Genetic Deletion or Pharmacological Inhibition of Dipeptidyl Peptidase-4 Improves Cardiovascular Outcomes After Myocardial Infarction in Mice

Meghan Sauvé, Kiwon Ban, M. Abdul Momen, Yu-Qing Zhou, R. Mark Henkelman, Mansoor Husain, and Daniel J. Drucker

OBJECTIVE—Glucagon-like peptide-1 (7-36)amide (GLP-1) is cleaved by dipeptidyl peptidase-4 (DPP-4) to GLP-1 (9-36)amide. We examined whether chemical inhibition or genetic elimination of DPP-4 activity affects cardiovascular function in normoglycemic and diabetic mice after experimental myocardial infarction.

RESEARCH DESIGN AND METHODS—Cardiac structure and function was assessed by hemodynamic monitoring and echocardiography in DPP-4 knockout (Dpp4−/−) mice versus wild-type (Dpp4+/+) littermate controls and after left anterior descending (LAD) coronary artery ligation-induced myocardial infarction (MI). Effects of sustained DPP-4 inhibition with sitagliptin versus treatment with metformin were ascertained after experimental MI in a high-fat diet–streptozotocin model of murine diabetes. Functional recovery from ischemia-reperfusion (I/R) injury was measured in isolated hearts from Dpp4−/− versus Dpp4+/+ littermates and from normoglycemic wild-type (WT) mice treated with sitagliptin or metformin. Cardioprotective signaling in the murine heart was examined by RT-PCR and Western blot analyses.

RESULTS—Dpp4−/− mice exhibited normal indexes of cardiac structure and function. Survival post-MI was modestly improved in normoglycemic Dpp4−/− mice. Increased cardiac expression of phosphorylated AKT (pAKT), pGSK3β, and atrial natriuretic peptide (ANP) was detected in the nonischemic Dpp4−/− heart, and HO-1, ANP, and pGSK3β proteins were induced in nonischemic hearts from diabetic mice treated with sitagliptin or metformin. Sitagliptin and metformin treatment of wild-type diabetic mice reduced mortality after myocardial infarction. Sitagliptin improved functional recovery after I/R injury ex vivo in WT mice with similar protection from I/R injury also manifest in hearts from Dpp4−/− versus Dpp4+/+ mice.

CONCLUSIONS—Genetic disruption or chemical inhibition of DPP-4 does not impair cardiovascular function in the normoglycemic or diabetic mouse heart. Diabetes 59:1063–1073, 2010

Type 2 diabetes is associated with an increased risk of cardiovascular disease, hence there is considerable interest in strategies that reduce cardiovascular morbidity and mortality in diabetic subjects. Although aggressive treatment of blood pressure and dyslipidemia reduces cardiovascular events in both nondiabetic and diabetic patients, whether reduction in blood glucose alone reduces cardiac events in subjects with established diabetes remains controversial (1). Moreover, pharmacotherapy of diabetes using agents with unique antidiabetic mechanisms may be associated with differential and occasionally unexpected adverse effects on cardiovascular outcomes, independent of effects on glucose control (2,3). Therefore, a detailed understanding of the unique cardiovascular benefits and risks of each antidiabetic drug used to treat diabetes seems prudent.

The two most recently approved drug categories for the treatment of type 2 diabetes, GLP-1 receptor (GLP-1R) agonists and dipeptidyl peptidase-4 (DPP-4) inhibitors, exert their antidiabetic actions largely through potentiation of GLP-1R activation (4). Because these agents have only been used clinically for several years, there are scant data on cardiovascular outcomes associated with these incretin-based therapies. GLP-1 improves endothelial function in subjects with type 2 diabetes (5), and transient GLP-1 administration improves cardiovascular outcomes in subjects with myocardial infarction (MI) or congestive heart failure (CHF) (6,7). Moreover, preclinical data demonstrate that GLP-1 is cardioprotective when administered before the induction of ischemia (8–10). Furthermore, therapy with the GLP-1R agonists exenatide and liraglutide is associated with blood pressure reduction in the majority of treated subjects (11,12). Hence, although limited, the available data support the hypothesis that antidiabetic therapy with GLP-1 may be associated with beneficial effects on cardiovascular outcomes. Nevertheless, as GLP-1R agonists may produce weight loss (13), the extent to which the salutary effects of GLP-1R activation on the cardiovascular system in vivo reflect the beneficial consequences of weight loss remains unclear.

In contrast, much less is known about the cardiovascular biology of DPP-4. Unlike therapy with GLP-1R agonists, the use of sitagliptin, saxagliptin, or vildagliptin has not been associated with weight loss or sustained improvement in lipid profiles (4). Moreover, inhibition of DPP-4 enzyme activity modulates the activity of cardioactive peptides such as brain natriuretic peptide, neuropeptide Y, and stromal cell–derived factor-1 (SDF-1) (14) via non–GLP-1 mechanisms of action. More recently, GLP-1 (9-36), a peptide metabolite derived from native GLP-1 (7-
36) amide after cleavage by DPP-4, has been shown to exert cardioprotective actions in rodents (15, 16) and improve cardiovascular function in dogs with CHF (17). Accordingly, to delineate the importance of DPP-4 for cardiovascular biology in vivo, we studied cardiovascular function in Dpp4-/- mice in vivo, in isolated perfused Dpp4-/- and Dpp4-/- hearts ex vivo, and in wild-type diabetic mice subjected to experimental MI and treated with the DPP-4 inhibitor sitagliptin.

**RESEARCH DESIGN AND METHODS**

**Animal models and drug treatments.** Experimental procedures adhered to approved protocols of the University Health Network and Mt. Sinai Hospital Animal Care Committees. Mice were housed under pathogen-free conditions in micro-isolator cages and maintained on a 12-h light (0700)/dark (1900) cycle with access to standard rodent food and water ad libitum, except where noted. All experiments used age- and sex-matched littermates. Dpp4-/- mice were inbred on the C57BL/6 background (18). Experimental animals were derived by crosses between heterozygous Dpp4-/- mice to generate Dpp4-/- and Dpp4-/- littermate mice. All genotypes were confirmed by PCR analyses of tail DNA.

C57BL/6 mice, 4 weeks old, were purchased from Taconic (Germantown, NY) and housed as described above, but placed on a high-fat diet (HFD; 45% kcal from fat; Research Diets, New Brunswick, NJ). After 5 weeks of HFD, mice were fasted for 5 h and then injected with a single dose of streptozotocin (STZ; Sigma) (75 mg/kg i.p.) as a freshly prepared solution in 0.1 mmol/L sodium citrate, pH 5.5. Mice were then randomized to treatment with either HFD alone, HFD plus a DPP-4 inhibitor (sitagliptin, 250 mg·kg−1·day−1) (19), or HFD plus metformin (450 mg·kg−1·day−1). The dose of metformin was chosen based on related studies (20) and following pilot studies demonstrating optimal antidiabetic actions without significant effects on food intake or body weight. This dose of sitagliptin does not affect food intake, yet results in 90% inhibition of DPP-4 (19, 21). Sitagliptin and metformin were supplied by Merck Research Labs (Rahway, NJ).

For RNA and protein analyses by real-time quantitative PCR and Western blot, respectively, heart tissue was obtained from separate groups of normoglycemic Dpp4-/- or Dpp4-/- mice fed regular food, or wild-type C57BL/6 mice exposed to HFD for 4 weeks, given a single dose of STZ (75 mg/kg), and then treated with either HFD alone, or HFD plus 1) metformin, 2) sitagliptin, or 3) the GLP-1R agonist transliragide (Novo Nordisk, Novo Alice, Bagsvaerd, Denmark) (10), 75 µg/kg i.p. twice daily for an additional 7 days. All animals were killed by exposure to carbon dioxide.

Isolated heart preparations were from 12-week-old male Dpp4-/- and Dpp4-/- littermates or separate groups of wild-type C57BL/6 mice. Only isolated mouse hearts exhibiting a heart rate >350 bpm were used. Wild-type C57BL/6 mice were treated with an intraperitoneal injection of either sitagliptin (20 mg/kg) or metformin (125 µg/kg) or saline at 24 h and 1 h before heart excision.

**Metabolic measurements.** Oral glucose tolerance tests were performed in sitagliptin- or metformin-treated wild-type C57BL/6 mice and untreated controls. Mice were fasted for 16 h and administered glucose (1.5 mg/kg) via oral gavage. A1C and blood glucose levels were measured on whole blood using the DCA 2000+ Analyzer and a Glucometer (both Bayer, Toronto, ON, Canada). GLP-1 (7-36)amide levels were measured by a Meso Scale Discovery
TABLE I
Echocardiography-defined dimensional and functional parameters in Dpp4+/− and Dpp4−/− mice

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<td>Aortic flow</td>
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<td>Aortic valve ejection time (ms)</td>
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<td>Deceleration time (ms)</td>
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<td>LV chamber dimensions by M-mode</td>
<td>Left atrium size (mm)</td>
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<td>LV end diastolic diameter (mm)</td>
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<td>LV end systolic diameter (mm)</td>
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<td>LV outflow tract diameter (mm)</td>
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<td>Anterior wall thickness (mm)</td>
<td>0.74 ± 0.30</td>
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<td>Posterior wall thickness (mm)</td>
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<tr>
<td>Fractional shortening (%)</td>
<td>56.42 ± 2.17</td>
<td>52.85 ± 1.04</td>
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Data are means ± SE. Genetic elimination of DPP-4 activity is not associated with abnormalities in parameters of cardiovascular function. Inbred and outbred Dpp4+/− mice were studied. Inbred mice showed a significant reduction in LV wall thickness and systolic function, while outbred mice showed no significant changes.

RESULTS
Cardiac structure and function in Dpp4−/− mice. We first verified that Dpp4−/− mice used in our studies retained the phenotype of improved glucose tolerance and reduced DPP-4 activity as originally described (18). Consistent with previous findings, oral glucose tolerance was improved and plasma DPP-4 activity was markedly reduced in Dpp4−/− mice (supplemental Fig. IA–C, available in an online appendix at http://diabetes.diabetesjournals.org/cgi/content/full/db00-0965/DC1). Heart weights did not differ between 12-week-old sex-matched Dpp4+/− and Dpp4−/− mice (Fig. 1A, P = NS). High-resolution echocardiography did not detect any differences in LV wall thicknesses, LV end systolic and end diastolic dimensions, and aortic and flow velocities, LV systolic and diastolic areas, LV outflow tract diameter, ejection time, left atrial size, or fractional shortening between Dpp4+/− and Dpp4−/− mice (Table 1, NS for all comparisons). Hence, genetic disruption of the Dpp4 gene in mice is not associated with baseline abnormalities in cardiac structure or function.

Myocardial infarction outcomes in normoglycemic Dpp4+/− mice. To determine whether disruption of the Dpp4 gene modifies the response to cardiac injury, we induced MI in non-diabetic 12-week-old male and female Dpp4+/− and Dpp4−/− mice via permanent surgical LAD ligation (Fig. 1B). At the predefined end point of 4 weeks post-MI, Dpp4+/− mice exhibited a ~20% absolute increase in survival compared with Dpp4+/− littermate controls (Fig. 1C). Post-MI, the hearts of Dpp4−/− and

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Dpp₄⁺/⁺ mice underwent similar compensatory hypertrophy (Fig. 1D). Although infarct size was reduced in Dpp₄⁻/⁻ mice, this difference was not statistically significant (Fig. 1E).

To explore mechanisms mediating the increased survival of Dpp₄⁻/⁻ mice post-MI, we analyzed cardiac mRNA and protein levels of known cardioprotective genes. Normoglycemic nonischemic Dpp₄⁻/⁻ mice exhibited small but nonsignificant increases in Akt1, Gsk3β, Ppara, Pi3k, and Ho1 transcripts (Fig. 2B–F). Moreover, hearts from Dpp₄⁻/⁻ mice contained higher levels of phosphorylated Akt (pAKT), pGSK3β, and ANP (Fig. 2G–I), proteins known to be regulated by GLP-1R agonists (10) and associated with cardioprotection in vivo (24–27).

**Treatment of diabetic mice with metformin or silitaglaptin pre- and post-MI.** Because Dpp₄⁻/⁻ mice are resistant to the development of STZ-induced diabetes (28), we assessed whether reduction of DPP-4 activity is car-

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**FIG. 2.** Dpp₄⁻/⁻ hearts express increased levels of proteins associated with cardiomyocyte survival. A: Experimental outline for analysis of basal RNA and protein expression in Dpp₄⁻/⁻ and Dpp₄⁺/⁺ mice. Relative levels of mRNA transcripts for Akt1 (B), Gsk3β (C), Ppara (D), Pi3k (E), and Ho1 (F) in nonischemic hearts from 12-week-old Dpp₄⁻/⁻ versus Dpp₄⁺/⁺ mice assessed by quantitative real-time PCR and normalized to levels of β-actin transcripts in the same samples. n = 6 per group. Relative levels of pGSK3β (G), ANP (H), and pAKT (I) determined by Western blot analysis of protein extracts from hearts of 12-week-old Dpp₄⁻/⁻ and Dpp₄⁺/⁺ mice are shown. *P < 0.05, n = 3 for each genotype.
FIG. 3. Sitagliptin and metformin reduce blood glucose levels and increase plasma GLP-1 (7-36)amide levels in diabetic mice. Male C57BL/6 mice were placed on HFD (45% fat) for 4 weeks (A). At the start of week 5, mice were injected with a single dose of STZ (75 mg/kg) and then randomized into three treatment groups: 1) HFD/STZ alone, 2) HFD/STZ + sitagliptin (250 mg/kg), or 3) HFD/STZ + metformin (450 mg/kg) for an additional 8 weeks. At week 12, mice underwent LAD ligation or control sham surgery. At week 16, surviving mice were killed and infarct size was measured. Levels of random fed blood glucose (B) and A1C (C) were significantly reduced in mice treated with sitagliptin or metformin. Oral glucose tolerance (D and E) was significantly improved in sitagliptin-treated (n = 29) or metformin-treated (n = 23) mice compared with HFD alone (n = 23). F: Body weight (BW) in mice treated with HFD/STZ alone, sitagliptin, or metformin (n = 22-23 per treatment). Plasma active GLP-1 is increased in mice treated with sitagliptin or metformin (n = 14 per treatment) (G). *P < 0.05, ***P < 0.001 vs. the untreated HFD/STZ group. AUC, area under the curve.

Dioprotective in diabetic Dpp4+/+ mice. Wild-type mice were placed on an HFD for 4 weeks, rendered diabetic with STZ, and maintained for an additional 12 weeks on HFD alone or on HFD plus either sitagliptin or metformin (Fig. 3A). After 8 weeks on drug treatment or HFD alone, mice were subjected to LAD ligation and observed for an additional 4 weeks (Fig. 3A). Random blood glucose (Fig. 3B), levels of A1C (Fig. 3C), and oral glucose tolerance (Fig. 3D–E) were improved to a similar extent with body weights remaining comparable (Fig. 3F) in mice treated with sitagliptin or metformin. Moreover, plasma levels of active GLP-1 (7-36)amide were increased to a similar extent in sitagliptin- versus metformin-treated mice (Fig. 3G).

Cumulative survival assessed up to 4 weeks after LAD ligation was improved in mice treated with either sita-
gliptin or metformin, compared to mice on HFD/STZ alone (Fig. 4A). A significant increase in heart-to-body weight ratios post-MI was observed only in diabetic mice treated with metformin (Fig. 4B). No differences in infarct size were observed between the three groups (Fig. 4C).

To identify potential mechanism(s) underlying improved survival post-MI in sitagliptin- and metformin-treated diabetic mice, we assessed cardiac mRNA and protein levels of candidate pro-survival genes in separate groups of diabetic animals treated for 1 week with either sitagliptin, metformin, or the GLP-1R agonist, liraglutide. No significant changes were detected in mRNA levels of Pp3k, Akt, Hsl1, Mmp9, and Pparg after treatment with these antidiabetic agents (Fig. 5B–F). In contrast, sitagliptin, metformin, and liraglutide increased expression of ANP (Fig. 5G). Sitagliptin and liraglutide, but not metformin, activated the prosurvival kinase AKT (Fig. 5H), whereas all three drugs increased levels of HO-1 (Fig. 5I). A modest but nonsignificant increase in levels of phospho-GSK3β was also observed with all three antidiabetic agents (Fig. 5J).

We next examined whether metformin or sitagliptin treatment of HFD-fed mice produced changes in gene and protein expression in the mouse heart after LAD ligation (Fig. 6A). Levels of Hsl1 and Gsk3β RNA transcripts were modestly increased (Fig. 6D and E), whereas Akt1, Pp3k, and Pparg RNA transcripts were significantly increased in the post-ischemic heart after metformin treatment (Fig. 6B, C, and F). In contrast, sitagliptin treatment was not associated with significant changes in levels of cardioprotective mRNA transcripts post-MI (Fig. 6B–F). Neither metformin nor sitagliptin treatment produced detectable changes in levels of AKT, ANP, GSK3β, or HO-1 proteins assessed at day 5 post-MI (Fig. 6G–J). Similarly, although heart rate was increased in metformin-treated mice, we did not detect other significant differences in parameters of cardiac function in sitagliptin- versus metformin-treated mice after myocardial infarction (Table 2).

Ischemia-reperfusion injury and metformin versus DPP-4 inhibition in normoglycemic mouse hearts. To determine whether cardioprotective actions of sitagliptin are observed in the normoglycemic murine heart, we acutely administered sitagliptin, metformin, or saline (PBS) to nondiabetic wild-type mice in vivo before assessing recovery of LVDP after I/R injury to their hearts ex vivo (15). Parallel experiments included I/R injury in hearts from normoglycemic Dpp4–/– and Dpp4+/+ animals, as well as testing cardioprotective actions of an acute ex vivo sitagliptin infusion (20 min) versus placebo (PBS) immediately before wild-type hearts undergoing I/R injury (Fig. 7A).

Acute administration of metformin or sitagliptin in vivo improved recovery from subsequent I/R injury in normoglycemic mice (Fig. 7B). Recovery of LVDP was also greater in Dpp4–/– hearts versus Dpp4+/+ littermate controls (Fig. 7B). By contrast, sitagliptin administered to the coronary circulation ex vivo (and immediately before I/R injury) exerted no direct cardioprotective actions in isolated mouse hearts (Fig. 7C). Taken together, these data show that the cardioprotective effects of genetic or pharmacological inhibition of DPP-4 activity are not strictly glucose dependent and depend on one or more DPP-4–dependent actions in vivo.

**DISCUSSION**

Analysis of the cardiovascular profile of antidiabetic agents involves ascertainment of the effects of each drug on the myocardium and endothelium, and on secondary risk factors such as control of blood pressure and cholesterol. Although preclinical studies may be useful in generating hypotheses about the putative cardiovascular actions of different drug classes, the results of subsequent clinical studies have not always been concordant with predictions made from preclinical analyses. For example, although both thiazolidinediones, pioglitazone and rosiglitazone, exert beneficial effects on inflammation and endothelial function (29), pioglitazone, but not rosiglitazone, is associated with reduced cardiovascular events in human studies (3,30). Similarly, although data from both preclinical (31) and clinical studies (32) suggests that metformin therapy may be cardioprotective, the mechanisms through which metformin therapy is associated with cardioprotec-
FIG. 5. Treatment of diabetic C57BL/6 mice with sitagliptin, metformin, or liraglutide leads to increased expression of cardioprotective proteins. Diabetes was induced in HFD-fed STZ-treated WT C57BL/6 mice (A). Levels of mRNA transcripts in hearts from mice were treated with HFD/STZ alone, or HFD/STZ plus sitagliptin, metformin, or liraglutide, for 1 week. Relative levels of *PI3k (B), Akt1 (C), Ho1 (D), Mmp9 (E), and Ppara (F)* were assessed by quantitative real-time PCR and normalized to levels of β-actin transcripts in the same samples. *n* = 6 per group. Western blot analysis is shown for ANP (G), AKT (H), HO-1 (I), and pGSK3β (J) using heart extracts from wild-type mice on HFD/STZ alone, or HFD/STZ plus either sitagliptin, metformin, or liraglutide. *P < 0.05, ***P < 0.001, n = 3 per treatment.*
FIG. 6. Gene and protein expression after LAD occlusion in hearts from wild-type mice treated with metformin or sitagliptin. Nondiabetic 10-week-old mice were treated with sitagliptin or metformin for 1 week (A), and cardiac levels of mRNA transcripts for Akt1 (B), PI3K (C), Hof (D), Gsk3β (E), and Ppara (F) were determined by real-time PCR, normalized to the values of Gapdh mRNA transcripts in the same sample. Western blot analysis was used to ascertain levels of pAKT (G), ANP (H), pGSK3β (I), and HO-1 (J) 5 days after LAD ligation. HSP90 was used as an internal control protein. ***P < 0.001, n = 5 mice per group.
Data are means ± SE. The 12-week-old C57Bl/6 mice treated with control, sitagliptin, or metformin for 1 week before experimental cardiac ischemic injury were subjected to high-frequency ultrasound imaging. Aorta and LV chamber dimensions were determined in a short-axis view using the M-mode method. The peak A velocity represents the maximum velocity caused by atrial contraction in late diastole. Acquisition of images and analysis of data were carried out as previously described (44,45). *P < 0.05 vs. control.

We have now investigated the cardiovascular consequences arising from genetic elimination or pharmacological inhibition of DPP-4 activity. DPP-4 has three major functions: adenosine deaminase binding, peptidease activity, and extracellular matrix binding, all of which potentially influence the activity of the immune and/or endocrine systems (34). Although DPP-4 cleaves and inactivates several cardioactive peptides, including neuropeptide Y, brain natriuretic peptide, SDP-1, and GLP-1, there is little information on the cardiovascular consequences of reduced or absent DPP-4 activity. Normoglycemic Dpp-4−/− mice exhibit normal cardiac function and structure and function in the basal state, yet increased survival after experimental MI. Whether the increased survival after LAD ligation is directly due to loss of DPP-4 activity per se in cardiomyocytes or blood vessels, or indirectly due to the subsequent upregulation of cardioprotective molecules such as GLP-1 (18) or SDF-1 (35), cannot be inferred from the present study. Zaruba et al. (35) also observed modest improvements in survival after experimental MI in Dpp-4−/− mice or in WT mice treated with a DPP-4 inhibitor, and more robust improvements in survival were observed after administration of G-CSF, findings attributed to SDF-1-dependent mobilization of cardiac stem cells. Our studies extend their observations through examination of the cardiovascular effects of DPP-4 inhibition in diabetic mice and by demonstrating that direct sitagliptin administration into the circulation of the ischemic mouse heart is not directly cardioprotective ex vivo, suggesting that acute reduction of cardiac DPP-4 activity is not sufficient to produce cardioprotection.

Our findings demonstrating that both sitagliptin and the GLP-IR agonist liraglutide upregulated levels of cardioprotective proteins in the nonischemic myocardium suggest a possible role for GLP-1 in the context of enhanced survival after DPP-4 inhibition and experimental MI. Nevertheless, we did not observe a sustained induction of cardioactive proteins in sitagliptin-treated murine hearts when the same proteins were examined after MI. Moreover, we did not detect significant changes in infarct size or cardiac function after MI that might directly account for the improved survival seen with genetic or chemical reduction of DPP-4 activity. Hence, the precise mechanisms mediating the improvements in survival observed after pharmacological treatment with sitagliptin in diabetic mice or genetic reduction of DPP-4 activity in normoglycemic Dpp-4−/− mice require further investigation, ideally through examination of whether DPP-4 inhibitors exert cardioprotective actions in GIP−/− mice.

Western blot analysis of proteins in nonischemic hearts demonstrated that both sitagliptin and metformin therapy induced an overlapping set of cardioprotective proteins. Metformin is thought to exert its cardioprotective actions through distinct mechanisms requiring activation of AMP kinase and endothelial nitric oxide (31). Intriguingly, administration of metformin has also been associated with reduction of DPP-4 activity (36) and increased circulating levels of GLP-1 in both rodent (37) and clinical studies (38), and we detected increased levels of GLP-1 in both metformin- and sitagliptin-treated mice. Accordingly, the extent to which therapy with sitagliptin and metformin produces an overlapping spectrum of actions reflecting similarities in their mechanism(s) of action through enhanced levels of GLP-1 requires further clarification.

Both metformin and sitagliptin significantly increased survival in diabetic mice, possibly due to a comparable reduction in blood glucose achieved with either agent. Hyperglycemia is a risk factor for a poor outcome after MI in humans (39), and there is considerable interest in determining whether intensive glucose control safely and consistently improves outcomes post-MI (40). Similarly, hyperglycemia is known to be associated with reduced survival and impaired LV function in mice after MI (41-43), and it seems likely that reduction in the severity of

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<td>441 ± 17</td>
<td>423 ± 19</td>
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hyperglycemia contributes to improved survival, perhaps independent of the antiadipotic mechanisms unique to each agent under study.

In contrast, the increased survival observed in normoglycemic Dpp4−/− mice after MI supports the concept that reduction of DPP-4 activity may be cardioprotective in the absence of hyperglycemia (35). Similarly, our observations demonstrating that genetic or chemical inhibition of DPP-4 is associated with enhanced recovery of LVDP in the normoglycemic ischemic murine heart ex vivo suggest that DPP-4 modifies cardiovascular outcomes independent of
glucoregulation and provide a useful model for future studies. Given the increasing interest in using strategies based on DPP-4 inhibition for the treatment of diabetes, a more detailed understanding of the role of DPP-4 in the normal and diabetic cardiovascular system is clearly warranted.

ACKNOWLEDGMENTS
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