Dipeptidyl-peptidase IV inhibition improves pathophysiology of heart failure and increases survival rate in pressure-overloaded mice

Ayako Takahashi,1,4 Masanori Asakura,2 Shin Ito,1 Kyung-Duk Min,1 Kazuhiro Shindo,1,4 Yi Yan,4 Yulin Liao,6 Satoru Yamazaki,1 Shojo Sanada,5 Yoshihiro Asano,4,5 Hatsue Ishibashi-Ueda,3 Seiji Takashima,4,5 Tetsuo Minamino,3 Hiroshi Asanuma,7 Naoki Mochizuki,1 and Masafumi Kitakaze2

1Department of Cell Biology, 2Department of Clinical Research and Development, and 3Division of Pathology, National Cerebral and Cardiovascular Center, Osaka, Japan; 4Department of Molecular Cardiology and 5Department of Cardiovascular Medicine, Osaka University Graduate School of Medicine, Osaka, Japan; 6Department of Cardiology, Nanfang Hospital, Southern Medical University, Guangzhou, China; and 7Department of Cardiology, Kyoto Prefectural University School of Medicine, Kyoto, Japan

Submitted 12 June 2012; accepted in final form 25 January 2013

Dipeptidyl-peptidase IV inhibition improves pathophysiology of heart failure; impaired glucose tolerance; dipeptidyl-peptidase IV inhibitor

HEART FAILURE (HF) is a leading cause of death in humans worldwide (1, 20, 37, 56), and is often linked to impaired glucose tolerance or diabetes mellitus (DM) (21, 53). DM is a major risk factor for cardiac dysfunction; Lind et al. (28) reported that poor glycemic control among patients with type 1 DM led to a high incidence of cardiovascular events. The energetic substrate utilization of cardiomyocytes under hypoglycemic conditions shifts from glucose to fatty acid oxidation, leading to HF (38). In DM, oxidative stress also causes endothelial dysfunction and decreases endothelial NO release, inducing microangiopathy (13, 31). Either glucose abnormalities or diabetes commonly exists in patients with HF, but as previously reported, patients with diabetes have no worse outcome of HF (50). Our previous clinical study revealed that ~90% of patients with chronic HF had impaired glucose tolerance (21).

Incretin hormones have recently been proposed as new targets for DM treatment. Glucagon-like peptide-1 (GLP-1) is an incretin hormone secreted from the lower intestines and colon, which stimulates insulin secretion from pancreatic beta cells. Its receptors are ubiquitously expressed, including in the cardiovascular system (8). GLP-1 is thought to possess cardioprotective properties because of the following three reasons: 1) GLP-1 receptors localize to cardiomyocytes and endothelial cells (3, 57); 2) activation of GLP-1 receptors increases phosphoinositide 3 (PI3)-kinase, serine/threonine protein kinase Akt (Akt), and extracellular signal-regulated kinase phosphorylation, potentially mediating cardioprotection (6, 19); and 3) activation of GLP-1 receptors stimulates p38 mitogen-activated protein (MAP) kinase and endothelial nitric oxide synthase via protein kinase A activation, putatively affecting cardioprotection (5, 59) and plasma glucose normalization (16). As dipeptidyl-peptidase IV (DPP-IV) rapidly degrades GLP-1, which has a biological half-life of approximately 1.5–5 min (11, 18), both GLP-1 analogs and DPP-IV inhibitors have been developed as new drugs to treat type 2 DM. GLP-1 analogs reportedly ameliorate not only DM but also HF and myocardial ischemia (15, 33, 34, 48, 59), suggesting that DPP-IV inhibitors function cardioprotectively. Indeed, DPP-IV inhibitors are reportedly effective against myocardial infarction in mice and pacing-induced heart failure in pigs (14, 42, 59), suggesting that DPP-IV inhibitors may also affect the survival rate. However, the effects of DPP-IV inhibitors on the pathophysiology of pressure-overloaded HF and survival after HF are unknown.

We aimed to clarify whether vildagliptin, a DPP-IV inhibitor, improves the pathophysiology of HF and increases survival rate in pressure-overloaded mice.

METHODS

All of the animal care procedures were performed according to the American Physiological Society “Guiding Principles in the Care and Use of Vertebrate Animals in Research and Training” and with the approval of the ethical committee of Osaka University.
Animal preparation. Male C57BL/6j mice (8 wk old, weighing 22–24 g) were purchased from CLEA Japan, (Tokyo, Japan). After 1 wk of observation, either transverse aortic constriction (TAC) or a sham operation was performed as previously described (24). In brief, the transverse aorta was isolated within the carotid arteries and constricted by a 7-0 silk suture ligature tied firmly against a 30-gauge needle. Sham-operated mice underwent a similar surgical procedure without aortic constriction. The needle was promptly removed, and the chest was closed with a 5-0 silk suture. Each surgical procedure was completed within 30 min to maintain the body temperature at 37°C.

Experimental protocol. Vildagliptin was gifted by Novartis Pharmaceuticals (Basel, Switzerland). The sham-operated or TAC mice were randomly divided into two subgroups; the sham-operated group (n = 10) or without vildagliptin (n = 10) and TAC with (n = 40) or without vildagliptin (n = 41). The vildagliptin treatment subgroups were provided with drinking water containing vildagliptin (10 mg·kg body wt·day−1) (39, 58), and the other groups received unsupplemented drinking water from 1 day postsurgery. The mice were allowed to drink ad libitum, and the drinking volumes were measured. The mice were fed a normal chow diet for 4 wk.

Echocardiography. Transthoracic echocardiography was performed before euthanasia as previously described (24). In brief, at 4 wk postsurgery, the mice were placed in a supine position without anesthesia. Short-axis, two-dimensional guided M-mode Doppler echocardiograms were captured and analyzed offline using a Vevo 770 High-Resolution in vivo Micro-Imaging System (VisualSonics, Toronto, Canada) equipped with a 15- to 45-MHz transducer. Left ventricular (LV) end-diastolic diameter (Dd), end-systolic diameter (Ds), and fractional shortening (FS) were measured. All measurements were made from leading edge to leading edge, according to the American Society of Echocardiography guidelines (22). Percentage FS was calculated as follows: %FS = ([LVdD − LVdV/LVdD] × 100. The investigator performing and interpreting the echocardiograms was blinded to the subgroups.

Hemodynamic assessment. To confirm pressure overload, four to five mice in each group were randomly selected for LV pressure measurement, as previously described (27). In brief, under pentobarbital anesthesia, an endotracheal tube was inserted and connected to a volume-cycled rodent ventilator. A 1.4-Fr micromanometer-tipped catheter (Millar Instruments, Houston, TX) was inserted into the right carotid artery, blood pressure and heart rate were measured simultaneously, and data were acquired using the PowerLab Data Acquisition System (AD Instruments, Bella Vista, NSW, Australia).

Analysis of intraperitoneal glucose tolerance test. Four weeks after either TAC or sham operation, about half of the surviving mice, namely five mice in each sham-operated group and 12 and 8 mice in TAC with and without vildagliptin groups, were randomly selected for an intraperitoneal glucose tolerance test following overnight fasting (12–16 h). As overnight fasting and glucose injection might be effect on additional deaths. Glucose (1 mg/kg body wt) was injected into the intraperitoneal cavity, as previously described (54). Blood was sampled from the tail prior to and at 30, 60, 90, and 120 min after glucose administration. Blood glucose concentrations were measured by a glucose meter using the glucose oxidase method (Glustest Ace R; Sanwa Kagaku Kenkyusho, Nagoya, Japan).

GLP-1 measurement [ELISA]. Four weeks after treatment, their chests were opened under anesthesia 1 h after feeding following overnight fasting for GLP-1 measurement by enzyme-linked immunosorbent assay (ELISA). Blood samples were obtained from the hearts and immediately collected in BD P700 tubes (Becton Dickinson, Franklin Lakes, NJ) containing EDTA and DPP-IV protease inhibitor cocktail. The tubes were centrifuged at 1,200 g for 10 min to extract plasma. The plasma samples were then stored at −80°C in a freezer until GLP-1 assay. Plasma GLP-1 levels were measured using a Glucagon-Like Peptide-1 (Active) ELISA Kit (Millipore, Billerica, MA) according to the manufacturer’s instructions (52). The GLP-1 ELISA measures biologically active GLP-1(7–37) and GLP-1(7–36)-NH2 but does not cross-react with glucagon, GLP-2, inactive GLP-1(9–37) or GLP-1(9–37)-NH2.

Histology and immunohistochemistry. Histological analysis was performed as previously described (25). Briefly, the surviving mice were euthanized after 4 wk of observation. The hearts were harvested, and cardiac tissues were fixed with 4% paraformaldehyde. The fixed samples were embedded in paraffin and sectioned at 4-μm thickness for picrosirius red staining. The extent of myocardial collagen was analyzed in five hearts from each group (30). The original images were digitized and transformed into binary images, and each area was calculated using ImageJ software (NIH, Bethesda, MD). The total myocardial collagen index was defined as the total area of collagen content in the entire microscopic field divided by the total connective tissue area plus the myocardial area. The terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay was performed using an ApoTag Peroxidase In Situ Apoptosis Detection Kit (Millipore), according to the manufacturer’s instructions. The number of TUNEL-positive cells was expressed as a percentage of total cells, as previously described (35).

Real-time quantitative polymerase chain reaction. Four weeks after TAC, murine ventricles were processed for total RNA isolation using TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer’s instructions. First-strand cDNA was synthesized from 1 μg total RNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). The primers and probes used to quantify transforming growth factor-β1 (Tgf-β1) and glyceraldehyde 3-phosphate dehydrogenase (Gapdh) were recommended by the manufacturer (Applied Biosystems). Real-time quantitative reverse transcriptase polymerase chain reaction (RT-PCR) was performed in a StepOne Real-Time PCR System (Applied Biosystems). From each amplification plot, a threshold cycle (Ct) value was calculated, representing the PCR cycle number at which fluorescence was detectable above an arbitrary threshold. Each sample was analyzed in duplicate, and the results were systematically normalized to GAPDH expression using the ΔΔCt method (29).

Western blot analysis. LV samples frozen at −80°C were placed on ice, homogenized, and lysed with lysis buffer [1% NP-40, 150 mM NaCl, 20 mM Tris pH 7.5, 2 mM EDTA, 50 mM NaF, 1 mM Na3VO4, plus protease inhibitor cocktail (Nacalai tesque, Kyoto, Japan)]. The supernatant was loaded onto 10%–15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels. Immunoblotting was performed as previously described (41). The ChemiDoc XRS System (Bio-Rad Laboratories, Hercules, CA) was used for chemiluminescence imaging. Primary antibodies against phospho-Smad2 (p-Smad2), p-Smad3, caspase-3, and cleaved caspase-3 primary antibodies were purchased from Cell Signaling Technology (Beverly, MA); anti-Smad2/3 primary antibody was purchased from BD Transduction Laboratories (Franklin Lakes, NJ); and anti-GAPDH (used as a loading control) primary antibody was purchased from Millipore. Target bands were identified using ECL prime and ECL Select Western blotting reagents (GE Healthcare, Little Chalfont, Buckinghamshire, UK). Protein bands were quantified by densitometry.

Statistical analysis. All of the data are expressed as means ± SE and were analyzed by repeated-measures analysis of variance (ANOVA) followed by Bonferroni test and Student’s t-test for paired and unpaired data, as appropriate. The differences in the number of surviving mice were analyzed by Kaplan-Meier method. P values of <0.05 were considered significant using JMP 8.0.1 software (SAS Institute, Cary, NC).

RESULTS

Hemodynamic measurements. The blood pressures and heart rates 4 wk after TAC were similar in the sham-operated groups with and without vildagliptin (71.2 ± 3.1 vs. 74.5 ± 3.2

Downloaded from http://ajpheart.physiology.org/ on October 15, 2017 by 10.220.33.215 on October 15, 2017

AJP-Heart Circ Physiol • doi:10.1152/ajpheart.00454.2012 • www.ajpheart.org
mmHg; 459 ± 36 vs. 451 ± 22 beats/min; P = 0.482 and P = 0.830, respectively; n = 5 in each group), and in the TAC groups with and without vildagliptin (101.4 ± 9 vs. 121.4 ± 10 mmHg; 394 ± 80 vs. 463 ± 27 beats/min; P = 0.193 and P = 0.400; n = 5 and n = 4, respectively).

Intraperitoneal glucose tolerance test and plasma GLP-1 levels. Figure 1A shows the results of the intraperitoneal glucose tolerance test. Blood glucose levels at 30 and 120 min after intraperitoneal glucose injections were higher in the TAC mice than in the sham-operated mice (TAC vs. sham operated: 476.4 ± 20.9 vs. 400.5 ± 11.2 mg/dl at 30 min, and 161.5 ± 9.4 vs. 127.3 ± 8.3 mg/dl at 120 min, n = 8 and 5; P < 0.05). This was consistent with our previous report (27) in which TAC mice exhibited impaired glucose tolerance. Vildagliptin administration decreased blood glucose levels at each time point after glucose injection in TAC mice (with vs. without vildagliptin: 345.1 ± 7.9 vs. 476.4 ± 20.9 mg/dl at 30 min, 245.5 ± 13.1 vs. 349.6 ± 25.3 mg/dl at 60 min, and 127.9 ± 8.5 vs. 161.5 ± 9.4 mg/dl at 120 min, n = 12 and 8; P < 0.05).

We evaluated the GLP-1 levels in the TAC mice with or without vildagliptin. Because ad libitum feeding could have affected the plasma GLP-1 levels, we conducted a preliminary experiment to identify the optimal conditions for GLP-1 measurement. Nine-week-old C57BL6/J mice were fed a normal chow diet, divided into two groups, and treated with or without vildagliptin for 4 wk, as described above. To evaluate whether feeding affected the plasma GLP-1 levels, the mice were fasted 12 h before blood sampling. We randomly separated each group into two subgroups; the two subgroups were fasted further, and the others were allowed to feed 1 h before sampling. Under fasting conditions, vildagliptin produced a statistically insignificant increase in GLP-1 levels (with vs. without vildagliptin: 5.19 ± 1.04 vs. 3.93 ± 0.70 pM, n = 5 each; P > 0.05). In the mice sampled 1 h after feeding, GLP-1 levels were elevated with in the vildagliptin group (with vs. without vildagliptin: 4.83 ± 0.50 vs. 1.41 ± 0.26 pM, n = 8 and 15; P < 0.05), but elevated in TAC mice with vildagliptin to the levels of sham-operated mice with vildagliptin (with vs. without vildagliptin: 9.13 ± 2.35 vs. 4.47 ± 0.87 pM, n = 4 and 5; P < 0.05), suggesting that this sampling time schedule, i.e., 1 h after food intake, was suitable for plasma GLP-1 measurement. Figure 1B shows the plasma GLP-1 levels under this time schedule. The GLP-1 levels were decreased in the TAC mice (sham operated vs. TAC: 2.37 ± 0.40 vs. 1.41 ± 0.26 pM, n = 8 and 15; P < 0.05), but elevated in TAC mice with vildagliptin to the levels of sham-operated mice with vildagliptin (with vs. without vildagliptin: 4.83 ± 0.50 vs. 1.41 ± 0.26 pM, n = 21 and 15; P < 0.05).

Echocardiography. Representative echocardiographic images are shown in Fig. 2. Echocardiographic analysis revealed enlarged Dd and Ds in the TAC mice both with and without vildagliptin (n = 27 and 17). Both LV dilatation and dysfunction in the TAC group were ameliorated by vildagliptin treatment (Fig. 3).
were examined. LVDd, LV end-diastolic dimension; LVDs, LV end-systolic dimension. Data shown are means ± SE. *P < 0.05 vs. sham operated, †P < 0.05 vs. TAC.

Water uptake and heart weight. Body weight was not statistically different between the groups: 24.3 ± 1.7 g and 23.4 ± 1.4 g in the sham-operated and TAC mice without vildagliptin (n = 10 and 17), 25.8 ± 1.7 g and 25.2 ± 2.4 g in the sham-operated and TAC mice with vildagliptin (n = 10 and 27). Heart weight-to-body weight ratio (HW/BW) markedly increased in the TAC group compared with the sham-operated mice without vildagliptin, 1.77 ± 0.4 and 1.35 ± 0.12; TAC with vildagliptin, 1.55 ± 0.27% vs. sham operated, 1.26 ± 0.12% (P < 0.05 vs. sham operated; †P < 0.05 vs. TAC; sham operated, 1.79 ± 0.22%; TAC, 12.12 ± 0.27%; TAC with vildagliptin, 8.02 ± 1.84%). We next analyzed expression of Tgf-1/ß, a fibrosis-related gene, using RT-PCR. Myocardial Tgf-1/ß expression significantly increased in the TAC group compared with that in the sham-operated group (P < 0.05 vs. sham operated) but significantly decreased in the groups with vildagliptin (P < 0.05 vs. TAC; sham operated, 1 ± 0.08; sham operated with vildagliptin, 0.98 ± 0.11; TAC, 1.85 ± 0.12; TAC with vildagliptin, 1.55 ± 0.06; Fig. 6C, n = 3 per each group).

Finally, we performed immunoblotting to verify the fibrotic changes that arose through increased levels of TGF-ß pathway proteins. We observed increased p-Smad2 and p-Smad3 protein levels in the pressure-overloaded murine hearts, which were partially restored by vildagliptin (Fig. 5, C and D, n = 4 per each group). These findings indicate that vildagliptin partly reduces myocardial apoptosis in pressure-overloaded murine hearts.

Figure 6A shows increases in myocardial collagen in the TAC group, also ameliorated by vildagliptin. Figures 6B shows fibrotic areas identified by picrosirius red staining. The total myocardial interstitial collagen area significantly increased in the TAC group compared with the sham-operated groups (P < 0.05 vs. sham operated, n = 5 per each group) but was decreased in the TAC with vildagliptin group (P < 0.05 vs. TAC) (sham operated, 1.79 ± 0.22%; sham operated with vildagliptin, 1.77 ± 0.20%; TAC, 12.12 ± 0.27%; TAC with vildagliptin, 8.02 ± 1.84%). We next analyzed expression of Tgf-1/ß, a fibrosis-related gene, using RT-PCR. Myocardial Tgf-1/ß expression significantly increased in the TAC group compared with that in the sham-operated group (P < 0.05 vs. sham operated) but significantly decreased in the groups with vildagliptin (P < 0.05 vs. TAC; sham operated, 1 ± 0.08; sham operated with vildagliptin, 0.98 ± 0.11; TAC, 1.85 ± 0.12; TAC with vildagliptin, 1.55 ± 0.06; Fig. 6C, n = 3 per each group).

Finally, we performed immunoblotting to verify the fibrotic changes that arose through increased levels of TGF-ß pathway proteins. We observed increased p-Smad2 and p-Smad3 protein levels in the pressure-overloaded murine hearts, which were partially restored by vildagliptin (Fig. 7, n = 4 per each group). These findings indicate that vildagliptin reverses myocardial fibrosis via the TGF-ß pathway in murine pressure-overloaded hearts.

Survival analysis. The number of TAC mice without vildagliptin was 41 and the number of those with vildagliptin was 40. Only 17 (41.5%) TAC mice without vildagliptin survived 28 days, whereas 27 (67.5%) TAC mice with vildagliptin survived 28 days (Fig. 8; P < 0.05). These data indicate that vildagliptin treatment is strongly protective. Vildagliptin did not affect the survival rate in the sham-operated mice.

DISCUSSION

This study was the first to demonstrate that a DPP-IV inhibitor improved survival rate in mice with pressure-overloaded HF. We presented the following experimental evidence: I) TAC exacerbated the development of impaired glucose metabolism. II) TAC exacerbated the development of myocardial fibrosis. III) TAC and vildagliptin treatment improved survival rate in mice with pressure-overload HF.
tolerance, which was attenuated by vildagliptin with an attendant increase in total GLP-1 levels; 2) TAC induced myocardial apoptosis and fibrosis, which were attenuated by vildagliptin; 3) TAC increased LVDd and LVDs, leading to FS decline, while vildagliptin attenuated increased LVDd and LVDs and increased LVFS. These effects may contribute to the improvement in survival rate generated by vildagliptin in mice with pressure overload-induced HF.

We demonstrated that TAC exacerbated the development of impaired glucose tolerance, which was attenuated by vildagliptin. This result implies that HF causes impaired glucose tolerance and improvement of impaired glucose tolerance may ameliorate HF severity. Glycemic control independently correlates with reduced LV contractile reserve and positivity for HF in diabetic patients (12, 28). We previously reported that HF is associated with impaired glucose tolerance in mice and dogs, and that correction of impaired glucose tolerance with voglibose or metformin reduces HF severity (26, 27, 41). Shimizu et al. (46) reported that systolic dysfunction induced by pressure overload exacerbates plasma glucose and hepatic insulin resistance via Akt and insulin signaling in rodents. In humans, chronic HF is associated with hyperinsulinemia (36, 51). Insulin resistance observed in HF is partly due to the lack of activity and increase in weight gain/fat redistribution. Stolen et al. (49) showed that exercise training improved insulin-stimulated myocardial glucose uptake in patients with dilated cardiomyopathy. Ashrafian et al. (2) proposed the other mechanism of HF-induced insulin resistance. Hyperadrenergic state of HF initiates the elevation of plasma free fatty acids (FFAs). The elevation of plasma FFAs induces insulin resistance due to increased triglycerides, increased cellular FFAs, and increased cytoplasmic fatty acid metabolites in hearts and skeletal muscle (43).

To our knowledge, this is the first study to evaluate an improvement in impaired glucose tolerance in animals with HF in the presence of DPP-IV inhibitors. Indeed, vildagliptin increased the plasma GLP-1 levels in animals with TAC-induced HF, suggesting that HF is attenuated by the correction...
of glucose intolerance by DPP-IV inhibitors. This hypothesis is supported by our findings that vildagliptin attenuates LV apoptosis and fibrosis in the TAC mice, which may explain the amelioration of LV dilatation and dysfunction. This evidence is consistent with previous studies in which sitagliptin was shown to attenuate HF severity induced by rapid pacing in pigs (14), ameliorate myocardial fibrosis in diabetic (db/db) mice (23), and improve diastolic dysfunction without altering ejection fraction in a rat model of uremic cardiomyopathy (9).

Intriguingly, GLP-1 reportedly has cardioprotective properties besides its ability to correct glucose intolerance in HF. GLP-1 receptors are expressed in the heart and activate PI3 kinase and Akt in addition to cyclic AMP (6, 19). Protein kinase A activation via accumulation of cyclic AMP may activate p38 MAP kinase, which may in turn mediate cardio-
Inhibitors (e.g., vesnarinone) improved pathophysiological parameters. Indeed, inotropic agents such as phosphodiesterase III inhibitors to HF patients.

HF patients (e.g., age, dyslipidemia, ischemia, etc.). Further the patients with HF, or does not mimic the background of the animal models for HF, this model is not the universal model for the HF severity with or without DM, suggesting that GLP-1 dosage was not enough to be cardioprotective. We observed that vildagliptin increased survival rate in the context of pressure overload-induced HF in mice, indicating that an adequate dose of DPP-IV inhibitors is ultimately cardioprotective against HF.

This study includes the limitations. Since TAC animals are fragile, especially when using the narrower size of needle (30 gauge) to create severe HF, the procedures of the examination such as glucose tolerance test at first, but 2 of 6 died because of the onset of acute severe HF (pulmonary edema shown by dissection). This is the reason that we shifted to the intraperitoneal glucose tolerance test, which did not cause severe HF leading to death. Importantly, the timing and number of procedures were identical in the groups with or without vildagliptin, suggesting that these additional stresses of examination to TAC does not largely affect the present results and conclusions.

In conclusion, vildagliptin, a DPP-IV inhibitor, improved the pathophysiology of HF in pressure-overloaded mice. This effect was mediated partly by improved glucose tolerance and partly by the cardioprotective effects of GLP-1, both of which ultimately improved survival following HF.

ACKNOWLEDGMENTS

We are thankful to Akiko Ogai, Chizuko Kimura, and Nobuyoshi Imai for excellent technical assistance. Vildagliptin was a gift from Novartis.

GRANTS

This work was supported by grants-in-aid from the Ministry of Health, Labor, and Welfare of Japan and the Ministry of Education, Culture, Sports, Science and Technology of Japan.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

DPP-IV INHIBITOR IMPROVES SURVIVAL IN MURINE HEART FAILURE

edited and revised manuscript; S.L., K.-D.M., Y.L., H.I.-U., and M.K. inter-
preted results of experiments; N.M. and M.K. approved final version of
manuscript.

REFERENCES

1. Abhayaratna WP, Warwick TH, Smith WT, Becker NG. Characteris-
tics of left ventricular diastolic dysfunction in the community: an echo-

2. Ashrafian H, Frenneaux MP, Opie LH. Metabolic mechanisms in heart

Cardioprotective and vasodilatory actions of glucagon-like peptide 1
receptor are mediated through both glucagon-like peptide 1 receptor-
dependent and -independent pathways. Circulation 117: 2340–2350,
2008.

Snyder K, Hilliard D, Tanaka W, Zeng W, Tamen M, Wang AQ, Chen 
L, Winchell G, Davies MJ, Ramael S, Wagner JA, Herman GA. Pharma-
cokinetic and pharmacodynamic properties of multiple oral doses of 
satiglitin, a dipeptidyl peptidase-IV inhibitor: a double-blind, random-
ized, placebo-controlled study in healthy male volunteers. Clin Ther 28: 
55–72, 2006.

5. Bhashyam S, Fields AV, Patterson B, Testani JM, Chen L, Shen YT, 
Shannon RA. Glucagon-like peptide-1 increases myocardial glucose up-
take via p38alpha MAP kinase-mediated, nitric oxide-dependent mecha-
nisms in conscious dogs with dilated cardiomyopathy. Circ Heart Fail 3: 

6. Bose AK, Mocanu MM, Carr RD, Brand CL, Yellon DM. Glucagon-
like peptide 1 can directly protect the heart against ischemia/reperfusion 

7. Brandt I, Lambeir AM, Ketelslegers JM, Vanderheyden M, Scharpe S, 
De Meester I. Dipeptidyl-peptidase IV converts intact B-type natri-

8. Bullock BP, Heller RS, Habener JF. Tissue distribution of messenger 
ribonuclease acid encoding the rat glucagon-like peptide-1 receptor. Endo-

Drewe J, Goke B, Schmidt H, Rohrer B, Lareida J, Gutzwiller JP, 
Beck T, Gottlieb SO, McGrew F, 3rd DeMets DL, White BG. Echocardiographic 
assessment of LV hypertrophy and function in aortic stenosis through 

10. Cohn JN, Goldstein SO, Greenberg BH, Lorell BH, Bourge RC, Jaski 
BE, Gottlieb SO, McGrew F, 3rd DeMets DL, White BG. Glucagon-like peptide-1 
in plasma does not reflect its long-lasting beneficial effects. Endocrinology's 
Guidelines and Standards Committee and the Chamber 
Quantification Writing Group, developed in conjunction with the Euro-
pean Association of Echocardiography, a branch of the European Society 
for chamber quantification: a report from the American Society of Echo-
cardiography's Guidelines and Standards Committee and the Chamber 
Quantification Writing Group, developed in conjunction with the Euro-
pean Association of Echocardiography, a branch of the European Society 
for chamber quantification: a report from the American Society of Echo-

Both subcutaneously and intravenously administered glucagon-like pep-
tide 1 are rapidly degraded from the NH2-terminus in type II diabetic 

12. Egstrup M, Kristorp CN, Schou M, Hofsten DE, Moller JE, Tuxen CD, 
Gustafsson I. Abnormal glucose metabolism is associated with reduced 
left ventricular contractile reserve and exercise intolerance in patients 

der Velden J, van Heerebeek L, Stienen GJ, Niessen HW, Leite-
Moreira AF, Paulus WJ. Diabetes mellitus worsens diastolic left ventricu-
lar dysfunction in aortic stenosis through altered myocardial structure 

14. Gomez N, Touihri K, Matheussen V, Mendes Da Costa A, Mahmoud-
Meester I, Bartunek J, Vanderheyden M, Meente K. Dipeptidyl-
peptidase IV inhibition improves cardiac function in overpacing-

15. Greig NH, Holloway HW, De Ore KA, Jari D, Wang Y, Zhou J, 
Garant MJ, Egli M. Once daily injection of exendin-4 to diabetic mice 
achieves long-term beneficial effects on blood glucose concentrations. 

Beglinger C. Glucagon-like peptide-1 promotes satiety and reduces food 
take in patients with diabetes mellitus type 2. Am J Physiol Regul Integ 

17. Halbirk M, Norreund H, Moller N, Holst JJ, Schmitz O, Nielsen R, 


