Distinct hemodynamic responses to (Pyr)Apelin-13 in large animal models

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ABSTRACT

This study tested the hypothesis that (pyr)apelin-13 dose-dependently augments myocardial contractility and coronary blood flow, irrespective of changes in systemic hemodynamics. Acute effects of intravenous (pyr)apelin-13 administration (10 nM to 1,000 nM) on blood pressure, heart rate, left ventricular pressure and volume, and coronary parameters were measured in dogs and pigs. Administration of (pyr)apelin-13 did not influence blood pressure ($P = 0.59$), dP/dt-max ($P = 0.26$) or dP/dt-min (P = 0.85) in dogs. However, heart rate dose-dependently increased >70% ($P < 0.01$) which was accompanied by a significant increase in coronary blood flow ($P < 0.05$) and reductions in left ventricular end-diastolic volume and stroke volume ($P < 0.001$). In contrast, (pyr)apelin-13 did not significantly affect hemodynamics, coronary blood flow, or indices of contractile function in pigs. Further, swine studies found no effect of intracoronary (pyr)apelin-13 administration on coronary blood flow ($P = 0.83$) or vasorelaxation in isolated, endothelium-intact ($P = 0.89$) or denuded ($P = 0.38$) coronary artery rings. Examination of all data across (pyr)apelin-13 concentrations revealed an exponential increase in cardiac output as peripheral resistance decreased across pigs and dogs ($P < 0.001$; $R^2 = 0.78$). Assessment of the Frank-Starling relationship demonstrated a significant linear relationship between left ventricular end diastolic volume and stroke volume across species ($P < 0.001$; $R^2 = 0.70$). Taken together, these findings demonstrate that (pyr)apelin-13 does not directly influence myocardial contractility or coronary blood flow in either dogs or pigs.

Keywords: Apelin, inotropy, coronary blood flow, dog, pig

New and Noteworthy

Our findings provide much needed insight regarding the pharmacologic cardiac and coronary effects of (pyr)apelin-13 in larger animal preparations. In particular, data highlight distinct hemodynamic responses of apelin across species which are independent of any direct effect on myocardial contractility or perfusion.

INTRODUCTION

Apelin is an endgenous peptide and ligand for angiotensin-like 1 (APJ) receptors (6, 35, 46) which are ubiquitously expressed throughout the body (29). Several active isoforms of apelin have been identified with aminoacid numbers ranging from 36 to 12 (16). Prior evidence supports that apelin-13 and its post-transcriptionally modified pyroglutamyl form ((pyr)-apelin-13) are the predominant circulating isoforms (3, 56). The apelin/APJ system has received a lot of attention in recent years as initial studies support that this pathway normally acts to oppose actions of the renin-angiotensin system (6, 52). As such, apelin has been implicated in the pathophysiology of numerous disease states, including obesity/diabetes (21-23, 32, 54), hypertension (1, 43, 53), coronary artery disease (8, 27), cardiac hypertrophy (11), and heart failure (4, 15, 25, 52).

APJ receptor expression has been demonstrated in cardiomyocytes, endothelial and vascular smooth muscle cells and corresponds with a number of cardiovascular effects (29). In particular, apelin has been reported to induce endothelial-dependent vasodilation (4, 19, 20, 24, 25, 33, 41), reductions in blood pressure (4, 9, 25, 30, 47) and increases in myocardial contractility (2, 5, 33, 44) in both normal and failing hearts. While these salutary influences support APJ receptor agonism as a potential therapeutic modality, closer inspection of prior studies reveals conflicting results regarding the cardiovascular actions of apelin across species (16). In particular, investigations in rodent models provide strong evidence of a positive inotropic effect of apelin, including increases in fractional shortening of isolated cardiomyocytes (12, 49), elevated developed pressure in isolated, isovolumic hearts (38, 44, 51), as well as augmented stroke volume with no changes in end-diastolic volume *in vivo* (2, 5). In contrast, studies in larger species show biphasic hemodynamic responses in sheep (7) and relatively modest (~5-10%) decreases in systemic vascular resistance that are associated with comparable increases in cardiac output in healthy humans (25) and in dogs (14, 50) and humans with heart failure (4, 25). Apelin-mediated increases in coronary blood flow have also been reported in human

subjects (25). However, the extent to which these responses are mediated by direct cardiac and/or vascular effects vs. consequences of peripheral vasodilation (reductions in afterload) remains a critical gap in our understanding of the apelin/APJ pathway.

Based on prior conflicting data regarding the cardiovascular effects of apelin across species, this study was designed to examine the cardiac and coronary effects of apelin in multiple large animal species. In particular, we tested the hypothesis that (pyr)apelin-13 dose-dependently augments myocardial contractility and coronary blood flow, irrespective of changes in systemic hemodynamics, in dogs and pigs. Experiments assessed the acute effects of intravenous (pyr)apelin-13 administration (10 nM to 1,000 nM) on blood pressure, heart rate, left ventricular pressure and volume, and coronary parameters in open-chest, anesthetized animals. Additional swine studies also examined cardiovascular responses to (pyr)apelin-13 in the presence of angiotensin II, during direct intracoronary administration of apelin, and vasomotor responses in isolated coronary artery rings. Our findings provide much needed insight in to the pharmacologic cardiac and coronary effects of (pyr)apelin-13 in larger animal preparations, which more closely mimic the underlying physiologic phenotype of humans (10, 36).

METHODS

All protocols were approved by the Institutional Animal Care and Use Committee in accordance with the *Guide for the Care and Use of Laboratory Animals* (NIH Pub. No. 85-23, Revised 2011) and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Mongrel dogs were administered morphine (3 mg/kg, s.c.) as a sedative, pre-anesthetic before inducing anesthesia with α-chloralose (100 mg/kg, iv). Pigs were initially sedated with Telazol (tiletamin-zolazepam, 5mg/kg sc), xylazine (2.2mg/kg sc), and ketamine (3.0 mg/kg sc). In order to avoid potential confounding differences in estrous cycle, only male animals were studied in this investigation. Following endotracheal intubation and attainment of venous access, anesthesia was maintained in both species with morphine (3.0mg/kg sc) and α-chloralose (100mg/kg, iv). All animals were mechanically ventilated (Harvard respirator) with O_2 supplemented room air. Following completion of experimental protocols, hearts were fibrillated and excised in accordance with recommendation of the American Veterinary Medical Association Guide on Euthanasia (June 2007).

Surgical preparation. Acute *in-vivo* experiments were conducted in open chest, anesthetized dogs and pigs. Catheters were placed into the right femoral artery and vein for systemic hemodynamic measurements and administration of supplemental anesthesia, heparin and sodium bicarbonate respectively. Blood gas parameters were maintained within normal limits through periodic arterial blood gas analyses and appropriate adjustments to breathing rate and bicarbonate supplementation as necessary to maintain values within the following limits (arterial PO_2 = 185 ± 8 mmHg; arterial PCO_2 = 44 ± 2; pH = 7.40 ± 0.02; hematocrit = 36 ± 1). A left lateral thoracotomy was performed, allowing for access to the heart. The left anterior descending coronary artery (LAD) was then isolated and a perivascular flow transducer (Transonic Systems Inc.) was placed around the vessel. Following flow probe placement, a

catheter was introduced into the coronary interventricular vein for coronary venous blood sampling. A pericardial cradle was then made to allow for adequate access to the heart apex and a purse string suture was placed at the apex through which an 18 gauge needle was passed into the left ventricular cavity to allow for introduction and securing of a pressure volume admittance catheter (Transonic Systems). Placement of this catheter allowed for determination of left ventricular pressure and volume in both dog (n = 8) and in a subset of pig studies (n = 5). All data were collected using IOX acquisition software (EMKA Technologies, Falls Church VA. USA). Prior to any measurements, heparin was administered (bolus; 500 U/kg, iv) to prevent formation of blood clots during the protocol.

Experimental protocols. Following the surgical preparation a stabilization period of at least 20 min was allowed before animals received continuous, graded intravenous infusions of vehicle (Hanks' Balanced salt solution and 0.1% bovine serum albumin) or increasing concentrations of (pyr)apelin-13 (10 nM to 1,000 nM) in sequence for 5 min at each dose in dogs (n = 8) and pigs (n = 13). (Pyr)apelin-13 was prepared by peptide synthesis and appropriate molecular weight and cellular activity determined by Eli Lilly & Company. Vehicle studies were performed in a select number of dogs (n = 5) and pigs (n = 6), and animals that received vehicle infusion were also subjected to (pyr)apelin-13 administration. Similar dose-response studies to (pyr)apelin-13 (10 nM to 1,000 nM) were also performed in pigs (n = 6) following the titrated administration of intravenous angiotensin II necessary to achieve an ~25-30 mmHg increase in mean blood pressure. Hemodynamic parameters, coronary blood flow (LAD), and ECG were continuously measured throughout the entire protocol. Arterial and coronary venous blood samples were simultaneously collected (EDTA tubes + 4 µl formic acid/ml blood), immediately sealed, and placed on ice at baseline and at each dose of (pyr)apelin-13. These samples were analyzed for pH, PCO_2, PO_2, O_2 content, and hematocrit with an Instrumentation Laboratories automatic blood gas analyzer (GEM Premier 3000) and CO-oximeter (682) system. Myocardial oxygen

consumption (µl O_2/min/g) was calculated using the Fick principle as [coronary blood flow x (arterial O_2 content – coronary venous O_2 content)]. Cardiac output was determined by the product of cardiac stroke volume and heart rate (data from admittance catheter). Total peripheral resistance was determined as mean blood pressure divided by cardiac output. LAD perfusion territory was estimated to be 30% of total heart weight, as previously described by Feigl (13).

Additional experiments were also performed in a small cohort of pigs (n = 3) in which the LAD was cannulated with a stainless steel cannula connected to an extracorporeal perfusion system (28). Coronary perfusion pressure was maintained at 100 mmHg throughout the experimental protocol by a servo-controlled roller pump. Hemodynamic parameters were allowed to stabilize for ~30 min before (pyr)apelin-13 (1,000 nM) was infused directly into the LAD perfusion circuit.

Functional assessment of isolated coronary arteries. Isometric tension studies were performed on 3 mm coronary artery rings (isolated from swine) that were mounted in organ baths filled with Ca^{2+}-containing Krebs buffer (131.5 mM NaCl, 5mM KCl, 1.2 mM NaH_2PO_4, 1.2 mM $MgCl_2$, 25mM $NaHCO_3$, 10 mM glucose, 4mM $CaCl_2$) maintained at 37°C. Once stabilized at optimal passive tension (~4 g), both endothelium intact and denuded coronary arteries were pre-contracted with the thromboxane A_2 mimetic U46619 (1 µM). Vascular effects were then assessed by the addition of graded concentrations of (pyr)apelin-13 (10 nM to 1,000 nM) to the tissue bath. Following completion of the apelin dose-response curve, viability of each coronary artery ring was verified by >75% relaxation to 10 µM adenosine. Data are reported as the percentage of relaxation for arterial rings from individual animals, with 100 percent relaxation defined as the loss of all active tension developed in response to U46619.

178 *Statistical analyses.* Data are presented as mean ± SE and were analyzed using SigmaPlot 12
179 software (Systat Software Inc.). Statistical comparisons for concentrations of (pyr)apelin-13
180 were made by t-test or one-way ANOVA as appropriate. For all comparisons, $P < 0.05$ was
181 considered statistically significant. When significance was found with ANOVA, a Student-
182 Newman-Keuls multiple comparison test was performed to identify differences between
183 treatment levels. Linear regression was also used to analyze relationships between variables of
184 cardiac function.
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RESULTS

Cardiovascular effects of acute (pyr)apelin-13 administration in dogs. Initial studies revealed no effects of the vehicle infusion (n = 5) from baseline to the highest infusion rate (equivalent to 1,000 nM dose) on mean blood pressure (95 ± 6 to 97 ± 9 mmHg; $P = 0.98$), heart rate (93 ± 18 to 94 ± 16 beats/min; $P = 0.99$), coronary blood flow (0.49 ± 0.03 to 0.49 ± 0.05 ml/min/g; $P = 0.98$), or left ventricular dP/dt-max (1,976 ± 140 to 1,965 ± 81 mmHg/sec; $P = 0.87$). Intravenous infusion of (pyr)apelin-13 (n = 8) did not influence blood pressure (**Figure 1A**; $P = 0.59$), dP/dt-max (**Figure 1E**; $P = 0.26$) or dP/dt-min (**Figure 1F**; P = 0.85). However, heart rate dose-dependently increased >70% (**Figure 1B**; $P < 0.01$) which was accompanied by a significant increase in coronary blood flow (**Figure 1C**; $P < 0.05$) and reductions in left ventricular end-diastolic volume and stroke volume (**Table 1**; **Figure 3A**; $P < 0.001$). Myocardial oxygen consumption averaged 40 ± 4 μl O_2/min/g at baseline and 49 ± 5 μl O_2/min/g at the 1,000 nM dose of (pyr)apelin-13 ($P = 0.16$), while coronary venous PO_2 was unaltered (**Figure 1D**; $P = 0.91$).

Cardiovascular effects of acute (pyr)apelin-13 administration in pigs. Vehicle infusion in pigs (n = 6) also demonstrated no change in blood pressure (109 ± 11 to 108 ± 11 mmHg; $P = 0.99$), heart rate (81 ± 15 to 83 ± 15 beats/min; $P = 0.99$), coronary blood flow (0.51 ± 0.01 to 0.49 ± 0.03 ml/min/g; $P = 0.88$), or left ventricular dP/dt-max (1,381 ± 143 to 1,319 ± 158 mmHg/sec; $P = 0.98$). Systemic adminstration increased plasma apelin concentration from 0.44 ± 0.23 ng/ml at baseline to 347 ± 58 ng/ml at the 1,000 nM dose (n = 5; $P < 0.001$). These changes in circulating apelin concentration were associated with an ~12 mmHg decrease in mean blood pressure (**Figure 2A**), however this reduction did not achieve statistical significance (n = 13; $P = 0.48$). Moreover, (pyr)apelin-13 administration did not significantly affect any key cardiovascular endpoint, including heart rate (**Figure 2B**; $P = 0.94$), coronary blood flow (**Figure 2C**; $P = 0.09$), coronary venous PO_2 (**Figure 2D**; $P = 0.21$), dP/dt-max (**Figure 2E**; $P = 0.88$), or dP/dt-min

(**Figure 2F**; *P* = 0.46). Myocardial oxygen consumption averaged 59 ± 4 μl O_2/min/g at baseline and 49 ± 3 μl O_2/min/g at the 1,000 nM dose of (pyr)apelin-13 (*P* = 0.50). Left ventricular filling and output also remained unchanged (**Table 1**; **Figure 3B**). Similarly, (pyr)apelin-13 did not influence any of these variables in the presence of angiotensin II mediated increases (~25-30 mmHg) in mean blood pressure (**Table 2**).

Additional experiments to assess the coronary effects of (pyr)apelin-13 were also performed *in vivo* and *in vitro*. Direct intracoronary adminstration of 1,000 nM (pyr)apelin-13 (n = 3) did not affect coronary blood flow (**Figure 4A**; *P* = 0.83) with coronary perfusion pressure held constant at 99 ± 1 by an extracorporeal perfusion circuit. (Pyr)apelin-13 also failed to alter isometric tone of endothelium-intact (n = 4; *P* = 0.89) or denuded (n = 4; *P* = 0.38) isolated coronary arteries (**Figure 4B**).

Hemodynamic and cardiac effects of acute (pyr)apelin-13 across species. Examination of the relationship of all cardiac output and total peripheral resistance data across (pyr)apelin-13 concentrations revealed an exponential increase in cardiac output as peripheral resistance decreased across pigs (n = 5) and dogs (n = 8) (**Figure 5A**; *P* < 0.001; R^2 = 0.78). Overall, this tight, continuous relationship is determined by apelin-mediated reductions in cardiac output in dogs and a modest ~15% decrease in total peripheral resistance in swine (from 68 ± 16 mmHg/L/min at baseline to 58 ± 9 mmHg/L/min at 1,000 nM (pyr)apelin-13; *P* = 0.63). Assessment of the Frank-Starling relationship from all data points across species demonstrated a significant linear relationship between left ventricular end diastolic volume and stroke volume (**Figure 5B**; *P* < 0.001; R^2 = 0.70).

DISCUSSION

The apelin/APJ system has been the focus of extensive investigation as a potential biomarker and therapeutic for cardiovascular disease and heart failure (16, 26, 52, 53). While there is evidence to support an inotropic effect of apelin-based compounds in small animal models (2, 5, 38, 44, 51), there are, at present, a paucity of data with regard to left ventricular pressure and volume relationships in species larger than rodents. Furthermore, data from humans suggest that apelin-induced increases in cardiac output and coronary blood flow could be secondary responses related to reductions in total peripheral resistance (25). Accordingly, this study was designed to specifically test the hypothesis that (pyr)apelin-13 dose-dependently augments cardiac inotropy and coronary blood flow, irrespective of changes in systemic hemodynamics in dogs and pigs. This hypothesis was examined through a series of integrative *in vivo* and *in vitro* studies that combined assessment of systemic hemodynamics, left ventricular pressure and volume as well as coronary reactivity to (pyr)apelin-13 administration. Our findings highlight distinct hemodynamic responses across species which are independent of any direct effect of apelin on myocardial contractility or perfusion.

Hemodynamic responses to (pyr)apelin-13 across species. One of the most surprising findings of this investigation was the marked, dose-dependent increase in heart rate (> 50 beats/min) documented in response to (pyr)apelin-13 administration in dogs (**Figure 1B**). This effect does not appear to be baroreceptor-mediated as blood pressure remained unchanged in these animals (**Figure 1A**). The tachycardia produced by apelin in dogs lies in stark contrast to the majority of reported responses in other species which typically demonstrate little/no effect in rodents (2, 5, 47) and modest ~10% (~ 5-8 beat/min) increases in pigs (**Figure 2B**), sheep (7), and healthy human subjects (4, 25). However, discrepancies in hemodynamic responses to apelin have been described in relation to differences between the specific apelin peptide studied, concentration, duration, and route of apelin exposure, conscious vs. anesthetized

preparations, as well as species and underlying disease condition(s) present (16, 52, 53). Although (pyr)apelin-13 administration failed to significantly affect any hemodynamic endpoint in pigs studied in this investigation (**Figure 2**), the overall magnitude of changes in blood pressure (106 ± 6 mmHg to 95 ± 5 mmHg) and heart rate (65 ± 8 mmHg to 71 ± 7 beats/min) from baseline to the 1,000 nM dose of (pyr)apelin-13 are quite consistent with previous reported responses (~10% change) in humans (4, 25), including in the presence of renin-angiotensin system activation (**Table 2**). Furthermore, it is important to point out that our measures of plasma apelin concentration in pigs at baseline (0.44 ± 0.23 ng/ml) are quite similar to previously reported values in humans (31) and that the relatively modest cardiovascular effects occurred in response to >750-fold increase in plasma apelin levels. Mechanism underlying the distinct species dependent effects remains unclear as there are currently a lack of data regarding specific expression patterns of APJ receptor in nodal vs. myocardium.

Effects of (pyr)apelin-13 on the coronary circulation. Prior evidence supports that apelin produces vasodilation as evidenced by increases in forearm (4, 24) and coronary blood flow (25) in humans and by vasorelaxation of isolated peripheral artery rings from humans (33, 41) and rodents (19, 20, 34). In particular, Japp *et al.* demonstrated that intracoronary administration of apelin-36 (200 nmol) increased both coronary blood flow and left ventricular dP/dt max in human subjects, an index of myocardial contractiltity (25). While these data suggest apelin may have direct coronary effects, it is critical to recognize that myocardial oxygen consumption (i.e. cardiac oxygen “demand”) is the primary determinant of coronary blood flow (18). Thus, any increase in myocardial contractile function, heart rate, etc. will be closely matched by increases in coronary blood flow in order to maintain an adequate balance between myocardial oxygen delivery and metabolism (18). This point is relevant not only for the human data in the Japp *et al.* study (25) but also accounts for the significant increase in coronary blood flow in response to (pyr)apelin-13 in dogs in the present study (**Figure 1C**), in

which heart rate was augmented by >50 beats/min by apelin (**Figure 1B**). These responses are in contrast with findings from our pig study in which coronary blood flow (**Figure 2C**) tended to decrease (from 0.50 ± 0.02 ml/min/g at baseline to 0.42 ± 0.02 ml/min/g at 1,000 nM (pyr)apelin-13) in proportion to the ~10 mmHg decrease in mean blood pressure (**Figure 2A**). Furthermore, administration of a pure coronary vasodilator compound is also expected (*a priori*) to significantly increase coronary venous PO_2, thereby definitively establishing that myocardial oxygen supply has exceeded underlying myocardial oxygen demand (48). Accordingly, the lack of any change in coronary venous PO_2 in response to systemic (pyr)apelin-13 infusion in dogs (**Figure 1D**) and pigs (**Figure 2D**) argues against any direct coronary effect of acute apelin administration. This conclusion is further supported by the lack of any effect of intracoronary (pyr)apelin-13 on coronary blood flow (**Figure 4A**) or vasorelaxation in isolated coronary artery rings (**Figure 4B**). While the studies in isolated coronary arteries were not conducted in resistance vessels, these data serve as an important functional assay which argues against direct endothelial-dependent effects of apelin in the coronary circulation. Taken together, our findings support that apelin-mediated alterations in coronary blood flow occur secondary to alterations in underlying metabolic demand (e.g. heart rate, blood pressure, stroke volume), independent of any direct effect on coronary endothelium or vascular smooth muscle.

Effects of (pyr)apelin-13 on myocardial contractility. A preponderance of the literature asserts that apelin-based compounds augment myocardial contractility (2, 5, 33, 44) and proposes apelin as the most potent positive inotropic substance yet identified (44). However, while load-independent measures of contractility support this contention in rodents (2, 5, 38, 44, 51), such measurements in larger species or humans are scant. The current study provides the first systematic assessment of the dose-dependent effects of (pyr)apelin-13 on the relationship between left ventricular pressure and volume across two different large animal species, which have both been extensive utilized for cardiovascular experimentation for decades. Our findings

do not support any inotropic action of acute (pyr)apelin-13 as indices of contractile function were not significantly elevated in either dogs (**Figure 1; Table 1**) or pigs (**Figure 2; Table 1**). More importantly, plotting of the Frank-Starling relationship for all data points across species revealed a highly significant linear relationship between left ventricular stroke volume and end diastolic volume (**Figure 5B**). Thus, aside from tachycardia-mediated reductions in ventricular fillling and stroke volume in dogs (**Table 1**), (pyr)apelin-13 failed to augment stroke volume independent of changes in diastolic filling volume in either species (**Figure 5B**). Moreover, it is apparent that absolute levels of cardiac output in response to (pyr)apelin-13 are largely attributable to the underlying degree of total peripheral resistance both within and between species (**Figure 5A**). Overall, our reported changes in these key variables in response to apelin are similar in magnitude (~10% change) to previously reported effects of apelin-based compounds on systemic hemodynamics and cardiac output in humans (4, 25). Taken together, these findings indicate these effects of apelin are likely a consequence of moderate alterations in peripheral resistance and/or reductions in cardiac afterload as opposed to direct inotropic effects per se. Importantly, this possibility was addresssed in the Japp *et al.* study which documented similar hemodynamic effects of apelin and glyceryl trinitrate (peripheral vasodilator compound) in humans (25). Accordingly, we postulate that reported benefits of apelin-based compounds similar to any afterload reducing agent and as such would avoid potential complications of chronic postive inotropic therapy (17).

Summary and conclusions. Results from this series of experiments fail to support the hypothesis that (pyr)apelin-13 dose-dependently augments cardiac inotropy or coronary blood flow in either dogs or pigs. Our findings indicate that reported increases in cardiac output and coronary blood flow (in larger animal models and humans) are likely the consequence of alterations in underlying cardiac loading conditions and not the result of direct effects of apelin/APJ signaling. While these results argue against the potential for apelin-based therapies to augment

myocardial inotropy in the setting of heart failure, future efforts to delineate the mechanisms underlying the cardioprotective effects against ischemia-reperfusion injury (39, 40, 42, 55), apoptosis (45, 54), fibrosis and maladaptive remodeling (16, 37) are warranted. Furthermore, future studies to examine the role of endogenous apelin in response to patho-physiologic stimuli such as hypoxia are also needed.

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REFERENCES

1. **Akcilar R, Turgut S, Caner V, Akcilar A, Ayada C, Elmas L, and Ozcan TO**. Apelin effects on blood pressure and RAS in DOCA-salt-induced hypertensive rats. *Clin Exp Hypertens* 35: 550-557, 2013.

2. **Ashley EA, Powers J, Chen M, Kundu R, Finsterbach T, Caffarelli A, Deng A, Eichhorn J, Mahajan R, Agrawal R, Greve J, Robbins R, Patterson AJ, Bernstein D, and Quertermous T**. The endogenous peptide apelin potently improves cardiac contractility and reduces cardiac loading in vivo. *Cardiovasc Res* 65: 73-82, 2005.

3. **Azizi M, Iturrioz X, Blanchard A, Peyrard S, De Mota N, Chartrel N, Vaudry H, Corvol P, and Llorens-Cortes C**. Reciprocal regulation of plasma apelin and vasopressin by osmotic stimuli. *J Am Soc Nephrol* 19: 1015-1024, 2008.

4. **Barnes GD, Alam S, Carter G, Pedersen CM, Lee KM, Hubbard TJ, Veitch S, Jeong H, White A, Cruden NL, Huson L, Japp AG, and Newby DE**. Sustained cardiovascular actions of APJ agonism during renin-angiotensin system activation and in patients with heart failure. *Circ Heart Fail* 6: 482-491, 2013.

5. **Berry MF, Pirolli TJ, Jayasankar V, Burdick J, Morine KJ, Gardner TJ, and Woo YJ**. Apelin has in vivo inotropic effects on normal and failing hearts. *Circulation* 110: II187-193, 2004.

6. **Chandrasekaran B, Dar O, and McDonagh T**. The role of apelin in cardiovascular function and heart failure. *Eur J Heart Fail* 10: 725-732, 2008.

7. **Charles CJ, Rademaker MT, and Richards AM**. Apelin-13 induces a biphasic haemodynamic response and hormonal activation in normal conscious sheep. *J Endocrinol* 189: 701-710, 2006.

8. **Chen T, Wu B, and Lin R**. Association of apelin and apelin receptor with the risk of coronary artery disease: a meta-analysis of observational studies. *Oncotarget* 8: 57345-57355, 2017.

9. **Cheng X, Cheng XS, and Pang CC**. Venous dilator effect of apelin, an endogenous peptide ligand for the orphan APJ receptor, in conscious rats. *Eur J Pharmacol* 470: 171-175, 2003.

10. **Dixon JA, and Spinale FG**. Large animal models of heart failure: a critical link in the translation of basic science to clinical practice. *Circ Heart Fail* 2: 262-271, 2009.

11. **Falcao-Pires I, Goncalves N, Gavina C, Pinho S, Teixeira T, Moura C, Amorim MJ, Pinho P, Areias JC, and Leite-Moreira A**. Correlation between plasma levels of apelin and myocardial hypertrophy in rats and humans: possible target for treatment? *Expert Opin Ther Targets* 14: 231-241, 2010.

12. **Farkasfalvi K, Stagg MA, Coppen SR, Siedlecka U, Lee J, Soppa GK, Marczin N, Szokodi I, Yacoub MH, and Terracciano CM**. Direct effects of apelin on cardiomyocyte contractility and electrophysiology. *Biochem Biophys Res Commun* 357: 889-895, 2007.

13. **Feigl EO, Neat GW, and Huang AH**. Interrelations between coronary artery pressure, myocardial metabolism and coronary blood flow. *J Mol Cell Cardiol* 22: 375-390, 1990.

14. **Feng JH, Li WM, Wu XP, Tan XY, Gao YH, Han CL, Li SQ, and Xie HN**. Hemodynamic effect of apelin in a canine model of acute pulmonary thromboembolism. *Peptides* 31: 1772-1778, 2010.

15. **Foldes G, Horkay F, Szokodi I, Vuolteenaho O, Ilves M, Lindstedt KA, Mayranpaa M, Sarman B, Seres L, Skoumal R, Lako-Futo Z, deChatel R, Ruskoaho H, and Toth M**. Circulating and cardiac levels of apelin, the novel ligand of the orphan receptor APJ, in patients with heart failure. *Biochem Biophys Res Commun* 308: 480-485, 2003.

16. **Folino A, Montarolo PG, Samaja M, and Rastaldo R**. Effects of apelin on the cardiovascular system. *Heart Fail Rev* 20: 505-518, 2015.

17. **Ginwalla M, and Tofovic DS**. Current Status of Inotropes in Heart Failure. *Heart Fail Clin* 14: 601-616, 2018.

18. **Goodwill AG, Dick GM, Kiel AM, and Tune JD**. Regulation of Coronary Blood Flow. *Compr Physiol* 7: 321-382, 2017.

19. **Gurzu B, Petrescu BC, Costuleanu M, and Petrescu G**. Interactions between apelin and angiotensin II on rat portal vein. *J Renin Angiotensin Aldosterone Syst* 7: 212-216, 2006.

20. **Han X, Zhang DL, Yin DX, Zhang QD, and Liu WH**. Apelin-13 deteriorates hypertension in rats after damage of the vascular endothelium by ADMA. *Can J Physiol Pharmacol* 91: 708-714, 2013.

21. **Hou X, Zeng H, He X, and Chen JX**. Sirt3 is essential for apelin-induced angiogenesis in post-myocardial infarction of diabetes. *J Cell Mol Med* 19: 53-61, 2015.

22. **Hou X, Zeng H, Tuo QH, Liao DF, and Chen JX**. Apelin Gene Therapy Increases Autophagy via Activation of Sirtuin 3 in Diabetic Heart. *Diabetes Res (Fairfax)* 1: 84-91, 2015.

23. **Hu H, He L, Li L, and Chen L**. Apelin/APJ system as a therapeutic target in diabetes and its complications. *Mol Genet Metab* 119: 20-27, 2016.

24. **Japp AG, Cruden NL, Amer DA, Li VK, Goudie EB, Johnston NR, Sharma S, Neilson I, Webb DJ, Megson IL, Flapan AD, and Newby DE**. Vascular effects of apelin in vivo in man. *J Am Coll Cardiol* 52: 908-913, 2008.

25. **Japp AG, Cruden NL, Barnes G, van Gemeren N, Mathews J, Adamson J, Johnston NR, Denvir MA, Megson IL, Flapan AD, and Newby DE**. Acute cardiovascular effects of apelin in humans: potential role in patients with chronic heart failure. *Circulation* 121: 1818-1827, 2010.

26. **Japp AG, and Newby DE**. Unlocking the Therapeutic Potential of Apelin. *Hypertension* 68: 307-309, 2016.

27. **Kadoglou NP, Lampropoulos S, Kapelouzou A, Gkontopoulos A, Theofilogiannakos EK, Fotiadis G, and Kottas G**. Serum levels of apelin and ghrelin in

patients with acute coronary syndromes and established coronary artery disease--KOZANI STUDY. *Transl Res* 155: 238-246, 2010.

28. **Kiel AM, Goodwill AG, Baker HE, Dick GM, and Tune JD**. Local metabolic hypothesis is not sufficient to explain coronary autoregulatory behavior. *Basic Res Cardiol* 113: 33, 2018.

29. **Kleinz MJ, Skepper JN, and Davenport AP**. Immunocytochemical localisation of the apelin receptor, APJ, to human cardiomyocytes, vascular smooth muscle and endothelial cells. *Regul Pept* 126: 233-240, 2005.

30. **Lee DK, Saldivia VR, Nguyen T, Cheng R, George SR, and O'Dowd BF**. Modification of the terminal residue of apelin-13 antagonizes its hypotensive action. *Endocrinology* 146: 231-236, 2005.

31. **Liakos CI, Sanidas EA, Perrea DN, Grassos CA, Chantziara V, Viniou NA, Barbetseas JD, and Papadopoulos DP**. Apelin and Visfatin Plasma Levels in Healthy Individuals With High Normal Blood Pressure. *Am J Hypertens* 29: 549-552, 2016.

32. **Ma WY, Yu TY, Wei JN, Hung CS, Lin MS, Liao YJ, Pei D, Su CC, Lu KC, Liu PH, Lin CH, Chuang LM, Kao HL, Lin JW, Chuang YJ, and Li HY**. Plasma apelin: A novel biomarker for predicting diabetes. *Clin Chim Acta* 435: 18-23, 2014.

33. **Maguire JJ, Kleinz MJ, Pitkin SL, and Davenport AP**. [Pyr1]apelin-13 identified as the predominant apelin isoform in the human heart: vasoactive mechanisms and inotropic action in disease. *Hypertension* 54: 598-604, 2009.

34. **Mughal A, Sun C, and O'Rourke ST**. Activation of Large Conductance, Calcium-Activated Potassium Channels by Nitric Oxide Mediates Apelin-Induced Relaxation of Isolated Rat Coronary Arteries. *J Pharmacol Exp Ther* 366: 265-273, 2018.

35. **O'Dowd BF, Heiber M, Chan A, Heng HH, Tsui LC, Kennedy JL, Shi X, Petronis A, George SR, and Nguyen T**. A human gene that shows identity with the gene encoding the angiotensin receptor is located on chromosome 11. *Gene* 136: 355-360, 1993.

36. **Oh JG, Kho C, Hajjar RJ, and Ishikawa K**. Experimental models of cardiac physiology and pathology. *Heart Fail Rev* 2019.

37. **Pchejetski D, Foussal C, Alfarano C, Lairez O, Calise D, Guilbeau-Frugier C, Schaak S, Seguelas MH, Wanecq E, Valet P, Parini A, and Kunduzova O**. Apelin prevents cardiac fibroblast activation and collagen production through inhibition of sphingosine kinase 1. *Eur Heart J* 33: 2360-2369, 2012.

38. **Perjes A, Skoumal R, Tenhunen O, Konyi A, Simon M, Horvath IG, Kerkela R, Ruskoaho H, and Szokodi I**. Apelin increases cardiac contractility via protein kinase Cepsilon-and extracellular signal-regulated kinase-dependent mechanisms. *PLoS One* 9: e93473, 2014.

39. **Pisarenko OI, Lankin VZ, Konovalova GG, Serebryakova LI, Shulzhenko VS, Timoshin AA, Tskitishvili OV, Pelogeykina YA, and Studneva IM**. Apelin-12 and its structural analog enhance antioxidant defense in experimental myocardial ischemia and reperfusion. *Mol Cell Biochem* 391: 241-250, 2014.

40. **Rastaldo R, Cappello S, Folino A, Berta GN, Sprio AE, Losano G, Samaja M, and Pagliaro P**. Apelin-13 limits infarct size and improves cardiac postischemic mechanical recovery only if given after ischemia. *Am J Physiol Heart Circ Physiol* 300: H2308-2315, 2011.

41. **Salcedo A, Garijo J, Monge L, Fernandez N, Luis Garcia-Villalon A, Sanchez Turrion V, Cuervas-Mons V, and Dieguez G**. Apelin effects in human splanchnic arteries. Role of nitric oxide and prostanoids. *Regul Pept* 144: 50-55, 2007.

42. **Simpkin JC, Yellon DM, Davidson SM, Lim SY, Wynne AM, and Smith CC**. Apelin-13 and apelin-36 exhibit direct cardioprotective activity against ischemia-reperfusion injury. *Basic Res Cardiol* 102: 518-528, 2007.

43. **Sonmez A, Celebi G, Erdem G, Tapan S, Genc H, Tasci I, Ercin CN, Dogru T, Kilic S, Uckaya G, Yilmaz MI, Erbil MK, and Kutlu M**. Plasma apelin and ADMA Levels in patients with essential hypertension. *Clin Exp Hypertens* 32: 179-183, 2010.

44. **Szokodi I, Tavi P, Foldes G, Voutilainen-Myllyla S, Ilves M, Tokola H, Pikkarainen S, Piuhola J, Rysa J, Toth M, and Ruskoaho H**. Apelin, the novel endogenous ligand of the orphan receptor APJ, regulates cardiac contractility. *Circ Res* 91: 434-440, 2002.

45. **Tao J, Zhu W, Li Y, Xin P, Li J, Liu M, Li J, Redington AN, and Wei M**. Apelin-13 protects the heart against ischemia-reperfusion injury through inhibition of ER-dependent apoptotic pathways in a time-dependent fashion. *Am J Physiol Heart Circ Physiol* 301: H1471-1486, 2011.

46. **Tatemoto K, Hosoya M, Habata Y, Fujii R, Kakegawa T, Zou MX, Kawamata Y, Fukusumi S, Hinuma S, Kitada C, Kurokawa T, Onda H, and Fujino M**. Isolation and characterization of a novel endogenous peptide ligand for the human APJ receptor. *Biochem Biophys Res Commun* 251: 471-476, 1998.

47. **Tatemoto K, Takayama K, Zou MX, Kumaki I, Zhang W, Kumano K, and Fujimiya M**. The novel peptide apelin lowers blood pressure via a nitric oxide-dependent mechanism. *Regul Pept* 99: 87-92, 2001.

48. **Tune JD**. *Coronary Circulation*. Williston, VT: Morgan & Claypool Life Sciences, 2014.

49. **Wang C, Du JF, Wu F, and Wang HC**. Apelin decreases the SR Ca2+ content but enhances the amplitude of [Ca2+]i transient and contractions during twitches in isolated rat cardiac myocytes. *Am J Physiol Heart Circ Physiol* 294: H2540-2546, 2008.

50. **Wang M, Gupta RC, Rastogi S, Kohli S, Sabbah MS, Zhang K, Mohyi P, Hogie M, Fischer Y, and Sabbah HN**. Effects of acute intravenous infusion of apelin on left ventricular function in dogs with advanced heart failure. *J Card Fail* 19: 509-516, 2013.

51. **Wang W, McKinnie SM, Patel VB, Haddad G, Wang Z, Zhabyeyev P, Das SK, Basu R, McLean B, Kandalam V, Penninger JM, Kassiri Z, Vederas JC, Murray AG, and Oudit GY**. Loss of Apelin exacerbates myocardial infarction adverse remodeling and ischemia-reperfusion injury: therapeutic potential of synthetic Apelin analogues. *J Am Heart Assoc* 2: e000249, 2013.

52. **Wysocka MB, Pietraszek-Gremplewicz K, and Nowak D**. The Role of Apelin in Cardiovascular Diseases, Obesity and Cancer. *Front Physiol* 9: 557, 2018.

53. **Yamaleyeva LM, Shaltout HA, and Varagic J**. Apelin-13 in blood pressure regulation and cardiovascular disease. *Curr Opin Nephrol Hypertens* 25: 396-403, 2016.

54. **Zeng H, He X, Hou X, Li L, and Chen JX**. Apelin gene therapy increases myocardial vascular density and ameliorates diabetic cardiomyopathy via upregulation of sirtuin 3. *Am J Physiol Heart Circ Physiol* 306: H585-597, 2014.

55. **Zeng XJ, Zhang LK, Wang HX, Lu LQ, Ma LQ, and Tang CS**. Apelin protects heart against ischemia/reperfusion injury in rat. *Peptides* 30: 1144-1152, 2009.

56. **Zhen EY, Higgs RE, and Gutierrez JA**. Pyroglutamyl apelin-13 identified as the major apelin isoform in human plasma. *Anal Biochem* 442: 1-9, 2013.

Figure Legends

Figure 1. Effects of systemic (pyr)apelin-13 infusion on systemic hemodynamics, coronary blood flow and indices of cardiac function in dogs (n = 8). Solid circles and lines demonstrate average (mean ± SE) responses and open circles represent individual data points under each condition. * $P < 0.05$ vs. baseline.

Figure 2. Effects of systemic (pyr)apelin-13 infusion on systemic hemodynamics, coronary blood flow and indices of cardiac function in pigs (n = 13). Solid triangles and lines demonstrate average (mean ± SE) responses and open triangles represent individual data points under each condition.

Figure 3. Representative left ventricular pressure-volume loops from dogs (Panel A) and pigs (Panel B) under baseline conditions and in response to systemic administration of 1,000 nM (pyr)apelin-13.

Figure 4. Effects of intracoronary (pyr)apelin-13 (1,000 nM) on coronary blood flow in pigs (n = 3; Panel A). Solid triangles and lines demonstrate average responses and open triangles and lines represent individual responses under each condition. Response of isolated coronary arteries with endothelium intact (n = 4) and denuded (n = 4) to increasing concentrations of (pyr)apelin-13 (Panel B).

Figure 5. Effects of (pyr)apelin-13 on the relationship between cardiac output and total peripheral resistance in dogs (n = 8) and pigs (n = 5) (Panel A). Frank-Starling relationship between left ventricular stroke volume and end diastolic volume in response to (pyr)apelin-13 in dogs (n = 8) and pigs (n = 5) (Panel B).

539

540 ***Table 1.*** *Cardiac effects of (Pyr)Apelin-13 in dogs and pigs.*

	Baseline	(Pyr)Apelin-13	P value
Dogs (n = 8)			
End diastolic volume (ml)	85 ± 4	55 ± 2	$P < 0.001$
Stroke volume (ml)	38 ± 4	17 ± 2	$P < 0.001$
Cardiac output (L/min)	2.8 ± 0.3	2.2 ± 0.3	$P = 0.54$
Ejection Fraction (%)	58 ± 4	48 ± 3	$P = 0.33$
Pigs (n = 5)			
End diastolic volume (ml)	74 ± 10	67 ± 10	$P = 0.45$
Stroke volume (ml)	36 ± 5	33 ± 5	$P = 0.43$
Cardiac output (L/min)	2.0 ± 0.3	1.9 ± 0.2	$P = 0.41$
Ejection Fraction (%)	49 ± 2	50 ± 3	$P = 0.97$

541 Values are mean ± SE

542

Table 2. *Hemodynamic and cardiac effects of (Pyr)Apelin-13 in presence of angiotensin II.*

	Baseline	Baseline + Ang II	Apelin 10 nM	Apelin 100 nM	Apelin 1,000 nM	P Value
Mean Blood Pressure (mmHg)	98 ± 9	126 ± 6	121 ± 8	119 ± 7	117 ± 8	*P* = 0.12
Heart Rate (beats/min)	75 ± 15	60 ± 9	65 ± 13	68 ± 12	68 ± 12	*P* = 0.94
Coronary Blood Flow (ml/min/g)	0.48 ± 0.08	0.61 ± 0.08	0.58 ± 0.08	0.54 ± 0.08	0.54 ± 0.08	*P* = 0.85
Coronary Venous PO_2 (mmHg)	17 ± 2	17 ± 1	15 ± 1	15 ± 1	16 ± 2	*P* = 0.58
dP/dt max (mmHg/sec)	1,488 ± 149	1,938 ± 229	1,822 ± 258	1,777 ± 240	1,802 ± 261	*P* = 0.72
dP/dt min (mmHg/sec)	-1,352 ± 135	-1,526 ± 80	-1,417 ± 99	-1,391 ± 140	-1,393 ± 132	*P* = 0.87

Values are mean ± SE for n = 6 pigs

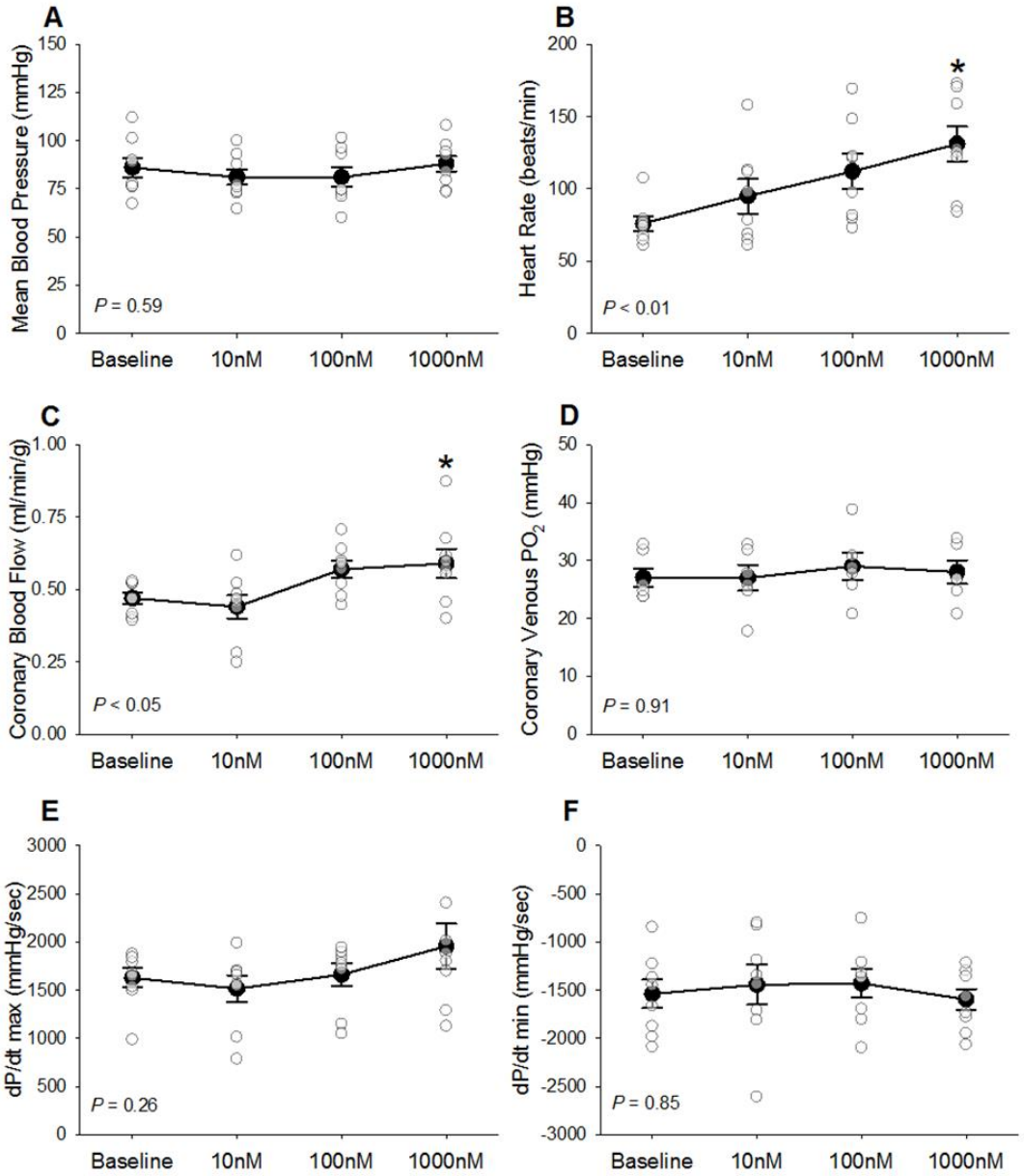
A
Mean Blood Pressure (mmHg)
150
125
100
75
50
25
0
P = 0.59
Baseline
10nM
100nM
1000nM
B
Heart Rate (beats/min)
200
150
100
50
0
*
P < 0.01
Baseline
10nM
100nM
1000nM
C
Coronary Blood Flow (ml/min/g)
1.00
0.75
0.50
0.25
0.00
*
P < 0.05
Baseline
10nM
100nM
1000nM
D
Coronary Venous PO2 (mmHg)
50
40
30
20
10
0
P = 0.91
Baseline
10nM
100nM
1000nM
E
dP/dt max (mmHg/sec)
3000
2500
2000
1500
1000
500
0
P = 0.26
Baseline
10nM
100nM
1000nM
F
dP/dt min (mmHg/sec)
0
-500
-1000
-1500
-2000
-2500
-3000
P = 0.85
Baseline
10nM
100nM
1000nM

Figure 1

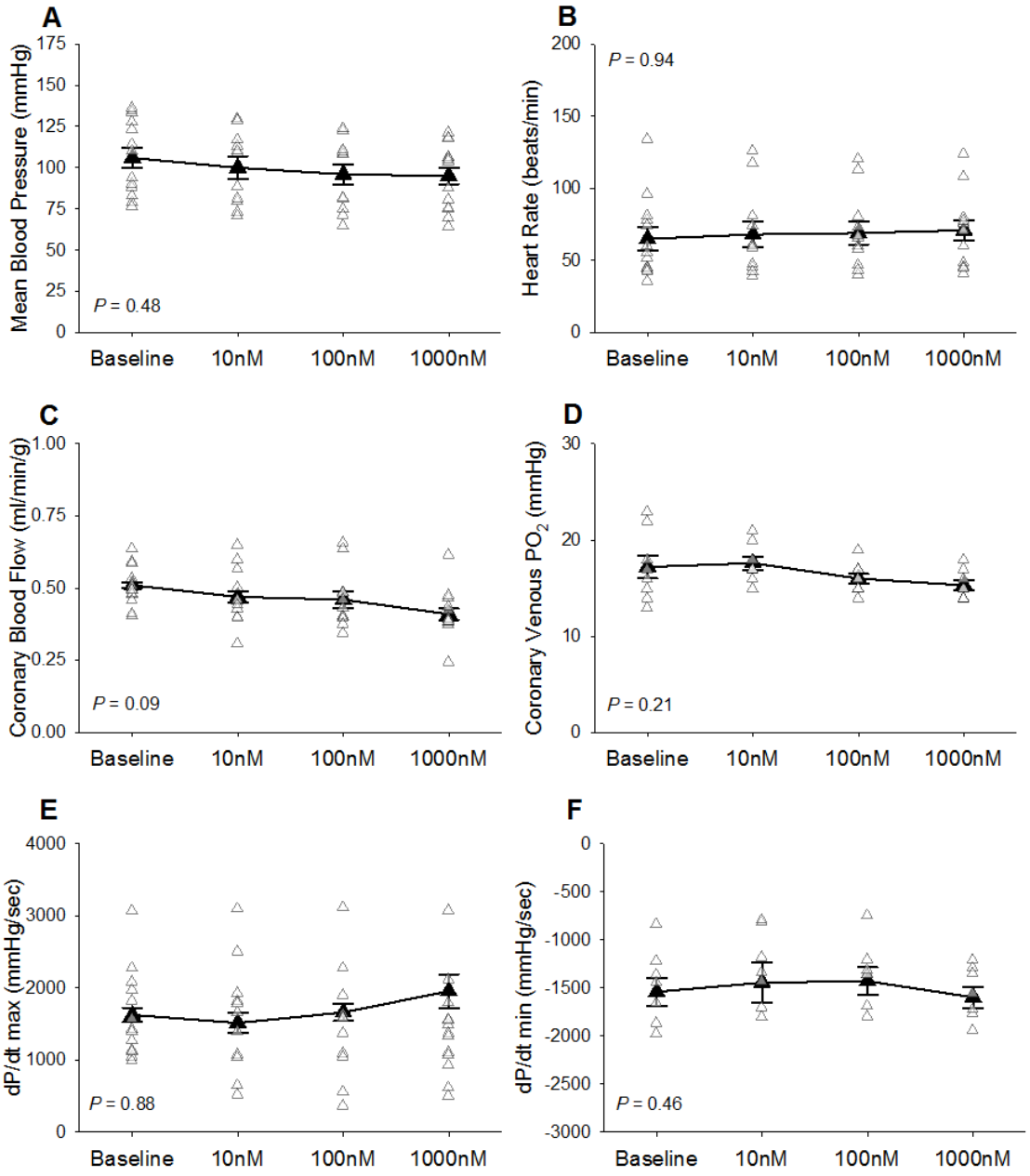
A
Mean Blood Pressure (mmHg)
175
150
125
100
75
50
25
0
P = 0.48
Baseline
10nM
100nM
1000nM
B
Heart Rate (beats/min)
200
150
100
50
0
P = 0.94
Baseline
10nM
100nM
1000nM
C
Coronary Blood Flow (ml/min/g)
1.00
0.75
0.50
0.25
0.00
P = 0.09
Baseline
10nM
100nM
1000nM
D
Coronary Venous PO2 (mmHg)
30
20
10
0
P = 0.21
Baseline
10nM
100nM
1000nM
E
dP/dt max (mmHg/sec)
4000
3000
2000
1000
0
P = 0.88
Baseline
10nM
100nM
1000nM
F
dP/dt min (mmHg/sec)
0
-500
-1000
-1500
-2000
-2500
-3000
P = 0.46
Baseline
10nM
100nM
1000nM

Figure 2

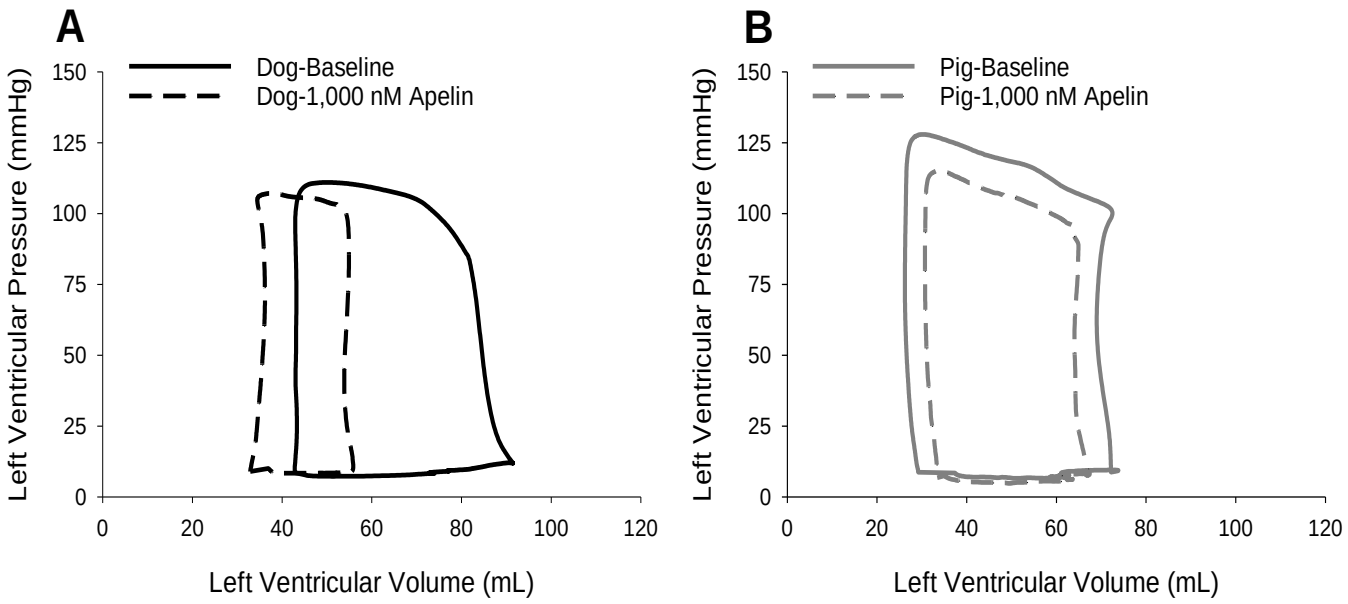
A
Dog-Baseline
Dog-1,000 nM Apelin
Left Ventricular Pressure (mmHg)
150
125
100
75
50
25
0
0
20
40
60
80
100
120
Left Ventricular Volume (mL)
B
Pig-Baseline
Pig-1,000 nM Apelin
Left Ventricular Pressure (mmHg)
150
125
100
75
50
25
0
0
20
40
60
80
100
120
Left Ventricular Volume (mL)

Figure 3

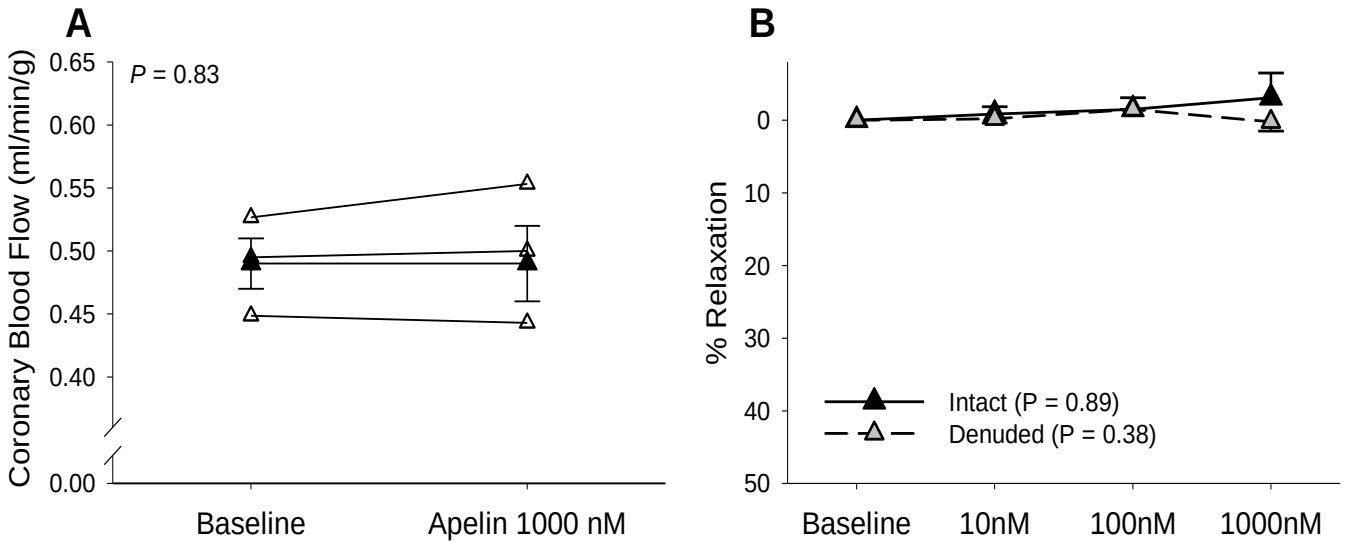
A
Coronary Blood Flow (ml/min/g)
0.65
0.60
0.55
0.50
0.45
0.40
0.00
P = 0.83
Baseline
Apelin 1000 nM
B
% Relaxation
0
10
20
30
40
50
Intact (P = 0.89)
Denuded (P = 0.38)
Baseline
10nM
100nM
1000nM

Figure 4

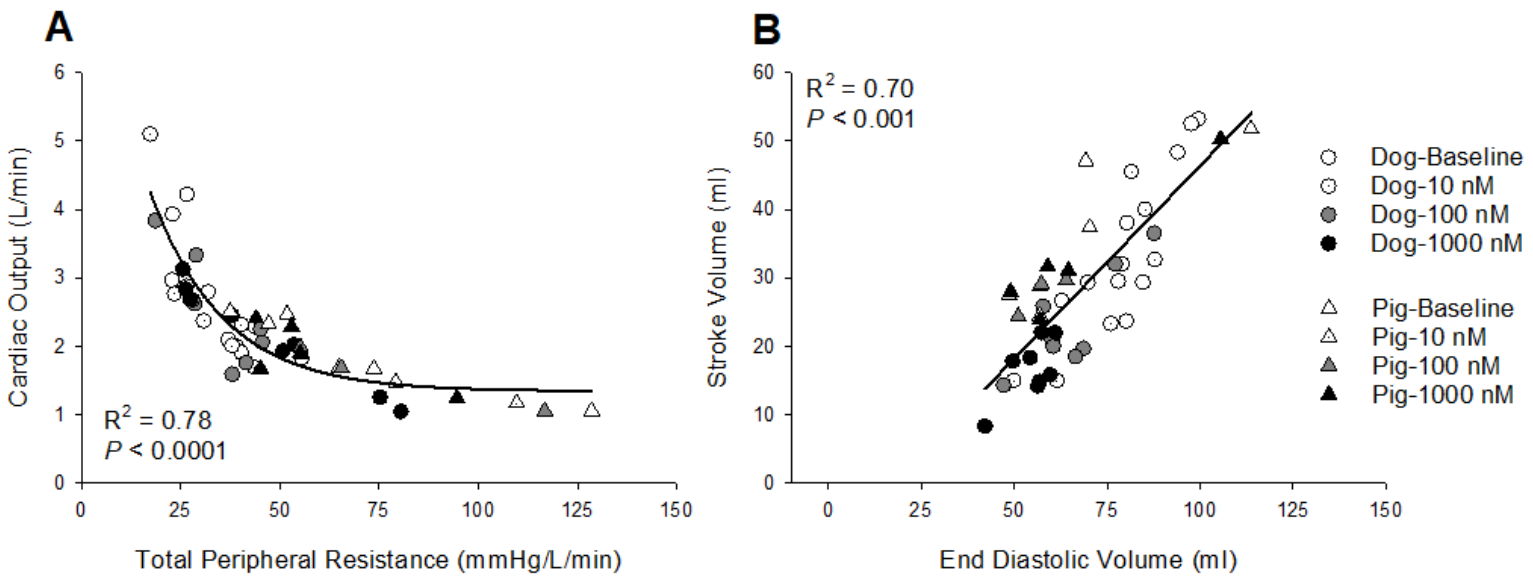
A
Cardiac Output (L/min)
Total Peripheral Resistance (mmHg/L/min)
R2 = 0.78
P < 0.0001
B
Stroke Volume (ml)
End Diastolic Volume (ml)
R2 = 0.70
P < 0.001
Dog-Baseline
Dog-10 nM
Dog-100 nM
Dog-1000 nM
Pig-Baseline
Pig-10 nM
Pig-100 nM
Pig-1000 nM

Figure 5