Recombinant Glucagon-Like Peptide-1 Increases Myocardial Glucose Uptake and Improves Left Ventricular Performance in Conscious Dogs With Pacing-Induced Dilated Cardiomyopathy

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Background—The failing heart demonstrates a preference for glucose as its metabolic substrate. Whether enhancing myocardial glucose uptake favorably influences left ventricular (LV) contractile performance in heart failure remains uncertain. Glucagon-like peptide-1 (GLP-1) is a naturally occurring incretin with potent insulinotropic effects the action of which is attenuated when glucose levels fall below 4 mmol. We examined the impact of recombinant GLP-1 (rGLP-1) on LV and systemic hemodynamics and myocardial substrate uptake in conscious dogs with advanced dilated cardiomyopathy (DCM) as a mechanism for overcoming myocardial insulin resistance and enhancing myocardial glucose uptake.

Methods and Results—Thirty-five dogs were instrumented and studied in the fully conscious state. Advanced DCM was induced by 28 days of rapid pacing. Sixteen dogs with advanced DCM received a 48-hour infusion of rGLP-1 (1.5 pmol · kg\(^{-1}\) · min\(^{-1}\)). Eight dogs with DCM served as controls and received 48 hours of a saline infusion (3 mL/d). Infusion of rGLP-1 was associated with significant (P<0.02) increases in LV dP/dt (98%), stroke volume (102%), and cardiac output (57%) and significant decreases in LV end-diastolic pressure, heart rate, and systemic vascular resistance. rGLP-1 increased myocardial insulin sensitivity and myocardial glucose uptake. There were no significant changes in the saline control group.

Conclusions—rGLP-1 dramatically improved LV and systemic hemodynamics in conscious dogs with advanced DCM induced by rapid pacing. rGLP-1 has insulinomimetic and glucagonostatic properties, with resultant increases in myocardial glucose uptake. rGLP-1 may be a useful metabolic adjuvant in decompensated heart failure. (Circulation. 2004;110:955-961.)

Key Words: cardiomyopathy • insulin • ventricles • glucose

Glucose, insulin, and potassium have been touted as useful metabolic adjuvants associated with improved regional and global left ventricular (LV) function in acute myocardial infarction.\(^1,2\) Their efficacy is based on the recognized preference of the ischemic myocardium for glucose as the metabolic substrate for oxidative phosphorylation.\(^3,4\) There is convincing evidence, both clinical\(^5-7\) and experimental,\(^8\) that the failing heart manifests a similar preference for glucose, even in the absence of ischemia. This shift is accompanied by a downregulation of cardiac metabolic pathways controlling fatty acid oxidation, such that the failing heart recapitulates a fetal phenotype at a molecular, cellular, and metabolic level.\(^9\) However, increasing evidence suggests that advanced stages of dilated cardiomyopathy (DCM) are accompanied by the development of whole-body\(^10-13\) and myocardial\(^14\) insulin resistance that may limit myocardial glucose uptake. Our laboratory has recently demonstrated that severe DCM is associated not only with myocardial insulin resistance and impaired glucose uptake\(^15\) but also with decreased myocardial ATP concentrations.\(^15,16\) Whether therapeutic strategies designed to overcome insulin resistance and enhance myocardial glucose uptake will improve cardiac performance in DCM remain untested.

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Glucagon-like peptide-1 (GLP-1) is a naturally occurring incretin that has been studied extensively in type 2 diabetes. GLP-1 is a novel insulinotropic peptide the actions of which are predicated on the ambient glucose concentration,\(^17-21\) mitigating the risks of hypoglycemia. GLP-1 has been administered as a continuous infusion in type 2 diabetic patients with impressive insulin-sensitizing effects and is associated...
with reduced insulin resistance in skeletal muscle and adipose tissue and improvements in insulin-mediated glucose uptake.\textsuperscript{19,20} Furthermore, GLP-1 can increase glucose uptake through non–insulin-dependent mechanisms.\textsuperscript{19,20}

Accordingly, the purpose of the present study was to examine the effects of a 48-hour infusion of recombinant GLP-1 (rGLP-1) on LV and systemic hemodynamics and myocardial substrate preference in conscious dogs with advanced DCM. A second goal was to examine the effects of GLP-1 on myocardial substrate uptake and the hormones that govern substrate preference in advanced DCM.

**Methods**

**Instrumentation**

Thirty-five mongrel dogs of either sex weighing 15 to 20 kg were instrumented as described previously in work from our laboratory.\textsuperscript{1,3} The dogs were allowed to recover from the surgical procedure for 2 weeks, during which time they were trained to lie quietly on the experimental table in a conscious, unrestrained state. Animals used in this study were maintained in accordance with the Guide for the Care and Use of Laboratory Animal Resources (Department of Health and Human Services publication No. [NIH] 86-23; revised 1996) and the guidelines of the Institutional Animal Care and Use Committee at Allegheny General Hospital.

**Experimental Protocol**

**Hemodynamic Measurements**

Control experiments consisted of hemodynamic recordings to determine LV contractility (LV dp/dt), stroke volume (SV), cardiac output (CO), and coronary blood flow (CBF). Arterial and coronary sinus blood samples were obtained to calculate myocardial oxygen consumption (MV\textsubscript{O}\textsubscript{2}) as the product of the left circumflex coronary artery blood flow and the myocardial arteriovenous O\textsubscript{2} content difference. Stroke work was calculated as (LV pressure−LV end-diastolic pressure)×SV×0.0136, as derived from LV pressure-volume loops. DCM was induced by rapid right ventricular pacing (240 min\textsuperscript{-1}) as described previously.\textsuperscript{15,16}

**Dose-Response Effects**

To determine whether rGLP-1 infusion was associated with short-term hemodynamic effects in conscious, chronically instrumented dogs, we conducted graded infusions at doses of 1.25, 2.5, 5, 10, and 20 pmol·kg\textsuperscript{-1}·min\textsuperscript{-1} for 10 minutes in 6 additional dogs before and after advanced DCM was induced. We compared those responses with those to a graded infusion of dobutamine (2.5, 5, 7.5, 10, and 15 μg·kg\textsuperscript{-1}·min\textsuperscript{-1}).

**Hemodynamic Effects of Continuous rGLP-1 Infusion in Conscious Dogs With Advanced DCM**

We conducted a 48-hour continuous infusion of rGLP-1 (1.5 pmol·kg\textsuperscript{-1}·min\textsuperscript{-1}, provided by D. Elahi) administered with a MiniMed pump. Rapid pacing was suspended during the 48 hours of infusion. In both groups, hemodynamics and metabolic parameters were measured before and at 1, 24, and 48 hours after rGLP-1 or saline administration.

**Metabolic Determinations**

All dogs were fed a standard diet with a fixed carbohydrate and fat content. Metabolic parameters were measured at 8 AM after an overnight fast. Transmyocardial substrate balance was calculated as the difference between arterial and coronary sinus content. Myocardial substrate extraction was calculated as the quotient of the substrate balance and the arterial content. Basal myocardial substrate uptake was calculated as the product of myocardial substrate balance and CBF.\textsuperscript{15} The measurements of plasma norepinephrine, insulin, glucagon, nonesterified (or free) fatty acids (NEFAs), lactate, and glucose were carried out as described previously from our laboratory.\textsuperscript{15}

In 5 additional dogs, myocardial insulin sensitivity was assessed (1) in the control state, (2) after development of DCM, and (3) after a 48-hour infusion of GLP-1 by the hyperinsulinemic-euglycemic clamp technique.\textsuperscript{19–20} In the fasting state, a primed, constant infusion of insulin (480 pmol·m\textsuperscript{-2}·min\textsuperscript{-1}) was administered for 120 minutes to create a steady-state concentration of plasma insulin (∼1000 pmol/L). Arterial glucose concentrations were measured every 5 minutes, and glucose was infused to maintain plasma glucose concentrations at 5 mmol/L ±10%. Myocardial glucose balance and CBF were sampled every 15 minutes to determine myocardial glucose uptake.

**Statistical Analysis**

Data are expressed as the mean±SEM. Differences in hemodynamic and metabolic responses over time between the groups were determined by repeated-measures ANOVA. A level of P<0.05 was considered statistically significant.

**Results**

**Dose-Response Effects of rGLP-1 on Resting Hemodynamics in Conscious Dogs**

Short-term administration of increasing doses of rGLP-1 (1.25 to 20 pmol·kg\textsuperscript{-1}·min\textsuperscript{-1}) in normal dogs and dogs with advanced DCM had no significant effects on LV systolic, end-diastolic, or mean arterial pressures. Figure 1 illustrates the LV dp/dt and heart rate responses to increasing doses of rGLP-1 compared with the response to intravenous dobutamine (2.5 to 15 μg·kg\textsuperscript{-1}·min\textsuperscript{-1}), a commonly used sympathomimetic amine the actions of which are desensitized in DCM. There were no significant effects of rGLP-1 on heart rate, mean arterial pressure, or LV dp/dt. In contrast, there were dose-related increases in response to dobutamine in both control and DCM dogs.

**Effects of 48-Hour Infusion of rGLP-1 in Normal, Conscious Dogs**

To determine whether there were hemodynamic effects in normal dogs after a continuous as opposed to a short-term infusion of rGLP-1, we examined the hemodynamic responses to rGLP-1 (1.5 pmol·kg\textsuperscript{-1}·min\textsuperscript{-1}), infused over 48 hours, in 5 conscious, chronically instrumented dogs before the onset of rapid pacing. There was a 10-fold increase in plasma GLP-1 levels (41±4 to 409±12 pmol/L). rGLP-1 had no effect on LV systolic (121±5 vs 117±1 mm Hg), LV end-diastolic (11±1 vs 11±1 mm Hg), or mean arterial (100±3 vs 99±2 mm Hg) pressure. Furthermore, LV dp/dt (3019±74 vs 2779±178 mm Hg/s) and CO (2.6±0.3 vs 2.1±0.4 L/min) tended to decrease, whereas systemic vascular resistance tended to increase, from 3077±234 to 3771±432 dyne·s\textsuperscript{-1}·cm\textsuperscript{-5}). There were no significant changes in plasma norepinephrine levels (0.34±0.08 to 0.45±0.10 mmol/L). Basal
myocardial glucose uptake increased from 1.3±0.6 to 5.3±0.8 μmol/min (P<0.03) during rGLP-1 infusion.

**Effects of 48-Hour Infusion of rGLP-1 in Conscious Dogs With Advanced DCM**

The data in Table 1 compare the baseline and hemodynamic perturbations associated with the development of DCM in the 16 dogs that received a continuous 48-hour infusion of rGLP-1 with those of the 8 dogs that received equal volumes of saline (3 mL/d) as a control. There were no significant differences between the 2 groups at baseline or after 28±2 days of rapid pacing.

Continuous infusion of rGLP-1 (1.5 pmol·kg⁻¹·min⁻¹) resulted in a 10-fold increase in plasma GLP-1 levels (from a baseline of 37±6 pmol/L), which was evident within 1 hour (412±21 pmol/L), and that was maintained at 24 (398±26 pmol/L) and 48 hours (387±31 pmol/L). In contrast to what was observed in normal dogs, rGLP-1 resulted in significant LV, systemic, and metabolic improvements in conscious dogs with advanced DCM. Figure 2 illustrates that rGLP-1 significantly increased LV systolic pressure by 9±2 mm Hg and the LV dP/dt by 960±47 mm Hg/s while reducing the LV end-diastolic pressure (−11±2 mm Hg) and heart rate (−34±5 min⁻¹). There were no significant changes in these parameters in the saline control group. Figure 3 demonstrates that rGLP-1 significantly increased LV ejection fraction (from 28±1% to 38±5%), SV (14±3 mL), and CO (548±39 mL/min) while lowering systemic vascular resistance (−1122±139 dyne·s·cm⁻⁵). There was no significant effect with regard to these parameters in the saline control group. The improvements in global LV performance after rGLP-1 occurred without LV remodeling (LV end-diastolic diameter).
volume before infusion, 60±3 mL; after infusion, 60±3 mL) or a change in mean arterial pressure (before infusion, 82±3 mm Hg; after infusion, 83±3 mm Hg). Figure 4 illustrates the improvement in stroke work and external efficiency after 48 hours of continuous infusion of rGLP-1. The mechanical improvements after rGLP-1 did not occur at the expense of excessive MV\(\dot{O}_2\) consumption.

Effects of rGLP-1 on Myocardial Substrate Uptake

The data in Table 2 show the effects of DCM and continuous rGLP-1 infusion on plasma levels of substrate and glucoregulatory hormones compared with those in the saline controls. Development of DCM was associated with significant increases in plasma NEFA in both groups but no change in plasma glucose or lactate concentrations. With the development of DCM, both groups manifested a 2.5-fold increase in plasma insulin, a 1.6-fold increase in plasma glucagon, and a 2- to 3-fold increase in plasma norepinephrine levels. These findings are consistent with an insulin-resistant state. Interestingly, continuous rGLP-1 infusion had no effect on plasma insulin levels, nor did the infusion suppress plasma NEFAs. However, rGLP-1 did reduce plasma glucagon levels and was associated with reduced plasma norepinephrine levels. There were no significant effects in the saline control group.

The data in Table 3 summarize the effects of rGLP-1 on basal myocardial substrate extraction and uptake. Development of DCM was associated with significant \((P<0.05)\) decreases in NEFA extraction and uptake in both groups. Myocardial lactate uptake was also reduced \((P<0.05)\) in the DCM group, whereas there was a trend toward diminished

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**Figure 2.** Effects of continuous infusion of rGLP-1 (1.5 pmol · kg\(^{-1}\) · min\(^{-1}\)) for 48 hours on LV systolic and end-diastolic pressures (EDP), LV dP/dt, and heart rate in 16 conscious dogs with advanced DCM compared with 8 conscious dogs that received equal amounts (3 mL/d) of saline as control. CHF indicates congestive heart failure. All other abbreviations are as defined in text.

**Figure 3.** Effects of continuous infusion of rGLP-1 (1.5 pmol · kg\(^{-1}\) · min\(^{-1}\)) for 48 hours on stroke volume, cardiac output, LV ejection fraction, and systemic vascular resistance in 16 conscious dogs with advanced DCM compared with 8 conscious dogs that received equal amounts (3 mL/d) of saline as control. CHF indicates congestive heart failure. All other abbreviations are as defined in text.
myocardial glucose uptake in both groups. rGLP-1 infusion significantly \((P<0.05)\) increased myocardial glucose extraction (from \(4.3\pm0.1\%\) to \(4.8\pm0.1\%\)) and uptake (from \(5.4\pm0.8\) to \(7.9\pm0.5\) \(\mu\text{mol/min}\)), whereas there was no effect in the saline control group. rGLP-1 had no significant effect on NEFA or lactate extraction. There was a trend toward increases in NEFA and lactate uptake after rGLP-1 infusion, but these did not reach statistical significance and could be attributable solely to increases in CBF (Table 3). Similar trends were seen in the control group and were likely also related to increases in CBF after cessation of rapid pacing. The increase in myocardial glucose uptake seen with rGLP-1 infusion was associated with a significant \((P<0.05)\) increase in \(\dot{MVO}_2\) that was not observed in the control group.

The data in Figure 5 depict the effects of DCM and rGLP-1 infusion on myocardial insulin sensitivity during hyperinsulinemic-euglycemic clamps. Development of DCM was associated with a marked impairment in myocardial glucose uptake compared with the same dogs studied under control conditions, consistent with myocardial insulin resistance. This state was associated with a markedly attenuated \(\dot{MVO}_2\) and a CBF response to hyperinsulinemia in DCM. rGLP-1 infusion was associated with significant increases in both basal and insulin-stimulated myocardial glucose uptake, with associated increases in \(\dot{MVO}_2\) and CBF.

### Discussion

In this investigation, we have demonstrated that a 48-hour infusion of rGLP-1 was associated with significant improvements in LV and systemic hemodynamics in conscious dogs with advanced DCM. The significant improvements were associated with increased myocardial glucose uptake and decreased plasma norepinephrine and glucagon levels. The increases in myocardial contractility and peripheral vasodilatation and the decrease in plasma hormones were not evident in normal dogs after a similar 48-hour infusion of rGLP-1.

Prior studies exploring the cardiovascular effects of GLP-1 have been limited. In vitro, GLP-1 was shown to have negative inotropic effects in rat cardiomyocytes despite increases in cAMP.\(^{22}\) In vivo, GLP-1(7–36) amide has been shown to increase heart rate and blood pressure in sedated, normal rats.\(^{23,24}\) Recently, the effects of exendin-4, a long-acting GLP-1 receptor agonist, were assessed in normal rats, in which dose-dependent increases in mean arterial pressure

### Table 2. Effects of rGLP-1 on Myocardial Substrate Availability and Glucoregulatory Hormones in DCM

<table>
<thead>
<tr>
<th>Substrate concentration</th>
<th>Control (n=6)</th>
<th>rGLP-1 (n=9)</th>
<th>DCM + rGLP-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEFAs, (\mu\text{mol/L})</td>
<td>276±76</td>
<td>601±66*</td>
<td>589±56*</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>4.6±0.7</td>
<td>4.9±0.6</td>
<td>5.0±0.4</td>
</tr>
<tr>
<td>Lactate, (\mu\text{mol/L})</td>
<td>0.8±0.1</td>
<td>0.6±0.1</td>
<td>0.7±0.2</td>
</tr>
<tr>
<td>Plasma hormones</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin, (\mu\text{mol/L})</td>
<td>30±1</td>
<td>70±5*</td>
<td>72±8*</td>
</tr>
<tr>
<td>Glucagon, pg/(\mu\text{L})</td>
<td>20±3</td>
<td>36±4*</td>
<td>31±6</td>
</tr>
<tr>
<td>Norepinephrine, (\mu\text{mol/L})</td>
<td>0.47±0.04</td>
<td>1.15±0.09*</td>
<td>1.04±0.11*</td>
</tr>
</tbody>
</table>

Abbreviations are as defined in text.

\*\(P<0.05\) compared with baseline.

†\(P<0.05\) compared with response in control.
and heart rate were noted and attributed to increased gene expression in central nervous system regions that subtend sympathetic outflow. In our dose-response studies in both normal, conscious dogs and dogs with advanced DCM, there were no short-term hemodynamic effects at doses comparable to those used in normal rodents. Furthermore, long-term rGLP-1 infusion decreased plasma norepinephrine levels in dogs with DCM. These hemodynamic and neurohumoral differences are likely attributable to the excited state of the conscious rodent, as evidenced by the significant hemodynamic effects of the vehicle. Alternatively, these discrepancies may be attributable to differences between the GLP-1 receptor agonist (exendin-4) and the peptide itself or to the action of rGLP-1 (9–36) amide, a metabolite of GLP-1. Ours is the first study to demonstrate the efficacy of rGLP-1 in a relevant, large-animal model of DCM, wherein impaired myocardial insulin responses were documented by hyperinsulenic-euglycemic clamps (Figure 5) and have been documented previously from our laboratory in association with decreased ATP levels. With this in mind, we hypothesized that rGLP-1 would improve contractility and ventricular performance by stimulating myocardial glucose uptake and oxidative phosphorylation. In our study, we measured myocardial substrate uptake but did not measure substrate oxidation. Thus, although there were significant increases in both basal and insulin-stimulated myocardial glucose uptake after rGLP-1 in DCM, we could not determine the metabolic fate of the substrates after uptake. During rGLP-1 infusion, we did observe increases in MV\(\dot{O}_2\) in association with increases in myocardial glucose uptake, consistent with increased oxidative phosphorylation. It is conceivable that the improvements in ventricular performance were attributable to peripheral effects of rGLP-1. We did observe a significant decrease in systemic vascular

The Effects of GLP-1 on Myocardial Glucose Uptake

![Graph showing the effects of GLP-1 on myocardial glucose uptake and MV\(\dot{O}_2\)](image)

**Figure 5.** Effects of hyperinsulenic-euglycemic clamps on myocardial glucose uptake, oxygen consumption, and CBF in 5 dogs studied under control conditions, after development of DCM, and after 48-hour continuous infusion of rGLP-1. Impaired myocardial glucose uptake in response to hyperinsulinemia at normal plasma glucose concentrations is consistent with myocardial insulin resistance in DCM. rGLP-1 improves myocardial glucose uptake, oxygen consumption, and CBF responses at matched levels of hyperinsulinemia, consistent with insulinomimetic effect. Abbreviations are as defined in text.
resistance and plasma norepinephrine levels. Whether these are a cause or consequence of improved ventricular performance remains to be determined. Nonetheless, the overall effects of rGLP-1 infusion in improving contractility, ventricular performance, peripheral resistance, and circulating neurohormones are all highly beneficial in advanced DCM. Furthermore, these hemodynamic benefits were not seen in normal dogs after rGLP-1 infusion, despite increases in whole-body and myocardial glucose uptake. These data suggest that rGLP-1 has unique benefits in DCM.

Although we observed increased myocardial glucose uptake in association with significant hemodynamic improvements after rGLP-1 infusion, these effects were not associated with increased plasma insulin concentrations. The absence of an insulinotropic effect of rGLP-1 is consistent with the normal plasma glucose levels in late DCM. This suggests that the mechanism of benefit may be attributable to the insulinomimetic effects of rGLP-1.19,20 These insulinomimetic effects are supported by the evidence of improved myocardial glucose uptake during matched hyperinsulinemia achieved during the clamp studies. rGLP-1 also improves skeletal muscle insulin sensitivity and increases glucose uptake in an insulin-independent fashion.19,20 These effects may be attributable to the metabolite, GLP-1(9–36) amide, which is biologically active and may increase glucose uptake, independent of increased insulin levels.26 Furthermore, the metabolite is a potent suppressor of glucagon activity.26 We did observe a significant suppression in plasma glucagon levels. Glucagon decreases fructose 2,6-biphosphate and thereby inhibits phosphofructokinase-1, the rate-limiting step in glycolysis. Thus, the rGLP-1–induced suppression of glucagon while maintaining plasma insulin levels has the synergistic effect of shifting the metabolic preference toward glycolysis. However, the precise metabolic and cellular mechanisms of rGLP-1 actions on the myocardium remain to be elucidated.

Taken together, these data demonstrate a novel metabolic approach to treating advanced DCM, with its attendant insulin resistance and preference for glucose as a substrate. In conscious dogs, the benefits include LV and systemic effects as well as metabolic and hormonal improvements. The relative contribution of myocardial metabolic effects to the overall improvement remains to be elucidated. Importantly, the salutary effects of rGLP-1 are devoid of the complexities associated with glucose-insulin-potassium infusion or hypoglycemia, rendering rGLP-1 an attractive therapeutic agent in DCM.

Acknowledgments

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References

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