Sustained Cardiovascular Actions of APJ Agonism During Renin-Angiotensin System Activation and in Patients with Heart Failure

Barnes et al: Sustained APJ Agonism in RAS Activation and Heart Failure

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Abstract

Background—To assess cardiovascular actions of APJ agonism in man during prolonged (Pyr1)apelin-13 infusion and renin-angiotensin system activation.

Methods and Results—Forty-eight volunteers and twelve patients with chronic stable heart failure attended a series of randomized placebo-controlled studies. Forearm blood flow, cardiac index, left ventricular dimensions and mean arterial pressure were measured using bilateral venous occlusion plethysmography, bioimpedance cardiography, transthoracic echocardiography and sphygmomanometry respectively during brief local (0.3-3.0 nmol/min) and systemic (30-300 nmol/min) or prolonged systemic (30 nmol/min) (Pyr1)apelin-13 infusions in the presence or absence of renin-angiotensin system activation with sodium-depletion or angiotensin II co-infusion. During sodium depletion and angiotensin II co-infusions in the presence or absence of renin-angiotensin system activation with sodium-depletion or angiotensin II co-infusion, (Pyr1)apelin-13 induced vasodilatation was preserved ($P<0.02$ for both). Systemic intravenous (Pyr1)apelin-13 infusion increased cardiac index whilst reducing mean arterial pressure and peripheral vascular resistance index ($P<0.001$ for all) irrespective of sodium depletion or angiotensin II (0.5 ng/kg/min) co-infusion ($P>0.05$ for all). Prolonged 6-hour (Pyr1)apelin-13 infusion caused a sustained increase in cardiac index with increased left ventricular ejection fraction in patients with chronic heart failure (ANOVA; $P<0.001$ for all).

Conclusions—APJ agonism has sustained cardiovascular effects in man that are preserved in the presence of renin-angiotensin system activation or heart failure. APJ agonism may hold major promise to complement current optimal medical therapy in patients with chronic heart failure.

Clinical Trial Registration—URL: http://www.clinicaltrials.gov. Unique identifiers: NCT00901719, NCT00901888, NCT01049646, NCT01179061.

Key Words: apelin; AJP; human; vasodilatation; heart failure
Identified in 1993,\(^1\) the APJ receptor is widely expressed throughout the body and, in particular, on the endothelium, vascular smooth muscle cells and cardiomyocytes.\(^2\) It is a G-protein coupled receptor, remaining orphaned until its endogenous ligand, apelin, was discovered in 1998.\(^3\) Although various apelin peptide fragments exist, the pyroglutamated 13-amino acid form of apelin, (Pyr\(^1\))apelin-13, is the most potent\(^3,4\) and abundant form in cardiac tissue,\(^5\) and stimulates the APJ receptor to cause vasodilatation\(^6,7\) and positive inotropism.\(^8-11\)

A key emerging aspect of the apelin-APJ system is its interaction with the renin-angiotensin system.\(^12\) The APJ and angiotensin II type I (AT1) receptors tend to co-localise and share similar sequence homology; around 50% in the transmembrane domains. These systems mediate opposing actions on vascular tone,\(^13\) fluid regulation\(^14\) and inflammation,\(^15\) suggesting a reciprocal relationship. Transcription of apelin is reduced during angiotensin II elevation,\(^16\) whilst inhibition of angiotensin II type 1 receptor transcription increases APJ expression.\(^17\) The two receptors also have the potential to heterodimerise, altering their cellular activation pathways.\(^15\) The effects of APJ agonism may therefore be attenuated by renin-angiotensin system activation.

The apelin-APJ system is required to maintain cardiac function. Apelin-deficient mice exhibit reduced cardiac contractility with ageing and severe cardiac failure in response to increased afterload.\(^18\) Concordant with a role in maintaining normal cardiac function, ventricular APJ expression is reduced in heart failure,\(^19\) and this may theoretically limit its utility as a therapeutic target. Furthermore, in keeping with many G-protein coupled receptors, the potential for receptor desensitisation and tachyphylaxis would limit the clinical utility of any potential therapeutic strategy targeting chronic APJ receptor agonism. Exogenous apelin increases cardiac output in preclinical models of heart failure heart\(^20,21\) and patients with
chronic stable heart failure. There are limited data assessing prolonged APJ agonism and presently clinical studies in heart failure are restricted to acute infusions.

The aims of this study were to assess the cardiovascular actions of (Pyr^1)apelin-13 in a range of physiological and pathophysiological settings. Specifically, to determine the effect of renin-angiotensin system activation on the vascular and systemic actions of (Pyr^1)apelin-13, and to define the cardiovascular actions of prolonged systemic (Pyr^1)apelin-13 administration in healthy volunteers and patients with heart failure.

Methods
Study Participants
Forty-eight healthy volunteers and twelve patients with chronic stable heart failure were recruited. Healthy volunteers were not taking any regular medication and had no history of clinically significant disease and were recruited by advertisements placed throughout the University of Edinburgh campus. One apparently healthy volunteer was withdrawn having been coincidentally found to have a bicuspid aortic valve on screening. Patients were included if they had stable chronic heart failure with a fractional shortening <20%, left ventricular ejection fraction <30%, and New York Heart Association grade II-IV symptoms. Patients were maintained on maximally tolerated doses of evidenced-based therapies including renin-angiotensin and beta-adrenergic inhibitor therapy, although they abstained from their regular medications on the morning of study. Cases notes of patients known to the community heart failure service were reviewed and 38 patients were contacted as they fulfilled inclusion and exclusion criteria. Of these, 24 patients declined to participate and 2 patients were withdrawn because of inability to obtain stable bioimpedance recordings. All participants abstained from alcohol and caffeine for 24 hours, and were fasted for 4 h prior to
the study. Studies were performed in a quiet temperature-controlled room (22-25°C). The study was performed with the informed written consent of all subjects, the approval of local research ethics committee and in accordance with the Declaration of Helsinki.

**Forearm Plethysmography**

Healthy volunteers underwent brachial artery cannulation with a 27-standard-wire-gauge steel needle under controlled conditions and the rate of infusion was kept constant at 1 mL/min. Blood flow was measured at 6-min intervals throughout the study in the infused and non-infused forearms by bilateral forearm venous occlusion plethysmography using mercury-in-silastic strain gauges as described previously. Heart rate and blood pressure were monitored throughout using a semi-automated non-invasive oscillometric sphygmomanometer (HEM 705CP, Omron, Tokyo, Japan).

**Impedance Cardiography and Sphygmomanometry**

Cardiac index was measured noninvasively with thoracic bioimpedance (HOTMAN Medical, U.S.A.). At each time point, cardiac output was taken as the mean of 3 recordings, each recording representing the average of 15 consecutive heartbeats. The electrocardiogram was monitored continuously, and blood pressure and heart rate were recorded with a semi-automated non-invasive oscillometric sphygmomanometer (HEM 705CP, Omron, Tokyo, Japan).

**Echocardiography**

Echocardiography was performed using a Philips IS30 ultrasound scanner with a 3 MHz transducer. Left ventricular dimensions were assessed by 2-dimensional and M-mode
echocardiography in studies of patients with chronic heart failure. All studies were performed and analyzed by British Society of Echocardiography-accredited sonographers (SA, AW).

**Sampling and Assays**

Venous cannulae (17-gauge) were inserted into large antecubital veins of both arms to allow drug infusion and sampling of venous blood. Blood samples were drawn into ethylene diamine tetraacetic acid and centrifuged to obtain plasma that was stored at -80°C prior to analysis. Both healthy volunteers and patients voided their bladder prior to commencing all studies and urine was collected throughout the systemic studies. Urinary sodium concentration was determined using an ion selective electrode.

**Drugs**

The effects of APJ agonism were assessed with synthetic good manufacturing practice-grade (Pyr1)apelin-13 (Genscript, California, U.S.A.). (Pyr1)apelin-13, acetylcholine (Novartis AG, Basel, Switzerland), angiotensin II (Bachem, Switzerland) and sodium nitroprusside (Mayne Pharma Plc, Warwickshire, UK) were administered after dissolution in 0.9% saline (Baxter Healthcare Ltd, Thetfold, Norfolk, UK) under aseptic conditions.

**Study Design**

**Protocol 1: Sodium Depletion and Peripheral Resistance Vessels**

Twelve healthy volunteers attended on two occasions at least one week apart having been randomised in a single blind cross-over design to a sodium-deplete or normal sodium-replete diet as described previously.²⁴ Briefly, for sodium depletion, healthy volunteers were asked to adhere to a diet containing <12 mmol of sodium per day for three days prior to the study visit that they were not blinded to. To ensure prompt sodium depletion, subjects were given a
single oral dose of furosemide (20 mg) on day one of the diet. For all sodium depletion studies, urine was collected from each individual 24 h before each visit to assess sodium excretion.

After a 30-min baseline 0.9% saline infusion, forearm blood flow was assessed following intra-arterial infusion of (Pyr1)apelin-13 (0.3, 1.0 and 3.0 nmol/min), acetylcholine (5, 10 and 20 μg/min) and sodium nitroprusside (0.5, 1.0 and 2.0 μg/min) given in a double-blind randomised manner, for 6 mins at each dose.7,8 The sequence of vasodilator administration was randomised between subjects but was kept constant for each individual subject to maintain a consistent order for both study visits. As sodium nitroprusside degrades if exposed to light all infusions were covered to ensure that blinding was maintained.

**Protocol 2: Local Angiotensin II Infusion and Peripheral Resistance Vessels**

Twelve healthy volunteers attended on one occasion and local vascular responses to (Pyr1)apelin were assessed during acute intra-brachial angiotensin II infusion (Figure 1). The dose of angiotensin II (5-30 pmol/min) was up titrated until forearm blood flow was reduced by ~50%. Once sufficient and stable vasoconstriction was achieved, (Pyr1)apelin-13 (0.3, 1.0 and 3.0 nmol/min) and sodium nitroprusside (2, 4 and 8 μg/min) were co-infused with angiotensin II in a randomised double-blind manner, for 6 mins at each dose,7,8 maintained as above.

**Protocol 3: Sodium Depletion and Systemic Hemodynamics**

Following local vascular studies, the same twelve healthy volunteers progressed to systemic vascular studies (Figure 1). In a double-blind randomised cross-over manner, subjects received ascending doses of intravenous (Pyr1)apelin-13 (30, 100 and 300 nmol/min) or
matched saline placebo, administered for 10 min at each dose. (Pyr1)apelin-13 is colorless and is indistinguishable from saline in appearance, maintaining blinding. The sequence of apelin and placebo administration was randomised between subjects but was kept constant for each individual subject to maintain a consistent order for both study visits (Figure 1).

**Protocol 4: Systemic Angiotensin II Infusion and Systemic Hemodynamics**

Twelve healthy volunteers attended on a further two occasions at least one week apart (Figure 1). In a double-blind randomised crossover design, subjects received 2-hour infusions of intravenous angiotensin II at a sub-pressor dose (0.5 ng/kg/min) or matched 0.9% saline placebo as described previously. Subjects then received ascending doses of intravenous (Pyr1)apelin-13 (30, 100 and 300 nmol/min) or matched saline placebo, administered for 10 min at each dose and again given in a double-blind (maintained as above) randomised crossover manner. As before, the sequence of apelin and placebo administration was randomised between subjects but was kept constant for each individual subject to maintain a consistent order for both study visits.

**Protocol 5: Prolonged Systemic Apelin Infusion**

Twelve healthy volunteers and 12 patients with chronic heart failure attended on two occasions separated by at least one week having been randomised in a double-blind (maintained as above) cross over design to (Pyr1)apelin-13 or matched saline placebo (Figure 1). Briefly, 6-hour infusions of (Pyr1)apelin-13 or 0.9% saline placebo were commenced after a 30-min run period when all hemodynamic variables were stable (consecutive readings within 10%). Cardiac index, peripheral vascular resistance index and heart rate were assessed using thoracic cardiac bioimpedance and mean arterial pressure calculated from blood pressure using a semi-automated sphygmomanometer at 5-min intervals throughout the first
hour of study and in the second half of each subsequent hour for the following five hours.
Left ventricular end-diastolic and end-systolic diameters were assessed every 15-min
intervals for the first hour in patients with chronic heart failure.

**Data and Statistical Analysis**

Variables are reported as mean ± standard error of mean and analyzed using repeated-
measures analysis of variance (ANOVA) with post-hoc Bonferroni corrections and 2-tailed
Student’s t-test as appropriate (Graph-Pad Prism, GraphPad Software, Inc., San Diego,
California). Residual plots from ANOVA and regression models were visually inspected for
each data set acquired with no evidence of non-normality or heterogeneity of variance.
Forearm blood flow was calculated from plethysmographic data as described previously.\(^{22}\)
Mean arterial pressure was defined as the sum of the diastolic blood pressure and a third of
the pulse pressure. Peripheral vascular resistance index was calculated as mean arterial
pressure divided by cardiac index. Left ventricular ejection fraction was calculated by the
Teichholz method. Statistical significance was taken as two-sided P<0.05. Based on power
calculations derived from previous studies\(^8\) and a significance level of 5%, the sample sizes
(n=12) will give 90% power of detecting the clinically meaningful differences of 0.7 mL/100
mL/min and 0.6 L/min in forearm blood flow and cardiac output respectively. We have
previously described the influence of a range of factors on regional and systemic vascular
beds using sample sizes of 8-12 subjects.\(^{7,8,24}\) For additional detail regarding statistical and
analytic methods, please see Supplementary Materials.
Results

Study Participants

Healthy volunteers were male, aged 21±0 yr with a body mass index of 22±1 kg/m². Patients were elderly and mostly male with heart failure predominantly of ischemic origin and maintained on maximally tolerated optimal medical therapy (Table).

Infusions were well tolerated with no serious adverse events. Studies were stopped prematurely in two patients with heart failure: one patient because of persistent hypertension (systolic blood pressure >200 mmHg; not present at baseline) and the other because of acute exacerbation of long-standing back pain.

Renin-Angiotensin System Activation

Sodium Depletion

Healthy volunteers adhered to the sodium-deplete diet with a marked reduction in urinary sodium excretion (34±6 vs 175±21 mmol/day; \( P<0.0001 \)) and increases in both plasma renin activity (4.2±0.9 vs 0.8±0.2 ng/mL/hr; \( P<0.01 \)) and plasma angiotensin II concentrations (11.6±1.9 vs 5.1±1.0 pg/mL; \( P<0.01 \)) in comparison to the sodium-replete diet.

Sodium Depletion and Peripheral Resistance Vessels

There were no changes in heart rate, blood pressure or non-infused forearm blood flow throughout all studies.

Baseline forearm blood flow was unchanged by sodium-depletion (1.9±0.2 versus 1.9±0.2 mL/100 mL/min; \( P>0.05 \)). Both (Pyr¹)apelin-13 and sodium nitroprusside caused vasodilatation in the infused arm (\( P\leq 0.001 \) for all) and these responses were similar under
both dietary conditions ($P>0.05$ for sodium-deplete versus sodium-replete diet; Figure 2A).

Acetylcholine caused dose-dependent vasodilatation under both dietary conditions ($P<0.001$ for all) that was attenuated, but not abolished, during sodium depletion ($P<0.001$, sodium-deplete versus sodium-replete diet; Figure 2A).

**Local Angiotensin II Infusion and Peripheral Resistance Vessels**

Angiotensin II was infused at a mean rate of $29\pm4$ ng/min with resultant increase in local angiotensin II concentrations (95.6±16.0 versus 4.5±1.0 pg/mL; $P<0.0001$) and reduced blood flow (from 2.5±0.4 to 1.3±0.3 mL/100 mL/min; $P=0.001$) in the infused forearm. In the setting of local vasoconstriction, both (Pyr1)apelin-13 ($P=0.02$) and sodium nitroprusside ($P<0.0001$) caused vasodilatation (Figure 2B).

**Sodium Depletion and Systemic Hemodynamics**

Compared to matched placebo, intravenous (Pyr1)apelin-13 infusion increased cardiac index ($P<0.001$ for both), reduced mean arterial pressure ($P<0.02$ for both) and decreased peripheral vascular resistance index ($P<0.001$ for both) with no differences between the sodium-deplete and sodium-replete diets ($P>0.05$ for all; Figure 3). A trend to increased heart rate was evident following the sodium-replete diet ($P=0.06$) that was not present during sodium depletion. In keeping with our previous work,$^8$ (Pyr1)apelin-13 rapidly achieved its peak systemic hemodynamic effects.

**Systemic Angiotensin II Infusion and Systemic Hemodynamics**

Intravenous angiotensin II infusion (0.5 ng/kg/min) increased systemic angiotensin II concentrations (from 3.7±0.4 to 7.3±0.8 pg/mL; $P=0.001$). Although it was our intension to administer a sub-pressor dose of angiotensin II,$^25$ we observed a small increase in both mean
arterial pressure (from 82±1 to 86±1 mmHg; \(P=0.0014\)) and peripheral vascular resistance index (from 1420±80 to 1529±91 dyne·s·cm\(^{-5}\)·m\(^{-2}\); \(P<0.0001\)) with a small reduction in cardiac index (from 4.6±0.2 to 4.4±0.2 L/min/m\(^2\); \(P=0.0288\)) during angiotensin II infusion. Compared to placebo, angiotensin II infusion had no effect on the increase in cardiac index or the reduction in mean arterial pressure or peripheral vascular resistance index observed during systemic (Pyr\(^1\))apelin-13 infusion (ANOVA; \(P>0.05\), Figure 4). There appeared to be an initial increase in heart rate following apelin infusion (ANOVA; \(P<0.02\)) during angiotensin II co-infusion although this was not significant with the placebo infusion.

**Prolonged Systemic Apelin Infusion**

**Healthy Volunteers**

Compared to placebo, (Pyr\(^1\))apelin-13 caused an increase in cardiac index during the first hour (ANOVA; \(P<0.0001\)) that was sustained throughout the 6-hour infusion (ANOVA; \(P<0.0001\)). There was an apparent increase in heart rate during the first hour (ANOVA; \(P=0.11\)) that became statistically significant during the remaining 5 hours of infusion (ANOVA; \(P=0.0002\)). Peripheral vascular resistance index was reduced during the first hour of infusion (ANOVA; \(P<0.0001\)) although this was not sustained by the end of infusion (ANOVA; \(P=0.12\)). Mean arterial pressure was unchanged throughout the (Pyr\(^1\))apelin-13 infusion (Figure 5) and there were no changes in urinary volume or sodium excretion (Students \(t\)-test; \(P>0.05\))

**Patients with Chronic Heart Failure**

Intravenous (Pyr\(^1\))apelin-13 infusion increased cardiac index during the first hour (ANOVA; \(P=0.003\)) and this was sustained throughout the 6-hour infusion (ANOVA; \(P=0.0003\)). Both mean arterial pressure and peripheral vascular resistance index were reduced during the...
initial hour of infusion (ANOVA; \(P=0.008\) and \(P=0.0001\) respectively) and this was maintained during the 6-hour infusion (ANOVA; \(P=0.007\) and \(P=0.002\) respectively). There was an apparent trend for an increase in heart rate during the first hour of infusion (ANOVA; \(P=0.051\)) although this did not persist or reach statistical significance during the 6-hour infusion (ANOVA; \(P=0.42\): Figure 6) and there were no changes in urinary volume or sodium excretion (Students \(t\)-test; \(P>0.05\)). Fractional shortening and left ventricular ejection fraction were markedly increased following apelin infusion (ANOVA; \(P<0.0001\): Figure 7).

Discussion

In these randomized clinical studies, the \textit{in vivo} cardiovascular effects of APJ agonism with (Pyr\textsuperscript{1})apelin-13 have been assessed in a range of complementary physiologic and pathophysiologic contexts. We have demonstrated that APJ agonism induces peripheral vasodilatation and increased cardiac output in both healthy volunteers and patients with chronic heart failure that is unaltered by renin-angiotensin system activity. In patients with heart failure, we present novel data showing that apelin causes sustained hemodynamic benefits associated with improved left ventricular performance. These studies suggest that therapeutic strategies targeting APJ agonism hold major promise to complement current optimal medical therapy in patients with chronic heart failure.

Increasing evidence suggests interaction between the APJ-apelin and renin-angiotensin systems. Transcription of apelin is reduced during angiotensin II elevation,\textsuperscript{16} whilst inhibition of angiotensin II type 1 receptor transcription results in increased APJ expression.\textsuperscript{17} In APJ-deficient animal models, there is an exaggerated pressor response to angiotensin II whilst double AT\textsubscript{1} and APJ knock out animals have elevated blood pressure relative to AT\textsubscript{1} deficient mice.\textsuperscript{26} Moreover, angiotensin II-induced myocardial fibrosis can be prevented by
apelin through inhibition of plasminogen activator inhibitor type-1 production.\textsuperscript{27} Finally there is \textit{in vitro} evidence of heterodimer formation by the APJ and angiotensin II type 1 receptors with consequent alteration of downstream signaling from each receptor.\textsuperscript{15} These findings suggest an important interaction between these hormonal systems. However, we have demonstrated that the hemodynamic cardiovascular effects of apelin are preserved despite achieving a range of plasma angiotensin II concentrations using different methods of local and systemic renin-angiotensin system activation. This suggests that there is no major functional interaction \textit{in vivo} in man although we cannot exclude interactions in other settings.

We have used different methods to activate the renin-angiotensin system. The application of a sodium-deplete diet increased plasma renin activity, with corresponding fall in urinary sodium excretion, which more than doubled plasma angiotensin II concentrations. Equally, angiotensin II infusions resulted in 21-fold and 2-fold increases in local and systemic plasma angiotensin II concentrations respectively that resulted in effects on both local and systemic hemodynamic variables. Thus, in all phases of the study, we successfully achieved a range of renin-angiotensin system activation and do not believe that the lack of effect is due to a failure to augment plasma angiotensin II concentrations.

We acknowledge that dietary modification will impact on other hormone systems, such as vasopressin, especially given its reciprocal relationship with apelin.\textsuperscript{28} Equally, infusion of angiotensin II may impact on other hormone systems that could possibly confound our findings. However, \textit{in vivo} in man, the ability to assess elevated angiotensin II in isolation is limited and alterations in the renin-angiotensin system will inevitably activate other neurohumoral systems. Furthermore, diseases states that have increased renin-angiotensin
activity, such as heart failure, will have corresponding alterations in other neurohumoral systems.

In contrast to apelin, acetylcholine-induced vasodilatation was impaired following sodium-depletion and renin-angiotensin system activation suggesting the presence of endothelial dysfunction. *In vitro* angiotensin II promotes superoxide anion generation when incubated with vascular smooth muscle cells.\(^29\) Furthermore in whole animals treated with angiotensin II, superoxide generation is increased and vasorelaxation to acetylcholine is impaired.\(^30\) The effect of dietary sodium on endothelial function has previously been assessed in both healthy individuals and patient populations leading to diverse findings. Some report the induction of vascular dysfunction with sodium-loading\(^31\) and no effect of sodium depletion,\(^32,33\) most likely reflecting different cohorts and protocols.

Apelin is the most potent *in vitro* inotrope, being effective at subnanomolar concentrations. Whilst the cellular mechanisms mediating its inotropic effects are not fully characterized, sodium-hydrogen channel activation, with resultant increase in pH and increase myofilament calcium sensitivity, are important\(^10\) in addition to increased intracellular calcium concentrations. Apelin-APJ expression and plasma apelin concentrations\(^34\) are reduced in heart failure. Animal models demonstrate reduced ventricular APJ receptor expression in heart failure,\(^16\) and this is consistent with expression studies in human myocardium.\(^19\) There are concerns that myocardial APJ receptor density in heart failure will be insufficient to evoke a response that does not readily saturate. Furthermore preclinical data demonstrate that surface APJ receptor activation leads to its rapid internalisation to perinuclear compartments.\(^35\) Additionally, acute (Pyr\(^1\))apelin infusion exhibits a prompt plateau in the dose-response relationship.\(^7,8\) Taken together, this could suggest rapid receptor
desensitization and tachyphylaxis: a common feature of other G-protein coupled receptor signaling pathways.\textsuperscript{36} Our study demonstrated persistent hemodynamic effects throughout a 6-hour infusion, suggesting sustained cardiovascular effects without evidence of major tachyphylaxis. Indeed, the response in patients with heart failure is of comparable magnitude to that observed in healthy volunteers. Moreover, we demonstrate marked improvements in echocardiographic-derived measures of left ventricular performance suggesting a direct effect on myocardial contractility. However, it is difficult to delineate whether this resulted from direct inotropic effects or a response to peripheral vasodilatation. Our previous data from studies of acute peripheral, systemic and intracoronary apelin infusions in man suggest that both effects are likely to be significant.\textsuperscript{8} Whatever the mechanism, the observed improvement in indices of left ventricular function with apelin infusion in the context of left ventricular systolic impairment are extremely encouraging. To address load-independent effects on contractility would be challenging and require highly invasive techniques including insertion of left ventricular conductance catheters and inferior vena caval obstruction.

Although there are well established effective medications in heart failure that improve patient outcome,\textsuperscript{37, 38} mortality and economic burden remains unacceptably high. As such, there is a need to improve current therapy further and we believe that apelin may have such a role. The emerging data from preclinical studies support a causative role in the pathogenesis of heart failure and APJ agonism can restore activity of a system that directly contributes to ventricular function. Current pharmacological interventions largely target maladaptive neurohumoral responses whereas apelin may present an exciting opportunity to further optimize endogenous adaptive responses. The combination of increased cardiac output with minimal changes in mean arterial pressure (peak reduction of 4 mmHg in our studies) or heart rate would be a highly attractive addition to the current armamentarium of therapy for
chronic heart failure. For many clinicians, the up titration of current therapies, or the addition of new therapies, is commonly hindered by concerns over hypotension and further vasodilatation. The cardiovascular profile of apelin makes it a very attractive potential novel candidate that could be added to current optimal therapy without concerns over severe hypotension or reflex tachycardia. We would like to explore the effects of more prolonged APJ agonism over several days, weeks or months. However, there are currently no available oral or parental preparations that can easily deliver prolonged APJ agonism to assess the potential sustained therapeutic benefits of this intervention in chronic heart failure.

Whilst we believe that the hemodynamic profile of apelin would be beneficial, there are obvious concerns with any positive inotrope in the setting of chronic heart failure. Previous agents have caused significant increases in mortality that has been attributed to sudden arrhythmic death. In our limited early studies, we have not seen any pro-arrhythmic effect but there remains the possibility that long-term APJ agonism will have this undesired effect on the myocardium.

In conclusion, we have shown that the local and systemic cardiovascular effects of apelin are sustained and not altered by renin-angiotensin activation or heart failure. We believe that this system warrants further assessment as a novel therapy in this important area that continues to be associated with major morbidity and mortality.

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Disclosures

None.

References

endogenous peptide apelin potently improves cardiac contractility and reduces cardiac loading in vivo. Cardiovase Res. 2005;65:73–82.
Table. Baseline characteristics of patients with chronic heart failure

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Figure Legends

Figure 1
Experimental protocols 1-5.

Figure 2

A. Sodium Depletion and Peripheral Resistance Vessels
Infused forearm blood flow with a normal (open symbols) or sodium-deplete diet (closed symbols) during intrabrachial (Pyr$^1$)apelin (upper panels, circles; 2-way ANOVA $P>0.05$), acetycholine (middle panels, triangles; 2-way ANOVA $P<0.001$) and sodium nitroprusside (lower panels, squares; 2-way ANOVA $P>0.05$).

Bonferroni post test: *$P<0.05$, **$P<0.01$, ***$P<0.0001$

B. Local Angiotensin II Infusion and Peripheral Resistance Vessels
Infused forearm blood flow following vasoconstriction with angiotensin II during intrabrachial infusion of (Pyr$^1$)apelin (circles; ANOVA, $P=0.02$) or sodium nitroprusside (squares; ANOVA, $P<0.0001$).

Bonferroni post test: *$P<0.05$, **$P<0.01$, ***$P<0.0001$

Figure 3
Sodium Depletion and Systemic Hemodynamics
Systemic hemodynamic response to (Pyr$^1$)apelin-13 (closed) or placebo (open) during normal diet (circles) or sodium-deplete diet (squares). Two-way ANOVA with repeated measures.

Bonferroni post test: *$P<0.05$, **$P<0.01$, ***$P<0.0001$
Figure 4

Systemic Angiotensin II Infusion and Systemic Hemodynamics

Systemic hemodynamic responses to (Pyr1)apelin-13 (closed symbols) or placebo (open symbols) during intravenous placebo (circles) or angiotensin II co-infusion (squares; 0.5 ng/kg/min). Two-way ANOVA with repeated measures.

Bonferroni post test; *P<0.05, **P<0.01, ***P<0.0001

Figure 5

Prolonged Systemic Apelin Infusion, healthy volunteers.

Systemic hemodynamic response to (Pyr1)apelin-13 (closed) and placebo (open) presented during first hour and subsequent 6 hours of infusion in healthy volunteers. Two-way ANOVA with repeated measures.

Bonferroni post test: *P<0.05, **P<0.01, ***P<0.0001

Figure 6

Prolonged Systemic Apelin Infusion, heart failure.

Systemic hemodynamic response to (Pyr1)apelin-13 (closed) and placebo (open) presented during first hour and subsequent 6 hours of infusion in patients with chronic stable heart failure.

Two-way ANOVA with repeat measures.

Bonferroni post test: *P<0.05, **P<0.01, ***P<0.0001

Figure 7

Prolonged Systemic Apelin Infusion, healthy volunteers.
Echocardiography data for left ventricular dimensions fractional shortening during the first hour of (Pyr\textsuperscript{1})apelin-13 infusion (closed) and placebo infusion (open).

Two-way ANOVA with repeat measures.

Bonferroni post test: *$P<0.05$, **$P<0.01$, ***$P<0.0001$
Figure 1

Protocol 1

- (Pyr^1)Apelin-13 (nanomol/min)
- Acetylcholine (µg/min)
- Sodium Nitroprusside (µg/min)

Saline 0.3 1.0 3.0 Saline 2.5 5.0 10.0 Saline 0.5 1.0 2.0

Protocol 2

- (Pyr^1)Apelin-13 (nmol/min)

Saline 0.3 1.0 3.0 Saline

Local angiotensin II infusion

Protocol 3

- (Pyr^1)Apelin-13 (nmol/min)

Saline 30 100 300 Saline

Protocol 4

- (Pyr^1)Apelin-13 (nmol/min)

Saline 30 100 300 Saline

Systemic angiotensin II infusion

Protocol 5

Saline

(Pyr^1)Apelin-13 30nmol/min or saline

-30mins 0 1hr 2hr 3hr 4hr 5hr 6hr

Legend:
- Light gray: Left ventricular measurements
- Dark gray: Hemodynamic measurements
- White: Mobilization period
Figure 3

**Cardiac Index**

**Heart Rate**

**Peripheral Vascular Resistance Index**

**Mean Arterial Pressure**

% Change

<table>
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<tr>
<th>Time (mins)</th>
<th>Normal diet</th>
<th>Sodium-deplete diet</th>
<th>Normal vs Sodium-deplete diet</th>
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<td>15</td>
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</tbody>
</table>

\( P < 0.0001 \)

\( P < 0.0001 \)

\( P < 0.001 \)

\( P < 0.006 \)

\( P < 0.001 \)

\( P < 0.0001 \)

\( P < 0.0001 \)

\( P < 0.0018 \)

\( P < 0.007 \)
Figure 4

Cardiac Index

Heart Rate

Peripheral Vascular Resistance Index

Mean Arterial Pressure

% Change

Saline  Angiotensin II  Saline vs Angiotensin II

Saline  Angiotensin II  Saline vs Angiotensin II

Time (mins)

Time (mins)
Figure 5

Cardiac Index

Heart Rate

Peripheral Vascular Resistance Index

Mean Arterial Pressure

% Change

% Change

0 15 30 45 60 120 180 240 300 360

0 15 30 45 60 120 180 240 300 360

Time (min)

Time (min)

P<0.0001

P=0.0002

P=0.11

P=0.0002

P<0.0001

P=0.12

P=0.40

P=0.42
Figure 7

Left Ventricular End-systolic Diameter

Left Ventricular End-diastolic Diameter

Fractional shortening

Ejection Fraction

Diameter change (cms)

Absolute change %

Time (mins)

Time (mins)

P=0.005

P=0.05

P<0.0001

P<0.0001
Sustained Cardiovascular Actions of APJ Agonism During Renin-Angiotensin System Activation and in Patients with Heart Failure


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Supplemental Material

Additional detail regarding methods; randomization, blinding and recruitment

A suitably trained researcher within the department, competent in Good Clinical Practice, performed randomization for each protocol. This blinding code was held securely in the Welcome Trust Clinical Research Facility and investigators had no access to this code. A protocol was in place to ensure that emergency unbinding could be undertaken if required. This was not necessary throughout this clinical trial.

For those participating in the salt restriction protocol, each of the local and systemic studies was performed on the same day and as such under the same dietary conditions. The order of drug infusion in the local and systemic infusion was independent of each other. The volunteers were responsible for their own dietary modification, following specific guidelines that were provided, and therefore were not blinded to the salt restriction design in this protocol. All investigators remained blinded during this protocol.

All infusions are colorless and as such there is no way visual way of knowing what medication is infused. Sodium nitroprusside is colorless, however is sensitive to ultra-violet light that requires the syringe to be covered. For each of the forearm plethysmography studies, all infusions were covered in order to maintain blinding. For systemic hemodynamic studies, blinding was maintained by virtue of (Pyr\(^{1}\))apelin-13 being colorless and indistinguishable from saline.

Each of the protocols was completed in series and participants recruited for each study after completion of the preceding protocol. For healthy volunteer recruitment participants responding to advertisements displayed on campus at the University of Edinburgh, as agreed under the Ethics Committee approving these trials. In order for enrolment all inclusion and exclusion criteria were fulfilled and informed consent obtained.

Patients with chronic stable heart failure known to the clinical cardiology service at the Royal Infirmary of Edinburgh and community heart failure service were contacted and provided with information relevant to the trial. A screening visit proceeded entry
to the trial to ensure inclusion and exclusion criteria were fulfilled and to obtain informed consent.

**Additional detail regarding statistical and analytic methods**

The ANOVA model used to analyze these data from each protocol was a mixed model ANOVA with both time and dose as fixed effects and subject as a random effect.

None of the terms is nested. All patients have been recruited from the same site and in each of the study protocols used a cross-over design such that individuals have acted as their own controls. These studies were not powered to assess the effects of differing term that may have been present within the populations.

Interactions of treatment were assessed by time and were non-significant. Given the small samples sizes, we did not anticipate identifying any interactions.

For analysis an arbitrary dose was assigned rather than a true numerical value. No specific modeling of dose-response was performed. The aim of these studies was to assess the effects of APJ agonism compared to placebo or under varying levels of renin angiotensin activation rather that to construct a dose response curve. The repeated measures ANOVA models assume compound symmetry (constant variance and covariance over time).

There are no individual contrasts between specific time points or dose levels and this was not pre-specified in our analysis. We have used Bonferoni corrections to provide adjustment of p-values and assess differences that may be present.