Downregulation of Myocardial Myocyte Enhancer Factor 2C and Myocyte Enhancer Factor 2C–Regulated Gene Expression in Diabetic Patients With Nonischemic Heart Failure

Background—In animal studies, diabetes has been shown to induce changes in gene expression of key regulators in cardiac energy metabolism and calcium homeostasis. In the present study, we tested the hypothesis that metabolic gene expression in nonischemic failing hearts of diabetic patients differs from that in nonischemic failing hearts of nondiabetic patients.

Methods and Results—Left ventricular tissue was obtained from nonfailing hearts (n=6) and from nonischemic failing hearts of patients with or without type 2 diabetes. Myocardial transcript levels of key regulators in energy substrate metabolism (glucose transporter 1, glucose transporter 4, pyruvate dehydrogenase kinase 4, peroxisome proliferator–activated receptor α, muscle carnitine palmitoyl transferase-1, medium-chain acyl-CoA dehydrogenase, and uncoupling protein 3), calcium homeostasis (sarcoplasmic reticulum Ca²⁺-ATPase [SERCA2a], phospholamban, and cardiac ryanodine receptor), and contractile function (myosin heavy chain α) were measured using real-time quantitative reverse transcription–polymerase chain reaction. In addition, we measured myocyte enhancer factor 2C (MEF2C) and SERCA2a protein levels. Only MEF2C regulated transcripts (glucose transporter 4, SERCA2a, and myosin heavy chain α) were lower in the diabetic group compared with the nondiabetic group. MEF2C protein content was also decreased.

Conclusion—MEF2C and MEF2C-regulated genes are decreased in the failing hearts of diabetic patients. This transcriptional mechanism may contribute to the contractile dysfunction in heart failure patients with diabetes. (Circulation. 2002;106:407-411.)

Key Words: diabetes mellitus ▪ cardiomyopathy ▪ metabolism ▪ polymerase chain reaction

Earlier studies, mainly in rodents, have shown that diabetic cardiomyopathy is associated with impairment in numerous cell functions, including energy substrate metabolism, calcium homeostasis, and cell signaling.1 At the same time, we have observed profound changes in the gene expression profile of hearts from insulin-deficient rats.2 These observations have led us to propose that the diabetic human myocardium may exhibit abnormalities in metabolic gene expression.

Methods

Patients

Clinical data were collected retrospectively. Nonfailing heart tissue (left ventricular apex) was obtained (up to 12 hours after death) from 6 male donors who died in motor vehicle accidents (age 35±8 years, no significant medical history). Left ventricular tissue (apex) of the heart failure patients was obtained during the implantation of a left ventricular assist device. All tissue samples were immediately frozen in liquid nitrogen. Informed consent was obtained from each patient or the next of kin, and the protocol was approved by the respective Institutional Review Boards.

Gene Expression

RNA was extracted by standard methods3 and analyzed by reverse transcription followed by real-time quantitative polymerase chain reaction for the transcripts of interest. The methodology of quantitative polymerase chain reaction has been previously described in detail.4 The nucleotide sequences for probes as well as forward and reverse primers have been previously published5 and are shown in the Table. The transcript for the constitutive gene product β-actin was used as a reporter gene for data normalization. Internal RNA standards were prepared using the T7 RNA polymerase method (Ambion). Because nonfailing human heart tissue was obtained up to 12 hours after death, we measured transcript levels of glucose transporter 4 (GLUT4) immediately after death and 12 hours thereafter in rat heart. There was no significant difference GLUT4 expression between the 2 time points (0.038±0.008 versus 0.043±0.005 GLUT4 mRNA/β-actin mRNA).
Protein Expression
Proteins from 8 nonischemic failing hearts (4 nondiabetic and 4 diabetic patients) were isolated as previously described. Briefly, samples were homogenized in extraction buffer and centrifuged (30 minutes at 15 000 g) and the supernatant was isolated. Proteins were fractionated by 6% PAGE and transferred to a nitrocellulose membrane. Antibodies were purchased from Santa Cruz Biotechnology (sc-313, sc-2030) and Affinity Bioreagents (MA3-919). The primary antibody against myocyte enhancer factor 2 (MEF2) binds to MEF2A and MEF2C. The different isoforms were identified by their primer/probe sequence.

Statistical Analysis
All data are presented as mean±SEM. Differences between the groups for normally distributed gene expression were calculated by 1-way ANOVA followed by the Bonferroni test. Nonnormally distributed data were analyzed by using the Kruskal-Wallis test versus 6.6

Results
Clinical Data of Heart Failure Patients
Clinical data of 8 (male/female: 4/4) nonischemic heart failure patients without diabetes and 7 (male/female: 6/1) nonischemic heart failure patients with type 2 diabetes of more than 5 years’ duration were retrospectively analyzed. There were no significant differences in age (47.9±6.3 versus 51.7±4.4 years), left ventricular diastolic diameter (6.8±0.2 versus 6.6±0.3 cm), or cardiac index (2.0±0.1 versus 2.2±0.4 L/min per m²) between nondiabetic and diabetic heart failure patients. Left ventricular ejection fraction was less than 20% in all patients. Fasting serum glucose levels and body mass index did not differ between the 2 groups (109±4 versus 157±22 versus mg/dL, P=0.10, and 25±2 versus 26±1 kg/m², P=0.61, respectively). All patients received diuretics, angiotensin-converting enzyme inhibitors, low-dose β-blockers, and positive inotropic agents. Five diabetic patients were treated with sulphonylureas, metformin, and insulin, and 2 only with insulin.

Gene Expression Data
Myocyte-Specific Marker
We assayed for transcript levels of the myocyte-specific marker cardiac troponin I to exclude the possibility of differences in gene expression between the 3 groups being caused by differences in the myocyte/nonmyocyte ratio. There was no significant difference in troponin I transcript levels between the groups (nonfailing: 401.9 versus 157±22 versus mg/dL, P=0.10, and 25±2 versus 26±1 kg/m², P=0.61, respectively). All patients received diuretics, angiotensin-converting enzyme inhibitors, low-dose β-blockers, and positive inotropic agents. Five diabetic patients were treated with sulphonylureas, metformin, and insulin, and 2 only with insulin.

Regulators of Energy Substrate Metabolism
Transcript level of GLUT1 did not significantly differ between the groups (Figure 1a). In contrast, gene expression of GLUT4 was significantly decreased in failing hearts of diabetic patients when compared with nonfailing and failing hearts of nondiabetics (Figure 1b). Transcript levels of pyruvate dehydrogenase kinase 4 were increased in failing
hearts of diabetics when compared with nonfailing hearts (Figure 1c).

Gene expressions of the transcription factor peroxisome proliferator–activated receptor α (PPARα) and 3 PPARα-regulated genes (muscle carnitine palmitoyl transferase-1, medium-chain acyl-CoA dehydrogenase, and uncoupling protein 3) are shown in Figure 1d through 1g. PPARα, medium-chain acyl-CoA dehydrogenase, and uncoupling protein 3 expressions were decreased in both failing groups when compared with the nonfailing group, but did not differ between nondiabetic and diabetic heart failure patients.

**Regulators of Calcium Metabolism**

Figure 1h through 1j shows transcript levels of genes regulating calcium homeostasis. Gene expression of sarcoplasmic reticulum Ca²⁺-ATPase (SERCA2a) was significantly depressed in the diabetes group with failing hearts when compared with the nonfailing and failing groups of nondiabetic patients (Figure 1h).

**Marker of Contractile Function**

Transcript levels of myosin heavy chain (MHC) α were significantly decreased in both heart failure groups when compared with the nonfailing groups. Heart failure patients with diabetes showed an even greater decrease when compared with heart failure patients without diabetes (Figure 1k).

**MEF2 and SERCA2a Protein Expression**

Previous studies have shown that MEF2 is involved in the transcriptional regulation of GLUT4, SERCA2a, and MHCα.①-③
During diabetes, myocardial glucose uptake and subsequent oxidation are impaired. The present study shows that nonischemic heart failure patients with diabetes decrease myocardial GLUT4, but not GLUT1, transcript levels when compared with nonischemic heart failure patients without diabetes. This distinction between glucose transporter isoform expression may be explained by differences in their transcriptional regulation. A recent study has shown that the transcriptional coactivator proliferator-activated-receptor gamma coactivator-1 mediates increased GLUT4 expression, in large part, by binding to and coactivating MEF2C. This is consistent with our findings of a concomitant decrease in MEF2C protein and GLUT4 gene expression.

Genes regulating fatty acid oxidation show no difference in the failing human heart of diabetic and nondiabetic patients. PPARα regulates genes encoding for enzymes, which are involved in fatty acid uptake and oxidation, and has been suggested to be involved in the regulation of cardiac hypertrophy. We have previously shown that myocardial PPARα expression is decreased in a rat model of cardiac hypertrophy and in a model of insulin deficient diabetes. The present study shows that PPARα transcript levels and 2 PPARα-regulated gene levels (medium-chain acyl-CoA dehydrogenase and uncoupling protein 3) are decreased in both failing groups but do not significantly change in heart failure patients with diabetes when compared with heart failure patients without diabetes. One explanation for this finding is that end-stage heart failure may itself maximally depress PPARα expression. Thus, the downregulation of PPARα expression suggests a transcriptional mechanism for the decrease in PPARα-regulated genes in the failing human heart.

**Disturbances of Myocardial Calcium Homeostasis and Contractile Function in Diabetes**

Heart failure in diabetics is characterized by early diastolic dysfunction followed by late systolic dysfunction. In the failing human heart, decreased levels of SERCA2a are associated with impaired cardiac function. The present study shows that diabetes decreases levels of SERCA2a in patients with nonischemic heart failure. These results are consistent with previous studies showing impaired sarcoplasmic calcium uptake in the diabetic rat heart.

MHCα expression is decreased in the failing human heart when compared with the nonfailing human heart. We confirmed the downregulation of MHCα in the failing human heart and showed that diabetes augments this depression. A recent report in rat cardiac myocytes showed that MHC isoform expression determines force-velocity relationships, velocity of loaded shortening, and overall power output-generating capacity. Thus, together the decrease in SERCA2a and MHCα gene expression observed in human failing hearts of diabetics may contribute to cardiac dysfunction.

**MEF2-Regulated Gene Expression**

Because MEF2C binds to the GLUT4, SERCA2a, and MHCα promoter, we focused our analysis on this transcription factor. Although the present data do not prove that the decrease in MEF2C protein expression causes the downregulation of GLUT4, SERCA2a, and MHCα transcript levels, they are consistent with previous animal studies showing a decrease in myocardial MEF2, GLUT4, SERCA2a, and MHCα expression with diabetes.

**Study Limitations**

We examined only myocardial tissue of nonischemic heart failure patients because ischemia is known to alter metabolic...
gene expression and therefore may mask diabetes-induced changes in transcripts of metabolic genes. This may explain why the present study, unlike previous reports, could not find any significant decrease for the glucose transporters muscle carnitine palmitoyl transferase-1 and SERCA2a in the nonischemic failing human heart. We cannot exclude the possibility that increasing the number of patients may lead to statistically significant differences between the groups for genes with high variances (eg, phospholamban).

Conclusions
The downregulation of MEF2C and MEF2C-regulated genes (GLUT4, SERCA2a, and MHCα) in the failing hearts of patients with diabetes suggests a transcriptional mechanism that might contribute to the pathogenesis and contractile dysfunction of heart failure patients with diabetes.

Acknowledgment
This study was supported in part by grants from the US Public Health Service (RO1-HL/AG 61483 and F32HL-67609).

References
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Circulation. 2002;106:407-411; originally published online July 1, 2002; doi: 10.1161/01.CIR.0000026392.80723.DC
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2002 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/106/4/407

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