rate beyond 30 days after transplantation was 50% (4/8) in fructose-fed and 67% (6/9) in nonfructose-fed ($P$=NS) animals. All failed allografts and approximately half of all diabetic allografts surviving to the 60-day end point were found to have TxCAD. Of the 7 diabetic animals with beating allografts at 60 days, 4 had TxCAD. Overall survival of recipient animals did not differ significantly between experimental groups.

**Discussion**

The purpose of this study was to test a hypothesis generated from our observations in heart transplant patients. We proposed that the accelerated course of TxCAD is driven by the presence of diabetes and its associated dyslipidemia. By inducing these metabolic abnormalities in Lewis strain rats receiving heart transplants from MHC-mismatched donors (ACI strain), we confirmed the development of TxCAD in 75% to 89% of allografts within 30 to 60 days after transplantation.

In developing this model, we have made several observations: (1) Rapid development of atherosclerotic lesions oc-
curred exclusively in diabetic animals and was not observed in normoglycemic animals treated with CsA. However, both the incidence and severity of atherosclerotic lesions were significantly greater in allograft than isograft diabetic animals. (2) Fructose feeding had no impact on fasting glucose levels, but mildly raised total cholesterol, triglyceride, and insulin levels. Fructose feeding in the absence of diabetes did not alter the incidence of TxCAD but in the presence of diabetes showed a trend toward decreased severity of TxCAD. (3) Graft survival was significantly decreased in diabetic allografts compared with nondiabetic allografts and isografts; this difference was associated with a higher incidence and severity of TxCAD in the diabetic allografts. (4) Native hearts of diabetic transplant recipients showed no vascular disease.

Our first observation suggests that diabetes plays a significant role in the development of TxCAD. Specifically, diabetes augments and accelerates development of TxCAD in allograft animals. Furthermore, it appears to trigger development of mild TxCAD even in isografts. These observations suggest a relative order of importance for the effects of diabetes, alloimmunity, and vascular injury in accelerating the course of TxCAD in this model. That is, since the severity of diabetes augments and accelerates development of TxCAD in diabetic animals, (2) it did not increase the incidence of TxCAD but in the presence of diabetes showed a trend toward decreased severity of TxCAD. (3) Graft survival was significantly decreased in diabetic allografts compared with nondiabetic allografts and isografts; this difference was associated with a higher incidence and severity of TxCAD in the diabetic allografts. (4) Native hearts of diabetic transplant recipients showed no vascular disease.

Our results are consistent with our earlier observations in heart transplant patients, in whom total cholesterol and LDL were not correlated with TxCAD but rather elevated hypertriglyceridemia was an independent predictor of TxCAD.7 It suggests an important role for ischemia/reperfusion injury in the disease process of TxCAD and is consistent with the response-to-injury hypothesis proposed by Ross and Agius,11 ie, that endothelial injury at the time of transplant surgery, even in the absence of an alloimmune response, may contribute significantly to the development of TxCAD. This hypothesis is further supported by a recent report that questioned the need for an ongoing alloimmune response in the disease process of TxCAD. In that study, retransplantation of the cardiac allograft after 9 days back into the syngeneic animal resulted in progression of TxCAD in a fashion similar to that observed in the allogeneic recipient.22

Our second observation addresses the relative importance of each of the metabolic derangements inherent in the diabetic state. Although fructose feeding mildly raised total cholesterol, triglyceride, and LDL levels in diabetic animals (without further affecting glucose, VLDL, or HDL), it did not increase the incidence of TxCAD. In fact, it showed a somewhat surprisingly consistent, although nonstatistically significant, trend toward having a protective effect against the development of TxCAD. This finding, taken together with the lack of significant elevation in total cholesterol or LDL in the diabetic, non-fructose-fed animals, suggests that elevated total cholesterol and in particular elevated LDL do not contribute to the pathophysiology of TxCAD in this diabetic model. Similar findings were reported by Mennander et al23 in a rat aortic allograft model, in which hypercholesterolemia in the absence of hypertriglyceridemia did not enhance atherosclerotic changes in the allograft. Our results are also consistent with observations from another animal model, heart transplants in apo E–deficient mice.24 In this model, the character of the coronary vascular changes in the transplanted heart was distinctly affected by the lipid environment, but severity in terms of luminal encroachment was not markedly different. Further studies to investigate the cellular and molecular characteristics of our model are currently in place.

Our results are consistent with our earlier observations in heart transplant patients, in whom total cholesterol and LDL were not correlated with TxCAD but rather elevated hypertriglyceridemia was an independent predictor of TxCAD.7 It must be noted, however, that the type of diabetes induced in our rat model was type I, characterized by β-pancreatic islet cell failure and the absence of insulin. This situation differs from that of our heart transplant patients, who were typically glucose intolerant and insulin resistant and who had elevated plasma insulin levels. In these patients, a high plasma insulin level was equally prognostically significant to glucose intolerance for subsequent occlusive coronary artery disease.9 In the present study in rats, hyperinsulinemia was never present;

### TABLE 4. Correlation of Metabolic Abnormalities With Transplant Coronary Artery Disease (TxCAD)

<table>
<thead>
<tr>
<th>Metabolic Parameter</th>
<th>TxCAD</th>
<th>R</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>0.65</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td>−0.40</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Triglyceride</td>
<td>0.53</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.38</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>VLDL</td>
<td>0.43</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>LDL</td>
<td>0.29</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>HDL</td>
<td>0.41</td>
<td>0.04</td>
<td></td>
</tr>
</tbody>
</table>

Figure 5. Oil red O stain showing extensive lipid deposition in inflammatory cells, intima (arrows), and adventitia of a vessel with TxCAD.
partial normalization of hypoinsulinemia occurred with fructose feeding alone but not with diabetes and fructose feeding. In this rat model, both hyperglycemia and hypoinsulinemia were correlated with TxCAD by multiple regression analysis. Future studies are required to determine the role of hyperglycemia per se in the development of TxCAD in this model of type I diabetes. It will be important to determine whether treating these animals with insulin will completely inhibit the development of TxCAD.

Our third observation relating decreased graft survival with increased TxCAD provides compelling evidence that TxCAD plays a causal role in graft failure. We noted that all animals with early (<60 days) graft failure had significant TxCAD. Moreover, the group of animals with the highest mean grade of TxCAD—diabetic allografts—was the only group with compromised graft survival during the 60-day experimental period. These results are consistent with observations in heart transplant patients of decreased graft survival and poor outcome in hyperglycemia, hyperinsulinemia, and hypertriglyceridemia.

Our fourth observation, the absence of intimal thickening in all native hearts, regardless of their metabolic state, suggests an important role for vascular injury at the time of transplantation as a trigger for the disease process of TxCAD. Furthermore, this observation suggests that TxCAD is unlikely to have been triggered by a direct toxic effect of streptozotocin on cardiac vascular endothelium.

Conclusions
In this study, we reported the development and initial characterization of a rat heterotopic heart transplant model with diabetes and dyslipidemia leading to significant TxCAD. To the best of our knowledge, this is the first report of a model of TxCAD induced by diabetes and its associated dyslipidemia. Despite the relatively small number of animals in this pilot study, the strong correlation between the metabolic derangements and the incidence and severity of TxCAD and graft survival suggest a significant role for diabetes in the pathophysiology of TxCAD. The similarity of the metabolic profiles achieved in this rat model of TxCAD, as well as the similar histological appearance of TxCAD lesions, to heart transplant patients indicates that further study in this model may contribute significantly to our understanding of the mechanisms of TxCAD.

Study Limitations
The use of streptozotocin to produce diabetes results in type I rather than type II diabetes, wherein hyperglycemia, hyperinsulinemia, and obesity with insulin resistance characterize the disease process. This digression from the human cardiac transplant scenario limits our ability to draw parallels between the two systems. Further studies to clarify the role of hyperglycemia per se in this rat animal model would be useful in this regard.

Our model demonstrates a combined effect of diabetes and alloimmunity with requisite immunosuppression. We were unable to assess the effect of diabetes and alloimmunity in the absence of Cs treatment, since the allograft would have soon failed in this strongly MHC-mismatched model. Preliminary studies of CsA-untreated diabetic isograft animals, however, showed an incidence similar to that of TxCAD in CsA-treated diabetic animals, suggesting that the doses of CsA used in this study had no significant effect on the development or severity of TxCAD.

The trend toward a protective effect with a high-fructose diet for TxCAD is intriguing and was consistently found in all

![Figure 6. Graft survival according to experimental groups. Non-diabetic animals had 100% graft survival compared with a 41% average survival in diabetic animals (P<0.02). DM indicates diabetes mellitus; Non-DM, nondiabetic; Iso, isograft; Allo, allograft; +D, with diabetes (streptozotocin induced); and +F, with fructose feeding.](image-url)
our animal subjects, independent of both their metabolic and alloimmune status. This may be worth further exploration in future experiments. Finally, our observations were made qualitatively with regard to the degree of TxCAD and histological rejection. Morphometry for quantitative assessment of TxCAD, immunohistochemical analysis of the tissue inflammatory infiltrate, and phenotypic characterization of the composition of the vascular intimal lesion are currently in place.

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