Novel Management of Heart Failure

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Work Performed Personally by Candidate

For the UNICORN study, the candidate participated in planning meetings to refine the protocol and oversaw the implementation of the study under the oversight of Associate Professor Richard Troughton and Dr Ian Crozier. The candidate performed daily screening throughout the trial period, and had personally recruited and consented 52 out of 53 participants for the study. The majority of data collection (clinical observations, examination, venesection, echocardiography) and plasma processing for storage prior to assaying were performed by the candidate except on occasions when there were simultaneous tasks, assistance were provided by nursing staff. The echocardiography protocol was designed by the candidate with input from Associate Professor Richard Troughton and personally performed all but one inpatient (150/151) echocardiography studies and performed all echocardiography measurements for the trial. For the right heart catheter sub-study, the candidate either personally inserted or assisted in the insertion of the twenty-two Swan-Ganz catheters with equipment set up by cardiac electrophysiology technician. All invasive haemodynamic data were obtained by the candidate personally. The candidate reviewed all participants in the 30-day follow-up and performed all clinical examination, echocardiography and venesection with occasional nursing assistance. All data (clinical, blood, echocardiography) were graphed, analysed and interpreted by the candidate with the advice from Associate Professor Christopher Frampton, biostatistician. As the first author, the candidate was responsible for drafting and critically revising a manuscript aimed for publication in a peer-reviewed journal.

For the TOUCHÉ study, the candidate participated in planning meetings to refine the

protocol and oversaw protocol implementation under the oversight of Associate Professor Chris Charles and Associate Professor David Jardine. The candidate performed six pre-trial infusions to ensure the targeted infusion dosage was achieved with the right carrier. The candidate personally screened, recruited and consented all trial participants. All venesections and plasma processing were performed by the candidate. All haemodynamic data were collected by the candidate. All data (haemodynamic and microneurographic) were graphed, analysed and interpreted by the candidate with advice from Associate Professor Christopher Frampton, biostatistician.

In zLAP, the candidate was primarily responsible for the three-monthly follow-up including clinical assessment, non-invasive device calibration and therapy adjustment for the main study. In the zLAP-CRT sub-study, the candidate contributed to refinement and oversaw the protocol implementation. Echocardiography was performed by an echocardiography technician approved by the trial committee. All echocardiographic measurements were performed by the candidate with the raw data offline. The left atrial pressure data were obtained by the candidate and nursing assistance, these data were extracted offshore by staff from St Jude Medical. The extracted left atrial pressure data and echocardiography data were graphed and analysed by the candidate with guidance provided by Associate Professor Christopher Frampton. As the first author, the candidate was responsible for drafting and critically revising a manuscript aimed for publication in a peer-reviewed journal.

All studies described in this thesis were undertaken whilst the candidate held a Research Fellow/ Clinical Teaching Fellow position at the Christchurch Hospital/University of Otago, Christchurch between November 2009 and February 2013 under the joint supervision of Associate Professor Richard Troughton, Professor

Mark Richards and Dr Ian Crozier.

Abstract

This thesis explores novel therapies in both acute decompensated heart failure and chronic heart failure.

Heart failure (HF) continues to be a major medical problem worldwide. In this complex condition, haemodynamic and neurohormonal imbalances occur simultaneously. The presentation of HF is heterogeneous and prognosis, despite therapeutic advances remains poor. Opportunities to intervene arise in each stage of HF with unique targets according to the stage of disease. Despite proven therapies, including angiotensin converting enzyme inhibitors/ angiotensin receptor blockade, beta blockers and mineralocorticoid receptor antagonists, the five year mortality remains above 50% therefore the search for new and improved therapies remains necessary.

Chapter 1: A literature review which discusses the epidemiology, pathophysiology and available treatments for HF and their limitations. In this literature review, an overview of the urocortin peptides is also provided. Urocortin-2, has potential as a therapeutic agent and is the key element involved in two studies discussed in this thesis.

Chapter 2: Describes key methodologies incorporated in the three studies.

Chapter 3: Reports the results of a study on the effects of urocortin-2 as an adjunct to conventional therapy in 53 patients hospitalised with acute decompensated HF. In this double-blind placebo-controlled randomised trial, urocortin-2 produced favourable haemodynamic effects in the acute decompensated setting without major adverse

effects. Urocortin-2 warrants further investigation to explore its full potential on renal and hormonal effects as a therapeutic agent in acute decompensated heart failure.

Chapter 4: Explores the effect of urocortin-2 on muscle sympathetic nerve activity (SNA) in eight healthy volunteers and four patients with stable HF. The results do not support the hypothesis that urocortin-2 inhibits muscle SNA in man as opposed to inhibition of cardiac SNA in conscious sheep. The response does not differ between healthy volunteers and HF patients.

Chapter 5: Explores left atrial pressure and left atrial waveform effects of varying cardiac resynchronisation therapy (CRT) settings in eight patients with stable HF. Optimal CRT settings corresponded to lower left atrial pressure and more favourable waveform characteristics. The study demonstrated that it was feasible to use this implantable left atrial pressure sensor to guide CRT optimisation.

Chapter 6: Summarises the key findings learned from the studies presented in Chapter 3-5. Areas of interests learned from these studies and proposed research direction are discussed.

Chapter 7: References quoted in this thesis.

Acknowledgement

left atrial pressure data extraction

Published Abstracts and Presentations

UNICORN

- Chan W, Troughton R, Frampton C, Crozier I, Richards M. Integrated Effects of Urocortin-2 Infusion in Patients with Acute Decompensated Heart Failure: Findings from a Randomised Controlled Clinical Trial.

    o Oral presentation Cardiac Society of Australia and New Zealand, New Zealand annual scientific meeting, Auckland, June 2012. *Heart Lung Circ 2012; 21 (8): Pg 483*

    o Oral presentation Cardiac Society of Australia and New Zealand, Australia annual scientific meeting, Brisbane, August 2012. *Heart Lung Circ 2012; 21 (Suppl 1): S84*

- Chan W, Troughton R, Frampton C, Crozier I, Richards M. Urocortin-2 has Favorable Hemodynamic Effects in Acute Decompensated Heart Failure: Findings from the UNICORN trial.

    o Oral presentation American Heart Association annual scientific meeting, Los Angeles, November 2012. *Circulation 2012; 126 (21 Suppl): A13781*

TOUCHÉ

- Chan W, Jardine D, Butler J, Charles C, Troughton R, Richards M. Urocortin-2 Increases Muscle Sympathetic Nerve Activity in Healthy Man.

    o Poster presentation Cardiac Society of Australia and New Zealand, New Zealand annual scientific meeting, Auckland, June 2012. *Heart Lung Circ 2012; 21 (8): Pg 482*

    o Mini-oral presentation Cardiac Society of Australia and New Zealand, Australia annual scientific meeting, Brisbane, August 2012. *Heart Lung Circ 2012; 21 (Suppl 1): S98*

zLAP-CRT

- W Chan, A Blomqvist, I Melton, K Noren, I Crozier, R Troughton. Effects of Atrio-ventricular Delay and Interventricular Delay on Continuous Left Atrial Pressure Waveforms in Ambulant Heart Failure Patients: A Novel Method of Cardiac Resynchronisation Therapy Optimisation.

  - Oral presentation Cardiac Society of Australia and New Zealand, New Zealand annual scientific meeting, Hawke's Bay, June 2011. *Heart Lung Circ 2011; 20 (6): Pg 378-9(Abstract 6)*

  - Mini-Oral presentation Cardiac Society of Australia and New Zealand, Australia annual scientific meeting, Perth, August 2011. *Heart Lung Circ 2011; 20 (Suppl 2): S63(Abstract 157)*

  - Poster presentation Heart Failure Society of America, annual scientific meeting, Boston, September 2011. *Journal of Cardiac Failure 2011; 17 (8 Suppl 1): S56 (Abstract 179)*

- Chan W, Blomqvist A, Melton I, Noren K, Crozier I, Troughton R. Left Atrial Pressure Parameters: a Novel Method for Cardiac Resynchronisation Therapy Optimisation.

  - Poster presentation International Congress of Cardiology, Hong Kong, February 2012. *Eur Heart J Suppl 2012; 14(Suppl A): A22 (Abstract P065)*

- Melton I, Chan W, Blomqvist A, Crozier I, Noren K, Troughton R. Parameterization of Continuous Left Atrial Pressure Waveforms: A Novel Technology for CRT interval Optimization in Heart Failure Patients (presented by Dr Iain Melton)

  - Poster presentation Heart Rhythm Society, annual scientific meeting, Boston, May 2012. *Heart Rhythm 2012; 9(8 Suppl): S373(Abstract PO05-22)*

Manuscripts in Preparation

- Urocortin-2 Infusion in Acute Decompensated Heart Failure: Findings from the UNICORN Study (Submitted)

- Effects of AV Delay and VV Delay on Left Atrial Pressure and Waveform in Ambulant Heart Failure Patients: Novel Insights Into CRT Optimization (Submitted)

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Table of Content

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Abbreviation List

A	Mitral inflow peak late filling velocity
ACC	American College of Cardiology
ACE	Angiotensin converting enzyme
ACEI	Angiotensin converting enzyme inhibitor
ACTH	Adrenocorticotropin
ADHF	Acute decompensated heart failure
AHA	American Heart Association
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
Ang I	Angiotensin I
Ang II	Angiotensin II
ANP	Atrial natriuretic peptide
ARB	Angiotensin receptor blocker
AT1	Angiotensin receptor type 1
AVD	Atrioventricular delay
BMI	Body-mass index
BNP	Brain natriuretic peptide
CAD	Coronary artery disease
CaMK II	Calmodulin-dependent protein kinase II
cAMP	Cyclic adenosine monophosphate
CI	Confidence interval
CNS	Central nervous system
CRF	Corticotrophin releasing factor
CRF-BP	Corticotrophin releasing factor-binding protein

CRT	Cardiac resynchronisation therapy
CRT-D	Cardiac resynchronisation therapy defibrillator
cTPR	Calculated total peripheral resistance
CV	Coefficient of variation
DCM	Dilated cardiomyopathy
E	Mitral inflow peak early filling velocity
e'	Early diastolic mitral annular velocity
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
ERK	Extracellular signal-regulated kinases
ESC	European Society of Cardiology
ET-1	Endothelin-1
ET_A	Endothelin A receptor
ET_B	Endothelin B receptor
GFR	Glomerular filtration rate
GSK	Glycogen synthase kinase
HF	Heart failure
HFPEF	Heart failure with preserved ejection fraction
HFREF	Heart failure with reduced ejection fraction
HPA	Hypothalamic-pituitary-adrenal
HPLC	High performance liquid chromatography
LAP	Left atrial pressure
LVEF	Left ventricular ejection fraction
LVOT	Left ventricular outflow tract
MAPK	Mitogen-activated protein kinase
MDRD	Modification of diet in renal disease

MIBG	^{123}I-meta-iodobenzylguanidine
MR	Mineralocorticoid receptors
NT-proBNP	N-terminal pro brain natriuretic peptide
NYHA	New York Heart Association
PAM	Patient advisory module
PAP	Pulmonary artery pressure
PCWP	Pulmonary capillary wedge pressure
PKA	Protein kinase A
PKC	Protein kinase C
PRA	Plasma renin activity
RAS	Renin-angiotensin-system
RAAS	Renin-angiotensin-aldosterone system
S'	Doppler mitral annular systolic velocity
SNA	Sympathetic nerve activity
SNS	Sympathetic nervous system
VT	Ventricular tachycardia
VTI	Velocity time integral
VVD	Interventricular Delay

1 Literature Review[a]

1.1 History and Definition of Heart Failure

1.1.1 Definition

Heart failure (HF) is defined as an abnormality of cardiac structure or function that leads to the heart being unable to meet the metabolic requirements of tissues except at the expense of increased filling pressures.[1] HF affects multiple systems. To put this into clinical context, HF is recognised as a complex syndrome in which patients have typical symptoms and signs of fluid retention resulting from an abnormality of the structure or function of the heart.[1]

1.1.2 History

The feature of oedema (dropsy) associated with the condition later recognised as HF was well described in ancient Egypt, India and Greece and for centuries, treatment was focussed on removal of excess fluid by blood letting and leeches with little change in approach other than the introduction of Southey's tubes (small cannulas inserted into the lower limbs as trocars to drain oedema) in the 19th century.[2, 3] William Harvey's description of the circulation in 1628 had opened up a new era in the understanding of the haemodynamic changes in HF and there was a growing interest in the study of abnormal structure in the failing hearts.[4] By the 19th century, the association of left ventricular dilatation and a poor prognosis was established.[4] By this time, the term "heart failure" was introduced.[4]

Diuretics as treatment were first introduced in the 1920s, however their use was

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[a] This literature review encompasses literature published prior to January 2013

limited by the toxicity of the mercurial-based agents. Modern diuretic treatment (thiazide diuretic) was not introduced until 1958.[2]

Knowledge of the pathophysiology of HF has evolved over the latter part of the 20th century including consideration of impaired myocardial contractility[5], understanding the importance of concept of neurohormonal activation[6] which later formed a cornerstone of HF treatment and more recently the understanding of genomic and epigenetic changes[4]. HF is now recognised as a multi-organ syndrome.

1.2 Epidemiology

Heart failure is a common medical problem. The lifetime risk of developing HF is 20-30% for both men and women based on large longitudinal studies in western countries.[7, 8] HF is associated with high morbidity and mortality and comprises a major health care burden worldwide.[9-11] In the United States, 5.8 million people were affected by HF, and 1.1 million hospitalisations were attributed to HF in 2006.[9] The health care costs including treatment and hospitalisations and indirect cost (lost productivity resulting from HF related morbidity and mortality) to the United States health care system in 2010 was estimated at $39.2 billion.[9]

HF is predominantly a disease of older persons. The average age of hospitalised HF patients based in large epidemiological studies is over 70 years.[10, 12-14] As a result, HF is an emerging "epidemic" coincident with the aging population worldwide.[15] The incidence of HF was clearly on the increase from the 1970s but began to plateau in the late 1990s and early 2000s.[16-19] More recent data have indicated some cause for optimism with epidemiological data from mid 2000 onwards showing a falling incidence worldwide, in particular for first hospitalisation.[18, 20] This is likely to reflect,

at least in part, a decreased incidence and improvement in treatment of coronary artery disease (CAD)[21-23] (one of the commonest causes of HF), improvements in the treatment of systolic HF[18, 20] and changes in admission practice[24]. Despite reductions in HF incidence and rates of hospitalisation[13, 24] HF prevalence remains high and prognosis poor for those requiring hospitalisation.[10-14, 25, 26] The New Zealand experience parallels worldwide trends. The majority of patients admitted to hospital are elderly -- the median age in 2008 was 78.1 years.[27] Hospital admissions increased from 7,500 in 1988 to a peak of over 12,000 HF admissions in 1999 then gradually fell to just under 11,000 HF admissions in 2008. Amongst those with the diagnosis of chronic HF, the number of days alive out of hospital has increased.[27] Long-term mortality following hospitalisation for HF remained high over recent decades with over a quarter of patients dead within twelve months from their index HF admission and 5-year mortality exceeding 50% in most series.[12, 26, 27] Post discharge mortality rates appear relatively static since 2000.[27]

In New Zealand Maori and the Pacific Islanders, are over-represented in HF statistics compared with other ethnicities. Maori are affected by HF on average 10-15 years earlier than non-Maori.[28] Maori were 4-times more likely to be hospitalised or to die from HF in 1996 than non-Maori.[29] A bigger discrepancy is observed in the younger age group, mortality from HF in Maori males is almost 9-times higher than non-Maori in the 45-64 year age group and 3.5-times higher in Maori than non-Maori in those aged 65 years and over.[30] Younger Maori are also more likely (8 to 9-times in those aged 45-64 years and 4-times for 65years and over) to be hospitalised with HF than non-Maori. Unfortunately, little has changed a decade later, Maori remained more than twice likely to die from HF than non-Maori.[31]

1.3 Aetiology of Heart Failure

Causes	Examples
Hypertension	
Coronary artery disease	Myocardial infarction, angina
Valvular	Aortic stenosis, mitral regurgitation
Metabolic and endocrine	Obesity, diabetes mellitus, hyper/hypothyroidism
Myocardial disease	Familial /acquired dilated/restrictive/hypertrophic cardiomyopathy, myocarditis
Congenital heart disease	
Drugs	Anthracycline, alcohol
Infiltrative disease	Amyloidosis, sarcoidosis, haemochromatosis, connective tissue disease
Pericardial disease	
Systemic illness	Anaemia, sepsis, autoimmune
Other causes	Tachyarrhythmia, peripartum cardiomyopathy, cor pulmonale, idiopathic, stress-induced cardiomyopathy

Table 1.1. **Aetiology of heart failure**. Modified from Dickstein et al. ESC guidelines for the diagnosis and treatment of acute and chronic heart failure 2008.[32]

The aetiology of HF is diverse (Table 1.1). *Hypertension* leads to increased myocardial hypertrophy, ventricular fibrosis and altered coronary flow which eventually results in left ventricular systolic and more so diastolic dysfunction.[33] Indeed hypertension has repeatedly been shown to be the predominant chronic background cause of HF in large observational population studies, followed closely by *coronary artery disease* (CAD) which is the single most prominent proximate cause of HF.[34, 35] In the Framingham study, 75% of patients who developed HF had an

antecedent history of hypertension.[34] In the hospital setting, the background role of hypertension may be under appreciated as many patients do not have a prior diagnosis of hypertension and blood pressure has often fallen with the onset of HF or treatment of HF. Almost half of hospitalised HF populations have co-existing CAD.[12, 36, 37] In the Western world, HF is attributable to CAD in up to 50% of cases based on meta-analyses of published HF trials between 1989-1990.[38] These analyses may have underestimated the importance of hypertension for the reasons given above in addition to the fact that trials may exclude older population where hypertension is more prevalent. In prospective observations of community populations and also patients hospitalised for HF in France, West London and Denmark, the risk attributed to CAD is lower -- 36-46%.[36, 37, 39] The risk of developing HF is increased when both hypertension and CAD are present and of course hypertension is a major risk factor pre-disposing the development of CAD.[7] Several studies have highlighted the importance of antecedent hypertension in the risk of the onset of HF after acute myocardial infarction.[40-42]

Studies often dichotomise HF into ischaemic and non-ischaemic origin for the ease of analysis. *Dilated cardiomyopathy* (DCM) is a generalised term used to describe ventricular dilatation associated with impaired ventricular contractility.[43] Often DCM is used interchangeably with idiopathic cardiomyopathy when the cause is unknown. It could also be a manifestation of advanced hypertensive heart disease or CAD. Genetic predisposition is more common than previously recognised and may account for a large proportion of idiopathic cardiomyopathy. In approximately 1/3 of patients with DCM, at least one other family member was also found affected by DCM during prospective screening.[44] There are more than 20 genes associated with DCM identified with a range of inheritance patterns.[45] However, inherited DCM is subject

to variable penetrance and significant locus and allelic polymorphism.[44] Post-infectious causes (for example viral, bacterial or protozoa myocarditis) are common and accounted for approximately 13% of cases of DCM as diagnosed by endomyocardial biopsies in one study.[43, 46]

Obesity, now reaching epidemic proportions is associated with the development of HF. The Framingham data indicated that, each increase in 1 kg/m^2 body-mass index (BMI) was associated with 5% increased risk of developing HF in men and 7% in women, in particular for HF with preserved left ventricular systolic function.[47] The age-adjusted 10-year cumulative incidence of HF for normal women (BMI 18.5-24.9) was 3.4%, overweight (BMI 25-29.9) was 3.7% and 6.8% for obese women (BMI ≥30) and a clear stepwise increase in incidence observed in men, age-adjusted 10-year cumulative incidence of HF was 4.9% for normal (BMI 18.5-24.9), 6.1% for overweight (BMI 25-29.9) and 10% for obese men (BMI ≥30).[47] This observation was confirmed in a large Swedish prospective cohort.[48] The mechanisms contributing to obesity related cardiomyopathy remain a topic of continued study, but human and experimental rat studies provide evidence of the accumulation of amyloid deposits in myocardium secondary to proteotoxic hyperamylinemia, which contributes to myocardial diastolic and systolic dysfunction.[49] There is also an emerging concept that intra-cardiac lipid deposits termed "cardiac steatosis" directly causes myocardial impairment.[50, 51] This lipid toxicity is observed to be reversible by magnetic resonance imaging in obese diabetic patients who lost weight following caloric restriction in which the myocardial triglyceride content was reduced and diastolic function improved.[50]

There is a growing awareness of chemotherapy-induced cardiomyopathy in oncology patients. Symptomatic cardiac dysfunction occurs in 1-5% of cases and up to 20% of

patients have asymptomatic cardiac abnormality identified by electrocardiography or echocardiography.[52] This increased observation of chemotherapy related cardiomyopathy may reflect the improved and more frequent cardiological surveillance incorporated into cancer therapy protocols, improved survival in cancer[52, 53] and the increased use of cardiotoxic immune-target therapy such as trastuzumab in breast cancer , alone or in combination with other known cardiotoxic chemotherapy.[52, 54]

Other causes of HF include familial *hypertrophic cardiomyopathy* which has a prevalence of 1 in 500 in the general population, although the majority are not clinically affected. *Valvular heart disease*, *congenital heart disease*, tachyarrhythmia-related cardiomyopathy, diabetes mellitus, peripartum cardiomyopathy, alcohol-related cardiomyopathy, or cardiomyopathy related to systemic infection or illness make up the other causes of cardiomyopathy and heart failure.[37, 43, 46, 55-61] Indeed the list of aetiology of HF is extensive and expanding. Despite the long list of potential causes, in 11-34% cases of HF, the aetiology is unknown.[36, 37, 39, 62] Inflammatory response may be under appreciated as a cause of HF in "idiopathic cardiomyopathy".[63] In two separate case series, inflammation was present in endocardial biopsies and pointed towards myocarditis being the underlying cause in a significant proportion of patients, with otherwise unexplained cardiomyopathy, including 51% who developed cardiomyopathy in the peripartum period.[46, 64] Notably, in this study, cardiac function did not improve with immunosuppressant therapy.[64] In a case-controlled study, a significantly higher percentage of DCM patients were alcohol abusers compared to a random population, strongly suggestive of alcohol as a cause of the otherwise unexplained DCM.[56] Alcohol abuse fosters development of hypertension and abnormal adrenal function and may be associated

with dietary deficiencies (e.g. thiamine) as well as possessing direct dose-related cytotoxic effects; all of which provide mechanisms whereby alcohol may predispose to HF.

1.4 Acute Decompensated Heart Failure

Acute decompensated heart failure (ADHF) is defined as gradually or rapidly worsening HF symptoms or signs that necessitate urgent therapy, and for which hospitalisation is often required.[65] ADHF can be triggered by a number of conditions, and the list is not restricted to cardiac conditions; respiratory illness, acute cardiac ischaemia and cardiac arrhythmia have been identified as the three most common precipitants in one of the largest ADHF registries.[66] Comorbidities are common in ADHF and confer a worse outcome. For example, more than half of patients have some degree of renal impairment at presentation,[67] and renal function frequently worsens during the course of admission.[68] It is very likely that renal dysfunction contributes to the pathophysiology of ADHF and multiple large observational studies have repeatedly confirmed the association of renal dysfunction with increased mortality in HF.[67, 69]

The clinical presentations of ADHF are heterogeneous, however the majority present with symptoms and signs of pulmonary congestion (i.e. dyspnoea), while some may present predominantly with systemic congestion (i.e. peripheral oedema).[69, 70] In apparent contradiction to the conventional belief that HF is characterised by depressed cardiac contractile power, approximately 25-50% of patients with ADHF have hypertension and relatively preserved left ventricular systolic function at presentation.[12, 71] Decompensated HF is associated with broad neurohormonal

activation. By definition (because this is "decompensation" we are describing) compensatory vasodilatory effectors such as the natriuretic pepetides and adrenomedullin are overwhelmed by the neurohormonal systems that cause vasoconstriction and fluid retention; the renin-angiotensin-aldosterone system (RAAS) and sympathetic nervous system (SNS) (see Section 1.8). This contributes to elevation of left ventricular filling pressure and a rise in pulmonary venous pressure. High hydrostatic pressure causes transudation of fluid from the vascular compartment into the alveolar space at a rate exceeding the capacity of lymphatic drainage leading to pulmonary oedema.[72] Left ventricular failure with secondary increases in pulmonary arterial pressures leads in turn to systemic venous congestion (raised right atrial pressure) and systemic congestion with peripheral oedema and weight gain. Elevated left ventricular diastolic pressure also contributes to a vicious cycle of subendocardial ischaemia and adverse left ventricular remodelling, further impairment of left ventricular efficiency, energy deficiency, reduction in cardiac output and further adverse neurohormonal activation, with this spiral manifesting in high rates of ADHF and mortality.[71]

1.5 Classification of Heart Failure

Multiple methods are available to categorise HF, based on aetiology, anatomical abnormality or symptoms. The most widely used symptomatic classification is by New York Heart Association (NYHA) functional class (Table 1.2).[73]

NYHA class	Definition
I	No limitation of physical activity.
II	Slight limitation of physical activity.
III	Marked limitation of physical activity. Experiences heart failure symptoms with less than ordinary activity.
IV	Symptoms at rest and increased with any activity.

Table 1.2. Definition of New York Heart Association functional class. NYHA, New York Heart Association.[1]

The NYHA classification has proven prognostic implications, with increasing functional class associated with greater risk of death or hospitalisation.[74, 75] Limitations of NYHA classification include the lack of specificity of symptoms for HF and the subjective nature of grading meaning relatively low reproducibility between physicians.[76] In 2001 the ACC/AHA guideline writing committee developed a new approach to classification of HF stages, intended to complement the NYHA classification (Figure 1.1). Staging of HF is based on structural and/or functional abnormality of the heart reflecting the development and progression of the disease together with consideration of symptoms.[73] Stage A (high risk for developing HF based on presence of vascular risk factors or conditions associated with HF without evidence of cardiac structural changes on imaging) and B (structural heart disease is present but without overt symptomatic HF) represent pre HF phases of the syndrome and are aimed at identifying those at risk of progressing to overt HF. Stage C (structural heart disease with symptoms of HF) and D (advanced structural heart disease and refractory symptomatic HF) comprise patients with symptomatic (NYHA class II-IV) HF.

The classification systems highlight HF as a complex and progressive syndrome with heterogeneous presentations. Although the treatment goals differ, there are various

interventions available that target different stages of the disease.
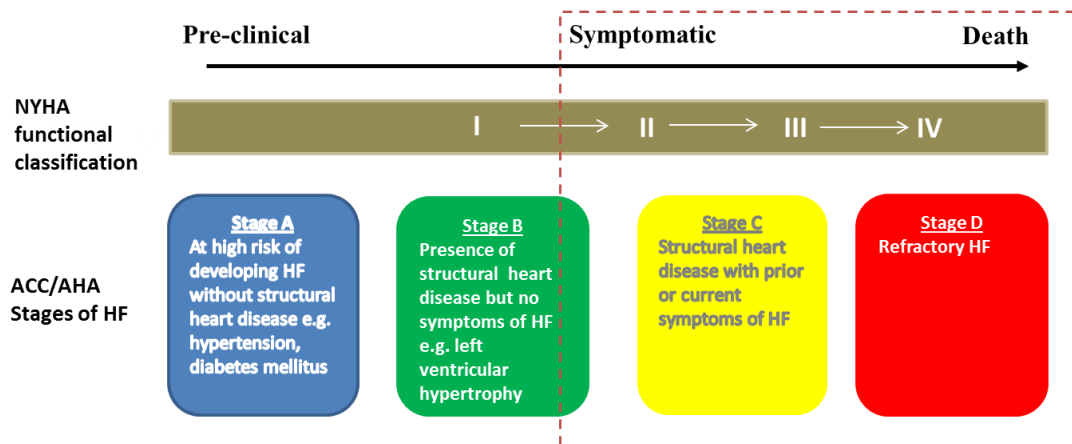
Figure 1.1. Diagramatic presentation of ACC/AHA stages of HF and its correlation with NYHA functional class. Dashed box indicated presence of symptoms. Modified from Hunt et al.[77]

1.6 Heart Failure with Preserved Ejection Fraction

There are two major components of left ventricular dysfunction: systolic dysfunction – related to impairment of ventricular contractility and the ability of the heart to eject a normal stroke volume; and diastolic dysfunction – related to impairment of ventricular relaxation and filling. The left ventricular ejection fraction (LVEF) has long been recognised as an indicator of global myocardial systolic function.[78] Reduced LVEF is a marker of reduced myocardial contractility. However, reduced LVEF to less than 50% is not universal in clinical HF, being present in between 30 and 55% of the population diagnosed with HF.[35, 79, 80] In HF patients with preserved LVEF, key abnormalities including prolonged left ventricular relaxation, slowed left ventricular filling and increased diastolic stiffness (indices of diastolic function) can be identified.[81] The pathophysiology of diastolic dysfunction includes excessive collagen accumulation in the myocardium, abnormal cytoskeleton protein that

increases left ventricular stiffness and reduced myofilamentary calcium sensitivity causing impaired relaxation. As diastolic dysfunction is also present in systolic HF, it is more appropriate to address this group of patients as HF with preserved ejection fraction (HFPEF). These patients are more likely to be older, female, have hypertension, more likely to be in atrial fibrillation and less likely to suffer from CAD although this must be stressed that these characteristics are very common at both ends of the LVEF spectrum in HF and do not provide sharp separation of HFPEF and HF with reduced ejection fraction (HFREF) populations.[80] They are just as likely to be re-admitted to hospital after an episode of decompensation as subjects of HFREF but have been shown to have a lower in-patient mortality.[80] Whilst they symptomatically respond to diuretic therapy, unfortunately, none of the treatments of proven efficacy in HFREF have been shown to improve clinical outcome in randomised controlled trials of HFPEF. The use of angiotensin converting enzyme inhibitor (ACEI) or angiotensin receptor blocker (ARB) has failed to demonstrate mortality benefit in HFPEF.[82-84] Although reduction in HF hospitalisation was observed with ACEI/ARB, it did not reach statistical significance in the "Perindopril in Elderly People with Chronic Heart Failure" (PEP-CHF) study due to the low study power and a high proportion on open-label ACEI[83] and in the "Candesartan in Heart Failure: Assessment of Reduction in Mortality and morbidity" (CHARM)-Preserved study, it was criticised for including about 30% patients with LVEF between 40-50%[82]. The benefit of beta-blockers in HFPEF is more conflicting. Nebivolol significantly reduced mortality or cardiovascular hospitalisation in elderly in "Study of Effects of Nebivolol Intervention on Outcomes and Rehospitalization in Seniors with Heart Failure" (SENIORS), however in the sub-group analysis of HFREF and HFPEF, the beneficial effect was no longer present.[85] The recently published "Japanese diastolic

heart failure" study was neutral in the overall study, but significantly reduced composite of death or HF readmission in sub-group of patients taking >7.5mg per day of carvedilol.[86] The difference in response to therapy may reflect differences in the underlying pathophysiology between HFPEF and HFREF although flawed trial designs and incorrect case selection may partially underlie the lack of therapeutic response observed in randomised controlled trials. Recently, analysis from the Swedish Heart Failure Registry has shown contrasting results to randomised controlled trials.[87] In this study, the all-cause mortality was compared in an unselected group of patients with HFPEF treated with or without renin-angiotensin-system (RAS) antagonist. The cohort was age and propensity score-matched to adjust for selection bias or confounding factors for the use of RAS antagonist. Patients who were on RAS antagonists were associated with a small but significantly reduced all-cause mortality compared with patients not on RAS antagonists and the association was dose-dependent.[87] The study suggested a benefit of RAS antagonist treatment in the "real world" setting but would require a carefully designed and powered randomised trial to confirm this observation. Other potential therapies include exercise and phosphodiesterase-5 inhibition; however the demonstrated benefit thus far is only limited to surrogate endpoints.[88, 89]

1.7 Haemodynamic Model of Heart Failure

This conceptual approach or hypothesis regarding HF prevailed in the 1960s and 70s. The output of a normal heart is determined by the interaction of preload (end-diastolic volume), afterload (the resistance against ejection of blood) and contractility (the maximum rate of pressure rise). The heart adapts to changes in loading conditions to

maintain cardiac output. When afterload is increased, stroke volume is reduced and end-diastolic volume increased. Subsequently contractility increases to restore preload and afterload. Increases in both preload and contractility augment stroke volume.[90]

In HF, the pump function is inadequate to provide adequate cardiac output for the needs of the body (unless the heart operates under increased intra-cardiac filling pressures) and this activates a number of adaptive and maladaptive responses. The combination of inadequate pump function and compensatory changes results in a smaller stroke volume and a higher end-diastolic volume.[90] Volume overload increases ventricular diastolic volume and initial compensation is by an increase in ventricular compliance. In pressure overload, the initial response is to increase myocardial contraction to maintain the stroke volume. However with time, cardiac contractility may decrease and HF develop.[91] There is also baroreceptor-mediated maladaptation in the peripheral circulation in the form of excessive peripheral vasoconstriction to accommodate for the perceived hypotension or reduced tissue, especially renal, perfusion in HF, this in turn further increases the afterload and contributes to the vicious cycle outlined above.[92]

If HF is purely a result of haemodynamic imbalance, then in theory, increasing cardiac contractility or altering the afterload by peripheral vasodilatation should be beneficial. Results from a number of clinical trials have put this hypothesis to the test. The use of inotropic agents to increase myocardial contractility may temporarily improve cardiac function but had no long-term benefit and in most cases where randomised controlled trials have been performed they have increased arrhythmia-related sudden death and all-cause mortality.[93-95] Although these negative results might partially reflect case selection bias with, more severe HF cases receiving inotrope therapy (this does not apply to randomised controlled trials) or increased

cardiac work that altered myocardial energy balance, the failure to alter outcome despite haemodynamic restoration reflects the fact that HF is caused by more than simple reversible derangements of circulatory tone and capacity.

The first major randomised controlled trial of vasodilatation in HF reported in 1986 was the "Veterans Administration Cooperative Study" (V-HeFT), in which prazosin resulted in a greater blood pressure fall but had no mortality benefit whilst a combination of hydralazine and nitrate produced only a modest blood pressure drop but had significant mortality benefit (8.7% over 2 years) over placebo.[96]

1.8 The Neurohormonal Hypothesis in Heart Failure

Of note, neurohormones are significantly elevated in patients in early stages of HF who are relatively symptom free compared to healthy subjects.[97] Furthermore, the subsequent landmark trials including "Studies of Left Ventricular Dysfunction" (SOLVD), the second V-HeFT study and "Cooperative North Scandinavian Enalapril Survival study" (CONSENSUS) in which inhibition of the RAAS demonstrated superior survival benefits confirmed the important role of neurohormones in the pathophysiology of HF and neurohormonal blockade as a key therapeutic strategy over and above targeting haemodynamic improvement.[97-99]

Complex neurohormonal systems are in place to maintain pressure and volume homeostasis in health.[6] In HF, maladaptive processes occur including activation of neurohormonal systems that induce vasoconstriction along with salt and water retention which further burden the heart and lead to adverse left ventricular remodelling setting the scene for spiralling deterioration in cardiac structure and function.[100-103] Plasma neurohormones are prognostic.[99, 104, 105] In addition to

neurohormonal changes that cause vasoconstriction and fluid retention, counter-regulatory hormonal mechanisms co-exist which produce vasodilation and diuresis.[91] Natriuretic peptides enhance diuresis and natriuresis, they are vasodilators and their secretion is increased in HF.[6] Other vasodilators include endothelial hormones: prostaglandins and nitric oxide that are powerful vasodilators that oppose the effects of vasoconstrictor hormones on endothelial cells.[6] By definition the presence of overt HF indicates adaptive neurohormonal responses (such as that of natriuretic peptides) have been overwhelmed by the extent of underlying cardiac dysfunction in concert with the burden from maladaptive responses (such as that from sympathetic and RAA systems). Treatments that antagonise RAAS and sympathetic activation result in improved haemodynamics, reduced adverse ventricular remodelling and improved outcome.[99, 100, 106] This literature review will focus on the hormones that are important in HF and were measured in the studies reported in this thesis. The urocortins are key components of this thesis and will be discussed in more detail in the following section.

1.8.1 *Renin-angiotensin-aldosterone system*

The RAAS represents one of the primary mechanisms responsible for maintaining circulatory volume and blood pressure for organ perfusion. Renin is predominantly synthesised by the juxtaglomerular cells in the kidneys in response to changes in afferent glomerular arteriole perfusion and pressure coupled with sodium sensing by macula densa and sympathetic drive to the juxtaglomerular cells apparatus. Renin cleaves circulating angiotensinogen, primarily produced in the liver, to release angiotensin I (Ang I) which in turn is converted to the main effector, angiotensin II (Ang II) by angiotensin converting enzyme (ACE) in the pulmonary circulation.[107] There are two receptor subtypes for Ang II, with the more important one in the

context of HF being angiotensin receptor type 1 (AT1). AT1 is abundant in the vasculature, mediates both the powerful peripheral vasoconstrictor action of Ang II and the stimulation of aldosterone and arginine vasopressin release causing salt and water retention.[107, 108] Ang II is involved in myocardial remodelling, intracellular calcium handling and adrenergic stimulation, which all contribute to the pathophysiology of HF.[109, 110] Ang II is also generated locally in the myocardium independent of the ACE pathway.[111] More recently a related enzyme, ACE2, has been discovered that converts Ang II to angiotensin (1-7), which has been shown to counteract some of the effects of Ang II and participates in cardiac remodelling.[109, 112, 113]

Aldosterone is synthesised in the adrenal glomerulosa. Its release in health is primarily determined by diurnal shifts in adrenocorticotropin (ACTH), potassium levels and by Ang II which often becomes the prime stimulus in HF. Its effects are mediated by mineralocorticoid receptors (MR) which mediate altered Na/K ATPase function in the distal tubules of nephron, and also in the colon, salivary and sweat glands, to promote salt and water retention and potassium excretion.[114] In HF maladaptive MR activation in heart and vasculature by both aldosterone and glucocorticoids mediates inflammation and both vascular and myocardial fibrosis and adverse ventricular remodelling independent of the presence of hypertension or left ventricular hypertrophy.[114] Aldosterone also enhances the vasopressor effect of norepinephrine[115]. In HF, its level is increased not only due to increased Ang II stimulation but also increased stimulation from corticotrophin and decreased metabolic clearance. Elevated aldosterone level during hospitalisation is associated with a poor prognosis post myocardial infarction with reduced short and long-term survival[116-118] and increased subsequent risk of developing HF requiring

hospitalisation[117]. It is notable that even increments in plasma aldosterone within the accepted normal range are associated with worse outcomes in HF and over the long term following acute myocardial infarction.[117]

In HF, the RAAS is activated in response to multiple stimuli including systemic or renal hypoperfusion, activation of the SNS, decreased sodium concentration at macula densa, prostaglandins and diuretics use.[108] The low grade activation of RAAS is noted in the early stages of HF, and increases with HF progression.[99, 119] Plasma renin activity (PRA), Ang II and aldosterone increase in response to normal physiological stress such as exercise. This response is relatively preserved in mild HF but is augmented in more advanced HF[119], likely secondary to reduced baroreceptor sensitivity[120]. In acute HF decompensation, PRA and aldosterone promptly increase in response to a fall in systemic blood pressure.[121, 122] This leads to extracellular volume expansion in an attempt to restore blood pressure and, often results in the development of oedema, the hormone levels then gradually fall to near normal level until a new steady state is reached for chronic stable HF.[121]

1.8.2 Endothelins

The endothelins are potent vasoconstrictors with at least four isoforms identified with the predominant bioactive form in the context of HF being endothelin-1 (ET-1).[108] The major source of ET-1 synthesis is the vascular endothelium. ET-1 is also widely expressed in a wide variety of other tissues including cardiac myocytes and vascular smooth muscle and exerts a variety of actions in the cardiovascular, renal, pulmonary and endocrine systems.[123] Increased vascular shear stress or vasoactive substances including norepinephrine stimulate synthesis of ET-1 while factors that relax the vascular endothelium including nitric oxide, prostacycline and the natriuretic peptides

inhibit it.[123] The cellular action of ET-1 is mediated by at least two receptor subtypes. The endothelin A receptor (ET_A) found in vascular smooth muscle, cardiac myocytes and fibroblasts, mediates vasoconstriction, inotropy and mitogenesis. The endothelin B receptor (ET_B), found on vascular endothelial cells, mediates vasodilatation via an endothelial-derived relaxing factor-dependent pathway.[124] However, less is known about ET_B and its vasodilatory role in HF remains uncertain. ET-1 is elevated in advanced HF, but relatively normal in asymptomatic or mild HF.[125-127] Observations from human and experimental HF suggest that endothelins contribute to the pathophysiology of HF by both fostering maladaptive vasoconstriction[128, 129] and depressing myocardial contractility[101]. Early evidence has demonstrated endothelin receptor antagonism resulted in reduced pulmonary pressures, calculated pulmonary vascular resistance and increased cardiac output.[129] Disappointingly, larger clinical trials were either discontinued prematurely due to adverse events[130] or failed to demonstrate any improvement in clinical course[131].

1.8.3 Natriuretic Peptides

The natriuretic peptides are a family of peptide hormones that possess vasodilatory and natriuretic effects and they also suppress RAAS, dampen sympathetic outflow, and reduce hypertrophic and fibrotic changes in the heart. There are at least six members identified in this family.[132] All natriuretic peptides are synthesised as preprohormones, cleaved early in translation to form prohormones which in turn are further processed to release the biologically active mature hormones. All mature natriuretic peptides have a common disulphide-linked ring structure with a highly conserved amino acid sequence which is important for natriuretic receptor binding and bioactivity.[133]

Atrial natriuretic peptide (ANP) was first identified in 1981 following the observation that injection of atrial extracts into rats induced a marked natriuresis and diuresis.[134] ANP is predominantly stored in atrial granules and is released in response to atrial wall stretch.[135] In addition, endothelin[136], Ang II[113, 137] and adrenergic stimulation[138] can also stimulate its release. ANP induces a number of haemodynamic actions, diuresis and natriuresis[134, 139, 140] and inhibits the RAAS[141, 142]. ANP circulates at low picomolar concentrations in normal man but levels are significantly elevated in patients with HF when constitutive ventricular synthesis and release also contributes to plasma ANP levels.[103, 143-145] The level is higher in patients with lower cardiac output and more advanced HF.[103, 140, 143, 145] Survival is inversely proportional to natriuretic peptide levels and treatments for HF which reduce plasma natriuretic peptide levels are associated with improved survival.[99, 105] Natriuretic peptides have been powerfully and independently associated with prognosis in many published series in multiple settings including acute and chronic HF and acute and chronic coronary heart disease. However, unlike brain natriuretic peptide (BNP) (see below), ANP is not widely used as a clinical diagnostic marker; compared to BNP, it is inferior to predict left ventricular impairment post myocardial infarction[146]. In stable HF, short term ANP infusion exerted similar blood pressure lowering effect and improvement in cardiac output as seen in experimental HF[140, 147-149], but the renal and neurohormonal response was less consistent and short-lived[140, 148, 149] and there was a possible rebound effect on aldosterone observed upon cessation[140, 150]. The use of ANP as a therapeutic agent in ADHF is feasible.[151-153] In one study, ANP infusion in ADHF was suggested to have an improved prognosis at 500days.[151] However, its therapeutic use in ADHF has not been subjected to large randomised controlled trials and its use as a therapeutic agent is predominantly restricted to Japan.

Brain natriuretic peptide (BNP) was initially discovered in porcine brain extract which produced diuretic, natriuretic and hypotensive effects similar to ANP when intravenously injected into rats.[154] BNP gene expression and secretion was subsequently found to be predominantly in the human ventricular myocardium although a small amount is co-stored with ANP in atrial granules. BNP is predominantly constitutively secreted with levels and secretion greatly increased in HF in response to myocardial diastolic wall stress.[155-158] Hence, it is now more commonly called B-type natriuretic peptide. Whilst the major stimulant of BNP production in both health and HF is myocardial wall stress, other factors include Ang II[113], ET-1[132, 159], endogenous adrenergic drive and cytokine levels[160]. Plasma levels are also affected by concomitant cardiac ischaemia, atrial fibrillation, pulmonary embolism, age, gender, renal function, drug treatment, obesity, thyroid and glucocorticoid status.[146, 161-163] The physiological actions of BNP overlap with ANP as both hormones activate the natriuretic peptide receptor-A.[133] Physiological actions include vascular smooth muscle relaxation, inhibition of sympathetic nerve activity, RAAS, thirst, cardiac hypertrophy and fibrosis, diuresis and natriuresis.[133, 154, 164] Overall the natriuretic peptide properties are primarily cardioprotective.[165] Plasma BNP levels are elevated in HF in proportion to severity of cardiac dysfunction irrespective of the cause.[161, 166] Plasma BNP levels or those of the BNP fragment of 98 amino acid N-terminal proBNP (NT-proBNP) are progressively greater in severe HF than in the healthy state and much higher in acute decompensation.[154, 155, 166, 167] B-type natriuretic peptide levels correlate marginally more strongly with measurements of left ventricular function than ANP.[146] BNP has had the most extensive development among biomarkers as a diagnostic, prognostic and monitoring aid (with either a single or serial measurements) in HF[104, 167-172], and also has prognostic

significance in some non-HF cardiac conditions[146, 162]. More recently studies have shown it has a potential role in therapeutic monitoring and treatment guidance.[173, 174] Although BNP (neseritide) gained U.S. Food and Drug Administration approval as a therapeutic agent, a recent large randomised controlled trial did not demonstrate any clinical benefit (see later) and hence its appropriate use or otherwise in ADHF is currently unclear.[175]

1.8.4 Sympathetic Nervous System

The sympathetic nervous system (SNS) participates in homeostasis in a beat-to-beat manner to maintain tissue perfusion through modulation of heart rate and myocardial contractility, vasoconstriction (including modulation of intra-renal vascular tone) and activation of RAAS.[176] Increased sympathetic adrenergic bioactivity at adrenergic receptor level results from a combination of increased norepinephrine release from augmented neural firing, reduced organ norepinephrine clearance and depleted norepinephrine stores.[177, 178] Chronic agonist exposure leads to redistribution, mainly down-regulation, of β_1 and β_2-adrenergic receptors.[179] In sufficiently severe myocardial impairment, sustained sympathetic overdrive may prove unable to compensate for derangements of cardio-circulatory function. This maladaptive process stimulates apoptosis which contributes to decreased myocardial contractility, subsequent remodelling and calcium mishandling mediated through various G-protein coupled protein kinases including cyclic adenosine monophosphate (AMP) dependent protein kinase A (PKA), calmodulin-dependent protein kinase II (CaMKII) and increased production of reactive oxygen and nitrogen species.[179] These changes result in increased cardiac preload, afterload and cardiac work, salt and water retention, reduced renal blood flow and loss of baroreflex sensitivity which all exacerbate HF. Activation of SNS occurs early in the disease course when patients are minimally

symptomatic[97, 180-182] and before activation of RAAS.[97, 121]

To assess the SNS, microneurography is the only method that directly assesses the efferent post ganglionic sympathetic nerve traffic in humans.[183] It requires insertion of microelectrodes into nerve fibres innervating the skin or target organ and permits direct quantification of sympathetic firing and reflex control.[183] Although good correlation has been observed between direct muscle sympathetic nerve activity (SNA) and cardiac SNA assessed by indirect methods[184, 185] as discussed below, this method can only assess superficial nerves and may not truly represent the SNA of central target organs such as the heart. Due to the invasive nature of the study, microneurography has not been applied to routine clinical use. There are number of methods to assess SNA indirectly. Measuring arterial or venous plasma norepinephrine and epinephrine is the easiest. However, plasma catecholamines lack sensitivity to the target organ of interest and do not distinguish between increased release and reduced clearance.[183] In addition, circulating norepinephrine accounts for only 5-10% of total neurotransmitter secreted from nerve terminals.[186] To illustrate, sympathetic activity is known to be elevated in essential hypertension[187, 188] but the plasma norepinephrine is usually normal[187, 189]. A more organ-specific method is to measure the tissue clearance of a radiolabelled norepinephrine following intravenous infusion, subtracted from total body plasma norepinephrine to calculate the regional norepinephrine, the spillover technique.[186, 190] However, this method is influenced by blood flow to the target organ and is also invasive.[190] Single-photon emission computed tomography using ^{123}I-meta-iodobenzylguanidine (MIBG), a norepinephrine analogue, tracer imaging is a non-invasive imaging technique to assess cardiac SNA.[191] This method quantifies the heart-to-mediastinum ratio of MIBG uptake. In HF, cardiac MIBG uptake is reduced and is associated with

increased likelihood of ventricular arrhythmia in HF.[191] Although this non-invasive method has potential in risk stratification and treatment monitoring, its application in clinical use is limited due to the absence of prospective outcome data, expense, limited access to the method and the need for radiation exposure.[192] A surrogate marker of SNA is heart rate variability.[183]

In HF, sympathetic activation is non-uniform as shown in the norepinephrine spillover technique with preferential increases in cardiac and renal SNA in early stage HF, at a time when the total body SNA is relatively unaffected.[180, 193, 194] Direct microneurography in patients with severe HF also shows significant increase in muscle SNA, which is well correlated with cardiac SNA[195, 196], whilst skin SNA remains quiescent[197]. With disease progression, activation of the SNS becomes generalised and proportional to functional class and raised intra-cardiac pressures[178, 180, 182, 198-200] but the major target remains the heart as evidenced by the profound disproportionate increase in cardiac SNA[178, 193, 199]. Elevated SNA and more specially increased cardiac SNA is consistently shown to be associated with both increased cardiac and non-cardiac death.[99, 201, 202] In particular, patients with increased cardiac SNA were more likely to develop malignant ventricular arrhythmia[191, 203], sudden cardiac death and death from HF progression[204]. The risk is proportional to plasma norepinephrine concentration[171] and reduction of plasma norepinephrine levels with time, either spontaneously or with treatment is associated with better prognosis.[171]

1.9 Urocortins

Corticotrophin releasing factor (CRF), isolated in 1981,[205] has a pivotal role in the hypothalamic-pituitary-adrenal (HPA) axis to modify the behavioural, neuroendocrine

and autonomic response to stress.[206, 207] The low affinity and the anatomical mismatch of CRF and the CRF_2 receptor subtype led to the search for other cognate ligand(s). The first alternative ligand discovered was urocortin-1 which was cloned from cDNA of rat midbrain in 1995.[208] However, discrepancy remained between the distribution of urocortin1-immunoreactive projections and CRF_2[209]. In 2001, two separate groups almost simultaneously cloned urocortin-2 and urocortin-3 from mouse and human genome databases.[210-212] One group initially named urocortin-2 "stresscopin-related peptide" and urocortin-3 "stresscopin". Thus the CRF family consists of four members in mammals, CRF and urocortins 1-3, with other peptides present in non-mammals, including urotensin I in fish[213] and sauvagine in frog[214]. CRF and urocortins 1-3 are all present in humans. The peptides are structurally similar with α-helical structures (Figure 1.2) forming the backbones and variation of these helices at the N- and C-terminals regions accounting for the difference in selectivity and affinity for the CRF receptors, with subsequent difference in bioactivity.[210, 215] The exact structure of these peptides was uncertain due to the unknown cleavage site at the N-terminus.[210]

Another component of the circulating CRF peptide family is the CRF-binding protein (CRF-BP).[216] CRF-BP binds equally to CRF and urocortin-1 but has no appreciable affinity to urocortin-2 or urocortin-3.[212] No difference in hormonal or behavioural response was noted in CRF-BP overexpressing mice[217] but increased anxiety has been reported in CRF-BP knockout mice[218]. It has been suggested that CRF-BP participates in its ligand-bound complex clearance however its exact role in health and disease remains unknown.[219]

The metabolism of the urocortins is not well defined but renal clearance is not believed to be a major contributor. Pharmacokinetic studies in sheep suggest a two-

compartment model for urocortin-1 but a one-compartment model for urocortin-2 and urocortin-3, a smaller volume of distribution for urocortin-1, with the clearance rate being faster in urocortin-2 than urocortin-1 and even faster in urocortin-3.[220] In man pharmacokinetics of both urocortin-1 and urocortin-2 are consistent with a one-compartment model, with the half-life of urocortin-1 and urocortin-2 being approximately 50min and urocortin-2 15min respectively.[221-224]
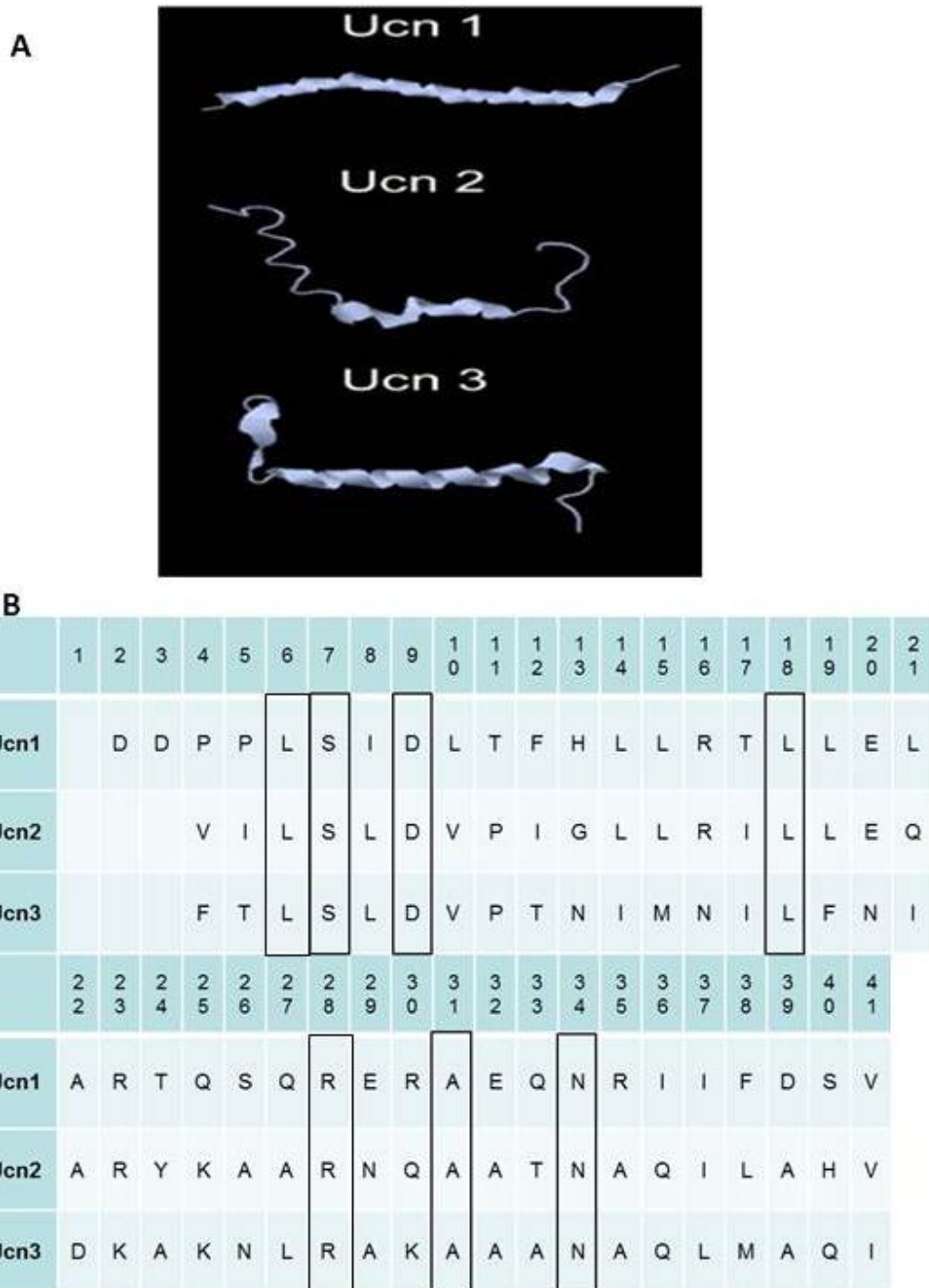
Figure 1.2 A) Urocortins 1-3 have similar α-helical structures. B) Amino acid sequence of Ucn1-3. The boxed sequences show similarities amongst all three peptides. Adapted from Davidson et al and Venkatasubramanian et al.[225, 226] Ucn1, urocortin-1; Ucn2, urocortin-2; Ucn3, urocortin-3.

1.9.1 Urocortin-1

Urocortin-1 is a 40-amino acid peptide, with 43% homology of amino acid sequence to rat and human CRF.[208] Urocortin-1 is expressed in the brain, mainly midbrain in

the Edinger-Westphal nucleus, lateral superior olivary nucleus and the supraoptic nucleus in the hypothalamus.[208, 209] It is also widely distributed in peripheral tissues. Urocortin-1 mRNA has been detected in thymus, spleen, gastrointestinal tract, reproductive system, liver, heart, vasculature, kidney and pituitary.[227-230] While CRF preferentially binds to CRF_1 receptor, Ucn1 binds non-selectively to both CRF_1 and CRF_2 receptors.[208]

1.9.2 Urocortin-2

Urocortin-2 is a 38-amino acid peptide that is a highly selective agonist for the CRF_2 receptor[210, 211] exhibiting 1000-fold less affinity to the CRF_1 receptor subtype[211]. There is no appreciable affinity to CRF_1 receptor in normal physiological concentrations but Ucn2 could activate CRF_1 receptor when present at high dose.[212] Amidation at the C-terminus has a crucial role in urocortin-2 bioactivity; non-amidated urocortin-2 has a markedly reduced potency in stimulating cAMP.[210] In the central nervous system (CNS), urocortin-2 is predominantly expressed subcortically, in the paraventricular, supraoptic and arcuate nuclei of the hypothalamus, locus of coeruleus, motor nuclei of brainstem and spinal cord and is heavily concentrated in the regions of the brain associated with response to stress.[211, 231] In peripheral tissues, urocortin-2 is also widely distributed, and has been detected in heart, vasculature, peripheral blood cells, lung, pituitary, adrenal, skeletal muscle, skin, gastrointestinal tract, reproductive system and fetal tissues.[210, 230-233] Peptide distribution varies between species, with humans showing high urocortin-2 expression in the cardiovascular system, brain, adrenal and peripheral blood cells.[210]

1.9.3 Corticotropin Releasing Factor Receptors

The actions of CRF and the urocortins are mediated via two subtypes of seven

transmembrane CRF G-protein- coupled receptors, CRF_1 and CRF_2. Genes encoding these two receptors have been identified in humans on separate chromosomes with different location than in the mouse.[234-237] The CRF_1 receptor is distributed extensively in the CNS, the pituitary gland, aortic endothelium, gastrointestinal tract, adrenal gland and reproductive tissue.[230, 238-240]

The CRF_2 receptor is found in discrete areas of the CNS, including the lateral septal nucleus, hypothalamic ventromedial and paraventricular nuclei and amygdala.[240] However, the CRF_2 receptor is more abundantly expressed in the periphery, in particular the cardiovascular system[229, 230, 241, 242], gastrointestinal tract and skeletal muscle[243]. In addition, three splice variants for CRF_2 receptor have also been identified: $CRF_{2(a)}$, $CRF_{2(b)}$ and $CRF_{2(c)}$. In rodent, $CRF_{2(a)}$ receptor mRNA is primarily expressed in the brain and $CRF_{2(b)}$ receptor in the periphery, most abundantly in the myocardium.[243] In contrast, $CRF_{2(a)}$ receptor mRNA is expressed both centrally and peripherally in humans. It is the dominant receptor subtype identified in the human cardiovascular system, with the highest concentration in the left ventricle whilst $CRF_{2(b)}$ receptor is only detected in the left atrium.[229] The $CRF_{2(c)}$ receptor has only been identified in the human limbic region.[244]

1.9.4 Actions of Urocortins

The overlapping anatomical distribution of urocortins and CRF receptor expression is suggestive of autocrine or paracrine roles for these peptides. Since urocortin distribution is extensive throughout the CNS and peripherally, it is not surprising that urocortins exhibit a diverse range of physiological actions. Not only are urocortins structurally similar, similar physiological actions are induced via activation of common receptor pathways. When urocortins 1-3 were administered centrally under

identical experimental conditions targeting the same CRF receptor all caused an increase in core temperature and differed only in the temporal profile of this effect.[245] The same was observed in normal and pacing-induced HF sheep. Administration of urocortins 1-3 peripherally consistently produced haemodynamic effects with similar magnitude but a different time-course (peak effect 120min for urocortin-1, 10-30min for urocortin-2 and 5min for urocortin-3).[220, 246-248] In rats, central administration of CRF induced hypertension mediated via the CRF_1 receptor, while intravenous CRF induced hypotension presumably via CRF_2 receptor activation.[249] Intravenous injection of urocortin-1 in mice induced a vasodepressor effect whilst this effect was absent in CRF_2 receptor deficient mice.[250] Urocortins induced vasodilatation in rat gut and hindlimbs but not renal artery.[251]

1.9.5 Central Nervous System

Urocortins regulate stress and anxiety response. Although no difference was seen in unstressed and acute stress in mice deficient of all three urocortins, these mice displayed increased anxiety-related behaviour 24 hours after a standardised stimulus compared to their wildtype littermates, suggestive of impaired stress recovery.[252] CRF_1 and CRF_2 receptor activation in the CNS appear to have opposing effects in anxiety-related behaviours. Decreased anxiety and impaired stress response were observed in CRF_1 receptor knockout mice[253] whereas the opposite was observed when CRF_2 receptor was absent[250]. Thus, one may assume that agonists for the CRF_1 receptor are stress-inducing or perpetuating while agonists for CRF_2 receptor alleviate or terminate stress and anxiety. However, this is not entirely clear as activation of both receptors by the same agonist can produce a synergistic effect e.g urocortin-1-induced anorexia is mediated via CRF_1 receptor in the early phase and CRF_2 receptor in the late phase.[254] Multiple studies have confirmed the anxiolytic property of

urocortin-2 via the CRF$_2$ receptor [255-257] , however controversy arose from a recent study that showed central urocortin-2 was anxiogenic[258], the difference in response might be related to the brain site involvement.

1.9.6 Cardiovascular System

The cardiovascular actions of urocortins are mediated via the CRF$_2$ receptor which is the most abundant CRF receptor in the heart and vasculature.

The importance of urocortin/CRF$_2$ receptor in the regulation of blood pressure homeostasis is reflected in hypertension observed in CRF$_2$ receptor knockout mice.[259] Furthermore, CRF$_2$ receptor blockade induces a rise in arterial pressure and calculated total peripheral resistance.[260] Systemic administration of urocortin-2 in both human and animal models has consistently demonstrated potent vasodilatory actions while co-administration of a CRF$_2$ receptor antagonist results in a reversal of these vasodilatory effects.[242, 261-265] Multiple studies have shown that the vasodilatory effects observed in human internal mammary and coronary arteries are not completely endothelium-dependent.[242, 261, 266] The mechanism underlying vasodilatation is not completely clear, but urocortins may act predominantly by altering intracellular calcium concentration.[267] In addition, mitogen-activated protein kinase (MAPK), adenylate-cyclase dependent PKA activation, prostagladins and nitric oxide release have all been implicated.[261, 264, 265, 268] Urocortin-2 also has an exaggerated hypotensive effect in chronic hypertension in hyperadrenergic mice by regulating catecholamine synthesis and release.[269] The hypotensive effect of chronic (5-8 weeks) administration of Urocortin-1 or -2 in spontaneous or diet-induced hypertensive rats appears to be long lasting, indicating no desensitisation with prolonged treatment.[270, 271] This observation is consistent with the absence of CRF$_2$ receptor down-regulation

in the vasculature.[270] The hypotensive effect of high dose urocortin-1 (7µg/kg/day) was equivalent to high dose enalapril (10mg/kg/day).[271] Furthermore, the prolonged treatment resulted in positive vascular remodelling and remarkably an absence of vascular fibrosis on electron microscopy.[271] A previous study by the same group showed urocortin-2 administration to spontaneously hypertensive rats reduced apoptosis in the mesenteric artery smooth muscle cells via L-type calcium channels unaffected by CRF_2 receptor blockade.[272] This suggested an alternative signalling mechanism independent of CRF_2 receptor. In a case-control study by Florio et al in pregnancy complicated by hypertension, significantly higher urocortin-1 was noted in the maternal circulation arising from the foetus. The amount correlated with umbilical arterial resistance and suggested a possible pathophysiological role.[273]

Urocortins exert positive inotropic and lusitropic effects in myocardium.[264, 274-276] This effect is absent in CRF_2 receptor deficient mice.[274] A study by Yang et al on mouse ventricular myocytes confirmed the positive inotropic and lusitropic effects of urocortin-2 were, like vascular endothelial cells, dependent on intracellular calcium transient concentration.[277] The mechanism was mediated by the CFR_2 receptor with downstream activation of PKA and CaMKII.[277] However, not all studies report the same mechanism of action. Whilst this study reconfirmed the earlier findings by Yang et al on rabbit ventricular myocytes that urocortin-2 increased contractility by altering intracellular calcium handling was dependent on PKA[278], Calderon-Sanchez et al showed in more than one study that the effect of urocortin-1 was PKA-independent in rat ventricular myocytes[276, 279]. Whether the PKA dependency is species-specific, or this is due to difference in study design (the PKA inhibitor dose and agent used were different) remains uncertain. Other secondary signalling pathways implicated included protein kinase C (PKC), and MAPK.[276, 279] As urocortin-2 alters intracellular

calcium homeostasis, Yang et al went on to investigate its potential effect in calcium-triggered arrhythmia.[277] Their study showed increased arrhythmogenicity with all three urocortins given at high doses to mouse ventricular myocytes and increased spontaneous contractions in human atrial trabeculae.[277] In contrast, in hypertensive rats with left ventricular hypertrophy, a study showed urocortin-2 shortened cardiac action potential duration and ventricular conduction time without affecting the repolarization time. These actions resulted in a raised ventricular fibrillation threshold and reduced likelihood of malignant arrhythmia and appear to be mediated by an improvement in intracellular calcium handling (without altering calcium release or reuptake from the sarcoplasmic reticulum).[275] More studies are warranted to investigate if urocortins are arrhythmogenic or anti-arrhythmic in an acceptable therapeutic dose.

Tachycardia is consistently observed with administration of exogenous urocortins in both humans and animals.[223, 224, 247, 251, 274, 280, 281] In the majority of these studies, tachycardia accompanied a significant blood pressure drop. However baroreflex-mediated increase in heart rate does not completely explain the tachycardia. In healthy man, tachycardia was induced by urocortin-2 without a significant fall in blood pressure.[223] It is also uncertain the tachycardia effect of urocortins in relation to SNS, as one would expect inhibition of SNS should result in a lower heart rate. Both urocortin-1 and urocortin-2 directly suppressed efferent cardiac SNA in normal conscious sheep, as assessed by a decrease in sympathetic nerve burst incidence, burst frequency and burst area recorded from electrodes implanted in cardiac efferent sympathetic nerve fibres in conscious sheep, without significant overspill of plasma norepinephrine, a marker of SNS activation. However the heart rates were increased in both studies.[280, 281]

Increasing evidence points towards urocortins having potent cardiac protective actions. In the setting of ischaemia, urocortins are believed to have an important beneficial pathophysiological role. Expression of urocortins is induced by hypoxia and the recent identification of a hypoxia-responsive element in the 3'-flanking region of human urocortin-2 mRNA and its up-regulation in hypoxia further support its significance in acute ischaemia.[282-284] Urocortin administration following ischaemia/reperfusion injury in experimental models minimized infarct size[284, 285], reduced apoptosis[283, 286], preserved contractility[287] and reduced occurrence of arrhythmia[285]. The cardioprotection was most prominent when urocortins were administered prior to the onset of ischaemia.[283] This is potentially relevant in future therapeutic application such as coronary artery bypass grafting. In ischaemia, urocortins upregulate genes that promote free radical scavenging. The pattern of gene recruitment differs between urocortins. Urocortin-2 is able to upregulate more protective genes thus possibly offering greater cardiopropection than urocortin-1.[288] The mechanism is believed to be CRF_2 receptor-mediated via the activation of extracellular signal-regulated kinases p42,44 (ERK1/2-p42,44) phosphorylation (secondary mediators of MAPK kinase) and Akt, a serine/threonine-specific protein kinase.[283, 284] Mice lacking the CRF_2 receptor genes are more susceptible to ischaemia/reperfusion injury. Urocortins induce hypertrophy of cardiomyocytes by increasing protein synthesis, resulted in induction of ANP and BNP gene expression (foetal gene activation) via phosphorylation of Akt and glycogen synthase kinase-3β (GSK-3β).[289, 290] Increased expression of urocortin-1 mRNA is noted in patients with hypertrophic cardiomyopathy but the cause and effect relationship is uncertain.[291]

1.9.7 Neurohormonal Effects

Urocortin-1 stimulates the HPA axis resulting in an increased secretion of ACTH and

cortisol via CRF_1 receptor pathways.[208, 246] Since urocortin-2 and urocortin-3 are selective CRF_2 receptor agonists, they should not induce secretion of ACTH under normal circumstances.[210] However, systemic administration of urocortin-2 and urocortin-3 increased ACTH and cortisol in normal and pacing-induced HF sheep.[247, 248] This may be explained, at least in part, by the concurrent rise in urocortin-1 observed in the sheep studies. It is also possible that urocortin-2 could activate CRF_1 receptor at high dose[212], but this would not explain the observation with urocortin-3 administration. The same investigators also studied administration of a CRF_2 receptor antagonist in sheep which resulted in an increase in plasma urocortin-1 concentrations.[260] This was associated with increased vasoconstriction, significant rises in vasoconstrictor hormones (PRA, aldosterone, ET-1) more evident in the HF state and rises in ANP and BNP in HF sheep. These results suggest that endogenous urocortins may have a compensatory role to antagonise multiple deleterious aspects of HF including neurohormonal activation, renal compromise and inappropriate vasoconstriction.

1.9.8 Gastrointestinal Tract and Energy Metabolism

Urocortins and CRF receptors are involved in a complex central and peripheral network regulating energy metabolism by balancing food consumption and expenditure.[292] All three urocortins induce hyperthermia[245] and decrease appetite even in a fasting state.[208, 210, 293] In a study that investigated the effect of all CRF related peptides in the same study, urocortin-1 was the most potent in inhibiting food intake, decreasing weight gain, lowering blood glucose and reducing visceral fat content in obese mice.[293] All these effects may be explained by urocortin-1 suppression of ghrelin.[222, 293, 294] In urocortin-2-null mice, insulin sensitivity was increased resulting in increased glucose utilisation in the skeletal muscle and these animals were

protected against fat-induced insulin resistance in skeletal muscle.[295] Since urocortin-1 suppresses ghrelin whilst urocortin-2 increases it[293], it is possible dysregulation of urocortins contributes to the development of obesity, but this requires further study. Gastrointestinal mobility alteration could be an autonomic manifestation of stress. All urocortins delay gastric emptying (weakly by urocortin-3) via CRF_2 receptor.[296, 297] Urocortin-1 and urocortin-2 increase colonic transit while urocortin-3 has no effect; the action is via CRF_1 receptor.[297] When rats were administered with urocortins centrally, similar response of delayed gastric emptying and enhanced colonic motility were observed as when the animals were under acute stress by restraint suggesting urocortins may affect bowel function during acute stress.[297]

1.9.9 Other Urocortin Actions

Urocortins may play a role in modulating immune response but available literature is contradictory. In rats with chemical induced vasculitis in the femoral artery and in colonic epithelial cells of patients with acute colitis, mRNA of urocortin-1 and -2 and their corresponding CRF receptors were upregulated locally.[298, 299] Although the upregulation could result as a compensatory mechanism to local inflammation, urocortin-1's ability to induce synthesis and release of interleukin-6 in rat cardiomyocytes and urocortin-2 for interleukin-8 in human colonocytes[299, 300], along with attenuation of ischaemia caused by urocortin-1-induced vasculitis by blockade of the CRF_1 receptor[298] point towards urocortins being actively involved in mounting a local inflammatory response. This is in contrast to other studies showing urocortins induce anti-inflammatory cytokines, interleukin-4 and 10, production and suppress pro-inflammatory cytokines, tumour necrosis factor-α and interferon-γ, in placental trophoblasts and mice infected with Listeria monocytogenes.[301, 302] Thus, the role of urocortins in immune modulation remains to be fully elucidated.

Urocortins have demonstrated some regulatory role in the reproductive system. Urocortin-2 suppresses gonadotrophins secretion in the anterior pituitary in a paracrine manner.[303] Urocortin-2 and urocortin-3 are detected in the endometrium with varying levels throughout the secretory phase in different cell types.[232] Since CRF_2 receptor is also present in the endometrium, it is possible both urocortin-2 and urocortin-3 are involved but with different roles in local endometrial physiology.

Urocortin-2 may also have a role in cancer biology as urocortin-2 was injected to lung cancer cells implanted in mice, resulted in tumour growth blunting through angiogenesis suppression and direct anti-proliferative effect.[304] This observation is opposite of its mitogenic effect on cardiomyocyte[289] but shows potential therapeutic application in anti-cancer therapy.

1.9.10 Urocortins in Heart Failure

At present there are no commercially available assays for urocortins 1-3. Due to the difference in assay methodology, there is no available normal reference range for circulating urocortin levels. Based on locally developed research laboratory immunoassays, plasma urocortin-1 levels in healthy man has been reported to range from 3.9 to 68.8pmol/L.[222, 305, 306] Circulating urocortin-1 is elevated in HF patients and is shown to be proportional to severity of left ventricular dysfunction and BNP levels. [221, 305-307] Urocortin-1's correlation with symptomatic status and its interaction with age and renal function are inconsistent. While Wright's study showed positive correlation, this was not true for the study by Gruson et al.[305, 307] In contrast, Ng et al reported that while urocortin-1 levels were higher in HF than in health overall, paradoxically levels were higher in NYHA I-II than III-IV.[306] Possible explanations might simply be the wide overlapping range and a small sample size of the study.

Interestingly, immunochemical staining of urocortin-1 in endocardial biopsy of hypertrophic cardiomyopathy was noted to be less in more severe disease with more left ventricular dysfunction.[291] While urocortin-1 exerts positive influence on myocardial remodelling in the early stages of HF, it is possible urocortin-1 cannot retain its ability to compensate in the advanced stages of disease. Due to the overlap in plasma urocortin levels between health and sickness, urocortin-1 may not be a good diagnostic biomarker for HF. Little is known about plasma concentrations of urocortin-2 and -3 in humans. Davis et al studied infusion of urocortin-2 in health and stable HF man.[223, 224] In their studies, similar to urocortin-1, significant overlap was found between plasma urocortin-2 levels in healthy subjects and those with stable HF but with urocortin-2 levels higher in HF patients (55.6; range 9.5-99.6 pmol/L) compared with normal volunteers (21.3; range 11.9-40.3 pmol/L). These data were obtained using a prototype in-house radioimmunoassay which is yet to be validated. No studies have examined the plasma levels of, or the biological effects of urocortin-3 in man.

Exogenous urocortin 1-3 infusions in experimental ovine HF model consistently produced favourable haemodynamic responses with dose-dependent increase in cardiac output, decrease in mean arterial pressure, calculated total peripheral resistance (cTPR) and left atrial pressure (LAP) (Figure 1.3); augmentation of renal function with urine volume, sodium and creatinine excretion; and suppressed adverse neurohormonal activation with a decline in a range of vasoconstrictors and volume-retaining factors (Figure 1.4).[246-248]
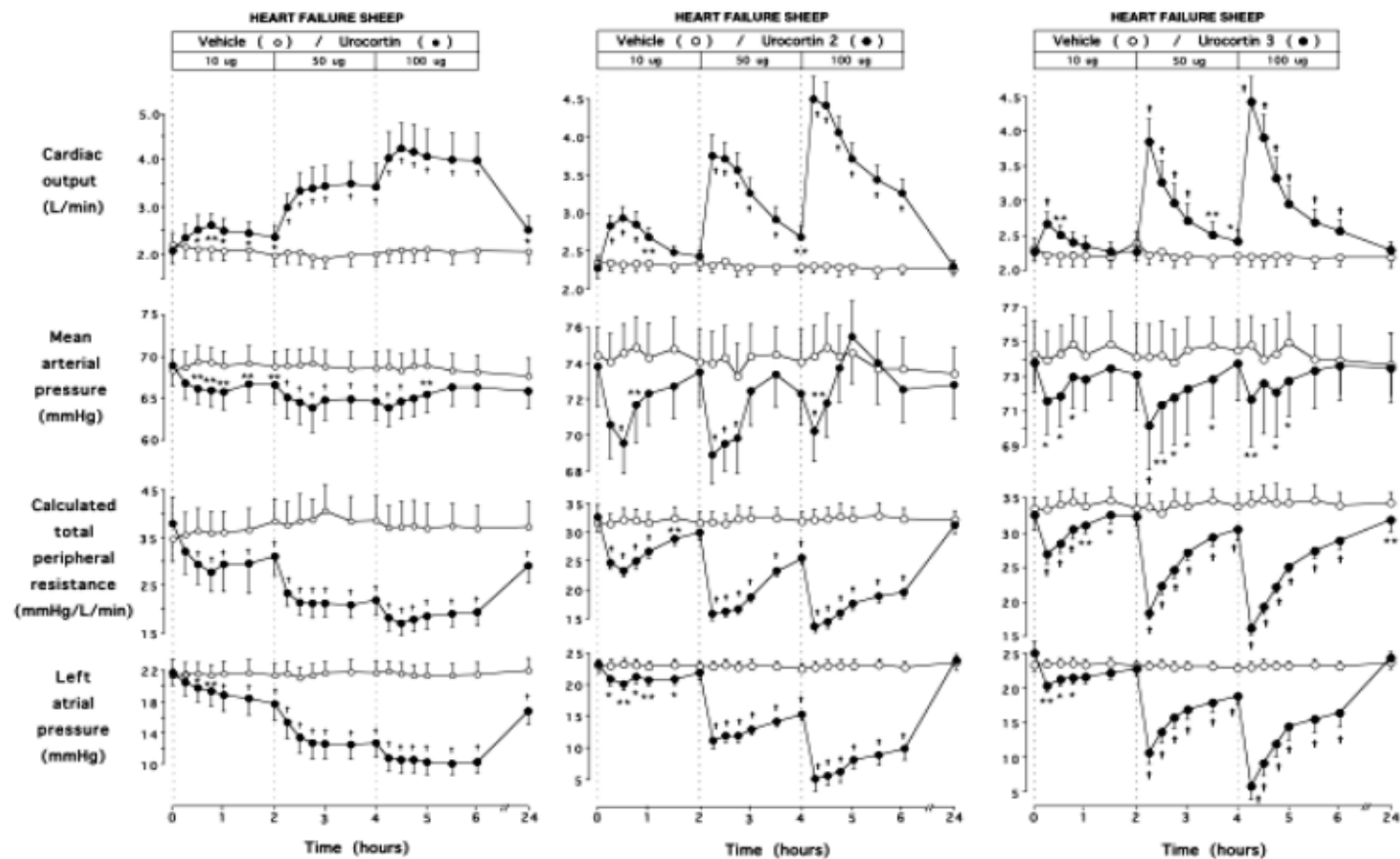
Figure 1.3. All three urocortins (black circles) produced similar dose-dependent haemodynamic effects compared to vehicle control (while circles) but at a different time scale when given in an identical dose-escalating regimen in ovine experimental heart failure. Urocortin-1, left column; urocortin-2, middle column; urocortin-3, right column. Diagram kindly provided by Associate Professor Miriam Rademaker.[246-248]
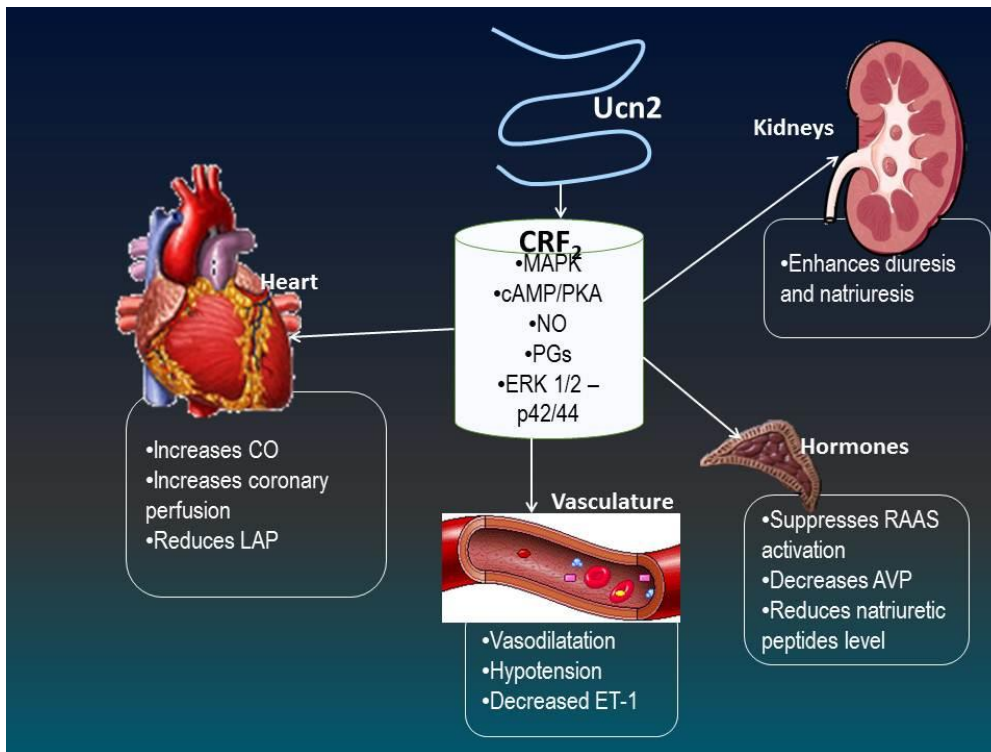
Figure 1.4. Actions of urocortin-2 in cardiac dysfunction. Ucn2, urocortin-2; CO, cardiac output; LAP, left atrial pressure; ET-1, endothelin-1; RAAS, renin-angiotensin-aldosterone system; AVP, arginine vasopressin; CRF_2, corticotrophin releasing factor type 2 receptor; MAPK, mitogen activated protein kinase; cAMP, cyclic adenosine monophosphate; NO, nitric oxide; PGs, prostaglandins; ERK, extracellular signal-regulated kinases.[247, 274]

Effects observed in HF were either absent or much reduced in magnitude in normal sheep.[246-248] In experimental (ovine) HF, haemodynamic, hormonal and renal effects persisted with no signs of dampened response when urocortin-1 or urocortin-2 were given as a continuous infusion for four days.[308, 309]

In humans, low dose exogenous urocortin-1 infusions did not affect haemodynamics or renal function but stimulated the HPA axis in both normal and stable HF state.[221, 222] In contrast, exogenous urocortin-2 infusion significantly increased cardiac output and reduced mean arterial pressure in normal man and patients with stable HF.[223, 224] The blood pressure lowering effect was more pronounced in HF patients and was associated with a persistent reduction in cTPR that outlasted the duration of the infusion.[224] While high dose urocortin-2 increased renin, Ang II and aldosterone in healthy man, likely secondary to concurrent lowering of blood pressure, no impact

was noted with lower dose or in HF patients and minimal renal effects were observed.[223, 224] Comparing the temporal profile amongst urocortins in the ovine model and the effect on humans, urocortin-2 stands out from its family members with potential in HF treatment (Figure 1.4). Further studies by Rademaker et al in experimental HF in the ovine model have evaluated the effects of co-administration in urocortin-2 with standard HF therapies. When combined with frusemide, urocortin-2 produced an enhanced diuretic and natriuretic response in a more sustained manner without worsening renal function. The combination also resulted in improvements in haemodynamics and suppression of PRA that were not seen with frusemide alone.[310] When urocortin-2 was co-administered with an ACEI, the combination produced a more favourable haemodynamic profile than that seen with separate ACE inhibition, including additional augmentation of cardiac output and vasodilatation, but without a further reduction in mean arterial pressure which is required to maintain peripheral perfusion.[311] Urocortin-2 co-treatment also produced significant inhibition of adverse neurohormone systems and improvements in renal function relative to ACE inhibition alone.[311] When combined with a beta-blocker, the haemodynamic effect was more favourable with reversal of tachycardic effect by urocortin-2 alone whilst urocortin-2 counterbalanced the increased cTPR observed with beta-blocker alone, without compromising the increased cardiac output.[312] Thus urocortin-2 works well in combination with established evidence-based therapies for HF which further supports a potential role for urocortin-2 as a novel adjunctive HF therapy. It is possible that the lack of neurohormonal effects in humans (compared to those seen in the ovine model) could be secondary to the relatively quiescent state of neurohormonal activation when HF was stable, thus the true potential of urocortin-2 in human HF may not have been seen.

1.10 Treatment of Heart Failure

The long-term of goals of HF therapy are to delay or reverse disease progression, inhibit adverse cardiac remodelling, improve quality of life and symptoms, prevent hospitalisation and reduce mortality. A multi-disciplinary approach is often deployed with a primary emphasis on pharmacological therapy to achieve these goals Figure 1.5). The majority of the evidence-based treatments that have been shown to successfully alter the natural history of HF, are based on antagonism of neurohormonal activation in HF. However, so far the success of neurohormonal antagonists is limited to the setting of HFREF. In contrast, neurohormonal antagonists have had limited or no efficacy in the setting of HFPEF, which may in part be related to a different pattern in neurohormonal activation.[313, 314] In ADHF, patients are symptomatically fluid overloaded, frequently associated with haemodynamic instability and renal dysfunction. Early initiation of evidence-based therapy may not be feasible. The aim of therapy in the ADHF setting is to rapidly relieve symptoms for which oxygen and morphine are commonly used, treat fluid overload, restore haemodynamic stability and then introduce (or increase) established therapies that have shown to reduce death or rehospitalisation as soon as appropriate.

Diuretics to relieve symptoms/signs of congestion[a]

+

ACE inhibitor (or ARB if not tolerated)[b]

ADD a beta-blocker[b]

Still NYHA class II–IV?

Yes → **ADD a MR antagonist**[b,d] No[c] →

Still NYHA class II–IV?

Yes → *LVEF ≤35%?* No →

Yes → *Sinus rhythm and HR ≥70 beats/min?* No →

Yes → **ADD ivabradine**[e] No →

Still NYHA class II–IV and LVEF ≤35%?

Yes → *QRS duration ≥120 ms?* No →

Yes → **Consider CRT-P/CRT-D**[f] No → **Consider ICD**[g]

Still NYHA class II–IV?

Yes No[g] →

No further specific treatment[c]
Continue in disease-management programme

Consider digoxin[h] **and/or H-ISDN**[i]
If end stage, consider LVAD and/or transplantation

ACE = angiotensin-converting enzyme; ARB = angiotensin receptor blocker; CRT-D = cardiac resynchronization therapy defibrillator; CRT-P = cardiac resynchronization therapy pacemaker; H-ISDN = hydralazine and isosorbide dinitrate; HR = heart rate; ICD = implantable cardioverter-defibrillator; LBBB = left bundle branch block; LVAD = left ventricular assist device; LVEF = left ventricular ejection fraction; MR antagonist = mineralocorticoid receptor antagonist; NYHA = New York Heart Association.
[a] Diuretics may be used as needed to relieve the signs and symptoms of congestion (see Section 7.5) but they have not been shown to reduce hospitalization or death.
[b] Should be titrated to evidence-based dose or maximum tolerated dose below the evidence-based dose.
[c] Asymptomatic patients with an LVEF ≤35% and a history of myocardial infarction should be considered for an ICD.
[d] If mineralocorticoid receptor antagonist not tolerated, an ARB may be added to an ACE inhibitor as an alternative.
[e] European Medicines Agency has approved ivabradine for use in patients with a heart rate ≥75 b.p.m. May also be considered in patients with a contraindication to a beta-blocker or beta-blocker intolerance.
[f] See Section 9.2 for details—indication differs according to heart rhythm, NYHA class, QRS duration, QRS morphology and LVEF.
[g] Not indicated in NYHA class IV.
[h] Digoxin may be used earlier to control the ventricular rate in patients with atrial fibrillation—usually in conjunction with a beta-blocker.
[i] The combination of hydralazine and isosorbide dinitrate may also be considered earlier in patients unable to tolerate an ACE inhibitor or an ARB.

Figure 1.5. Management algorithm of symptomatic heart failure with impaired systolic function. Figure reprinted from ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2012 with permission. Copyright Rightslink (2012).[1]

1.10.1 Drug Therapy for ADHF

Diuretics, predominantly loop diuretics are the mainstay of therapy for symptoms of

hypervolaemia and/or pulmonary congestion in acute and chronic HF. However, despite the pivotal role of diuretics in treating fluid overload and relieving symptoms of congestion, there is no evidence that diuretics alone improve prognosis in the intermediate and longer term. In stable HF patients, diuretic withdrawal has been shown to cause deterioration in haemodynamic and clinical status.[315] On the other hand, there is also concern that diuretics can stimulate RAAS and SNS[316, 317] which in turn cause vasoconstriction and fluid retention and contribute to the cardiorenal syndrome. Diuretic use, especially administering in high doses, has been associated with increased adverse outcomes including worsening of HF, HF hospitalisation and death in both acute and chronic settings.[318-321] Patients on higher dose diuretics tended to be older, had poorer left ventricular systolic function and higher plasma creatinine, i.e. a sicker group of patients. There is also ongoing debate over the best mode of administration. Recently, the "Diuretic Optimization Strategies Evaluation" (DOSE) trial has settled many questions in relation to the optimal dosing strategies for loop diuretics in ADHF.[322] The study showed similar efficacy in global assessment of symptoms with boluses or continuous infusion of frusemide but higher dose of frusemide (2.5 times previous oral dose equivalent) was associated with greater relief of symptoms, fluid loss, and fewer serious adverse events. Although there was transient worsening renal function, there was no sustained fall in estimated glomerular filtration rate (GFR).

Inotropic agents are sometimes deployed in ADHF to increase cardiac output and in turn end-organ perfusion and reduce intra-cardiac pressures by directly enhancing myocardial contractility.[323] The strongest indication is hypotension, often as a rescue therapy in cardiogenic shock. Patients who require inotropic therapy suffer higher in-hospital mortality.[93, 95] Despite the short-term improvement in haemodynamics, there

has been no proven advantage and possibly worse in-hospital or post-discharge survival, possibly partially explained by the arrhythmogenic nature of most clinically used positive inotropes.[94, 95, 324]

Recent trials on new drug therapies for ADHF have been disappointing. Rolofylline, a selective A_1 adenosine receptor antagonist showed greater dyspnoea relief, less frequent worsening of renal function and a trend to lower mortality or rehospitalisation with heart failure or renal failure than placebo in the pilot study.[325] However, in the phase 3 trial, up to 3 days of rolofylline 30mg daily did not increase HF treatment success or reduce the incidence of worsening renal function, and had no benefit on mortality or cardiovascular and renal rehospitalisation by 60 days compared to placebo whilst causing more seizures and strokes.[326] Tezosentan, a non-specific endothelin receptor antagonist, improved haemodynamic indices but showed no advantage over placebo in dyspnoea improvement, worsening HF or short and long term mortality.[327] Levosimedan, a calcium sensitiser, had a significantly greater reduction in BNP levels when compared to dobutamine or standard therapy in ADHF, however this has not translated into improved survival.[328, 329] Nesiritide, recombinant human BNP, showed promise in initial studies with significant improvement in haemodynamic and dyspnoea status compared to placebo and similar efficacy to other conventional vasoactive agents.[330, 331] However subsequent meta-analysis from a small collection of randomised controlled trials raised safety concerns of increased mortality and worsening renal function with nesiritide in ADHF.[332, 333] A later meta-analysis did not find increased risk of death at 30- and 180-days but concluded insufficient events occurred to properly address these concerns.[334] Recently results from the "Acute Study of Clinical Effectiveness of Nesiritide in Decompensated Heart Failure" (ASCEND-HF), a large randomised placebo controlled trial have

finally become available to address these concerns.[175] Although the safety of nesiritide use was confirmed without increases in death or worsening renal failure, its efficacy in symptom relief, death and rehospitalisation was disappointingly neutral. Finally, serelaxin, a recombinant of naturally occurring human relaxin-2 during pregnancy; the results from the "Relaxin in Acute Heart Failure trial" (RELAX-AHF) are recently published in what may be potentially the most significant breakthrough in the treatment of ADHF over the past two decades.[335] RELAX-AHF was a prospective, randomised, double-blind trial comparing serelaxin at 30μg/kg daily determined from an earlier phase 2 dose defining trial[336] to placebo for up to 48 hours in ADHF with mild to moderate renal dysfunction. Serelaxin resulted in mild improvement of dyspnoea and less likely to experience worsening HF at the first 14 days and fewer adverse renal events without increased in hypotensive adverse events at day 5. However, there was no reduction in recurrent HF and renal failure related readmission. In the post-discharge phase, there was a trend to reduced 30-day all-cause mortality in the serelaxin group (placebo 19 deaths, serelaxin 12 deaths; hazard ratio 0.63; 95% CI 0.3-1.29; P=0.20) and a significant 37% reduction in all-cause (placebo 65 deaths, serelaxin 42 deaths; hazard ratio 0.63; 95% CI 0.43-0.93; P=0.02) and cardiovascular (placebo 55 deaths, serelaxin 35 deaths; hazard ratio 0.63; 95% CI 0.41-0.96; P=0.028) mortality at 5-180 days. However as the authors have pointed out, the trial was not powered to study the impact on mortality, it is also unclear why there was a mortality benefit without reduction in HF rehospitalisation.

1.10.2 Non-Pharmacological Management of ADHF

Continuous positive airway pressure ventilation augments cardiac output and oxygen delivery, reduces respiratory effort and decreases left ventricular afterload.[337] These are all beneficial effects in ADHF with pulmonary oedema. Although bi-level positive

airway pressure ventilation is just as effective, safety concerns have been raised over the increased incidence of myocardial infarction.[338] A meta-analysis of 11 studies comparing continuous positive airway pressure with standard medical therapy showed favourable reduction in mortality and reduced need for invasive ventilation.[337]

Intra-cardiac pressures are elevated and cardiac index is often reduced in ADHF. Treatment guided by measurement of intra-cardiac pressures and cardiac output via pulmonary artery catheterisation successfully improved haemodynamic indices but did not improve 6 month outcome.[339] Whilst current guidelines recommend that pulmonary artery catheterisation should not be routinely used in ADHF, it may have value in selected patients whose volume status is difficult to judge by clinical non-invasive assessment.

Ultrafiltration reduces vascular volume by direct removal of excess fluid. The therapy has been adopted from nephrology from the treatment of renal replacement therapy for chronic renal failure. Its effectiveness and safety in ADHF remains controversial. Most reports in the literature are from case series. There are only four randomised trials, two of very small sample size (≤20 patients in each arm), and all had different protocols and endpoints with mixed success reported for weight loss, symptom relief, length of hospital stay and BNP reduction but none reported worsening plasma creatinine.[340-343] The "Ultrafiltration versus Intravenous Diuretics for Patients Hospitalized for Acute Decompensated Congestive Heart Failure" (UNLOAD) trial investigated longer term effects and reported reduced 90 day readmission. However the study has been criticised for the low diuretic use in the control arm.[342] Recently, a study of ultrafiltration initiated in ADHF patients to prevent worsening renal function, a common complication of ADHF, reported worse prognosis.[344] Ultrafiltration was no better than diuretic therapy and was associated with increased plasma creatinine. This

may reflect rates of fluid removal exceeding the vascular refilling rate leading to renal hypoperfusion. Thus, ultrafiltration is unlikely to be a routinely recommended part of treatment for ADHF until this issue is solved.

After initial stabilisation, diagnostic and other procedures may be necessary for best management of the underlying cause of HF. This includes revascularisation of CAD and valvular surgery.[32, 345] Although cardiac transplantation has never been subjected to any controlled trials, it is an excellent option in a selected group of end-stage heart failure patients with median post-transplant survival now exceeding 12 years. However, cardiac transplantation is not widely available and patients need to undergo detailed work-up for eligibility and often wait for a long time for suitable organs. These patients have severe cardiac insufficiency and may require mechanical circulatory support from ventricular assist devices as a bridge to definitive therapy.

1.10.3 Drug Therapy for Chronic Heart Failure

Once acute volume overload and haemodynamic instability are corrected in ADHF, introduction or increase in therapies proven to improve outcomes in chronic HF are indicated. These therapies have only been shown to improve outcomes in HFREF and the cut-off for LVEF to define HFREF vs HFPEF is variable between studies. The activation of the RAAS in HF has been discussed above. RAAS suppression reduces vasoconstriction, protects against and may reverse left ventricular adverse remodelling and improves endothelial function.[346, 347] Mortality benefit from ACEIs was evident in SAVE, SOLVD and CONSENSUS trials.[97, 99, 348] ACEI does not completely block Ang II generation due to alternative pathways in tissues, therefore ARBs may be useful. Treatment with ARBs also improves left ventricular remodelling and fibrosis.[349] In the "Optimal trial in myocardial infarction with the

angiotensin II antagonist losartan" (OPTIMAAL)[350] and "Valsartan in acute myocardial infarction trial" (VALIANT)[351] ARBs have been shown to be as effective as ACEI in reducing mortality in the post infarct setting and in symptomatic HF.[352] The Val-HeFT and the "Candesartan in heart failure: assessment of reduction in mortality and morbidity" sub-study (CHARM-added) evaluated concomitant use of ACEI and ARB. Benefit was modest (although importantly, significant reductions in HF readmissions were observed) and combined treatment was associated with a significant increase in plasma creatinine and hyperkalaemia.[351, 353] Thus, ARBs are a good alternative for those who are intolerant of ACEI in the long-term therapy of left ventricular dysfunction, whilst concurrent use requires careful weighing of the risk and benefits.

Mineralocorticoid receptors stimulated by aldosterone and, inappropriately, by glucocorticoids mediate toxic effects on the myocardium in HF. Aldosterone cannot be completely suppressed by ACEI or ARB and as stated above, much of the deleterious mineralocorticoid-mediated damage occurring in HF is due to abnormal stimulation by glucocorticoids which circulate at a thousand times the concentration of aldosterone (which is often present in normal plasma concentrations in many HF cases). The MR antagonist, spironolactone has been shown to suppress SNA in severe ischaemic cardiomyopathy.[354] Low dose spironolactone in addition to what was then considered optimal other therapy was safe and improved symptoms in severe HF[355] and could achieve a greater cardiac SNA suppression and BNP reduction than on Candesartan alone[356]. Selective mineralocorticoid antagonism has also been shown to reduce the incidence of atrial fibrillation or flutter, which is associated with a worse prognosis, in mild systolic HF presumed from its anti-fibrotic effect.[357] The landmark "Randomized Aldactone Evaluation Study" (RALES) study showed spironolactone

reduced mortality, cardiovascular hospitalisation and improved functional status in patients with severe chronic HF. The mortality benefit was close to the combination of ACEI and beta-blockers.[358] However, despite the majority of patients being on an ACEI, only ~10% of patients were on a beta-blocker (which was not part of the standard care at the time of the study) which later raised doubts about its possible benefit in addition to ACEI/ARB plus beta-blockade. This scepticism was mitigated when eplerenone, a selective aldosterone antagonist, reduced mortality (predominantly sudden cardiac death) and HF hospitalisation in patients with HF and left ventricular systolic dysfunction post acute myocardial infarction.[359] The magnitude of mortality reduction was somewhat smaller than RALES, however the majority of patients in this study were on both an ACEI and beta-blockers (standard care). Thus there is additional benefit from adding a MR antagonist to ACEI/ARB and beta-blockade in all stages of HFREF. Recently the benefit has been tested and extended to mildly symptomatic (NYHA II) patients with moderate to severe left ventricular dysfunction.[360]

Sympathetic nerve activity is elevated in HF, increases heart rate and work and is associated with a poor prognosis. The first large-scale randomised studies to investigate the benefit of beta-blocker in chronic HF were "Cardiac Insufficiency Bisoprolol Study" (CIBIS) and CIBIS-II.[184, 361] In CIBIS, bisoprolol compared to placebo in addition to vasodilator, predominantly ACEI, showed a non-significant trend of a 20% lower mortality and 30% fewer HF hospitalisations in NYHA III-IV patients with left ventricular systolic dysfunction.[184] The low number of events that occurred in CIBIS was believed to have contributed to the non-statistical significant result. The larger scale CIBIS-II trial demonstrated a significant (32%) mortality benefit and reduced HF hospitalisations regardless of the aetiology of HF or

functional class.[361] Later in the same year another large-scale multicentre randomised controlled trial in patients with less severe HF reported similar relative risk reduction for mortality.[362] Consistent reductions in mortality and HF hospitalisation were also seen in patients with more severe HF.[363] The concomitant use of beta-blockers, ACEI/ARB and mineralocorticoid antagonist are now incorporated into standard treatment for chronic HF, usually together with loop diuretics.

Amongst the three beta-blockers that have been trialled in chronic HF, bisoprolol and metoprolol are β_1 selective antagonists while carvedilol is a non-selective antagonist of α_1, β_1 and β_2 adrenoceptors. A few small studies have suggested superiority of carvedilol over metoprolol with improved haemodynamic, left ventricular remodelling and decreased cardiac norepinephrine production.[364, 365] The "Carvedilol or Metoprolol European trial" (COMET) is the only large trial comparing the two agents that showed a mortality advantage for carvedilol over metoprolol in moderate to severe chronic HF.[366] However, this study was heavily criticised due to the lower than optimal dose and use of the short acting formulation of metoprolol. Furthermore, beta-adrenergic receptor function is affected by genetic polymorphism and a subgroup of patients, based on a small observation study, may do better on metoprolol.[367] All studies that show mortality and morbidity benefits of beta-blockers are associated with significant heart rate reduction even when target dose is not reached. It is uncertain what proportion of benefit from beta-blocker is contributed by the heart rate modulation which in turn attenuates myocardial energy expenditure rather than direct beta-blocker-mediated reductions in cardiac energy demands. A post-hoc analysis from "Metoprolol CR/XL Randomized Intervention Trial in Congestive Heart Failure" (MERIT-HF) suggested it was the target heart rate achieved rather than the dose of beta-blocker that mattered.[368] The importance of heart rate reduction is further

reinforced by the recent trials of Ivabradine (a selective I_f channel blocker that lowers heart rate without other cardiovascular actions) showing additional reductions in cardiovascular death and first HF hospitalisation when given to chronic HF patients with heart rate sustained at over 70bpm despite maximum tolerated doses of beta-blocker.[369]

1.11 Cardiac Resynchronisation Therapy

Electrical conduction may be slowed in the failing myocardium. The resultant conduction delay is manifested as prolonged QRS duration, which in turn causes intra- and interventricular dyssynchrony. The principle of cardiac resynchronisation therapy (CRT), also known as biventricular pacing, is to restore synchronous systolic contraction on the left ventricular septal and lateral walls. CRT results in acute and sustained improvement in haemodynamics, cardiac remodelling, left ventricular systolic function, reductions in neurohormonal activation, reduced cardiac SNA, improved symptoms and improved quality of life.[370-380] CRT with or without a defibrillator significantly reduced mortality or hospitalisation by 20-37% as demonstrated in landmark trials including the "Comparison of Medical Therapy, Pacing, and Defibrillation in Heart Failure" (COMPANION) and the "Cardiac Resynchronisation – Heart Failure" (CARE-HF).[372, 373] The predominant benefit is driven by a substantial reduction in deaths and HF hospitalisations.[372, 373] CRT is cost-effective[381, 382] and has revolutionised the treatment of selected patients with NYHA class III-IV symptoms, severe left ventricular systolic impairment (EF ≤35%) and prolonged QRS duration (≥120ms) who are already on optimised medical therapy. The benefit of CRT has also been shown to extend to asymptomatic or mildly symptomatic patients, with delay or reversal of HF progression.[383-385]

However, echocardiographically apparent improvement in mechanical left ventricular function correlates poorly with symptom improvement.[383] Depending on the endpoints in each study, a significant proportion of patients do not benefit from CRT and approximately 30% of CRT patients are considered "non-responders".[371-373, 385] Subgroup analyses from large randomised trials have identified that patients with left bundle-branch block morphology[372, 386, 387], non-ischaemic aetiology[388], female gender[384] and QRS duration ≥150ms[384] are more likely to respond to CRT. The cause of CRT failure is usually multifactorial. Some of the device-related causes identified included inadequate biventricular pacing such as interruption by poor rate control atrial fibrillation, large myocardial scar burden, unfavourable coronary sinus anatomy, left ventricular lead placement discordant to dyssynchrony, suboptimal pacing settings and the disease being too advanced.[389-395]

Identifying CRT responders is challenging. Although the rationale of CRT is correction of electrical dyssynchrony, the presence of mechanical dyssynchrony poses additional risk.[396] Whilst electrical dyssynchrony is easily assessed by prolonged QRS duration on surface electrograms, mechanical dyssynchrony requires additional imaging assessments. Echocardiography is the most readily accessible modality to identify mechanical dyssynchrony. Although certain echocardiographic parameters have demonstrated ability to distinguish responders from non-responders in small scale studies, when undergoing head-to-head comparison in a large scale multicentre study, some parameters were not reproducible with very high (72%) inter-observer variability and none of 12 parameters assessed stood out as a good predictor with all exhibiting low sensitivity and poor specificity for predicting response to CRT.[397] Currently, many new echocardiographic parameters such as myocardial speckle tracking[398, 399], and other imaging modalities such as computed tomography[400, 401] are

being evaluated. However until new evidence is available, current screening tools cannot accurately prospectively identify CRT responders.

Opportunities to improve the CRT response rate post-implantation are limited. Most trials of CRT have excluded patients with atrial fibrillation and in general such patients tend to receive less benefit due to inadequacy of pacing. A systematic review suggested patients with atrial fibrillation and HF could benefit more from CRT if they also underwent AV nodal ablation.[402] CRT optimisation can partially compensate for suboptimal anatomical placement.[389]

1.11.1 CRT Optimisation

In a comprehensive assessment at a single centre in the United States, suboptimal CRT settings were noted to be the most common explanation for non-responders.[394] Fortunately suboptimal CRT settings are modifiable. There are two components of CRT optimisation, correction of atrioventricular delay (AVD) and interventricular delay (VVD). The aim of atrioventricular optimisation is to maximise left ventricular diastolic filling.
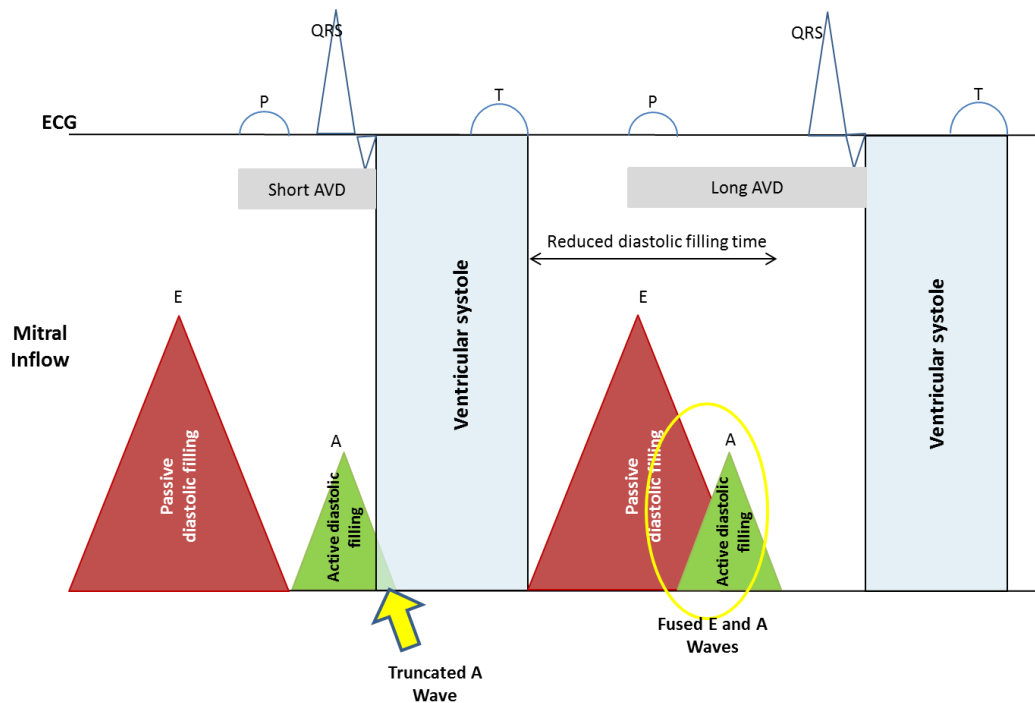
Figure 1.6. Diagrammatic presentation of when AV Delay is too short on the left and too long on the right. AVD, atrioventricular delay; E, E wave representing passive left ventricular diastolic filling; A wave, A wave representing active left ventricular filling from atrial kick. Adapted from Stanton et al.[403]

If the AVD is too short, the atrium contracts against a closed mitral valve and could paradoxically increase the pulmonary capillary wedge pressure, left ventricular diastolic filling also misses out on the active atrial systolic kick. On the other hand, if the AVD is too long, atrial contraction occurs much earlier than completion of passive left ventricular diastolic filling and the active filling becomes ineffective. (Figure 1.6) The importance of preserving atrioventricular synchrony was initially noted with the reduction of cardiovascular and thromboembolic events in patients who underwent atrial pacing than ventricular pacing for sick sinus syndrome.[404] Atrioventricular prolongation in first-degree heart block may not be as benign as once believed. During 5-year follow-up in a large prospective cohort with stable CAD, patients with PR interval >220ms were twice as likely to be hospitalised with HF and suffered a 1.6-fold increase in mortality.[405] In an early study, left atrial pressure (LAP) was raised with prolonged AVD.[406] Inappropriately timed AVD could also be detrimental,

such as in hypertrophic cardiomyopathy in which adequate cardiac stroke volume is heavily dependent on adequate diastolic filling.[407] The aim of interventricular optimisation is to reduce intraventricular dyssynchrony which in turn increases stroke volume. The reverse logic can be demonstrated by HF not uncommonly seen post right ventricular pacing from pacing induced dyssynchrony.[408] CRT optimisation has been shown in several studies to improve left ventricular haemodynamics, contractility and ejection fraction. The impact of CRT optimisation on outcome is questionable despite optimisation being routinely performed in the landmark trials.[372, 373] A major limitation is that CRT optimisation is normally performed in the resting state but not at heart rates occurring in exercise. While a physiological increase in heart rate shortens atrioventricular conduction, incremental atrial pacing prolongs it due to altered left atrial electromechanical conduction.[409, 410] Furthermore, the pattern of intraventricular dyssynchrony is also affected by increasing heart rate, and may require a different CRT setting during exercise.[409] More emphasis has been put on atrioventricular optimisation as the literature showing benefit on interventricular optimisation is weak.[411] Small observational studies suggested the potential value of atrioventricular optimisation using echocardiography on haemodynamics, functional status and possibly long-term outcome.[412, 413] Two large prospective randomised controlled trials testing automated electrogram-based optimisation do not support routine atrioventricular optimisation.[414, 415] In the "SmartDelay Determined AV Optimization" (SMART-AV) trial, no difference was shown in functional status, LVEF or dimensions between a fixed AVD and one determined by echocardiography, despite a greater than 30ms difference for more than half of the group.[415] However, a recent pilot study using automated algorithm to adjust peak endocardial acceleration (a haemodynamic variable) led to bigger proportion of patients with improved

functional status.[416] It is likely that for the majority of CRT recipients, the benefit of CRT is achievable without the need for optimised settings; however there may still be a role for optimisation in selected cases. Another problem with CRT optimisation is the lack of consensus regarding a gold standard methodology.[398, 417-419] Echocardiography-guided CRT optimisation is the most widely used method but is limited by being operator dependent and time consuming with no consensus on representative indices. An automated algorithm has its appeal, however, it is possible that like the SMART-AV trial, electrogram-based atrioventricular optimisation is not as reliable as haemodynamic variables or other surrogates (as in echocardiography) in maximising left ventricular diastolic filling. Although CRT optimisation could improve the response rate, no studies so far have investigated the isolated impact of CRT optimisation on mortality and HF hospitalisation and more studies are required to answer this question.

1.12 Heart Failure Monitoring

Regular monitoring of fluid status can allow early detection of deterioration. There is frequently a window of time between first presentation of symptoms and overt decompensation[420], which provides an opportunity to intensify medical therapy and potentially avert ADHF and hospitalisation.

1.12.1 Multidisciplinary Approach (Figure 1.7)
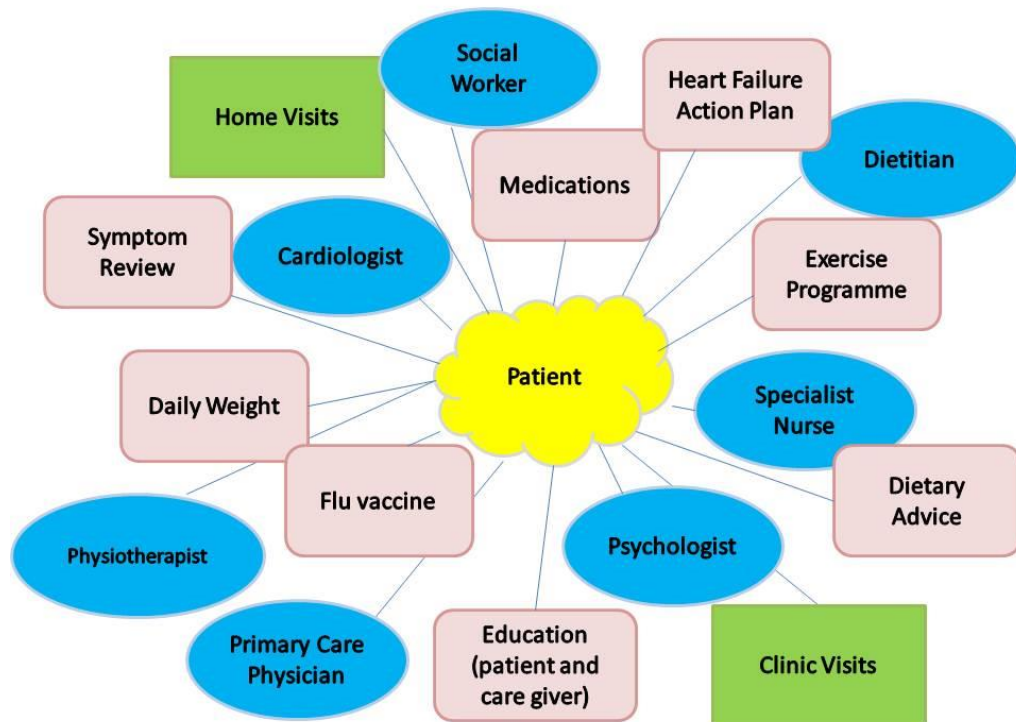
Figure 1.7. Diagrammatic presentation of multidisciplinary approach of heart failure management involves complex liaison of hospital and community personnel to achieve various tasks.

Most community-based heart failure management programmes are multidisciplinary and have proven benefits in reducing rehospitalisation rates, promoting greater uptake of proven medications, and are cost-effective.[421-425] These programmes are personnel and time-intensive, and often involve daily monitoring of clinical data like weight and blood pressure. Since development of telecommunication systems over the past decade, interest has been directed at applying this technology to achieve more frequent and reliable HF monitoring. In theory automated programmes could be as or more effective than nurse-led monitoring programmes in reducing mortality and hospital presentations.[426, 427] Interestingly results from the largest randomised controlled trial, the "Telemonitoring to Improve Heart Failure Outcomes" (Tele-HF) trial, were negative[428]. The main reason for this negative result was the marked drop

in patient participation from >90% participation at 1 week post discharge to 55% at 6 months.[428] This study highlights the key to success for community monitoring programme is ensuring patient compliance and suggests a need for inter-personal contact.

1.12.2 Brain Natriuretic Peptide

BNP secretion in HF is predominantly stimulated by myocardial wall stress.[155-157] Single time point measurement of BNP or NT-proBNP at various disease stages provides useful prognostic information on mortality and HF events.[104, 167] Change in BNP level with time can provide extra information about prognosis and response to therapy.[171, 429] Therefore there is a potential role for serial BNP measurements.

The first study that evaluated the feasibility of BNP-guided therapy was a pilot study from Christchurch.[430] In this study, patients with LVEF <40% receiving BNP-guided therapy had a significantly lower rate of cardiovascular death or HF decompensation requiring hospitalisation.[430] Following that multiple studies have shown there is an advantage in long-term BNP monitoring to guide therapy for chronic systolic HF thereby improving uptake of guideline-recommended therapy, and improving quality of life and has consistently shown to reduce HF death and HF hospitalisation (approximately halved in the "Systolic Heart Failure Treatment Supported by BNP" (STAR-BNP) study) over usual care.[173, 174, 431-433] Although reduction in all-cause mortality was not statistically significantly demonstrated in some studies, the trend was uniformly present. This could be due to insufficient power by individual study; pooled data in two subsequent meta-analyses were in concordance that BNP-guided therapy significantly reduced all-cause mortality.[434, 435]

Despite favourable outcomes reported, BNP-guided therapy appeared to be non-

beneficial in certain sub-groups and not universally accepted. First, the benefit of BNP-guided therapy was only demonstrated in patients <75 years. It is unclear why the discrepancy in relation to age occurs but the age effect clearly influences results in "Trial of Intensified vs Standard Medical Therapy in Elderly patients with Congestive Heart Failure" (TIME-CHF) and "NT-proBNP-assisted Treatment to Lessen Serial Cardiac Readmissions and Death" (BATTLESCARRED) trials.[174, 432] Other comorbidities often co-exist with advanced age that may have contributed to the lack of benefit in this age group. The elderly have higher plasma BNP and, perhaps the target BNP levels in the studies were not always appropriate. Secondly, patients with HFPEF do not seem to benefit.[432, 436] To date, none of the evidence-based medical therapies for HFREF has proven an equivalent efficacy in HFPEF which could account for the neutral result of BNP-guided therapy in this sub-group. In BATTLESCARRED 53% of patients aged >75 years had LVEF >40%, this may in part contribute to the lack of benefit in the >75 years age group.[432] As alluded to above, there is also a lack of consensus on the optimal target BNP levels. Target BNP in published trials varied widely. STAR-BNP set the lowest target at approximately 15pmol/L (100pg/mL) while target as high as 200pmol/L was noted in the Christchurch pilot study.[430, 431] TIME-CHF attempted to cater for higher BNP levels in the elderly with a separate target set for ≥75 years and the "Can Pro-brain-natriuretic Peptide Guided Therapy of Chronic Heart Failure Improve Heart Failure Morbidity and Mortality" (PRIMA) and "Swedish Intervention Study – Guidelines and NT-proBNP Analysis in Heart Failure" (SIGNAL-HF) studies have adopted a more individualised target approach.[436, 437] The "Heart failure outpatient monitoring evaluation" (HOME) study (http://www.clinicaltrials.gov, NCT01347567) of daily BNP monitoring has been halted due to slow recruitment and currently further such

trials are in the planning stage, the outcome of such trials could alter future management practice.

Overall, BNP-guided therapy positively impacts on all-cause and HF mortality and reduces HF decompensation requiring hospitalisation in young patients with left ventricular systolic dysfunction. It is likely that BNP monitoring will be incorporated in routine use in this sub-set.

1.12.3 Device Monitoring

Physical signs lack sensitivity to detect HF decompensation. In a small observational study only 11% of patients with an elevated pulmonary capillary wedge pressure of >22mmHg had pulmonary crepitations and 25% patients with elevated right atrial pressure had raised jugular venous pressure or peripheral oedema.[438] Haemodynamic disturbances in decompensated HF typically precede clinical signs by several weeks.[439, 440] There is potential for early detection of HF decompensation using sensing functions from devices already implanted for other clinical indications. This broadly divides into intrathoracic impedance monitoring and haemodynamic monitoring.

Intrathoracic impedance information can be obtained from implantable cardioverter defibrillators or CRT devices. Impedance is the degree of resistance of electrical current flow at a given voltage.[441] As water is a good electrical current conductor, increments in pulmonary fluid reduce intrathoracic impedance. Intrathoracic impedance is inversely proportional to capillary wedge pressure.[442] Impedance monitoring increases the sensitivity of detecting fluid accumulation compared to serial body weight alone.[443] However a fall in impedance is not specific to HF as other causes of increased in intrathoracic fluid, such as pneumonia, also influence

impedance. The results from a recent large scale double blind trial evaluating an impedance-based algorithm showed a sensitivity only 38% and 42% positive predictive value in detecting future HF events 6 months after implant[444] and from other smaller scale observational studies with less stringent criteria, the sensitivity of detecting worsening HF episode within 30 days was at best only 76%[443]. Similarly another recent prospective randomised controlled trial also did not show reduction in hospitalisation or outpatient presentations, instead, the use of impedance monitoring with a built-in alarm algorithm led to more hospitalisations and outpatient visits triggered by false alarms.[445] So far, the long-term performance of impedance in HF monitoring has been disappointing. Another large scale trial is ongoing using the same impedance algorithm but with a different alert notifying system.[446]
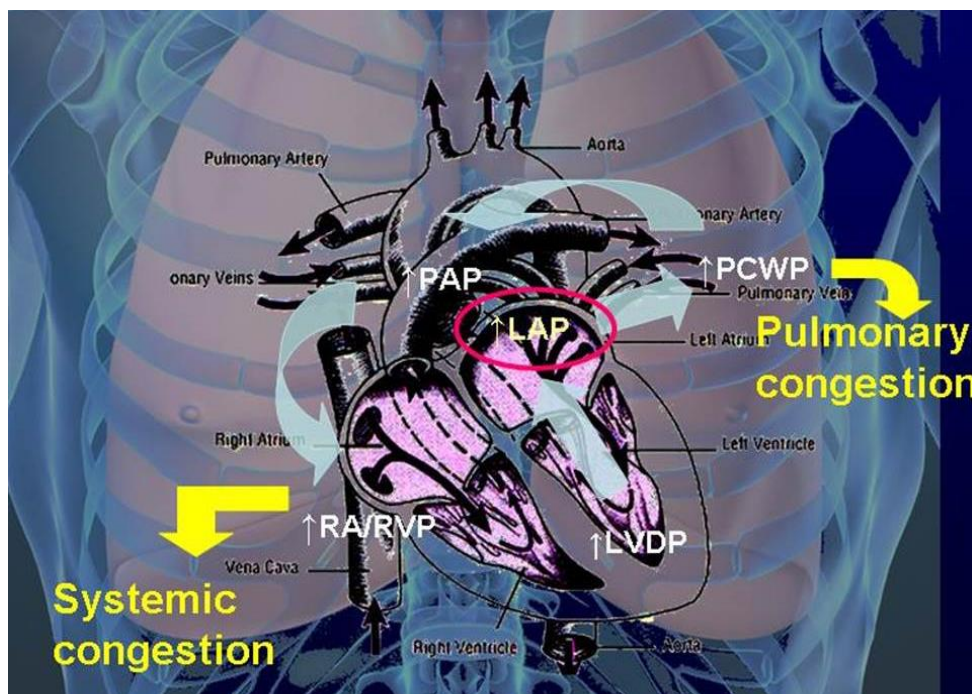
Figure 1.8. Raised left ventricular diastolic pressure leads to raised left atrial pressure which in turn leads to raised pulmonary capillary wedge pressure. The increase in hydrostatic pressure in the pulmonary capillary results in pulmonary congestion cumulating in fluid transudation into alveoli and pulmonary oedema. Back pressure from raised pulmonary capillary wedge pressure leads to raised pulmonary artery pressure then raised right atrial and ventricular pressure and eventually the development of systemic congestion and peripheral oedema. LVDP, left ventricular diastolic pressure; LAP left atrial pressure; PCWP, pulmonary capillary wedge pressure; PAP, pulmonary artery pressure; RA, right atrial; RVP, right ventricular pressure.

Direct haemodynamic monitoring provides immediate high-fidelity readings of intra-cardiac pressures. The concept of haemodynamic monitoring is based on detection of increased left ventricular filling pressure or its estimate (Figure 1.8). Different types of haemodynamic monitors have undergone or are currently under evaluation for routine use but none are commercially available. The first intra-cardiac haemodynamic monitor (Chronicle IHM) was placed in the right pulmonary ventricular outflow tract and measured systolic and diastolic right ventricular pressures and provided an estimate of the pulmonary artery diastolic pressure (itself a surrogate of left ventricular diastolic pressure).[447] The initial study was promising, with an apparent halving of HF hospitalisation.[439] However, this device failed to significantly reduce HF events when it was put to the test in a randomised trial, possibly due to the small study size and reduced power given the overall low event rate.[448]

CardioMEMS, a wireless pulmonary artery sensor, was first trialled in NYHA III patients compared to standard care and showed very few device related complications, a greater reduction in pulmonary artery pressure, improved quality of life, and a delayed and impressive 40% relative reduction in HF hospitalisation at 6 months.[449] However, the device is yet to gain approval from the U.S. Food and Drug Administration and may need to undergo larger scale efficacy trials to confirm its effectiveness.

Elevated LAP is the cardinal characteristic of decompensated HF. The HeartPOD™ device is a LAP sensor that enables ambulatory monitoring of LAP. Preliminary prospective observations suggest that dynamic changes in diuretic and other medications based on LAP recordings result in lower hospital admission rate, improved symptoms and increased use of evidence-based long-term therapy.[450]

Recent results from 48 months follow-up confirm long-term satisfactory sensor performance with regular non-invasive valsalva calibration.[451] At present, this LAP sensor is undergoing large scale randomised controlled trial, "Left Atrial Pressure Monitoring to Optimize Heart Failure" (LAPTOP-HF), to evaluate its effectiveness in reducing HF events (http://www.clinicaltrials.gov, NCT01121107).

1.13 Summary and Overview of Hypotheses and Aims of Thesis

HF is a major medical problem worldwide. Regardless of the aetiology, it poses a huge burden to the health system. Neurohormonal activation often leads to adverse left ventricular remodelling and as disease progresses; it further compromises haemodynamics, quality of life and increases risk of death. In ADHF hospitalisation is often required and treatment is often limited by haemodynamic instability and/or renal failure. There have been few advances in treatment for ADHF made over the past decade. Thus, with a view to improving overall outcome in HF, intervention could be targeted towards improved treatment of ADHF or to prevent ongoing deterioration of the chronic condition. This thesis is entitled "Novel Therapies for Heart Failure" and comprises three different projects that target different phases of HF intervention.

1.13.1 Urocortin-2 Administered in Addition to Conventional Care Compared with Placebo Administered in Addition to Conventional Care in Subjects with Acute Decompensated HeartFailure (UNICORN)

As discussed in (section 1.9) urocortin-2 is a vasoactive peptide that increasing

evidence suggests may be cardioprotective in hypertension, HF and cardiac ischaemia. In experimental ovine HF, it consistently exhibits favourable haemodynamic, renal and neurohormonal effects. In humans it has only been evaluated in healthy volunteers and patients with stable HF. While haemodynamic effects were seen, renal and hormonal effects were minimal. One possible explanation is the relatively quiescent state of neurohormonal activation in health and in stable treated compensated HF. In ADHF, haemodynamic compromise often limits the use of vasodilators. At present, the effect of urocortin-2 in ADHF remains unknown and there is little information on the impact of infusions longer than one hour in humans. In this thesis, the former question will be addressed by the first clinical study of urocortin-2 in ADHF.

In the UNICORN study, we hypothesise that 1) it is feasible to administer urocortin-2 infusion in ADHF patients and 2) urocortin-2 would exhibit the same beneficial augmentation of cardiac output and reduction in preload and afterload, enhancement of diuresis and natriuresis and suppression of adverse neurohormonal activation in ADHF patients, during a state of haemodynamic vulnerability and neurohormonal disharmony, as seen in experimental HF models. To test the hypotheses, the aims of the study are 1) to assess the feasibility and the safety of administering a short-term urocortin-2 infusion in ADHF and 2) to assess the effects of such infusions on haemodynamic, renal, neurohormonal parameters and symptoms over a 24-hour period.

1.13.2 The Effects of Urocortin-2 on Muscle Sympathetic Nerve Activity in Man (TOUCHÉ)

In the animal model, urocortin-2 directly inhibits cardiac SNA. This is of particular relevance in HF as SNA is intensified in HF and this can trigger a vicious cycle of adverse hormonal activation, arrhythmia and death. Whether urocortin-2 can suppress SNA in humans is unknown. We hypothesise that urocortin-2 would produce similar SNA inhibition as seen in sheep and as SNS is more active in HF, a more prominent response would be seen in HF patients. We aim to evaluate the integrated effects of urocortin-2 on haemodynamic, muscle SNA and plasma catecholamines in healthy subjects and stable HF patients.

1.13.3 CRT-based Heart Failure Monitoring Study CRT optimisation sub-study (zLAP-CRT)

In the chronic stage of HF multiple monitoring strategies aim at reducing HF events are available, including the HeartPOD™ LAP sensor device. CRT is an established therapy for severe left ventricular impairment and wide QRS duration however close to one-third of patients are non-responders. As discussed (in section 1.11.1) CRT optimisation could improve the response rate but there is no consensus on the best strategy to achieve this. The LAP sensor can be concomitantly implanted in patients with CRT. It is unknown if changes in CRT settings will be detected by this LAP sensor, it is also unknown if this LAP sensor can be used to guide CRT optimisation.

The zLAP-CRT study hypothesises that 1) changes in AVD and VVD during CRT optimisation would be associated with characteristic and reproducible changes in LAP and its waveform obtained by this LAP sensor and 2) these changes could provide an objective guide to CRT optimisation. The aims of the study are 1) to characterise the

changes in LAP and its waveform during CRT optimisation, 2) to assess the correlation of LAP to echocardiography-guided optimal CRT setting and 3) to determine if specific LAP waveform parameters can be used to guide CRT optimisation.

2 Methodology

This chapter describes the methodologies of the three studies that form this thesis. The key and common components are discussed in this chapter. As all three studies have very different trial design, details of patient selection and individual protocols, are discussed in the chapter devoted to the individual study.

2.1 Hormone Assays

Hormone assays form a key component in UNICORN and TOUCHÉ.

Blood samples were obtained intravenously from an indwelling cannula placed in a vein in the forearm contralateral to the infusion arm. Samples were drawn by the candidate for assay of urocortin-1, urocortin-2, ANP, BNP, NT-proBNP, ET-1, PRA, Ang II, aldosterone and cortisol for UNICORN and urocortin-2 and catecholamines, norepinephrine and epinephrine, for TOUCHÉ. Blood was collected into chilled tubes containing ethylenediaminetetraacetic acid (EDTA), except urocortin-2, which was collected in heparin and Ang II, which was collected in 0.125M EDTA, 0.05M o-phenanthroline, 2% ethanol, 0.2% neomycin sulphate and 0.03mg/mL enalkiren. Tubes were kept on ice and transferred for immediate separation at 4000rpm for 10 minutes in a refrigerated centrifuge. Aliquoted plasma was then stored at -80°C until assayed. All assays were performed by staff at Cardioendocrine Laboratory at the University of Otago, Christchurch under the supervision of Associate Professor Timothy Yandle, biochemist unless otherwise stated. When possible, all samples from each subject were assayed together to minimise the effects of inter-assay variation.

The assays for urocortin-1, ANP, BNP, PRA, Ang II, aldosterone and ET-1 are all in-house radioimmunoassays that have been well validated previously.[222, 452-461] In short, radioimmunoassay methodology was as follows: the hormones were extracted using assay-specific but standard methodology. Extracts were then assayed by adding a known quantity of antiserum followed by adding a known quantity of $[^{125}I]$-labelled hormone. After a period of incubation to allow equilibrium for antibody binding to be reached, the bound and free tracer were separated using solid phase methods with second antibody added followed by further incubation prior to separation by centrifugation. The bound fraction was then counted in a gamma counter and level derived from the standard binding curve.

Urocortin-1, the mean detection limit was 5.5pmol/L, reference range from 197 healthy volunteers was 2.7-14.1pmol/L. The intra-assay coefficient of variation (CV)s were 5.1% at 12.9pmol/L, 3.7% at 25.5pmol/L and 2.2% at 41.2pmol/L. The inter-assay CVs were 19.1% at 12.9pmol/L, 10.6% at 25.5pmol/L and 6.7% at 41.2pmol/L.

ANP, the mean detection limit was 2.4pmol/L, reference range from 92 healthy volunteers were 4-27pmol/L. The intra-assay CVs were 12% at 12.2pmol/L, 6.8% at 21.9pmol/L and 8% at 61.2pmol/L and the inter-assay CVs were 9.7% at 12.2pmol/L, 12.6% at 21.9pmol/L and 8.4% at 61.2pmol/L.

BNP, the mean detection limit was 2.3pmol/L and reference range from 92 healthy volunteers was 3-12pmol/L. The intra-assay CVs were 16.1% at 5.5pmol/L, 12.1% at 5.7pmol/L and 17.1% at 16.4pmol/L. The inter-assay CVs were 21.4% at 5.5pmol/L, 17.5% at 5.7pmol/L and 21.9% at 16.4pmol/L.

PRA, the mean detection limit was 0.04nmol/L/hr and 95% CI from the reference range of 86 healthy volunteers were 0.5-2.6nmol/L/hr. The intra-assay CVs were

5.8% at 0.39nmol/L/hr, 2.2% at 1.7nmol/L/hr and 3.6% at 3.7nmol/L/hr. The inter-assay CVs were 10% at 0.39nmol/L/hr, 3.5% at 1.7nmol/L/hr and 5.5% at 3.7nmol/L/hr.

Ang II, the mean detection limit was 2.8pmol/L, reference range from 160 healthy volunteers were 6-24pmol/L. The intra-assay CVs were 15.7% 5.9pmol/L, 11.1% 14.8pmol/L and 15.7% at 71.4pmol/L. The inter-assay CVs were 13.7% at 5.9pmol/L, 10.8% at 14.8pmol/L and 13.7% at 71.4pmol/L.

The aldosterone mean detection limit was 3.8pmol/L, reference range from 45 healthy volunteers were 100-800pmol/L. The intra-assay CVs were 7.6% at 164pmol/L, 7.7% at 425pmol/L and 7.4% at 846pmol/L. The inter-assay CVs were 7.5% 164pmol/L, 7.2% 425pmol/L and 10.4% at 846pmol/L.

The ET-1 mean detection limit was 2.03pmol/L, reference range from 153 healthy volunteers was 0.9-2.3pmol/L. The intra-assay CVs were 9.5% at 2.6pmol/L, 9.2% at 2.5pmol/L and 12.7% at 8.3pmol/L. The inter-assay CVs were 12.9% at 2.6pmol/L, 18.7% at 2.5pmol/L and 16.8% at 8.3pmol/L.

Urocortin-2 was measured in a 2-site chemiluminescent enzyme-linked immunosorbent assay (ELISA), set up locally under Associate Professor Yandle's supervision based on assay methodology developed by Neurocrine Biosciences, using an N-terminal-directed monoclonal antibody for plate coating and a C-terminal-directed rabbit polyclonal antibody.[223] Anti-sera was donated by Neurocrine Biosciences Inc. Chemiluminescent signal was generated using mouse anti-rabbit IgG-alkaline phosphatase conjugate plus CSPD® substrate. The assay detection limit (upper 95% confidence interval for the zero standard) was 0.09ng/ml. The inter-assay CVs (n=31) were 12.4% at 0.7ng/ml, 8.6% at 1.4ng/ml and 10.5% at 2.9ng/ml.

NT-proBNP was measured by a commercially available assay (Roche Diagnostic Corp, Indiana, USA).[462-464] Plasma was incubated in a sandwich-type complex with biotin-labelled anti-NT-proBNP antibody and a ruthenium-labelled anti-NT-proBNP antibody, both antibodies directed to the NT-proBNP (1-76) region. Streptavidin-coated microparticles were then added and coupled to biotin. In a measuring cell, particles were magnetically captured on an electrode. Voltage was applied to the electrode inducing chemiluminescence which was measured by a photomultiplier. The inter-assay CVs provided by Roche Diagnostics were 2.7% at 114pmol/L and 2.3% at 672pmol/L.

Plasma cortisol was measured in the steroid laboratory at Canterbury Health Laboratories, Christchurch Hospital using the ELISA method. ELISA plate wells were coated with cortisol-thyroglobulin conjugate and left overnight. The plates were then washed and blocked with buffer. Plasma samples were added and a locally raised mouse monoclonal antibody added to the mixture for incubation. The plates were then washed before anti-mouse immunoglobulin-peroxidase was added and then underwent further incubation. Absorbance was read at 492nm after addition of sulphuric acid to each well. The inter-assay CVs were 8.8% at 100nmol/L, 6.7% at 552nmol/L and 7% at 1017nmol/L.

The catecholamines assayed were epinephrine and norepinephrine using a high performance liquid chromatography (HPLC) technique.[465, 466] Catecholamines were extracted from plasma after addition of an internal standard (Sigma Chemicals, Connor, USA) by absorption onto alumina then eluted with dilute acetic acid. Extracted catecholamines were separated with ion pairing by sodium octane sulphonate. Eluted catecholamines are detected with a coulometric electrochemical detector. Plasma epinephrine and norepinephrine were derived from the standard

curve. The detection limit was 3-times baseline noise in the detector signal 100pmol/L for both epinephrine and norepinephrine. Reference range from 45 healthy individuals for epinephrine was <570pmol/L and norepinephrine 470-3800pmol/L. For epinephrine, at 219pmol/L intra-assay CV was 5.3% and inter-assay CV was 12.1%. For norepinephrine at 2197pmol/L, intra-assay CV was 2.6% and inter-assay CV was 4.1%.

2.2 Echocardiography

Echocardiography was performed in UNICORN and was a key component in zLAP-CRT. Images were obtained using Philips iE33 and a S5 probe (Philips, Andover, USA) and recorded with ECG trigger on R wave with the patient lying in the left lateral position during quiet respiration unless they were in the UNICORN right heart catheter sub-study for which images were obtained between a left lateral and supine position. All echocardiographic images were obtained according to the American Society of Echocardiography guidelines.[467-470] At least three cardiac cycles were recorded for two-dimensional images at 50Hz or higher frame rate; four cardiac cycles for M-mode, pulse-wave Doppler and Tissue Doppler indices. The sweep speed was set at 100m/s and sample volume at 2mm for pulse-wave Doppler. For Tissue Doppler indices, the sweep speed was set at 100m/s, sample volume at 5mm, scale between -15 to 15cm/s and gain adjusted to obtain clear tissue signals. The raw data were all analysed offline using Prosolv CardioVascular software (ProSolv, Indianapolis, Indiana) by the candidate with oversight from Associate Professor Richard Troughton. Measurements were an average of three cardiac cycles for patients in sinus rhythm or those who were paced with a regular R-R interval and five

cycles for patients in atrial fibrillation. Eleven studies were re-measured in UNICORN, the intra-observer measurement variability for two-dimensional indices was 9.6% and for Doppler indices was 5.1%. Two studies in zLAP-CRT were remeasured to determine the intra-observer variability. The variation for Doppler measurements was 8.2% and for two-dimensional measurements was 15.2%. Although only two studies were remeasured, this consisted of 25% of the entire study involving 242 measurements with acceptable intra-observer variations thus representative of the cohort.

2.2.1 UNICORN

In UNICORN, the key parameters of interest were left ventricular volumes, left ventricular systolic function and estimated left ventricular diastolic filling pressure. The protocol for serial echocardiography is described in Chapter 3. Two-dimensional images for volumetric analysis were obtained from standard apical four- and two-chamber views with the transducer placed at the apical impulse. For left ventricular end-diastolic and end-systolic volumes, the left ventricle was displayed in the most magnified presentation, the endocardial border was traced and volumes calculated using the modified biplane Simpson's method.[471] Left ventricular dimensions were acquired from M-mode images using standard two-dimensional parasternal long axis guidance where the transducer was placed on the left third intercostal parasternal space. The cursor was placed perpendicular to the left ventricular septum and posterior wall and M-mode derived volumes were calculated using the Teichholz formula.[472] LVEF is the most commonly used index for the assessment of left ventricular function. In this study, it was calculated by the difference of left ventricular end-diastolic and end-systolic volumes over left ventricular end-diastolic volume.[468] Pulse-wave tissue Doppler imaging was performed in the apical four-

chamber view for left ventricular longitudinal velocities with the sample volume positioned at the medial mitral annulus within 20 degrees angulation to the interatrial septum, perpendicular to the mitral annulus.[469] The velocities measured were mitral annular systolic velocity (S') and early diastolic mitral annular velocity (e'). Mitral annular systolic velocity (S') was measured to assess systolic function. This parameter has recently been shown to be useful in assessing left ventricular systolic dysfunction and has an advantage over LVEF in that it has a lower intra- and inter-observer variability.[473] Estimated left ventricular diastolic filling pressure was measured as the ratio of mitral inflow peak early filling (E) over e', where E was performed by colour flow imaging of the mitral valve to guide alignment of pulse-wave Doppler of mitral inflow in the apical four-chamber view with the sample volume placed at the mitral leaflet tips during diastole.[469] Multiple studies have shown this ratio can reliably detect elevated left ventricular filling pressure.[474-477]

2.2.2 zLAP-CRT

The zLAP-CRT study evaluated the changes in timing, synchrony, Doppler profiles of left ventricular diastolic filling and estimates of stroke volumes and severity of mitral regurgitation with an emphasis in this thesis on Doppler diastolic filling pattern and stroke volume estimates. As discussed in Section 1.11.1, there is no consensus on representative echocardiographic indices to guide CRT optimisation. For stroke volume estimates, the mitral inflow velocity time integral (VTI) and left ventricular outflow tract (LVOT) VTI were used. Both of these markers are commonly used in CRT optimisation.[417, 478] Mitral inflow VTI was obtained from pulse valve Doppler at level of the mitral annulus in the apical four-chamber. LVOT VTI was obtained from the apical five-chamber view with the sample volume placed within 5mm proximal to the aortic valve in the LVOT[470], a limitation of this method was that keeping the

sampling site constant throughout the CRT pacing protocol was challenging. In addition to stroke volume estimate, pulse-wave Doppler mitral inflow pattern provides additional information on diastolic function, where the E wave represents passive early diastolic filling and A wave represents active late diastolic filling. The parameters assessed in this study were: E/A ratio, peak A velocity, A duration and ratio of A/ total mitral inflow VTI; all reflected the atrial contribution in diastolic filling.[469] The iterative method (Figure 2.1) used in this study to determine optimal AVD was based on obtaining the largest A wave without truncation from the mitral inflow Doppler pattern.[412]
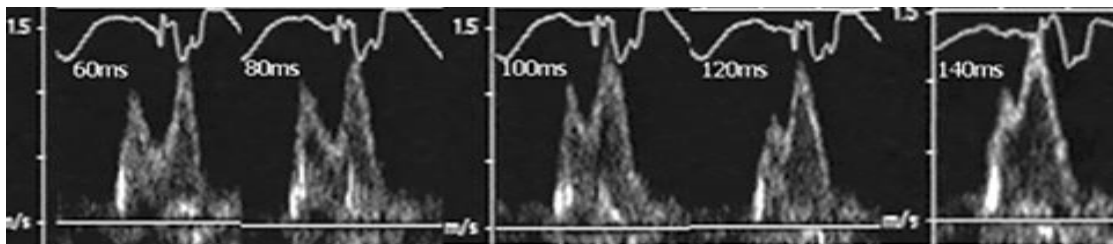
Figure 2.1. An example of iterative method for AV delay optimisation. E and A velocities are affected by various AV delay. The optimal AV delay is where E and A waves are well separated. In this example, the shorter AV delays appear to be more optimal with maximal E and A separation. Figure reprinted from Waggoner et al with permission, copyright Wiley (2013).[417]

Assessment of optimal interventricular delay involved visual intraventricular synchrony evaluated by a combination of visual two-dimensional method for septal-lateral dyssynchrony at the apical four-chamber view, anteroseptal-inferior dyssynchrony at the apical two-chamber view, but primarily on LVOT VTI and LVOT pre-ejection time measured from the onset of QRS to the beginning of flow in LVOT during pulse-wave Doppler of LVOT[467] and the severity of mitral regurgitation. Mitral regurgitation increases with dyssynchrony. This was evaluated by an integrated method including colour Doppler assessment of the mitral valve in the apical four-chamber view for proximal flow convergence, changes in pulse-wave transmitral inflow E velocities and pulse-wave Doppler pattern of pulmonary venous

flow with the sample volume placed ~1cm in the right upper pulmonary vein in the apical four-chamber view.

2.3 Microneurography

In TOUCHÉ, direct microneurography was used to evaluate the effect of urocortin-2 on muscle SNA. This method allows direct measurement of efferent postganglionic sympathetic nerve traffic, is more reproducible for assessment of dynamic short- and medium-term changes in sympathetic activity than plasma norepinephrine[479] and has good correlation with systemic SNA in HF.[196] This technique was developed in 1966 by Vallbo and Hagbarth.[480] All microneurography studies were performed by Dr David Jardine with the subject sitting in a semi-recumbent position in a "lazy-boy" chair. After locating the right superficial peroneal nerve adjacent to the fibular head using transcutaneous electrical stimulation, two 36 gauge insulated tungsten electrodes with a 1 to 5μm tip were inserted to the nerve supplying the distal leg muscle, one as the ground electrode and the other for recording nerve traffic (Figure 2.2). The nerve signal was amplified (x100000), filtered (700-2000Hz), integrated (time constant 0.1ms), and displayed on-line with blood pressure and electrocardiogram. Each subunit discharge of SNA was displayed as a narrow peak called a "burst". Bursts of sympathetic activity were identified, counted and measured each minute (burst frequency, burst incidence and burst area), by Dr Jardine. The nerve signal was accepted provided that the following criteria were met: the signal-to-background ratio was greater than three, the bursts were pulse-synchronous, burst amplitude was inversely proportional to diastolic blood pressure and skin activity was absent. To verify muscle but not skin SNA was obtained the nerve was tested with

electrical stimulation up to 2V at 0.2ms to elicit involuntary right foot dorsiflexion, manually dorsiflexing the right foot to increase nerve discharge and lack of stimulation with stroking over the skin.

To minimise the risk of nerve injury, the time required for initial needle placement was limited to 60 minutes. Mild nerve injury was observed in 9% out of 649 participants in a prospective study,[481] however 95% resolved by two weeks. In a much smaller study by Littell,[482] similar rate of neuropathy complication was reported, no one had permanent damage. If a satisfactory nerve field could not be obtained during this period, a repeat study day was rearranged with the subject's approval. The analogue output were fed into the computer together with haemodynamic data and converted digitally for analysis using in-house software. (Figure 2.3)
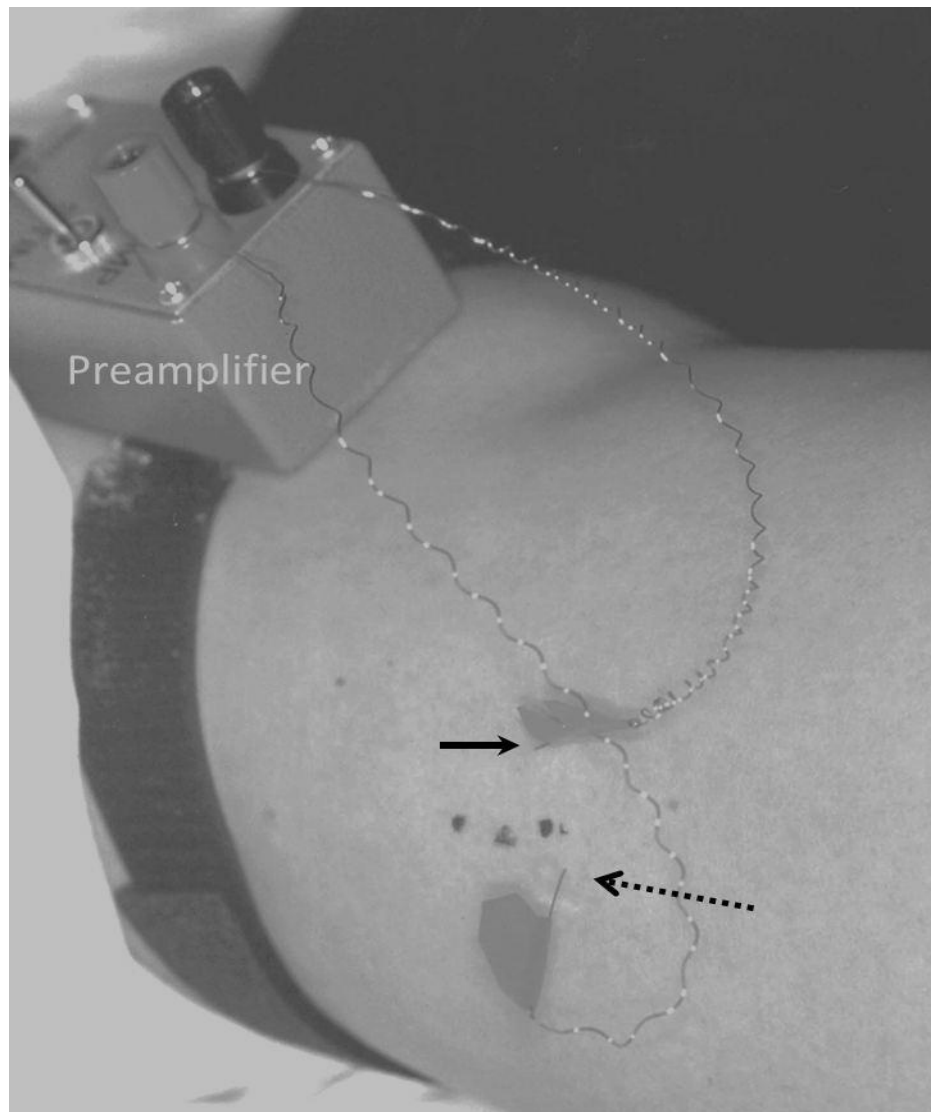
Figure 2.2. A preamplifier is strapped to a subject's right knee and connected to two electrodes. The black dots are surface markings of the right peroneal nerve. Solid-tail arrow is the ground electrode and dash-tail arrow is the nerve traffic recording electrode. Photo reprinted from Yucha with permission, copyright Springer (2013).[479]

Three components of muscle SNA were examined in the study: the rate of sympathetic discharge (burst frequency), the number of burst per 100 heart beats (burst incidence) and the mean amplitude over 1 minute determined by measuring the area under the curve (burst area). As the burst area is an arbitrary way of examining the change in amplitude, it is expressed as percentage change from baseline. In an historic study, muscle SNA was shown to be highly variable between individuals, however muscle SNA is relatively stable within an individual when recording on

different nerves simultaneously and was reproducible on repeated measurement over periods between 3 weeks and 21 months.[483] Amongst the eight individuals in this historic study who had simultaneous SNA recordings (median-peroneal or bilateral peroneal), the difference of total number of bursts was between 1.3-12.9%.[483] This validated the feasibility to study the muscle SNA of a group over time. As burst frequency can be affected by heart rate, we evaluated the measurement variability using burst incidence as the marker in our current study. Comparisons were made in each participant between study day and within the same using the two SNA recordings prior to trial drug infusion. The CV within the study day was 6% and between study days was 19%.
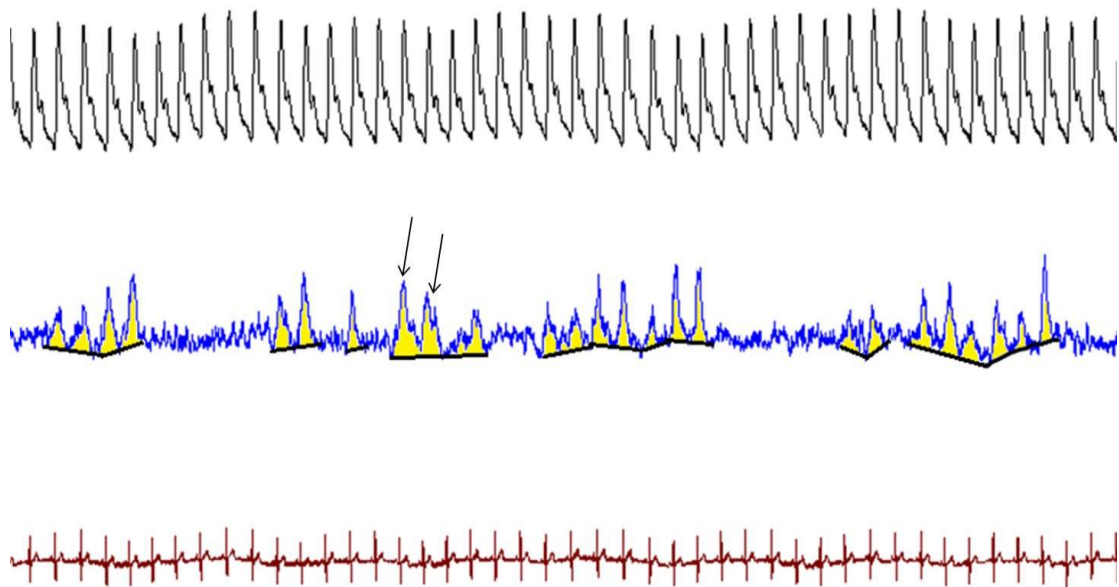
Figure 2.3. An example of continuous haemodynamic and microneurography tracing. Top row, arterial pressures; middle row in blue, muscle SNA; bottom row in red, ECG. Black arrows point towards representative bursts. Burst area is the area under curve shaded in yellow and bound by an arbitrarily drawn lower border.

2.4 Statistics

Raw clinical, biochemical and echocardiographic data were collected by the candidate and recorded in pre-set Case Report Forms (CRFs) for each study. These data were later transferred into a secure database where data could be systematically extracted for analysis. All data were analysed using SPSS version 19 (SPSS Inc. Chicago, IL, USA) by the candidate with advice from Associate Professor Chris Frampton, biostatistician. A two-tailed p-value of <0.05 was regarded as statistically significant. All continuous variables were expressed in mean ± SEM unless specifically stated otherwise. The non-normal distributions of the neurohormonal data in UNICORN necessitated $\log_e$-transformation for analysis and were subsequently expressed as geometric means with 95% confidence intervals.

The key statistical analysis performed in UNICORN and TOUCHÉ was repeated measures analysis of covariance (ANCOVA) with Greenhouse-Geisser adjustment made in TOUCHÉ for the small sample sizes. Randomised treatment was treated as a between-individual factor, the relevant baseline level as a covariate and time as a within-individual factor in UNICORN and in TOUCHÉ the relevant baseline level was a covariate and time and treatment were within-individual factors. The interaction between treatment and time was the key effect in these models. A repeated measures analysis of variance (ANOVA) was used in zLAP-CRT with Greenhouse-Geisser adjustment to examine the association of each of the echocardiographic and LAP waveform parameters with the CRT optimisation changes. The changes in CRT settings were treated as a within-individual factor.

The effect of urocortin-2 on haemodynamics, renal function and neurohormones over the 24hour period and in phases in reference to the commencement of the infusion were examined in UNICORN while analyses for TOUCHÉ and zLAP-CRT examined

effects over the entire study period. Where significant interaction effects between randomised treatment and time were identified these were further explored using Fisher's protected least significant difference (LSD) tests both within and between groups. In UNICORN, the Likert variables were compared between randomised groups using Mann-Whitney-U tests and categorical data were compared using Chi-square or Fisher's exact tests when expected cell frequencies were small. In zLAP-CRT, Pearson's correlation coefficients were used to test the association between LAP parameter derived optimal AVD and echocardiography derived optimal setting.

Over the three studies a considerable number of statistical comparisons have been undertaken on a large number of clinical, biochemical and echocardiographic results. In a manner typical of phase II studies while we have initially concentrated on the primary hypotheses associated with each study we have thoroughly explored the data looking for signals from the treatments, to generate hypotheses that would be further tested in future studies. No correction to the type I error level to allow for this multiple testing has been made. As a consequence, there is a possibility that some of the significant results identified and discussed may represent type I errors. To mitigate this to some extents, in the results and discussion we have focussed on consistent plausible patterns within a number of measures rather than identifying occasional, potentially spurious significance from single measures.

2.5 Ethics and Regulatory Approval

All three studies were approved by the Upper South B Regional Ethics Committee of New Zealand. Participants in all studies were able to provide signed informed consent and be compliant with the study protocol. The UNICORN study was also approved by

the Health Research Council's statutory Standing Committee on Therapeutic Trials after reviewing toxicology reports on urocortin-2 provided by Neurocrine Biosciences Inc.

2.6 Patient Selection

The specific patient selection criteria are discussed in the individual chapter of the study. An overview is outlined here. All three studies recruited adult patients only. In UNICORN, the target population was patients hospitalised within 36 hours with typical signs and symptoms and at least one objective measurement to support the diagnosis of ADHF. In TOUCHÉ, healthy volunteers with no past cardiac history, and not on vasoactive medications, were eligible. In the stable HF arm of TOUCHÉ, patients were eligible if they had HF with reduced LVEF, on stable HF therapy with minimal symptoms and in sinus rhythm. In zLAP-CRT, patients were eligible if they fulfilled conventional indications for CRT and had no contraindications for an implantable LAP sensor.

2.7 Protocol

An overview of the study protocols is provided here. Detailed study protocols are described in the individual study chapters.

UNICORN study was a double-blind, placebo-controlled randomised trial, a minimum of 25 patients per study arm was required. Patients were randomly allocated to a four-hour infusion of urocortin-2 or placebo as an adjunct to the conventional HF therapy as per the treating physician. Haemodynamic, renal and hormonal data

together with symptoms were monitored at predefined time points before, during and after infusion to 24-hours. Ten patients in each study arm underwent right heart catheterisation for a more detailed evaluation of the haemodynamic effects of urocortin-2. The effect of urocortin-2 on the integrated indices over the 24-hour period was assessed.

TOUCHÉ was a single-blinded crossover study. Participants received, in a random order on two separate occasions, a one-hour infusion of either 25μg urocortin-2 infusion or placebo. Haemodynamic and microneurography data were collected at 15 minutes intervals and plasma catecholamines at 30 minutes intervals from 15 minutes before commencement of infusion to one hour post cessation of trial infusion. The effects of urocortin-2 on haemodynamic and sympathetic nerve activity were assessed.

zLAP-CRT was a prospective observation study. After at least six weeks of the latest device implant, subjects underwent CRT optimisation with incremental AVD from 80ms to 200ms and VVD with left ventricular lead led by -20ms to +60ms at 20ms intervals. Echocardiography data, in particular measurements of transmitral Doppler diastolic filling, stroke volume estimates and synchrony parameters were collected at each CRT setting together with LAP and waveform data. The effect of change in CRT setting on LAP and correlation with echocardiography data were evaluated.

3 Urocortin-2 Infusion in Acute Decompensated Heart Failure

3.1 Introduction

Acute decompensated heart failure (ADHF) is a common cause for hospital admission and is associated with high mortality.[11, 484] Patients are often distressed by symptoms of fluid overload and the goal of treatment is to relieve symptoms, restore circulatory stability and to initiate or optimise evidence-based therapies that have been shown to prevent death or rehospitalisation. However, this is often limited by compromised haemodynamic status[485] and renal dysfunction[67]. Recent trials on new therapies in ADHF have been disappointing[175, 326-328, 335] and, to date, none have shown consistent effects concurrently to increase cardiac output, reduce intra-cardiac filling pressures, suppress adverse neurohormonal activation and maintain renal function. Hence, there is an ongoing need to search for new therapies.

The urocortins-1, 2 and 3 are small (38-40 amino acids) peptides that belong to the CRF family.[208, 210-212] Urocortins exert cardiovascular actions via the CRF_2 receptor which is highly expressed in the heart and vasculature. Urocortin-2 has been shown to have vasodilatory, inotropic and lusitropic properties.[223, 224, 264, 274, 275] Exogenous infusions of all three urocortin peptides in experimental (ovine) HF have consistently demonstrated fall in arterial blood pressure, increased cardiac output, decreased intra-cardiac pressures, reduced total peripheral resistance, enhanced diuresis and natriuresis and suppression of adverse neurohormones, with responses to the three peptides only differing in their temporal profile.[246-248] Urocortin-2 has a more favourable duration of action and demonstrates reproducible haemodynamic effects

without stimulating the HPA axis during infusion studies in man,[223, 224] compared with exogenous urocortin-1 administration in man.[221, 222] Thus, urocortin-2 stands out from its fellow family members as a potential therapeutic agent in HF. To date, our experience of urocortin-2 studies in man is limited to short-term (one hour) infusions in healthy volunteers and patients with stable HF. When given at high dose in healthy man, urocortin-2 stimulated the RAAS.[223] This was not observed with a lower dose and was most likely secondary to the magnitude of hypotension. The renal and neurohormonal effects were also minor in comparison with those observed in severe experimental HF.[224, 247] In stable HF, neurohormones are relatively quiescent.[121] Therefore, the true neurohormonal effect of urocortin-2 in man may not have yet been revealed. It is also uncertain if urocortin-2 produces the same haemodynamic, renal and neurohormonal profile in ADHF or how feasible it is to administer urocortin-2 to this group of haemodynamically vulnerable patients.

3.2 Hypothesis and Aims

Hypothesis:

1. It is feasible to administer urocortin-2 infusion in ADHF patients.

2. Urocortin-2 exhibits the same beneficial haemodynamic, renal and hormonal effects in ADHF patients, as seen in experimental HF models.

Aims:

1. To assess the feasibility and the safety of administering short-term urocortin-2 infusions in ADHF.

2. To assess the effect of such infusions on haemodynamic, renal, neurohormonal

parameters and symptoms over a 24-hour period.

3.3 Methods

3.3.1 Study Design

"Urocortin-2 administered in addition to conventional care compared with placebo administered in addition to conventional care in subjects with acute decompensated heart failure" (UNICORN), Australian New Zealand Clinical Trial Registry number: ACTRN12609000508279, was a randomised, double-blinded, placebo-controlled, single centre study.

The target sample size was 25 patients per study arm. This was based on the neurohormonal status of Ang II, PRA and NT-proBNP in a group of patients admitted to our institution who fulfilled the study enrolment criteria. This sample size would have 80% power (2-tailed, $\alpha = 0.05$) to confirm a 50% reduction in mean values of neurohormones from the admission values and 10% change in calculated GFR. Based on the preliminary experience in human infusion studies by our group that 8 per group in healthy and HF human showed highly statistically significant effects of urocortin-2 infusion,[223, 224] this sample size of 25 per arm was also adequately powered to detect 10% changes in key haemodynamic variables. Randomisation was derived from permuted blocks of four from a computer generated list which allocated a single numbered vial to each patient. A separate randomization sequence was established to ensure treatment allocation balance within the right heart catheter group.

3.3.2 Patient Selection

Participants were patients admitted with ADHF who were 18 years or older and

within 36 hours (and 24 hours of first dose intravenous frusemide) of an acute presentation with symptomatic left ventricular failure, with dyspnoea at rest or on minimal exertion; and supported by at least one clinical sign, tachypnoea at rest or bibasal crackles to at least the lower third of the chest; and at least one of the predefined objective investigation findings listed below to support ADHF or left ventricular systolic dysfunction:

1. Chest x-ray showing pulmonary congestion/ oedema.

2. BNP >115pmol/L or NT-proBNP >120pmol/L.

3. Pulmonary capillary wedge pressure (PCWP) >20mmHg.

4. LVEF <40% on echocardiography.

5. Mitral inflow early diastolic velocity/ Medial mitral annular myocardial velocity (E/E') >15.

Patients were excluded if any of the following was present:

1. Baseline systolic blood pressure was <100mmHg, unless chronically stable low blood pressure as determined by the investigator.

2. Admitted with acute coronary syndrome with a troponin I >0.15ug/L.

3. Had significant stenotic valvular lesion, hypertrophic, restrictive or constrictive cardiomyopathy or cardiac tamponade.

4. Unstable dosage of intravenous inotropes or nitrate for 3 hours before infusion start.

5. Patients with child bearing potential.

6. End-stage renal disease.

7. Significant chronic lung disease interfering with assessment of dyspnoea.

8. Non-cardiac disease with a life expectancy <six months.

9. BMI >35 due to the uncertainty of volume of distribution in obese patient.

10. On an investigational drug or device within last 30 days.

3.3.3 Protocol (Table 3.1)

After obtaining written informed consent, patients were transferred to the Coronary Care Unit for close observation of vital signs. Patients were randomised in a 1:1 fashion to a four-hour intravenous infusion of urocortin-2 or placebo, as an adjunct to conventional therapy. Each vial, containing 400µg urocortin-2 and 50mg mannitol or placebo (supplied by Neurocrine Biosciences Inc., San Diego, California), was diluted to a concentration of 2µg/mL with normal saline, 60mL drawn up in a syringe and delivered at 5ng/kg/min. Urocortin-2 is a particularly "sticky" substance, previous adsorption studies by Neurocrine Biosciences Inc. had demonstrated adding mannitol as a carrier could overcome the stickiness for target concentration delivery. Although Neurocrine Biosciences Inc. provided the trial peptide and the initial toxicology information for assessment by the Standing Committee on Therapeutic Trials, they made no other financial or intellectual contribution and the trial was entirely designed by the investigators and funded by a grant from the Health Research Council and all investigators declared no conflict of interest arose from conducting this study. Patients continued their standard care of HF management including intravenous diuretics as directed by the treating physician. Serial echocardiography, haemodynamic and clinical observations, blood collection for haematology, biochemistry and neurohormones, urine collection were carried out from one hour before infusion start to 24 hours following the commencement of the infusion at pre-specified time points

as below:

	Time (hour)																
	-1	-0.5	0	0.5	1	1.5	2	2.5	3	3.5	4	5	6	7	8	12	24
Haemodynamics	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Swan-Ganz		√	√	√	√		√		√		√	√	√	√	√		
Biochemistry			√				√				√		√			√	√
Neurohormones		√	√		√		√				√		√			√	√
Urine			←								→ ←				→	←	→
Likert Scale					√		√				√		√			√	√
Echocardiogram		√									√						√
HF Score	√																√

Table 3.1. Protocol of UNICORN study. Shaded area indicates the time of urocortin-2 or placebo infusion.

Haemodynamic and clinical observations including heart rate, heart rhythm, blood pressure, respiratory rate, oxygen saturation, were recorded every 30 minutes during infusion followed by hourly recording for 4 hours then at +12 and +24hours. A heart failure score was performed at baseline and +24hours to quantify HF severity. This consisted of a 7-point score that incorporates typical signs and symptoms of ADHF which has been routinely used in our centre in acute HF trials (Table 3.2).[430] Symptomatic status of dyspnoea was measured using the 7-point Likert Scale (Table 3.3) at +1, +2, +4, +6, +12 and +24hours.

Samples for full blood count, plasma sodium, potassium, creatinine and glucose were obtained at +0, +2, +4, +6, +12 and +24hours. Patients were not catheterised. To standardise urine collection, patients were requested to void within 15 minutes prior to commencement of infusions. Urine collections were divided into infusion phase (0 to +4hours), +4 to +12hours and +12 to +24hours for measurement of volume, creatinine, sodium and potassium. Creatinine clearance was calculated from UV/P (U = urine creatinine concentration, V = urine volume and P = plasma creatinine concentration) to estimate GFR.[486] As there was no pre-infusion collection of urine,

baseline estimated GFR was calculated using the Modification of Diet in Renal Disease (MRDR) equation.[487] Blood tests for hormonal assays were obtained at -0.5, +0, +1, +2, +4, +6, +12 and +24hours. Hormones tested in this study were urocortin-2, urocortin-1, PRA, Ang II, aldosterone, ANP, BNP, NT-proBNP, ET-1 and cortisol. The sample handling and assays are discussed in section 2.1.

Serial echocardiography were performed at baseline, end of infusion and +24hours. The echocardiography protocol and analysis are discussed in section 2.2.

Symptom/ Sign	Value
Orthopnoea	0.5
Paroxysmal nocturnal dyspnoea	1.0
Reduced exercise tolerance	0.5
Resting heart rate >100bpm	0.5
Jugular venous pressure >4cm	0.5
Positive hepatojugular reflex	1.0
Presence of 3rd heart sound	1.0
Basal crackles in chest	1.0
Hepatomegaly	0.5
Peripheral oedema	0.5

Table 3.2. Heart Failure Score for clinical assessment quantification.

Likert Score	Definition
1	Markedly better
2	Moderately better
3	Mildly better
4	No change
5	Mildly worse
6	Moderately worse
7	Markedly worse

Table 3.3. Likert scale for dyspnoea assessment.

3.3.4 Right Heart Catheter Sub-study

In addition to the above protocol, additional consent was obtained from the first twenty patients who agreed to take part in the right heart catheter sub-study. The patients, ten in each group, were randomly allocated to urocortin-2 or placebo. After 1% lignocaine local anaesthetic to the right supraclavicular fossa, a fluid-filled 7Fr Swan-Ganz catheter was placed in the pulmonary artery under fluoroscopic guidance via the right internal jugular vein while the patient was lying supine. PCWP was obtained by temporarily inflating the catheter balloon to occlude a proximal pulmonary branch artery and confirmed by typical waveform appearance. Right atrial pressure, pulmonary artery pressure (PAP) and PCWP were obtained after 30 seconds of quiet respiration at -0.5, +0, +0.5, +1hour then hourly until +8hours. Cardiac output was also recorded at each time point using the thermodilution method. For each measurement, 10mL of 5% dextrose in water at ambient temperature was injected from the proximal port and the cardiac output derived from the change in temperature detected by the temperature sensor at the catheter tip.[488] Each recording was an average of three measurements within a 10% range. Cardiac work was calculated by multiplying cardiac output and mean arterial pressure.

At 30 day post infusion follow-up, a physical examination, echocardiography and blood tests for neurohormones, haematology and renal function were performed. All major events (hospital or general practitioner visit) occurring since discharge were documented at this time.

3.3.5 Statistics

Methods for statistical analysis are described in details in Section 2.4.

3.4 Results

From Oct 2009 to Dec 2011, 832 patients were screened, 55 eligible patients gave informed consent to participate in the study (Figure 3.1). Two patients were withdrawn at right heart catheter insertion due to vasovagal episodes prior to the commencement of study drug infusion. Fifty-three patients started and completed trial infusions. Twenty-six received placebo and 27 received urocortin-2 infusions. The study population was predominantly males in their mid-60s. Approximately one-third of patients had preserved left ventricular ejection fraction. Demographics and baseline variables were well matched (Table 3.4 and 3.5).
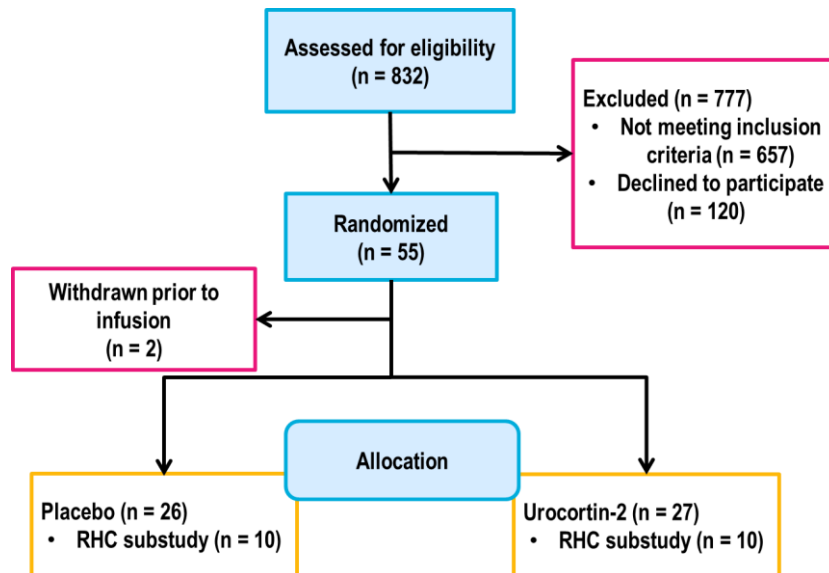
Figure 3.1. Patient flow diagram. RHC, right heart catheter.

	Placebo (n= 26)	Ucn2 (n=27)	P-value
Age (yrs) Mean ± SD	68 ± 12	63 ± 12	0.2
Male Gender	16	22	0.1
Ethnicity: Caucasian	25	22	0.09
BMI (kg/m^2)	25 ± 4	27 ± 4	0.2
LVEF (%)	42 ± 19	34 ± 17	0.1
Aetiology: ischaemic (%)	38	37	0.9
Non-ischaemic	62	63	
Past History (%)			
CAD	52	42	0.5
Prior MI	41	35	0.7
Prior PCI	8	26	0.08
Prior CABG	31	30	0.9
Hypertension	64	56	0.5
Atrial fibrillation	50	52	0.9
Diabetes mellitus	27	33	0.6
Hyperlipidemia	52	73	0.2
CVA	15	12	0.7
Smoking history	69	70	0.9
COPD	28	37	0.5
Frusemide prior 24hrs (mg)	195 ± 73	200 ± 115	1
Baseline Drug use (%)			
ACEI/ ARB	73	63	0.4
BB	50	52	0.9
MRA	15	30	0.2
Time presentation to start of infusion (hours)	21 ± 9	22 ± 8	0.7

Table 3.4. Patient demographics. Ucn2, urocortin-2; BMI, body mass index; LVEF, left ventricular ejection fraction; CAD, coronary artery disease; MI, myocardial infarction; PCI, percutaneous coronary intervention; CABG, coronary artery bypass grafting; CVA, cerebral vascular accident; COPD, chronic obstructive pulmonary disease; ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; MRA, mineralocorticoid receptor antagonist.

Baseline measurements	Placebo (n= 26)	Ucn2 (n=27)	P-value
Systolic BP (mmHg) mean ± SD	131 ± 31	123 ± 20	0.3
Heart rate (bpm)	83 ± 17	79 ± 15	0.4
Plasma creatinine (μmol/L)	109 ± 33	116 ± 35	0.5
Estimated GFR (mL/min)	58 ± 17	58 ± 15	1
BNP (pmol/L)	130 ± 112	105 ± 103	0.4

Table 3.5. Baseline haemodynamic and biochemical measurements. Ucn2, urocortin-2; BP, blood pressure; GFR, glomerular filtration rate; BNP, brain natriuretic peptide.

3.4.1 Haemodynamics

Urocortin-2 exerted a rapid and pronounced blood pressure lowering effect as compared to placebo, P<0.001. The onset of action was observed within 15 minutes of infusion commencement and the peak effect was noted between +1.5hours to +2.5hours. The reductions in systolic and diastolic blood pressure, were -18.9 ± 3.9mmHg and -16.1 ± 2mmHg respectively (both P<0.001 compared to baseline). Reductions in systolic and diastolic pressures were 16 ± 5.8mmHg and 12.9 ± 4.2mmHg greater than placebo respectively (both P<0.001). (Figure 3.2) There was no rebound and blood pressures remained lower than at baseline out to +24hours. The heart rate response to urocortin-2 was not significantly different to time-matched placebo (5.7 ± 3.8bpm); peak difference from baseline was 4 ± 1.8bpm for urocortin-2 and -0.1 ± 1.7bpm for placebo, P=0.07) (Figure 3.2). Approximately half of the study population had atrial fibrillation, post-hoc analysis did not find an impact of atrial fibrillation on blood pressure or heart rate response to urocortin-2.
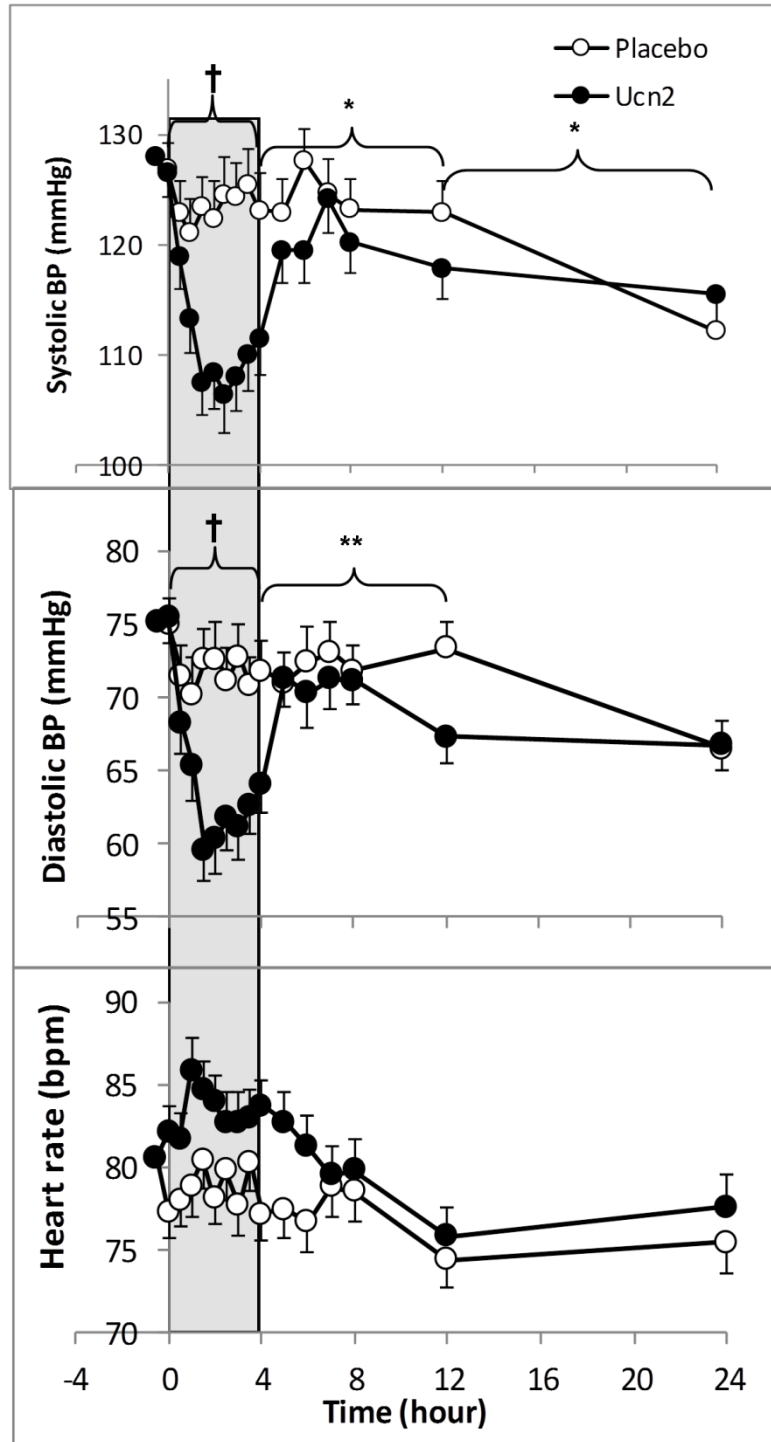
Figure 3.2. Blood pressure and heart rate effects during (shaded grey) and after trial drug infusions over 24 hours. * (P<0.05), ** (P<0.01) and † (P<0.001) indicate significant time-by-treatment interaction in the specific time phase. Ucn2, urocortin-2; BP, blood pressure.

3.4.2 Right Heart Catheter Sub-group

Urocortin-2 induced immediate and marked (50% from baseline, P=0.003) increase in cardiac output while placebo had minimal change (52 ± 19%, P<0.001 compared with placebo). The peak effect was reached at +1hours and cardiac output remained elevated throughout infusion (Figure 3.3). Cardiac output returned to baseline level within one hour after completion of the urocortin-2 infusion. The cardiac output was not significantly different from placebo during the post infusion period. With the increase in cardiac output, there was a corresponding reduction of cTPR in response to urocortin-2 compared with placebo (40 ± 8.4%, P=0.015). The maximum cTPR reduction was 47% from baseline P=0.001, inversely proportional to the increase in cardiac output (Figure 3.3). Following cessation of infusion, cTPR returned toward baseline levels indicating a persistent vasodilatory effect some hours after cessation of infusion. There was a biphasic response in calculated cardiac work during the observation period. During infusion, urocortin-2 increased the cardiac work, but cardiac work was reduced compared to time-matched placebo post infusion, P<0.001.

There was minimal change in right atrial pressure during infusion in both treatment arms. Mean PAP and PCWP both fell with time and there appeared to be a faster and greater peak effect with urocortin-2 compared to placebo during infusion but these differences did not reach significance, P=0.3 and P=0.096 for mean PAP and PCWP respectively.
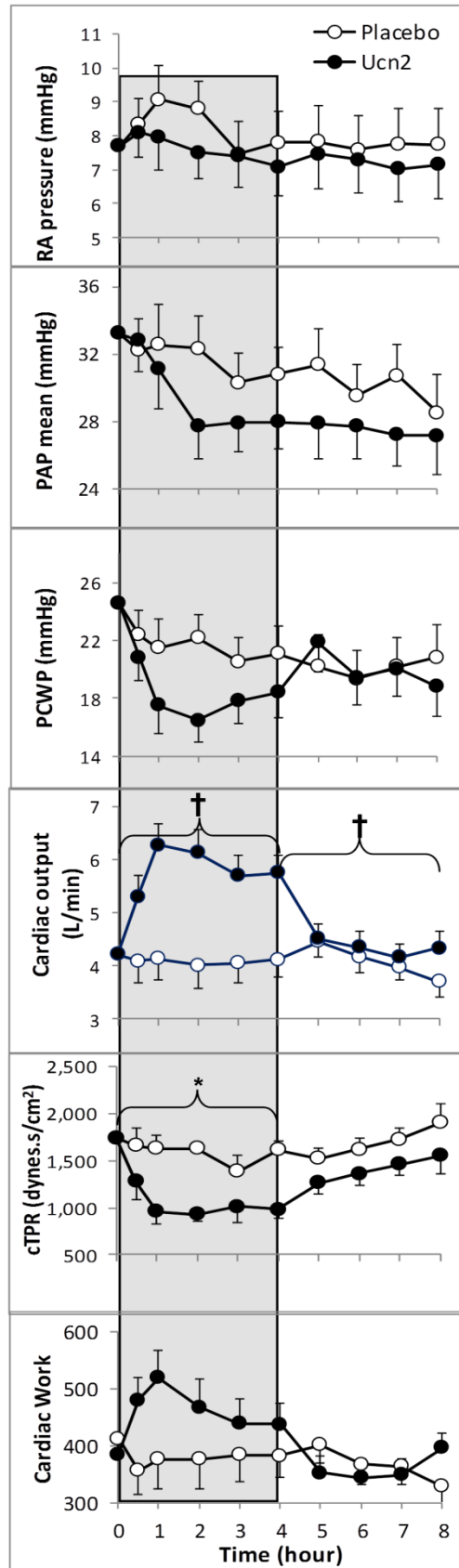
Figure 3.3. Invasive haemodynamic parameters during (shaded grey) and after trial drug infusions. * (P<0.05) and † (P<0.001) indicate significant time-by-treatment interaction in the specific time phase. Ucn2, urocortin-2; cTPR, calculated total peripheral resistance; PAP, pulmonary artery pressure; PCWP, pulmonary capillary wedge pressure; RA, right atrial.

3.4.3 Echocardiography

Urocortin-2 significantly reduced left ventricular end-diastolic (-13.4 ± 4.2mL, P=0.004) and systolic (-15.2 ± 3.2mL, P<0.001) volumes at +4hours relative to baseline resulting in a significant increase in ejection fraction (+4.4 ± 1.1%, P<0.001) with no significant changes observed with placebo Figure 3.4). The estimated diastolic filling pressure using E/E' decreased similarly in both treatment arms, P=0.46. For those patients who had S' measured at baseline and at end of infusion (n=17 in each arm), urocortin-2 did not significantly impact on myocardial contractility as compared with placebo, P=0.19.
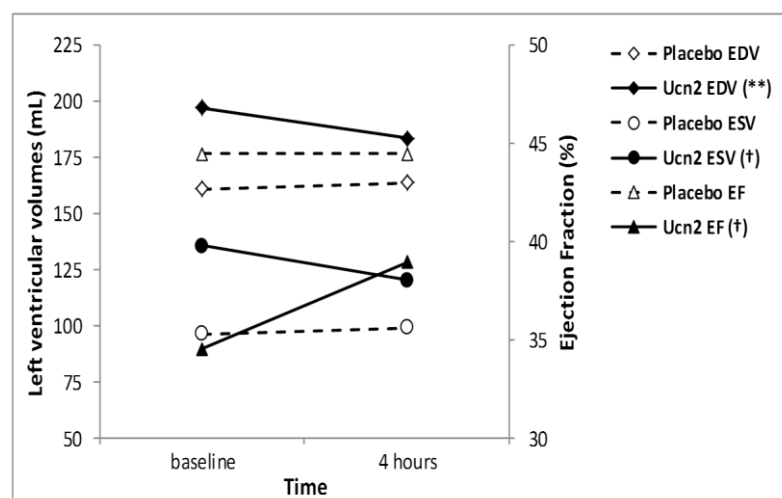
Figure 3.4. Change of left ventricular volumes and ejection fraction with trial drug infusions. EDV, end-diastolic volume; EF, ejection fraction; ESV, end-systolic volume; Ucn2, urocortin-2. ** (P<0.01) and † (P<0.001) indicate significant time-by-treatment interactions.

3.4.4 Renal Effects

Plasma creatinine (P=0.003), urine output (P=0.001), measured creatinine clearance (P=0.03) and sodium excretion (P=0.007) were all reduced with urocortin-2 relative to placebo during infusion but all renal indices rebounded post-infusion (Figure 3.5A). Although plasma creatinine and estimated GFR were marginally elevated from baseline, they were not significantly different between treatment arms at +24hours

(creatinine: urocortin-2 125μmol/L vs placebo 113μmol/L, P=0.2 and estimated GFR: urocortin-2 53mL/min/1.73m^2 vs placebo 56mL/min/1.73m^2, P=0.4). Cumulative urine volume, sodium and potassium excretion (urocortin-2 2440 vs placebo 2065mL, P=0.3; 190 vs 159mmol, P=0.4 and 54 vs 50mmol, P=0.5 respectively) over the 24hour period were similar with urocortin-2 or placebo.

Twelve patients in placebo group and sixteen patients in urocortin-2 group who were clinically assessed to have persistent symptoms and signs of pulmonary congestion received an extra intravenous dose of frusemide in the 8 hours post infusion as per the standard care for ADHF. The average dose was significantly higher with placebo (149 ± 30mg vs 85 ± 14mg, P=0.047). There was a marked increase in diuresis and natriuresis post frusemide in the 4-12hrs phase compared to 0-4hrs phase with urocortin-2 but minimal additional increase following frusemide with placebo or with urocortin-2 alone (Figure 3.5B).
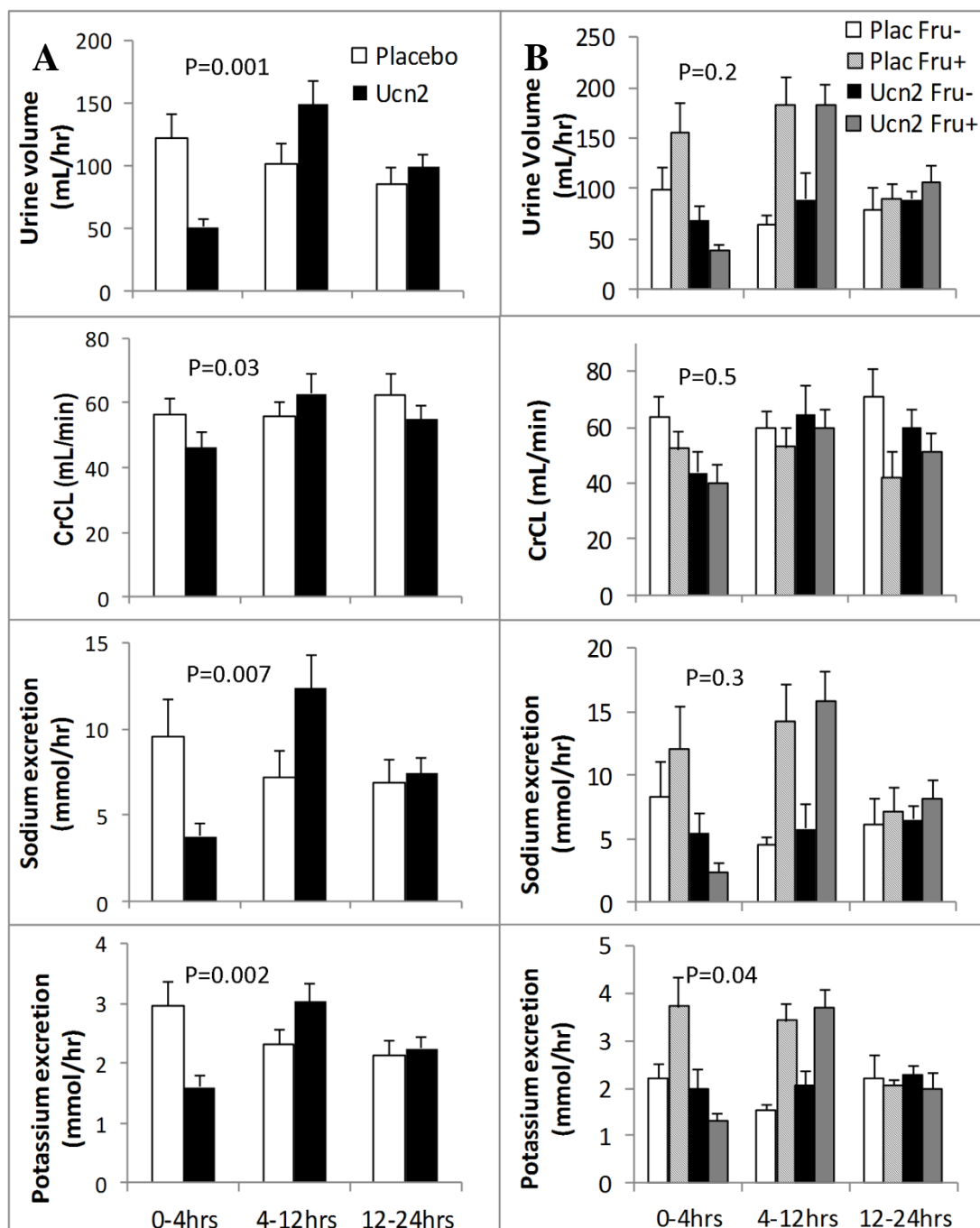
Figure 3.5A. (Left hand panels) show the renal response over 24 hours in phases with respect to the commencement of infusion. 3.4B. (Right hand panels) show the renal response comparing to a sub-group of patients who received an additional frusemide bolus (Fru+) to those without (Fru-) in the 4-12hour period. CrCL, creatinine clearance.

3.4.5 Neurohormones

Plasma urocortin-2 concentrations increased up to 7-fold over baseline level at +2hours into infusion, before returning to baseline within 2 hours after the infusion (Figure 3.6). Plasma urocortin-1 levels were unaffected by urocortin-2 infusion

(Figure 3.6).

The increase in PRA exceeded time-matched placebo by 46.3% during urocortin-2 infusion (P<0.001, Figure 3.6). However the absolute increase in PRA was modest at 1.1nmol/L/hr. The maximal change corresponded to the time of the lowest arterial blood pressure. There were no corresponding acute elevations in Ang II or aldosterone, resulting in a significant reduction in the aldosterone:renin ratio (P<0.001). PRA, Ang II and aldosterone all gradually increased similarly in both study arms from +12 to +24hours.

The B-type natriuretic peptides fell with time in both treatment arms over 24 hours (Figure 3.7). There was little difference between treatment arms during infusion for ANP, BNP and NT-proBNP. However, post infusion out to +12hours, urocortin-2 induced a significantly greater decrease in BNP and NT-proBNP levels compared to placebo (BNP -22.7% vs -9.2%, P=0.02; NT-proBNP -26.7% vs -10.5%, P=0.001). BNP (P=NS) and NT-proBNP (P=0.02) levels continued to diverge from placebo between +12 and +24hours.

Cortisol levels fell with time. The fall in cortisol was attenuated by urocortin-2 as compared to placebo, -17% vs -39%, P=0.04 (Figure 3.7). Although there appeared to be a transient tiny spike in ET-1 during the second half of the urocortin-2 infusion, the change was not statistically different from placebo. ET-1 levels were within normal reference range during the entire observation period.

Post-hoc analysis showed the neurohormonal changes did not differ significantly between participants with preserved compared with reduced LVEF.
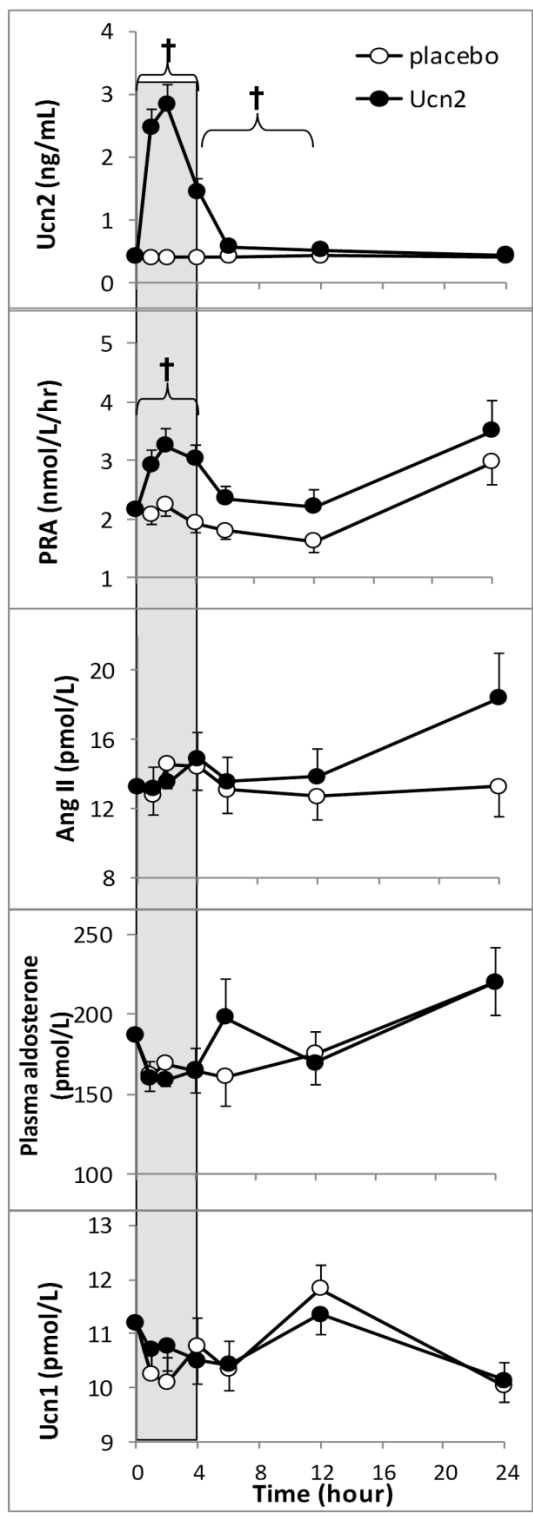
Figure 3.6. 24-hour plasma hormone levels: Ucn2, urocortin-2; PRA, plasma renin activity; Ang II, angiotensin II; aldosterone and Ucn1, urocortin-1 during (shaded grey) and after trial drug infusions. † (P<0.001) indicate significant time-by-treatment interaction in the specific time phase.
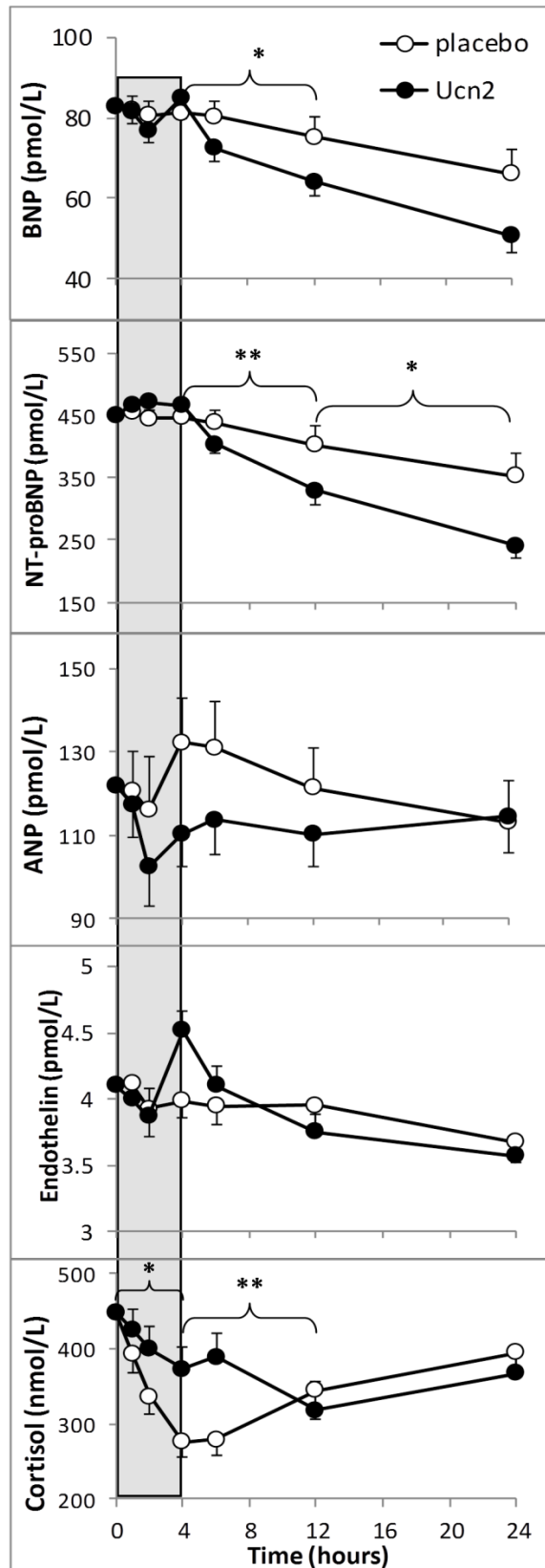
Figure 3.7. 24-hour plasma hormone levels: ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; cortisol; endothelin, endothelin-1 and NT-proBNP, N-terminal pro-brain natriuretic peptide during (shaded grey) and after trial drug infusions. * ($P<0.05$) and ** ($P<0.01$) indicate significant time-by-treatment interaction in the specific time phase.

3.4.6 Biochemistry and Haematology

There were no significant differences in plasma glucose, sodium and potassium over the study period between study arms (data not shown). White cell and platelet counts were not statistically different during the observation period (data not shown). There was a statistically significant but small inter-group difference in haemoglobin count which was increased by urocortin-2 but decreased with placebo with the maximal absolute change from baseline <4g/L (P=0.04).

3.4.7 Symptoms

63% of patients with urocortin-2 experienced an improvement in dyspnoea after the first hour of infusion compared to 46% of patients with placebo, although the difference did not reach statistical significance, P=0.2. For the remaining time points, the proportion that experienced an improvement in dyspnoea was similar between treatment arms. Both groups experienced similar improvement in HF scores at +24hours (urocortin-2 -2.3 ± 0.3 vs placebo -2.1 ± 0.2, P=0.7).

3.4.8 Adverse Events and Clinical Outcomes

Four patients required a downward titration of urocortin-2 infusion rate due to blood pressure falling below the pre-set threshold for ceasing infusion (systolic blood pressure <85mmHg), however, all were asymptomatic. Infusion was restarted at half the previous dose once systolic blood pressure was sustained above 90mmHg for at least 20min. Flushing was observed more often in the urocortin-2 group than with placebo, (15/27 vs 6/26 respectively; P=0.016), although only two patients were aware of flushing. Among those receiving urocortin-2, there was one episode of non-sustained atrial tachycardia and one episode of non-sustained ventricular tachycardia

(VT) whilst one patient in the placebo arm had non-sustained VT during infusion. Post-infusion, 5 patients, 2 in urocortin-2 and 3 placebo arm, had documented non-sustained VT on telemetry, predominantly in the 12-24hours period. All were asymptomatic and four had a prior history of VT.

Troponin I was added to the 24hour monitoring following the mid-term Data safety monitoring board recommendations. Fifteen patients with placebo and eleven patients with urocortin-2 had both admission and 24hours troponin I measurements. The Troponin I levels were identical to admission levels in both groups (0.03 for placebo and 0.02 for urocortin-2) and suggested urocortin-2 did not cause any myocardial injury.

Mean length of hospital stay was 7 days. In five patients in each study arm, heart failure worsened or failed to improve during the index admission, requiring an increase in diuretic dose compared to the prior 24hour period and/or introduction of intravenous diuretics or inotropes during any time of the admission. Two patients receiving placebo (day 3 and day 6) and one receiving urocortin-2 (day 3) received parental inotropic therapy.

There were two in-hospital deaths. A patient in the urocortin-2 arm died of progressive HF (day 5) and a patient in the placebo arm died of a non-cardiac cause (day 36). A further patient died of complication from a mechanical fall prior to the 30-day follow-up.

At 30-day follow-up, there was no significant change in LVEF from baseline in either group, P=0.8. The estimated GFR was similar in the two groups and did not differ significantly from baseline. Three patients in the urocortin-2 arm and three in the placebo arm received outpatient treatment for worsening HF or had signs of HF at 30-

day follow-up.

3.5 Discussion

This is the first report which confirms administering urocortin-2 as an adjunct to conventional therapy in ADHF is feasible and well tolerated, with only a small group (4/27) of patients requiring a reduction of urocortin-2 dose due to large but asymptomatic falls in blood pressure.

This study has reconfirmed urocortin-2 is a potent vasodilator with greater effects on afterload than preload.[247, 264, 309-311] Although the hypotensive effect was quite marked, this did not result in any further compromise in other haemodynamics indices. On the contrary, cardiac output was increased by approximated 50% likely secondary to a reduction in cTPR. This beneficial reduction in cTPR appeared to be persistent with lower levels maintained for a number of hours post-infusion. Although the increase in cardiac output is likely to be predominantly a result of vasodilatation, urocortin-2 is known to have mild positive inotropic effects,[274, 278] which may have contributed at least in part to the rise in cardiac output. Our study did not show a significant increase in the echocardiographic contractility index S', however the reduction in left ventricular volumes and increase in LVEF would suggest some degree of increased contractility. Cardiac work was increased during infusion, not readily explained by a modest (4 beats per min) and statistically non-significant increase in heart rate. This further supports the notion that urocortin-2 may increase myocardial contractility directly.

Given the magnitude of blood pressure drop, there was very little baroreflex-mediated tachycardia observed. The lack of increase in heart rate is unlikely to be related to

concurrent beta-blocker dosing as only 3 and 4 patients respectively in each arm received a dose of beta-blocker within the four hour period before and during infusion. This blunted tachycardic response contrasted with the reflex tachycardia to a fall in blood pressure reported in the literature[223, 224, 251, 312] and may be explained by the cardiac sympathetic nerve suppressant effects. However, to date, this has only been demonstrated in sheep[281] with no equivalent human data. In the next chapter, the results from the first study of urocortin-2 on the SNA in man are presented.

The pattern of response in plasma B-type natriuretic peptides over 24 hours suggests urocortin-2 facilitated cardiac decongestion which lasted beyond the period of urocortin-2 infusion.

The renin activation and reduction in urine output and calculated GFR during urocortin-2 infusion were unexpected findings and different from those observed in experimental heart failure[247]. However, these effects were short-lived and most likely occurred in response to the relatively large falls in systemic blood pressure and presumably renal perfusion pressure although direct renal suppressive effect by urocortin-2 is possible. The dose of urocortin-2 delivered each hour was equivalent to the low dose (25µg /hr) studied in stable HF patients[224] but for a longer duration, 4 hours, in this study. However the peak achieved plasma urocortin-2 concentration 2830 (2542-3512) pg/mL was twice that observed in previous study 1390 (1210-1590) pg/mL and the haemodynamic changes observed in these ADHF patients were closer to those observed in stable HF patients receiving a four-fold higher dose (100µg/hr). In that study, the higher dose also triggered an increase in renin and Ang II[223]. Although the overall RAAS effects amounted to brief intra-infusion increases of renin followed by return to time-matched placebo levels with little net effect on Ang II and aldosterone, in this study, any action of urocortin-2 to inhibit the RAAS and

enhance renal indices may well have been counterbalanced by the profound concurrent hypotensive effects. It is possible that further investigation with a lower dose of urocortin-2 (a quarter of 5ng/kg/min, an estimate based on observation of the current study to achieve the effect equivalent of 25μg over 4 hours) with less reduction in blood pressure may reveal net beneficial renal and neurohormonal effects in the setting of ADHF.

In sheep with experimental HF, plasma cortisol was raised by urocortin-2[247] while no change was seen in stable heart failure patients[224]. In the current trial, there was a significant treatment-by-time interaction with an attenuated fall in cortisol in those receiving urocortin-2 compared to those receiving placebo (Figure 3.6). Urocortin-2 has little affinity to CRF_1 receptors under normal physiological condition but could activate CRF_1 receptors at higher doses.[211, 212] It is also possible that central activation of CRF_2 receptor may contribute to urocortin-2 activation of cortisol.[258, 489, 490] Rademaker et al proposed that a concurrent rise in urocortin-1 levels through competitive inhibition of CRF_2 receptor by urocortin-2 may, at least in part, explain observed rises in plasma cortisol in the sheep model.[247] This does not appear a likely explanation in the current study as plasma urocortin-1 concentrations were unaffected throughout. The cortisol rise may not be a result of direct activation by urocortin-2 but rather, may reflect a more generalised stress reaction in response to the marked fall in blood pressure observed during infusion.

The lack of a significant difference in symptomatic improvement was perhaps not surprising. The infusion was started relatively late (mean 21 hours from admission) compared to the continuous conventional HF therapy administered to all patients throughout the hospitalisation period which commenced much earlier than the introduction of urocortin-2. The presentation for ADHF was also heterogeneous and

the sample size was small.

The plasma concentration of urocortin-2 at +4hours was almost halved from +2hours. The end-infusion hormone sample for 21 out of 27 patients was collected after the infusion was completed and the mean delay in obtaining this blood sample was +6.7 minutes, almost one half-life for plasma urocortin-2 and therefore the likely explanation for the decrease in plasma concentrations.

Urocortin-2 has a similar array of haemodynamic, renal and hormonal effects to those exhibited by endogenous vasodilators such as BNP and relaxin.[336, 491] However, urocortin-2 has a unique structure and activates a specific receptor, and has a distinctive combination of bioactivity including marked vasodilatation, increased cardiac output, diuresis and natriuresis, protection against myocardial ischaemia/reperfusion injury and suppression of cardiac sympathetic activity.[224, 246, 264, 274, 281, 284] No study has directly compared the effects of urocortin-2 to other vasodilators. Urocortin-2 appears to have similar if not earlier onset of vasodilatation compared to either BNP or relaxin.[331, 336, 491, 492] The rise in cardiac output is also greater in proportion to the vasodilatation achieved compared to these two agents. While the dose may need to be adjusted to unmask any beneficial renal and neurohormonal effects, the overall haemodynamic properties of urocortin-2 are very favourable in ADHF. Whilst inotropic agents have been associated with poorer long-term outcome and the inotropic-like action of urocortin-2 is as potent as isoprenaline[278], however urocortin-2 possesses other cardioprotective properties[274, 275, 288] and further investigation is warranted to determine if it has long-term benefits on outcome of ADHF.

3.6 Study Limitations

This pilot study included 53 participants. The wide variation of duration between admission and trial drug infusion made assessment of symptomatic improvement difficult. Furthermore, the study was not powered to detect changes in symptoms score. The study was also not powered to show difference in mortality or re-hospitalisation.

3.7 Conclusion

Urocortin-2 administration in the setting of ADHF was both feasible and safe and produced favourable haemodynamic responses. Modest and brief activation of PRA and short-lived decreases in renal indices were likely secondary to marked falls in systemic arterial pressure and, as such, the true potential for urocortin-2 to improve renal and neurohormonal indices in ADHF may yet to be seen. The role of urocortin-2 in ADHF, in particular administered at a dose with less blood pressure lowering effect, and its long-term effects warrant further research.

4 Urocortin-2 Infusion and Muscle Sympathetic Nerve Activity in Man

4.1 Introduction

The SNS is activated in HF with adverse long-term effects including myocardial remodelling and reduced contractility, increased preload and afterload, salt and water retention and exercise tolerance.[179] Abnormal activation of the SNS occurs early in HF,[97, 180-182] and is proportional to functional class and intra-cardiac pressures.[178, 198] Sympathetic activation is associated with increased risk of malignant arrhythmia, sudden cardiac death and death from HF progression.[177, 204] Treatments that reduce central sympathetic activation (as measured by plasma norepinephrine levels) or block peripheral adrenergic receptors such as beta-blockers, are associated with a reduction in all-cause mortality, sudden cardiac death, HF hospitalisation, and requirement for the use of inotropes and vasodilators.[171]

Urocortin-2 has multiple favourable cardiovascular effects via the CRF_2 receptor, and its actions have already been discussed in the earlier chapters of this thesis. Recently, urocortin-2 had been demonstrated to suppress efferent post-ganglionic cardiac SNA measured by direct microneurography in normal conscious sheep. Intravenous bolus administration of urocortin-2 induced a dose-dependent decrease in sympathetic nerve burst incidence, frequency and area, without significant effect on systemic plasma norepinephrine.[281] The suppression of SNA by urocortin-2 in sheep contrasts with an observed increase in plasma norepinephrine following a high dose one-hour infusion in healthy volunteers.[223] However urocortin-2 has been shown to suppress catecholamine secretion in rat and human adrenal chromaffin cells.[493] The explanation

for this result is that, change in plasma norepinephrine is a crude reflection of overall SNA, but is not informative with respect to sympathetic traffic to individual organs. There have been no studies of the effect of urocortin-2 on SNA measured by direct microneurography in healthy man or patients with HF. Measurement of cardiac SNA requires thoracotomy and therefore not is not possible in humans. However, muscle SNA can be measured in man by direct microneurography of the peroneal nerve.

4.2 Hypotheses and Aim

Hypotheses:

1. Urocortin-2 will produce similar inhibition of SNA in healthy man as that seen in sheep.

2. As the SNS is more active in HF, an exaggerated inhibition of muscle SNA will be seen following urocortin-2 infusion in HF patients.

Aim:

1. To evaluate the integrated effects of urocortin-2 on haemodynamics, muscle SNA and plasma catecholamines in healthy subjects and stable HF patients.

4.3 Methods

4.3.1 Study Design

"The effects of urocortin-2 on muscle sympathetic nerve activity in man" (TOUCHÉ), Australian New Zealand Clinical Trial Registry number: ACTRN12610000539033, was a placebo-controlled cross-over study. Eight male subjects were intended to be

recruited in each of the healthy volunteer and heart failure groups. The choice of sample size was pragmatic and reflected our observation of highly significant changes in haemodynamic variables in our previous studies in humans receiving similar doses of urocortin-2 as planned for TOUCHÉ.[223, 224]. A randomisation scheme using a balanced Latin-Square design was deployed to determine the sequence in which the treatment was delivered to the participants.

4.3.2 Patient Eligibility

This study was further divided into two sub-studies conducted in healthy volunteers and patients with stable HF. Participants were males, aged between 18-70 years, without any clinically significant illness in the healthy volunteer arm or any non-HF related illness in the stable HF arm and not exposed to any investigational drug in the one month before infusion. All participants had to be willing to comply with a caffeine-free metabolic diet for two days that controlled the amount of sodium and potassium intake.

To be eligible as healthy volunteers, participants

1. Could not have any unstable medical or psychiatric abnormalities that could interfere with the pharmacokinetic or pharmacodynamic evaluation of urocortin-2.

2. No drug/ alcohol abuse or known dependence.

3. Normal sinus rhythm was required.

4. Systolic blood pressure ≥100 mmHg at rest and not on any prescribed or over-the-counter remedies that might affect blood pressure or nerve activity.

5. No prior history of peripheral neuropathy.

6. No clinically significant abnormality detected at physical examination, on electrocardiogram, or in clinical laboratory tests during the screening visit and any time prior to infusion.

Stable HF patients had to have a diagnosis of HF at NYHA classification functional severity between I to III, left ventricular ejection fraction <50% and/or a BNP measurement >100 pmol/L within six months of study infusion. Exclusion criteria were as for the healthy volunteers with the additional stipulation that patients who had suffered an acute episode of decompensated HF within the prior six months were also excluded. Patients had to be in sinus rhythm on the day of infusion or had a permanent pacemaker implant for complete heart block. Any other rhythms including frequent premature beats (>10/min) was excluded as potentially interfering with haemodynamic and SNA recordings.

4.3.3 Protocol (Table 4.1)

The protocol was identical for healthy volunteers and patients with stable HF. All subjects underwent two days of controlled metabolic diet (sodium 120mmol/day, potassium 100mmol/day). The rationale behind this was that sodium and potassium imbalance could affect haemodynamic and hormonal status. The diet was prepared by a specialist dietician at the Human Nutrition Department, Christchurch Hospital. Subjects were asked to abstain from alcohol, caffeine and smoking during this period as these substances could stimulate sympathetic activity. On Day 3 (study day), subjects presented to study room in the morning following breakfast. HF patients were asked to withhold diuretics, vasoactive agents and beta-blockers if possible for 24 hours. Subjects were seated in a "LazyBoy" chair throughout the study period. An intravenous cannula was inserted on the right arm for trial drug infusion, and a second

intravenous cannula was placed in the contralateral arm for blood sampling. Microneurography was then performed on the right superficial peroneal nerve. Details of the microneurography technique are discussed in section 2.3. In brief, nerve signal was amplified, filtered, integrated and displaced on-line with blood pressure and electrocardiogram. The bursts of SNA were identified, counted and measured by a single investigator. Non-invasive beat-to-beat blood pressure was measured at the finger (Finometer Blood Pressure Monitor, Finapres Medical Systems B.V., Amsterdam, the Netherlands) with the hand held at the heart level. The measured analogue signal was digitally converted at 200Hz, and stored on a hard-disk for offline analysis. Systolic and diastolic arterial pressures were derived from the arterial pressure waveform. Mean arterial pressure was calculated from the integral of the arterial pressure waveform over one beat divided by the corresponding beat interval. Heart rate was computed as the inverse of the inter-beat interval and expressed as beats per minute. Beat-to-beat changes in stroke volume was estimated by modelling flow from the arterial pressure waveform (Modelflow, TNO Biomedical Instrumentation, Amsterdam, the Netherlands), expressed in mL.[494] Cardiac output expressed in L/min was the product of estimated stroke volume and heart rate. Total peripheral resistance was expressed in mmHg.s/mL, as Medical Units (MU) was derived from mean arterial pressure divided by the computed cardiac output. The Modelflow method estimated aortic flow from finger arterial pressure by simulating a non-linear model of aortic input impedance.[495] This pulse-wave analysis method corrects for individual differences in age, gender, height and weight, permitting group average data to be examined accurately in subjects without structural or functional heart disease.[496] Previous experiments have demonstrated good agreement between Modelflow and traditional estimates of cardiac output.[497, 498]

The -15min recording was measured after the nerve field was obtained and recordings had been stable for ten minutes. At time 0, the intravenous infusion of urocortin-2 (25µg from a 400µg vial with mannitol as carrier, made up to 50mL at 2µg/mL with normal saline, infused at 12.5mL/hr) or vehicle was administered over one hour. Data on microneurography, haemodynamics and hormones were continuously recorded, fed onto the computer and analysed later at pre-set time points as specified below:

	Time (minutes)									
	-15	**0**	**15**	**30**	**45**	**60**	**75**	**90**	**105**	**120**
Haemodynamics	√	√	√	√	√	√	√	√	√	√
MSNA	√	√	√	√	√	√	√	√	√	√
Neurohormones	√	√		√		√		√		√

Table 4.1. Study Protocol for TOUCHÉ. Shaded area indicates period of urocortin-2 or placebo infusion. MSNA, muscle sympathetic nerve activity.

Samples for indirect index of global SNA in the form of plasma venous norepinephrine and epinephrine assays, were obtained at baseline (-15min), +0 and at 30 minute intervals following the start of infusion. Five one-minute measurements of muscle SNA and haemodynamic recordings were averaged over five minute time intervals shown in Table 4.1.

The handling and assay of hormone samples are discussed in section 2.1.

After a minimum of 8 weeks following the first part of the study, the subject underwent the second part. Patient followed the same protocol of two days controlled metabolic diet. On Day 3, subjects presented to study room in the morning following breakfast. Subjects were seated in a "LazyBoy" chair throughout the study period. After insertion of intravenous cannulas for trial drug infusion and blood sample

collections, right peroneal microneurography was performed for muscle SNA recordings and non-invasive haemodynamic apparatus fitted to right middle finger. Participants then received trial drug infusion for one hour and continued SNA and haemodynamic recordings for a further hour post infusion completion. On this occasion, urocortin-2 or vehicle was administered opposite to what was administered in the first visit.

4.3.4 Statistics

Methods for statistical analysis are described in details in Chapter 2.

4.4 Results

Eight healthy Caucasian males participated in the healthy volunteer arm, mean age 48 (range 41-53) years. On completion of both infusion arms on the first four participants, it was suspected that there might be a problem with the urocortin-2 infusion, either dose or peptide content, as none of the expected haemodynamic changes were observed in either phase of the study. Thus, a formal interim analysis was performed with unblinding of data which confirmed there was no effect of urocortin-2 infusions on haemodynamic indices. This was an unexpected result given the investigators previous experience with similar doses of urocortin-2 in healthy man[223]. On further investigation it became apparent that the urocortin-2 in the four participants to date (source from Clinalfa AG, Weidenmattweg, Switzerland) was non-amidated and therefore not bioactive. It was decided that with the consent of the participants, a third infusion would be performed in these four participants using "bioactive" amidated urocortin-2 sourced from Neurocrine Biosciences Inc. The remaining four healthy volunteers and the HF patients did not receive the non-

amidated peptide and therefore only required the originally designed two infusion studies, namely placebo (vehicle) control and amidated urocortin-2. However, the need to unblind and repeat the urocortin-2 infusion in the first four participants inadvertently disrupted the proposed random order of infusions; therefore it was decided that the subsequent participants in the healthy volunteer arm of trial would have placebo infusion first infusion and urocortin-2 second.

4.4.1 Healthy volunteers

There were no significant differences in any baseline haemodynamic or SNA variables between study days (mean arterial pressure, $P=0.15$; heart rate, $P=0.7$; burst frequency, $P=0.5$; burst incidence, $P=0.6$).

The peak urocortin-2 concentration was achieved at +60min (Table 4.2). Facial flushing was noticeable with urocortin-2 infusion in all healthy subjects from +45min and faded by +90min (Figure 4.1).

Hormone	Infusion	unit	Time (minutes)						P-value
			-30	0	30	60	90	120	
Ucn2	placebo	ng/mL	0.38 ± 0.07	0.4 ± 0.08	0.39 ± 0.07	0.43 ± 0.09	0.41 ± 0.07	0.4± 0.07	P < 0.001
	active		0.26 ± 0.05	0.25 ± 0.06	0.62 ± 0.11	1.37 ± 0.22	0.33 ± 0.08	0.27 ± 0.06	
Epi	placebo	pmol/L	187 ± 28	151 ± 30	176 ± 28	151 ± 27	189 ± 29	152 ± 15	P = 0.3
	active		463 ± 260	362 ± 208	330 ± 181	357 ± 215	194 ± 130	165 ± 20	
NorE	placebo	pmol/L	2122 ± 160	1870 ± 79	1798 ± 81	1810 ± 126	1913 ± 99	1813 ± 115	P = 0.5
	active		2047 ± 176	1720 ± 110	1759 ± 137	1813 ± 115	1756 ± 143	1720 ± 137	

Table 4.2. Plasma urocortin-2 and catecholamines during and after trial drug infusion in healthy volunteers. Ucn2, urocortin-2; Epi, epinephrine; NorE, norepinephrine. The blue shaded section of the table indicates timing of infusion.

Figure 4.1. Photos of participant on the day of urocortin-2 infusion. Above: at baseline and below: with flushing induced by urocortin-2. Photo printed with permission from participant.

Haemodynamic effects (Figure 4.2)

The peak haemodynamic effects corresponded to peak plasma urocortin-2 concentrations. Urocortin-2 significantly reduced mean arterial pressure compared with an increase during time-matched placebo (urocortin-2 -6.5 ± 1.7mmHg vs placebo +4.5 ± 1.7mmHg, P<0.001) and remained significantly lower than baseline for a further 30min post infusion. Heart rate was significantly increased during urocortin-2 with minimal change during placebo (urocortin-2 8.4 ± 1bpm vs -1.9 ± 1bpm, P<0.001). The urocortin-2 induced fall in blood pressure corresponded to a significant reduction in cTPR (-23 ± 4%) compared with a small increase observed with time-matched placebo (10 ± 4%, P<0.001). Cardiac output was significantly increased by urocortin-2 (22 ± 4%) compared with a small reduction during placebo (-4 ± 4%, P<0.001).

Effects on muscle sympathetic nerve activity (Figure 4.3)

Urocortin-2 increased muscle SNA burst frequency and burst area compared with placebo (burst frequency 9.2 ± 2.2 burst/min vs 1.4 ± 2.2 burst/min, P=0.005; burst area 33 ± 7% vs 7 ± 7%, P=0.01 for urocortin-2 and placebo respectively). In contrast, the increment in burst incidence (corrected to heart rate) was not significantly different (urocortin-2: 6.3 ± 2.7 burst/100 beats vs placebo: 3.7 ± 2.7 burst/100 beats, P=0.08). Urocortin-2 had no significant effect on plasma norepinephrine or epinephrine (Table 4.2).

Healthy Volunteers **Heart Failure Patients**
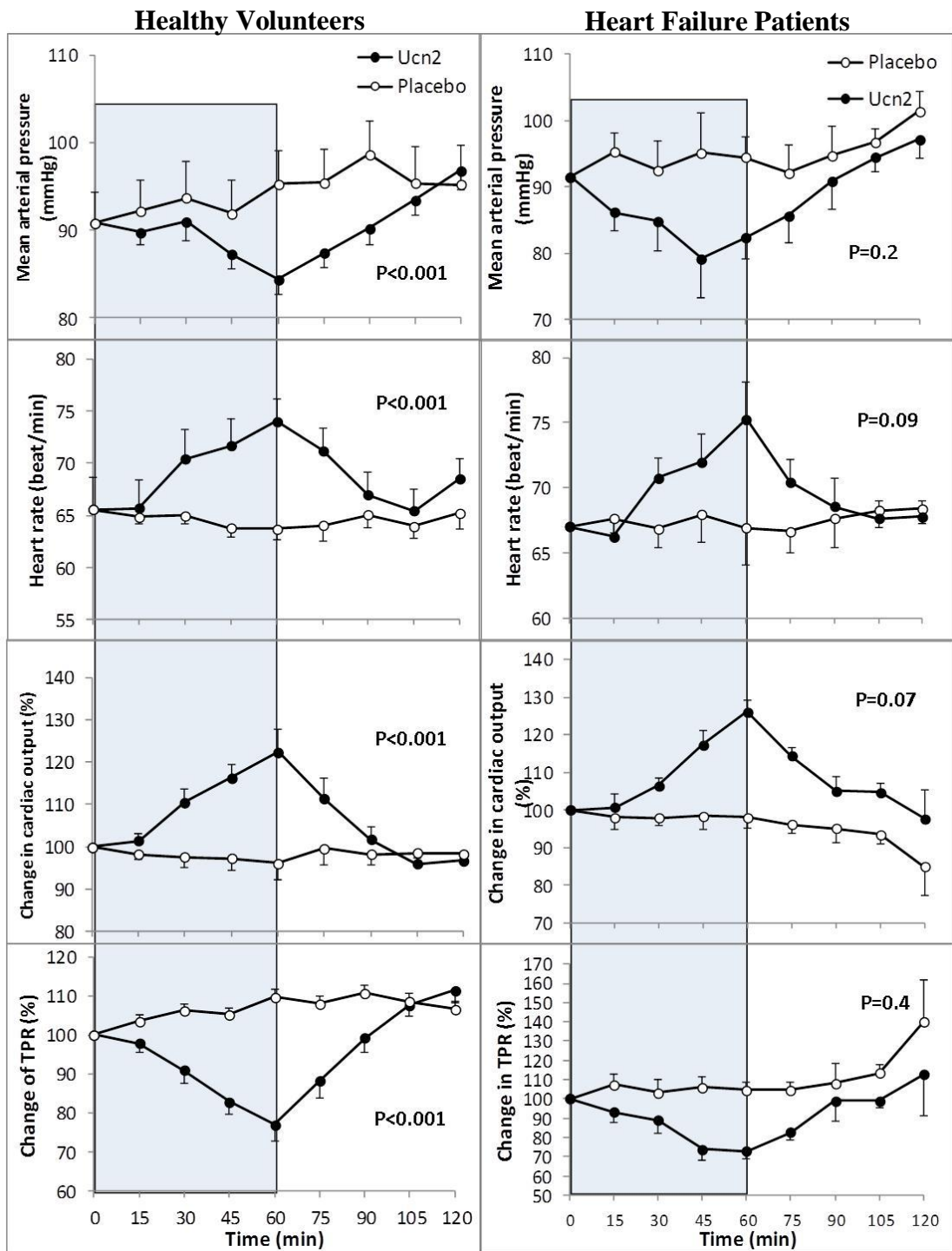
Figure 4.2. Change of haemodynamic parameters during and after urocortin-2 or placebo infusion in eight healthy volunteers on left hand panels and four heart failure patients on right hand panels. Ucn2, urocortin-2; TPR, total peripheral resistance. Shaded area indicates the period of trial drug infusion.
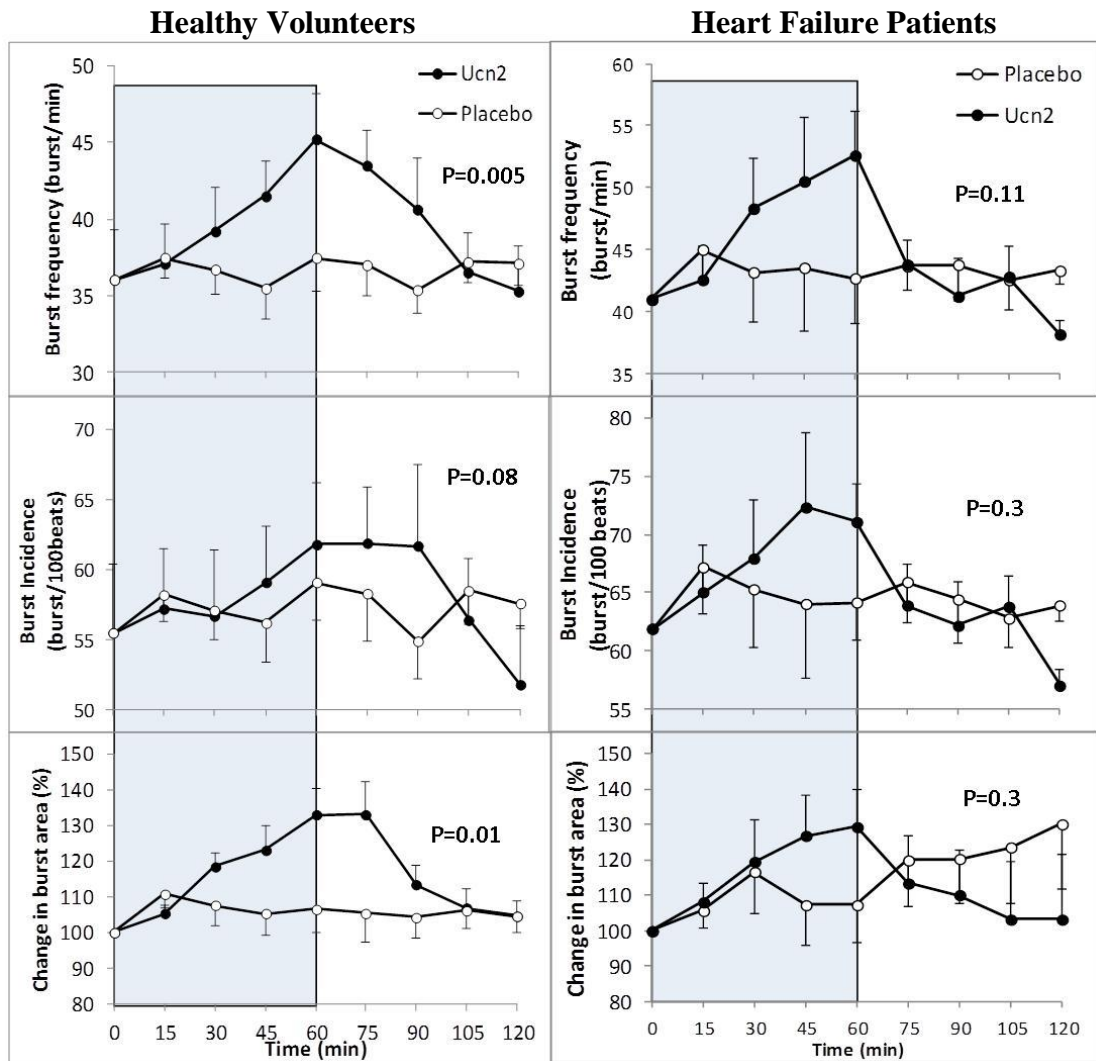
Figure 4.3. Muscle sympathetic nerve activity parameters during and after urocortin-2 or placebo infusion in eight healthy volunteers on left hand panels and four heart failure patients on right hand panels. Ucn2, urocortin-2. Shaded area indicates the period of trial drug infusion.

4.4.2 Stable Heart Failure Patients

The HF arm of the study is still ongoing, preliminary data is included in the thesis.

Four patients with stable HF (all NYHA I) were recruited in the HF arm, mean age 58 (range 51-68) years. Two patients had ischaemic cardiomyopathy and two dilated cardiomyopathy. The mean LVEF was 33.5 (range 29-35) %, mean BNP at screening was 47 (range 6-80 pmol/L or 50 to 680pg/mL). All patients were on ACEIs and beta-blockers, and only one patient was not on a MR antagonist. HF patients received trial

drug infusions in a balanced random order. There were no significant differences in any baseline haemodynamic or SNA variable between study days (mean arterial pressure, P=0.9; heart rate, P=0.9; burst frequency, P=0.4; burst incidence, P=0.4)

Haemodynamic effects (Figure 4.2)

The mean arterial pressure response to urocortin-2 in the four stable HF patients followed a similar trend to that observed in healthy volunteers. However, despite obvious numerical differences between active and placebo infusions, sample size precluded a statistical significance (urocortin-2 -12.4 ± 6mmHg vs placebo 3.6 ± 6mmHg, P=0.2). The peak effect was noted at one time-point earlier, +45min, and the magnitude was greater than with healthy volunteers. There was a trend for a corresponding rise in heart rate with the peak effect at +60min (urocortin-2 8.2 ± 2.8bpm vs placebo -1 ± 2.8bpm, P=0.09). Urocortin-2 augmented cardiac output in stable HF patients in similar magnitude to healthy volunteers (26 ± 3%) although again the difference compared to placebo (-2 ± 3%) did not achieve statistical significance (P=0.07). cTPR tended to be reduced by urocortin-2 (-27.2 ± 4.2%) compared to time-matched placebo (4.6 ± 4.2%, P=0.4). With the presence of diagnosis of HF as the between-individual factor on response, there was a strong trend towards HF status affecting the blood pressure response (P=0.08) but not on heart rate, cardiac output or cTPR.

Effects on muscle sympathetic nerve activity (Figure 4.3)

The effects of urocortin-2 on SNA in stable HF patients were qualitatively similar to healthy volunteers but none of the SNA indices reached statistical significance. Urocortin-2 tended to increase burst frequency, burst incidence and burst area compared to minimal change during placebo (burst frequency 11.6 ± 3.6 burst/min vs

placebo 1.6 ± 3.6 burst/min, P=0.11; burst incidence 10.5 ± 6.4 burst/100 beats vs 1.2 ± 6.4 burst/100beats, P=0.3; burst area 29 ± 11% vs 7 ± 11%, P=0.3). HF status had no impact on the response to urocortin-2 compared to healthy volunteers.

4.5 Discussion

This is the first report describing the effect of urocortin-2 on directly measured muscle SNA in humans. This study confirms the potent vasodilatation induced by urocortin-2 in healthy volunteers with resultant increase in cardiac output as previously observed in studies performed in healthy and stable HF subjects.[223, 224] Urocortin-2 significantly increased muscle SNA burst frequency in healthy volunteers presumably at least in part due to an expected baroreflex-mediated response to falls in arterial pressure.[499] When burst frequency was adjusted to heart rate (burst incidence), the change was no longer present. The increase in heart rate was reciprocal to the fall in mean arterial pressure. Qualitatively similar, but non-significant, changes were observed in the HF group. There was however a difference in temporal profile of heart rate and blood pressure response with urocortin-2 infusion between the two groups. In the healthy volunteer arm, heart rate increased from +15min, before a significant fall in blood pressure occurred. In the HF group, the mean arterial pressure gradually increased following its nadir at +45min, however heart rate continued to increase for another 15 minutes before its turnaround point. Similar observations were made in a sheep study where there was a biphasic increase in heart rate with the second phase of increase heart rate occurred without a fall in blood pressure.[281]

The consistent rise in muscle sympathetic burst frequency and burst area contrasts with earlier findings in conscious sheep where, following a transient spike in burst

frequency in response to falls in blood pressure, urocortin-2 produced a long lasting inhibitory effect on cardiac SNA as evidenced by a reduction in burst frequency, burst incidence and burst area.[281] The initial spike in the ovine study was believed to be baroreflex-mediated but the mechanism for subsequent inhibition of cardiac SNA was a urocortin specific effect. Although reduction in mean arterial pressure was similar in both normal sheep[281] and normal volunteers in the current study, the duration of hypotension was much longer in the current study (60-90 minutes in human compared with 30 minutes in sheep). The different time course of hypotensive response between sheep and man may reflect different modes of administration (bolus in sheep vs infusion in man), or may be due to differences between species. Thus, it is possible that any direct inhibitory effect of urocortin-2 on SNA in the present study was overpowered by a more prolonged baroreflex response to falls in blood pressure. However, it is not clear whether urocortin-2 modulated the baroreflex response in this study as there was no comparison with another vasodilator such as nitroprusside. It is also possible that muscle SNA response may not reflect cardiac SNA as, even though direct muscle microneurography can be a good surrogate for systemic SNA[196], it is well known that SNA directed at different tissue beds may be regionally differentiated.[500, 501] Also of note, urocortin-2 is known to have differential haemodynamic response in different target organs.[262] Plasma catecholamine levels, as shown in this study, is a crude and indirect measure of SNA and generally is not sensitive enough to detect subtle changes and is clearly not organ-specific. Although the plasma norepinephrine was increased with high dose urocortin-2 infusion in healthy volunteers, there was minor suppression of plasma epinephrine.[223] The proposed mechanism for the rise in norepinephrine was baroreflex-mediated; however the cause of the epinephrine suppression was not clear. The effect of urocortin-2 on

cardiac SNA in healthy man remains unknown.

In the HF arm, none of the changes in haemodynamic and sympathetic nerve parameters reached statistical significance. This was most likely due to the small study number (n=4) as the haemodynamic and SNA changes all parallel the changes seen in healthy volunteers. The HF patients were all well treated (NYHA I), and a possibly more marked effect could be seen in more symptomatic patients. The magnitude of hypotension tended to be greater in the HF arm than in healthy volunteer arm, but the degree of increase in heart rate was similar between two arms. The lower blood pressure may simply be a chance occurrence due to the small sample size unrelated to the HF status or from residual effect of background HF therapy. All patients in the HF arm withheld beta-blockers for only 24 hours; the blunted tachycardia response was most likely a result of residual beta-blocker effect. It is unclear why the duration of urocortin-2 effect on SNA was less persistent than healthy volunteers.

4.6 Study Limitations

This is a small study with only eight participants in the healthy volunteer arm and four relatively asymptomatic participants in the HF arm. The trial drug infusion was given in a non-random order in healthy volunteers and was not compared with another vasodilator. The cardiac SNA is known to be different between genders[200], it is uncertain if results from this study could be applied to women.

4.7 Conclusion

Urocortin-2 increases heart-rate dependent muscle SNA likely secondary to baroreflex unloading. The observed increase in muscle SNA contrasts with inhibition of cardiac SNA documented in sheep and may reflect differential regional sympathetic response to urocortin-2 or inter-species differences in urocortin-2 effects.

5 Cardiac Resynchronisation Therapy Optimisation Guided by Left Atrial Pressure

5.1 Introduction

Cardiac resynchronisation therapy (CRT) is an established treatment that significantly improves quality of life and clinical outcomes among patients who have symptomatic (NYHA class II-IV) HF with severe left ventricular systolic impairment (LVEF ≤35%) and QRS duration ≥120ms.[371-373, 384, 385, 502, 503] There is no universal definition of success; however regardless of the criteria used, approximately 30% of patients do not improve.[371-373, 385] These patients are labelled as "non-responders". In the pre-implant stage, there are no reliable predictors to identify non-responders at present.[397] In the post-implant setting, Mullens et al observed that suboptimal pacing settings accounted for almost half of all CRT failures.[394] Pacing settings can be reprogrammed and reprogramming has been shown to improve left ventricular haemodynamics, contractility and ejection fraction.[370, 375, 379] The two components of CRT optimisation are AVD, optimisation of which aims to enhance left ventricular diastolic filling which in turn also improves forward left ventricular stroke volume, and VVD optimisation of which aims to increase left ventricular stroke volume by reducing intraventricular dyssynchrony.

There is no gold standard for performing CRT optimisation. Echocardiography is the technique that is most widely used to guide CRT optimisation.[478] While echocardiography-guided optimisation is readily available, it is limited by being operator dependent, time-consuming, providing only indirect estimates of cardiac haemodynamics and requiring integration of a range of indices to determine optimal

settings.[418, 419]

The left atrium acts as a conduit during diastole, therefore LAP closely approximates left ventricular pressure especially at end-diastole, in the absence of mitral valve disease.[504] In the past, LAP could only be measured invasively thus it was not readily available for everyday clinical use. An implantable LAP sensor is now available and makes it feasible to measure LAP in an ambulatory setting.[505]

5.2 Hypotheses and Aims

Hypotheses:

1. Changes in AVD and VVD during CRT optimisation are associated with characteristic and reproducible changes in LAP and its waveform.

2. The changes in LAP and waveform provide an objective guide to CRT optimisation.

Aims:

1. To characterise changes in LAP and its waveform during CRT optimisation.

2. To assess the correlation of LAP to echocardiography-guided optimal CRT setting.

3. To determine if specific LAP waveform parameters can be used to guide CRT optimisation.

5.3 Methods

5.3.1 Study Design

This study was a single-centre substudy of CRT-based Heart Failure Monitoring Study (zLAP), US National Clinical Trial Registry number: NCT00632372, a prospective multi-centre non-randomised observational study which investigates whether there is any correlation between cardiac impedance obtained from CRT devices and LAP obtained by the implantable device and their ability to predict clinical events.

5.3.2 Patient Selection

Patient eligibility criteria followed the main study.

The inclusion criteria were:

1. Aged between 18 and 85 years.

2. Had indication as per ACC/AHA/HRS guideline at the time for CRT-D implant (Figure 5.1) or a pre-existing compatible device or suitable for an upgrade.

3. Patients had no identifiable contraindications for a permanent LAP sensor device (Figure 5.1) implant or already had one implanted from the previous study and had completed twelve months follow-up.

4. Capable of Valsalva manoeuvre with airway pressure sustained at >40 mmHg for ≥10 seconds.
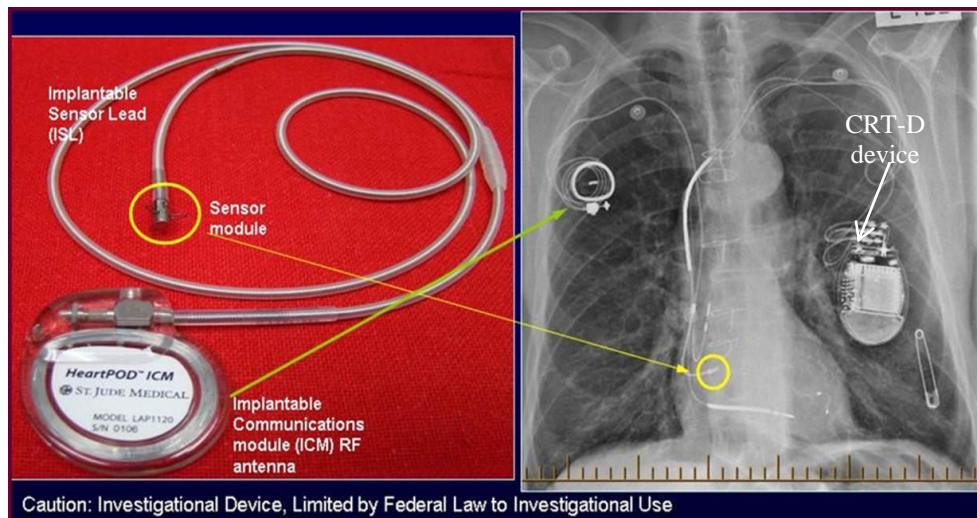
Figure 5.1. On the right, chest x-ray of patient with CRT-D device implant and LAP sensor (yellow circle and arrow) and module (green arrow) implant that are shown on the left panel.

Patients were excluded if they had any of the following:

1. A resting systolic blood pressure <90mmHg or >180mmHg.

2. Acute myocardial infarction or unstable ischaemic syndrome within previous six weeks.

3. Coronary revascularisation procedures planned or performed within six weeks.

4. Coexisting stenotic valve lesions/ vegetations, hypertrophic/ restrictive/ constrictive/ infiltrative cardiomyopathy, cardiac tamponade or moderate to large pericardial effusion.

5. History of thromboembolic disease or cerebral vascular disease including transient ischaemic attacks within six months or history of uncorrectable cerebral vascular disease.

6. Previous surgical correction of congenital heart disease involving atrial septum, intra-cardiac thrombus, atrial septal defect or a clinically significant patent foramen ovale.

7. Life expectancy of less than one year.

8. Gastrointestinal bleeding during the past six months.

9. Coagulopathy or uninterruptible anticoagulation therapy or unable to take antiplatelet medications.

10. Creatinine >212 umol/L.

11. Active systemic infection.

12. Already participating in an investigational drug/ device study that has not completed primary endpoint or interferes with the current study endpoint.

13. Any contraindication to emergency thoracotomy.

14. Hypersensitivity to a single 1.0mg dose of dexamethasone sodium phosphate or short term use of heparin.

15. Positive pregnancy test at enrolment or planning a pregnancy in the next 12 months.

16. Pacemaker dependent.

All patients enrolled in the CRT-based Heart Failure Monitoring Study in Christchurch Hospital were automatically enrolled in this optimisation sub-study.

5.3.3 Protocol

Echocardiography-guided CRT optimisation was performed at least six weeks following the latest device implantation. Patients omitted the morning diuretic on the day of procedure if possible. After the patient had rested in the left lateral supine position for five minutes, baseline echocardiography data were collected. Details of echocardiography data collection and analysis are described in section 2.2. To recap, the Doppler parameters assessed in the study were E/A ratio, peak A velocity, A duration, A/transmitral VTI, LVOT VTI and LVOT pre-ejection time. For AVD

optimisation, AVD was tested from 80ms to 200ms in 20ms increments while VVD was constant as chosen by the operator based on two-dimensional and Doppler images showing least dyssynchrony. For VVD, VVD range was tested between left ventricular-led activation by 0ms to 60ms at 20ms increment and right ventricular-led activation by 20ms while the AVD was at the optimal interval as chosen by the operator with the most favourable mitral filling pattern. There was a one minute wait after each change of setting to allow for equilibrium to be reached before acquiring echocardiogram images and two LAPs were collected at each CRT setting when zoomed in on the left atrium as per the echocardiogram data collection sequence. This routine provided an approximately five minute separation between LAP collections after each change of setting.

The implantable LAP sensor was activated by wireless radiofrequency transmission through a wand placed over the skin surface of the device and was attached to the Patient Advisory Module (PAM) which was manually operated.[505] Twenty seconds of intra-atrial electrocardiogram and LAP were acquired during quiet respiration, stored in PAM and later retrieved for analysis.

For the purposes of comparison, the optimal CRT setting was determined by Associate Professor Richard Troughton, who was blinded to the LAP data, based on a combination of transmitral filling pattern using the Iterative Method[412], LVOT VTI and two-dimensional synchrony parameters.

5.3.4 Left Atrial Pressure and Waveform Analysis

At each CRT setting, mean LAP was derived from the average of stable representative waveforms from two continuous 20 second acquisitions. LAP waveform analysis was performed offline with MatLab (MathWorks, Natick, Massachusetts). Multiple

waveform parameters were chosen reflecting LAP waveform during atrial contraction, passive atrial filling and the change in pressure during these phases, they were peak a, peak v, positive a-slope, negative a-slope, positive v-slope and negative v-slope (Figure 5.2). The ensemble averages were evaluated with respect to the stated hypothesis.
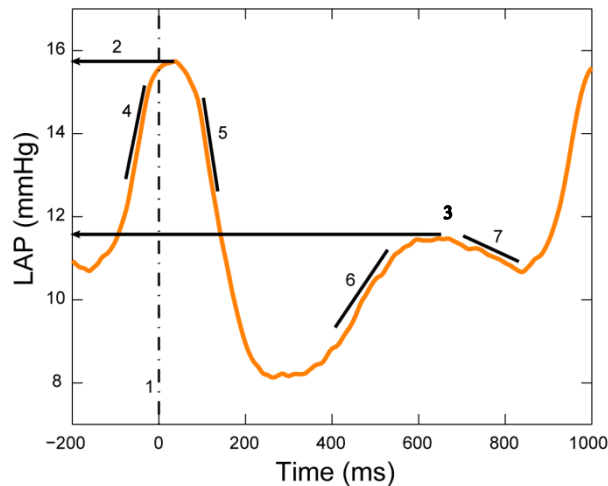
Figure 5.2. Representative left atrial pressure (LAP)-waveform over one cardiac cycle. 1—beginning of QRS, 2 – peak a wave, 3 – peak v wave, 4 –positive a-slope, 5 – negative a-slope, 6 –positive v-slope and 7 – negative v-slope.

5.3.5 *Statistics*

Details of statistics analysis is described in Chapter 2.

5.4 Results

Nine patients were enrolled in the main study. One patient with chronic atrial fibrillation, in whom atrioventricular optimisation was not performed, was excluded from analysis as there was no available atrioventricular optimisation data. For the remaining eight patients, the basic demographics are shown in Table 5.1.

		Range
Age (mean ± SD)	65 ± 13 years	45-78 years
Aetiology	Ischaemic 7 Non-ischaemic 1	
Hypertension	3	
Diabetes Mellitus	3	
NYHA class at time of study (mean ± SD)	1.8 ± 0.7	1-3
LVEF (mean ± SD)	35 ± 11%	11-46%
CRT duration (mean ± SD)	25 ± 19 months	13-68 months
Baseline LAP (mean ± SD)	12.9 ± 6.8mmHg	4.8-27.3mmHg
Daily frusemide (mean ± SD)	159 ± 162mg	20-500mg
On ACEI/ARB	7	
On Beta-blockers	7	
On MRA	2	

Table 5.1. Patient characteristics. NYHA, New York Heart Association; LVEF, left ventricular ejection fraction; ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; MRA, mineralocorticoid receptor antagonist.

5.4.1 Effect of Atrioventricular Delay and Interventricular Delay on Left Atrial Pressure and Waveform Morphology

LAP waveform changes during atrioventricular optimisation and the pattern of changes were consistent among patients during atrioventricular optimisation (Figures 5.3 and 5.4). Mean changes in LAP waveform variables are shown in Table 5.2 and magnitude of change in LAP and LAP and echocardiography-derived optimal AVD were shown in Table 5.3.
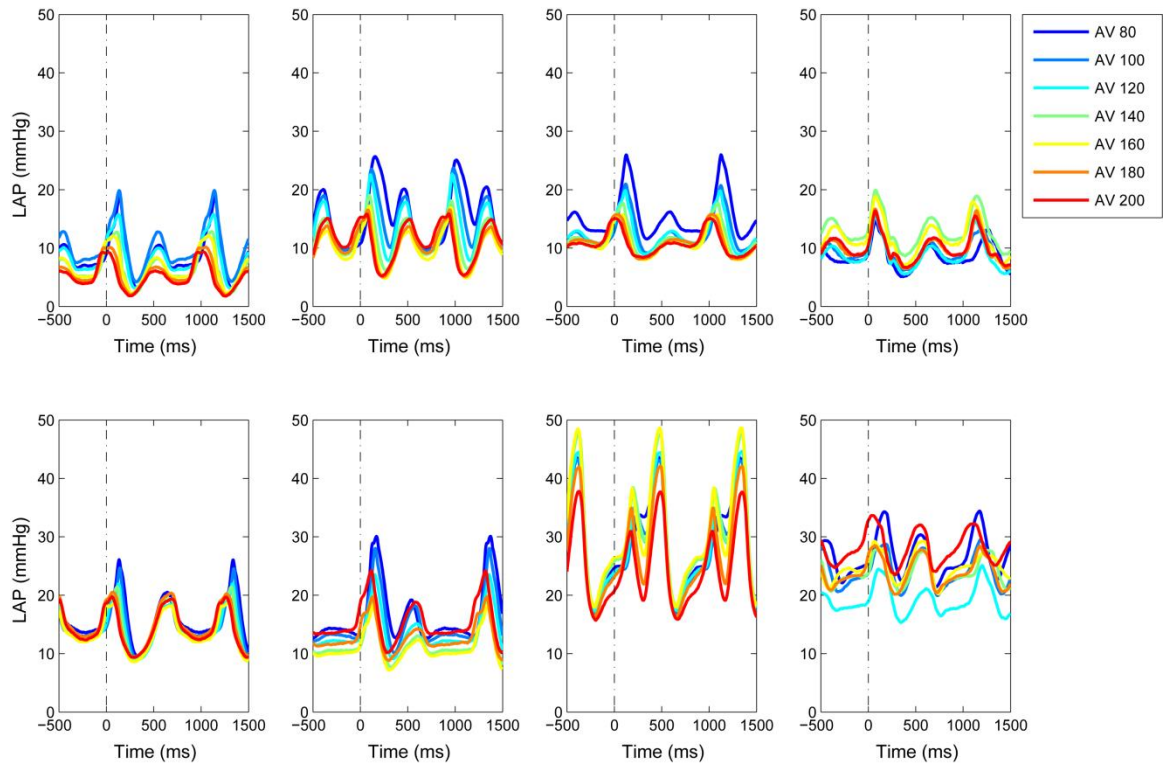
Figure 5.3. Left atrial pressure and waveform at varying atrioventricular (AV) intervals. Each panel represents an individual patient. LAP, left atrial pressure.

Figure 5.4. A selection of waveform parameters at varying atrioventricular (AV) intervals for individual patient. A slope+, positive a-slope; A peak, peak a velocity; LAP, left atrial pressure.

AVD (ms)	80	100	120	140	160	180	200	P-value
LAP (mmHg)	17 ± 3	16 ± 2	15 ± 2	16 ± 3	15 ± 3	14 ± 3	14 ± 3	0.2
peak a (mmHg)	25 ± 2	25 ± 2	22 ± 2	22 ± 3	22 ± 3	20 ± 3	20 ± 3	0.1
peak v (mmHg)	21 ± 4	20 ± 4	19 ± 4	20 ± 4	20 ± 5	19 ± 4	18 ± 4	0.3
a+ slope (mmHg/ms)	145 ± 23	134 ± 17	118 ± 14	110 ± 16	102 ± 14	93 ± 9	90 ± 8	0.2
a- slope (mmHg/ms)	-131 ± 20	-134 ± 17	-121 ± 12	-130 ± 14	-127 ± 8	-118 ± 11	-107 ± 10	0.5
v+ slope (mmHg/ms)	71 ± 14	78 ± 19	74 ± 22	76 ± 22	82 ± 27	67 ± 19	61 ± 15	0.09
v- slope (mmHg/ms)	-107 ± 38	-109 ± 36	-97 ± 39	-102 ± 44	-109 ± 45	-98 ± 41	-88 ± 32	0.4

Table 5.2. Mean ± SEM left atrial pressure waveform morphology during atrioventricular optimisation. AVD, atrioventricular delay; LAP, left atrial pressure; peak a, peak a velocity; peak v, peak v velocity; a+ slope, positive a-slope; a- slope, negative a-slope; v+, positive v-slope; v-, negative v-slope.

	Lowest LAP (mmHg)	Magnitude LAP Change (mmHg)	LAP=derived Optimal AVD (ms)	Echo-guided Optimal AVD (ms)
Patient 1	5	4.9	200	180
Patient 2	12.8	2.1	180	180
Patient 3	10.7	4.5	200	200
Patient 4	8.5	4.5	80	140
Patient 5	13.4	1.8	160	160
Patient 6	9.9	5.4	160	180
Patient 7*	23.4	6.8	225	225
Patient 8	18.1	8.7	120	150

Table 5.3. Parameters for individual patients on lowest left atrial pressure and magnitude of left atrial pressure change during atrioventricular optimisation, left atrial pressure and echocardiography-derived optimal atrioventricular delay. AVD, atrioventricular delay; Echo, echocardiography; LAP, left atrial pressure. *Due to favourable trend, atrioventricular optimization extended to AVD 275ms.

In general, at shorter AVDs where the left atrium contracted against a closed mitral valve, a large "cannon" a wave was observed. At longer AVD, the a and c waves were well separated. The amplitude of the a and v waves also trended to be smaller at

longer AVDs (Figure 5.5). At longer AVDs, there was also a trend toward smaller positive and negative slopes of the a and v waves. These changes in the a and v waves with longer AVDs were concurrent with lower mean LAP throughout the cardiac cycle; this was observed across all patients, but was especially prominent in patients with clinically evident volume overload. Over the whole group mean LAP tended to be lower with AVD prolongation, 17 (9–28) mmHg at 80 ms vs 14 (5–27) mmHg at 200 ms (P=0.16). The optimal AVD based on LAP varied between patients (range 80-225ms). Of note, the LAP with the longest AVD was neither the lowest mean LAP nor the most favourable LAP waveform.
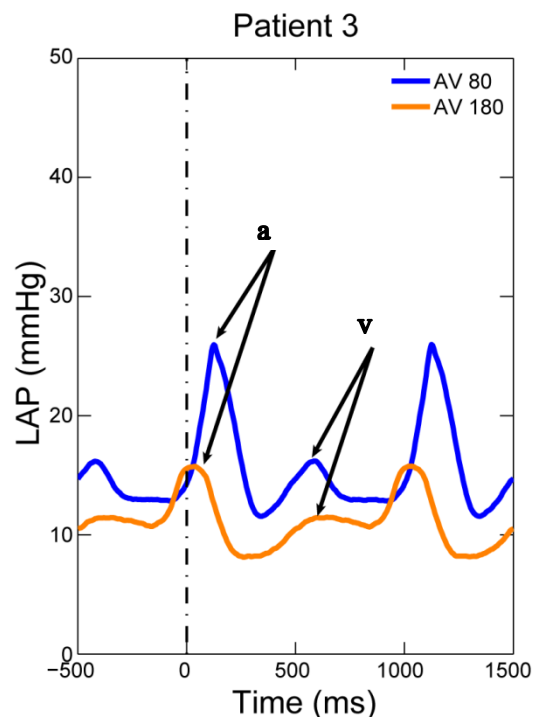
Figure 5.5. Example from Patient 3 demonstrating the effect of atrioventricular delay (AVD) on left atrial pressure (LAP) waveform. AVD 80ms and AVD 180ms are used here to illustrate changes in short and long AVD. At short AVD, 80ms, the a and c waves merged to form a cannon a wave. The longer AVD, 180ms was more optimal for this patient with smaller a and v waves and LAP was lower throughout the cardiac cycle.

With changes in VVDs, much smaller effects were seen on LAP and no discernible effect was seen on LAP waveform. (Figures 5.6 and 5.7). Whilst the lowest LAP was seen with varying degrees of left ventricular pre-excitation, in no case did right

ventricular pre-excitation give the lowest LAP. (Table 5.4).
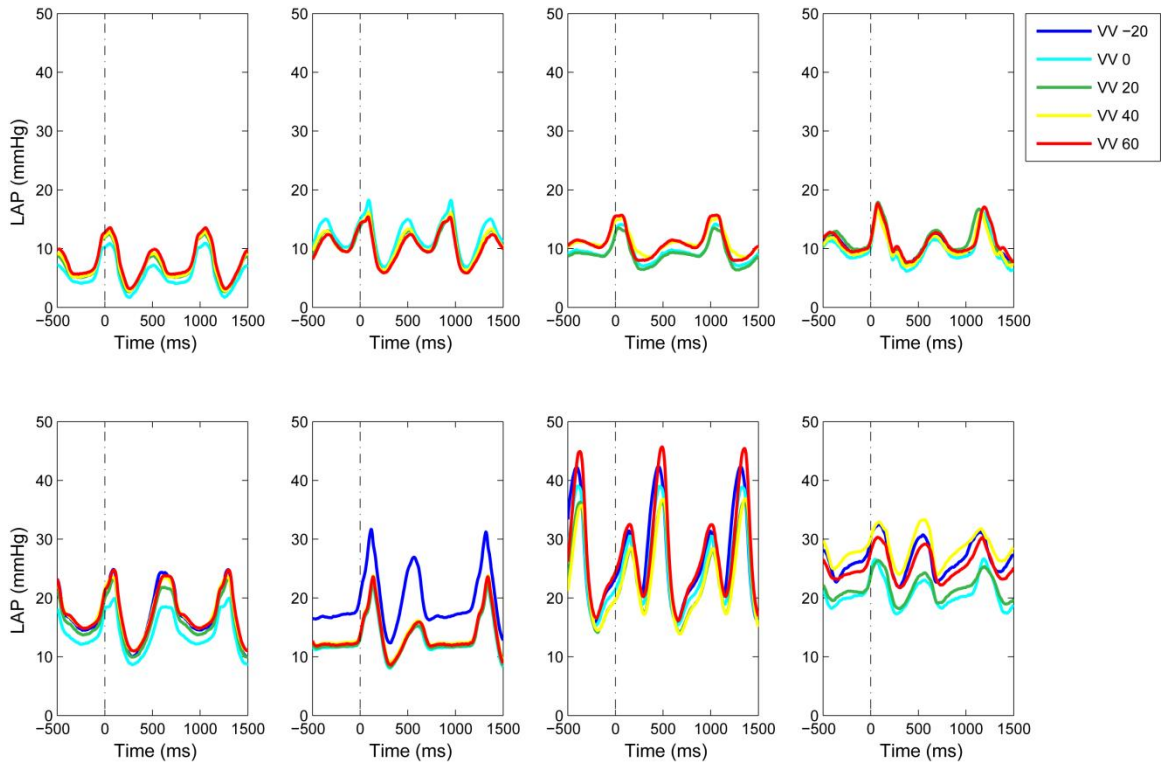
Figure 5.6. Left atrial pressure and waveform at varying interventricular (VV) intervals for individual patient. Each panel represents an individual patient. LAP, left atrial pressure.
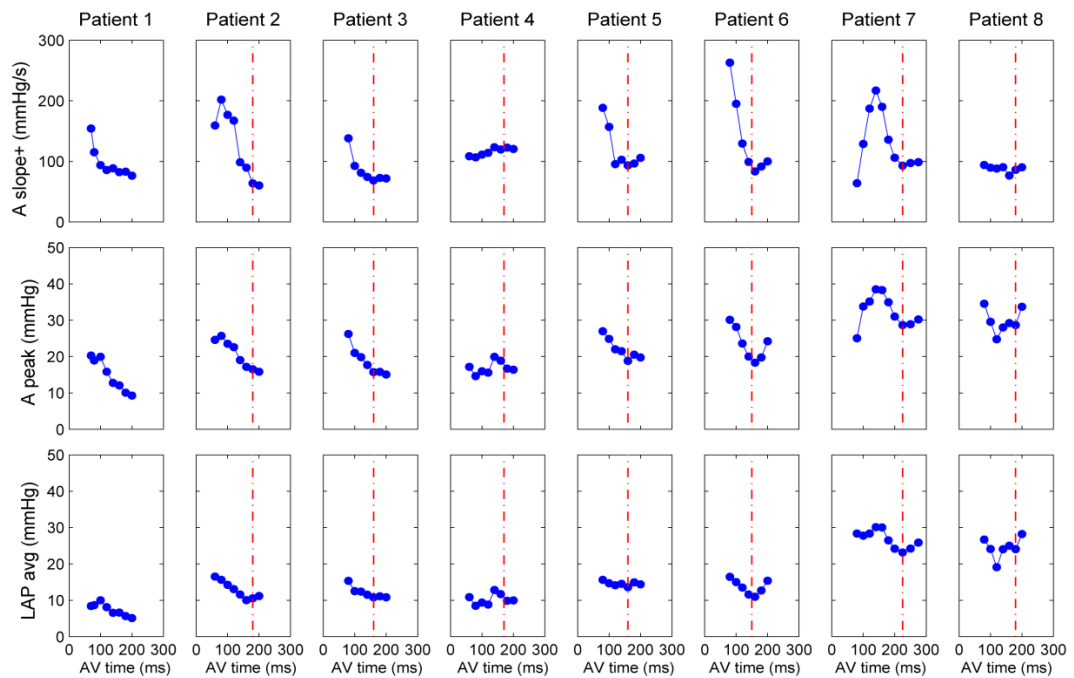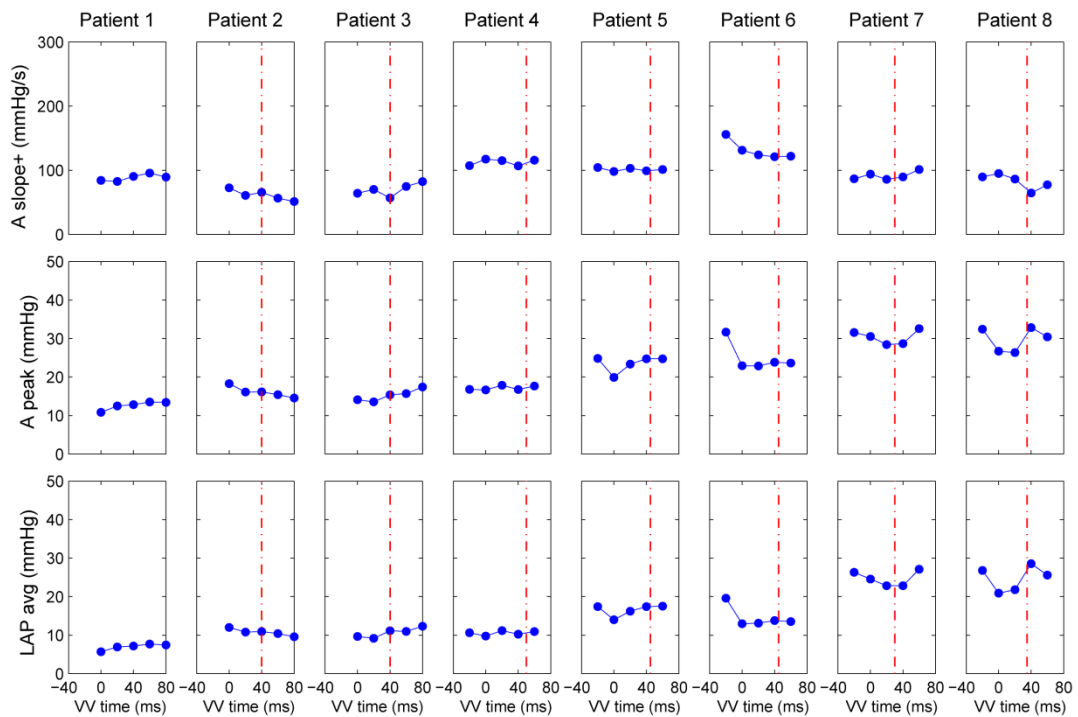

Figure 5.7. A selection of waveform parameters at varying interventricular (VV) intervals for individual patient. A slope+, positive a-slope; A peak, peak a velocity; LAP, left atrial pressure.

VVD (ms)	-20	0	20	40	60	P-value
LAP (mmHg)	19 ± 3	14 ± 2	14 ± 2	15 ± 3	16 ± 3	0.2
peak a (mmHg)	26 ± 3	20 ± 2	20 ± 2	21 ± 3	22 ± 3	0.3
peak v (mmHg)	26 ± 5	18 ± 4	18 ± 3	20 ± 4	21 ± 4	0.4
a+ slope (mmHg/ms)	93 ± 5	89 ± 6	87 ± 6	83 ± 6	89 ± 7	0.2
a- slope (mmHg/ms)	-140 ± 15	-127 ± 12	-133 ± 11	-128 ± 12	-119 ± 11	0.3
v+ slope (mmHg/ms)	94 ± 29	68 ± 15	63 ± 14	69 ± 15	70 ± 20	0.3
v- slope (mmHg/ms)	-135 ± 48	-85 ± 33	-88 ± 30	-99 ± 33	-108 ± 43	0.3

Table 5.4.Mean ± SEM left atrial pressure waveform morphology during interventricular optimisation.VVD, interventricular delay; LAP, left atrial pressure; peak a, peak a velocity; peak v, peak v velocity; a+ slope, positive a-slope; a- slope, negative a-slope; v+, positive v-slope; v-, negative v-slope.

5.4.2 Effect of Changing Cardiac Resynchronization Therapy Setting on Echocardiographic Parameters

Greater transmitral Doppler filling tended to be seen with longer AVD. (Table 5.5) There was a significant increase in atrial contribution to left ventricular diastolic filling with longer, more optimal AVD as evident by a smaller E/A ratio (P=0.04), higher peak A velocity (P=0.001), a larger VTI A/transmitral ratio (P=0.01) and a trend towards a longer A duration (P=0.06). Stroke volume, estimated from LVOT VTI, tended to increase with longer more optimal AVD (P=0.2). There were no significant changes with CRT optimization for stroke volume equivalent echocardiographic parameters or reduction in pre-ejection time with altering VVD (Table 5.6).

AVD (ms)	80	100	120	140	160	180	200	P-value
E/A	1.2 ± 0.3	1.2 ± 0.2	0.9 ± 0.2	0.8 ± 0.1	0.7 ± 0.1	1.1 ± 0.4	1.1 ± 0.4	0.04
A/ MV VTI (cm)	0.34 ± 0.08	0.36 ± 0.08	0.36 ± 0.06	0.41 ± 0.05	0.4 ± 0.04	0.52 ± 0.07	0.52 ± 0.07	0.01
LVOT VTI (cm)	21.1 ± 1.9	20.8 ± 2.1	20.5 ± 2.3	21.5 ± 1.7	22.2 ± 1.8	21.9 ± 2.1	22.3 ± 2	0.2
Peak A velocity (cm/s)	58 ± 10	54 ± 7	64 ± 9	71 ± 9	76 ± 9	66 ± 9	69 ± 10	0.001
A duration (ms)	152 ± 13	147 ± 11	167 ± 18	174 ± 15	158 ± 10	178 ± 10	166 ± 10	0.06

Table 5.5. Changes of echocardiographic parameters during atrioventricular optimisation. AVD, atrioventricular delay; MV, mitral valve; VTI, velocity time integral; LVOT, left ventricular outflow tract.

VVD (ms)	-20	0	20	40	60	P-value
MV VTI (cm)	17.1 ± 0.9	15.7 ± 1.4	16.1 ± 1.1	15.6 ± 1	15.4 ± 1	0.3
LVOT VTI (cm)	22.5 ± 3.4	21.2 ± 1.8	22.9 ± 2.3	22.6 ± 2.5	22.8 ± 2.1	0.2
LVOT PET (ms)	153 ± 10	141 ± 7	143 ± 7	137 ± 11	136 ± 12	0.3

Table 5.6. Changes of echocardiographic parameters during interventricular optimisation. VVD, interventricular delay; MV, mitral valve; VTI, velocity time integral; LVOT, left ventricular outflow tract; PET, pre-ejection time.

5.4.3 Correlation of Echocardiography and Left Atrial Pressure Derived Optimal Cardiac Resynchronization Therapy Setting

The mean optimal AVD derived by echocardiography was 177 (140–225) ms. One patient's A wave was absent until AVD >180ms leading to AVD testing beyond the 200ms. The mean optimal AVD derived by the lowest LAP was 166 (80-225) ms. Comparing the optimal AVD defined by the lowest mean LAP to the optimal echocardiography-guided AVD, there was a very strong correlation between the two modalities (r=0.91, P=0.001). The mean difference in optimal AVD determined by the two modalities was small, 16±21ms, and within one device setting for 6 out of 8 subjects. For the remaining LAP waveform parameters, optimal AVD identified by the peak of the a wave (r=0.92, P=0.003) and negative v-slope (r=0.79, P=0.03)

correlated well with echocardiography-guided optimal AVD (Table 5.7 and Figure 5.8). Despite the strong correlation demonstrated in this study, result should be interpreted in caution due to the small sample size.

	Pearson's coefficient	P-value
Lowest mean LAP	r = 0.91	P = 0.001
Lowest peak a	r = 0.92	P = 0.003
Lowest peak v	r = 0.5	P= 0.25
Lowest positive a slope	r = 0.67	P = 0.1
Highest negative a slope	r = -0.13	P = 0.78
Lowest positive v slope	r = 0.4	P = 0.38
Highest negative v slope	r = 0.79	P = 0.03

Table 5.7. Correlation of echocardiography-derived vs left atrial pressure waveform parameter-derived optimal atrioventricular delay. LAP, left atrial pressure.
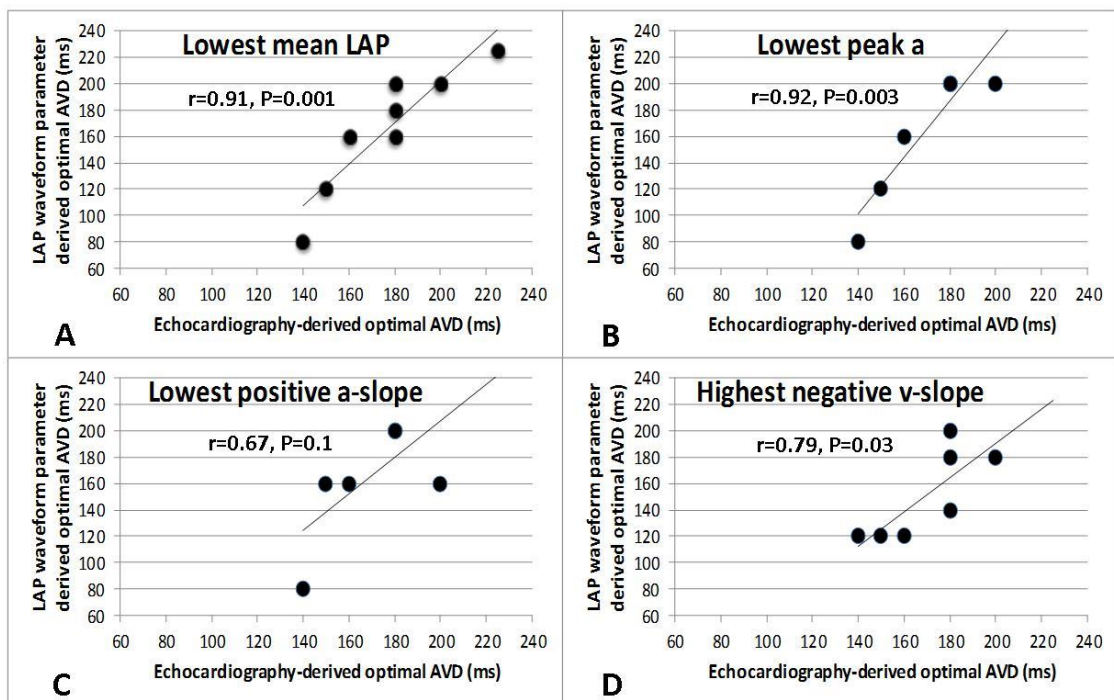
Figure 5.8. Left atrial pressure waveform parameters that had the best correlation with echocardiography-derived optimal atrioventricular delay. A – mean left atrial pressure, B – peak a, C – positive a-slope, D – negative v-slope. AVD, atrioventricular delay; LAP, left atrial pressure.

5.5 Discussion

This study provides the first description of shifts in directly recorded LAP and LAP waveform with changes in CRT settings. Changes in CRT settings consistently induced changes in LAP and LAP waveform which were readily detected by the implantable "HeartPOD™" LAP sensor in ambulant patients. Changes in AVD had more marked effects on LAP and waveform than VVD. As altering VVD mainly reduced dyssynchrony thus improving stroke volume, not diastolic filling, therefore it is not surprising that interventricular optimisation had little impact on LAP.

LAP waveform parameters provide valuable information on atrial function and haemodynamic status throughout the cardiac cycle (Figure 5.2). During atrial diastole, the mitral valve closes and the left atrium relaxes and fills from the pulmonary venous flow which leads to a gradual build-up of pressure and volume. The rate of pressure change is represented by the negative a-slope and positive v-slope and reflects atrial operating compliance. The v-wave is at its peak just before the mitral valve opens in diastole during passive left atrial emptying which is represented by the negative v-slope. The atrium contracts to complete the process of atrial emptying as represented by the positive a-slope and peak a wave before the mitral valve closes then the atrium relaxes (the negative a-slope) and the atrium gradually fills up again. This study demonstrates that optimal AVDs are associated with lower peak a and v waves and gentler a and v slopes consistent with reduced atrial work during atrial systole, more optimal active and passive left atrial emptying; and lower operating compliance of the left atrium. These changes are highly reproducible across patients. The optimal AVD determined by the LAP is also well correlated with echocardiographically-determined AVD, the most widely acceptable method for CRT optimisation.

Whilst the change in mean LAP across the group with varying AVD did not reach

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statistical significance, this is likely to be due to the small numbers involved in this pilot trial as the magnitude of the changes in LAP with varying AVD was considerable and likely to be clinically significant. Also this trial included both responders and non-responders, and our patients were by and large well treated, with a baseline mean LAP of 13mmHg.

In this study, the optimal AVD was considerably longer than the device nominal setting. The optimal AVDs by both methods also varied considerably between patients, the variation in optimal AVD and the marked effect on both LAP and waveform strongly suggest value in individual optimisation of AVD. However, the benefit of CRT optimisation has recently been challenged. No study has shown CRT optimisation alters mortality or HF hospitalisation, and routine use is not supported by recent large-scale studies.[414, 415] In the SmartDelay determined AV Optimisation (SMART-AV) trial, no difference was shown in functional status, LVEF or dimensions between a fixed AVD, one determined by echocardiography or an electrogram-based algorithm, despite a greater than 30ms difference for optimal AVD determined by these settings in more than half of the group.[415] Another study using an electrogram-based algorithm also showed no clinical benefit from frequent routine CRT optimisations.[414] On the other hand, a small pilot study, using a haemodynamic automated algorithm based on peak endocardial acceleration rather than electrical conduction showed a bigger proportion of patients improved NYHA class from optimisation although no significant changes in death or HF hospitalisation were noted.[416] The reasons for these varied results from a range of optimisation strategies have not been determined with certainty. However as the two large-scale negative trials both used electrogram-based algorithm to determine optimal AVD,[414, 415] it is possible that mechanical dyssynchrony may persist despite amelioration of electrical

dyssynchrony. Therefore it is possible that haemodynamically based CRT optimisation may be more clinically relevant and this is supported by the marked effects on LAP and waveform with CRT in the current study. In contrast to the innate limitations of echocardiography, an implantable sensor such as the one used in the current study is attractive for its ease of use, instantaneous feedback and most importantly provides a high fidelity direct haemodynamic measure. Furthermore, implantable sensors such as this LAP sensor have the potential to guide hemodynamic optimisation not only at rest but also under varying physiological conditions such as exercise. It is possible that a combination device containing both a CRT pacemaker and an implantable LAP sensor could allow for both automatic and dynamic CRT optimisation.

5.6 Study Limitations

This was a pilot trial with eight participants to explore the possible utility of LAP monitoring during CRT optimisation. In this study, only the acute effects of CRT optimisation under resting conditions were studied. The impact of CRT optimisation on LAP in other physiological settings such as exercise is uncertain.[506, 507] This study only assessed the immediate changes in LAP but the long-term outcome has not been addressed.

5.7 Conclusion

Optimisation of AVD settings in subjects with advanced HF and an implanted CRT device was associated with characteristic changes in mean LAP and LAP waveform.

The waveform changes were consistent across patients. Optimal AVD determined by left atrial indices (particularly mean LAP) varied considerably between patients and was longer than from electrogram-based analysis or optimisation. Changes in VVD produced only minor changes in LAP and no discernible effect on LAP waveform. The optimal setting derived from mean LAP correlated strongly with echocardiographically determined optimal settings. The role of LAP guided CRT optimisation warrants further investigation.

6 Summary and Future directions

Heart failure is a syndrome of broad spectrum and heterogeneous presentations. This thesis explores new therapies in HF and the three studies discussed in the thesis all target different stages of the syndrome. The UNICORN study investigated the effect of urocortin-2 therapy in ADHF. TOUCHÉ investigated the effect of urocortin-2 on muscle sympathetic nerve activity. zLAP-CRT explored the effects of changes in CRT settings on LAP and its waveform and assessed the feasibility for LAP to guide CRT optimisation.

6.1 UNICORN

The UNICORN study assessed the safety and feasibility of administering urocortin-2 infusion at 5ng/kg/min over four hours as an adjunct to conventional HF therapy in patients hospitalised with ADHF. At the same time, it also evaluated the effects of urocortin-2 administration on haemodynamic, renal, hormonal and symptomatic status.

This study proved administration of urocortin-2 in ADHF was feasible and, in general, symptomatically well tolerated. Administration of urocortin-2 in ADHF was associated with favourable haemodynamic effects, predominantly potent vasodilatation and a resultant augmentation in cardiac output. Despite marked reduction in blood pressure, the rise in heart rate was comparatively minor. There was a suggestion of increase in cardiac contractility by urocortin-2, although this index is difficult to measure in vivo and our study was not designed to assess this. Urocortin-2 produced a delayed and persistent reduction in B-type natriuretic peptides levels

consistent with reduced cardiac congestion.

There were a few unexpected results which deviated from the original hypotheses, with transient suppression of natriuresis and diuresis during urocortin-2 infusion and the small but significant rise in PRA, in contrast to significantly enhanced diuresis and natriuresis and suppression of the RAAS observed in experimental HF[247]. However, both of these changes were transient and may have been secondary to the marked blood pressure reduction caused by the selected dose regime employed in the current study which achieved plasma concentrations approximately double those observed with a similar hourly dose in stable HF patients[224]. The fall in plasma cortisol level was attenuated by urocortin-2 compared to placebo in the current study for which the mechanism was not clear. There was no significant improvement in symptoms as compared to placebo, possibly due to the relative delay in introduction of trial drug infusion over a background of standard HF therapy, the small sample size and that the study was a feasibility trial and therefore not adequately powered to detect improvement in symptoms and outcomes.

In summary, the current study is the first demonstration that urocortin-2 administration was feasible and safe, and produced a favourable haemodynamic effect, a transient adverse renal effect and a mixed hormonal effect in ADHF, the most sustained of which was reduction in plasma B-type natriuretic peptides over 24 hours consistent with a sustained decongesting effect from urocortin-2. These results are suggestive that urocortin-2 has a potential therapeutic role in ADHF. The following questions and issues arise from this study and provide directions for future investigation:

1. The unexpected renal suppression and the rise in PRA are likely to be secondary to the dose-dependent marked reduction in systemic blood pressure.

A trial of a lower dose may result in a lesser fall in blood pressure and may unmask the beneficial effects on renal function and renin suppression previously seen in experimental models of HF.

2. Inclusion of a third comparative arm in the study with a vasodilator such as Glycerine Trinitrate infusion to determine whether urocortin-2 is renal and hormonal protective when under similar condition of vasodilatation and hypotension.

3. Measurement of ACTH during urocortin-2 infusion including a comparison of background therapy that produces a similar fall in blood pressure. This could clarify if the proposed therapeutic dose activates the HPA independent of the effect of hypotension on this axis.

4. Commencing urocortin-2 infusion as close to the time of presentation as possible when patients are still significantly symptomatic from HF symptoms would enable better assessment of symptomatic effects.

5. A much larger sample size and a longer follow-up time are required to assess the long-term outcome or mortality benefits.

6. To assess the effect of urocortin-2 on cardiac contractility in HF: this could be assessed using invasive haemodynamic studies with generation of pressure-volume loops or non-invasively with echocardiography under conditions of controlled heart rate and blood pressure.

6.2 TOUCHÉ

The TOUCHÉ study evaluated the effect of urocortin-2 on muscle SNA in healthy

man and patients in stable HF. Both the stated hypotheses were rejected in this study.

The first hypothesis, that urocortin-2 would produce a similar inhibition of SNA in healthy man as that seen in sheep, was rejected as urocortin-2 increased muscle SNA in healthy man as opposed to the overall suppression in cardiac SNA observed previously in sheep.[281] This study confirmed urocortin-2 as a potent vasodilator. The observed increased in heart rate-dependent SNA was presumably predominantly a normal baroreflex-mediated response to systemic hypotension however in the absence of comparisons with other vasodilator challenges this study did not determine if there was any modulation of baroreflex sensitivity by urocortin-2. The difference in response compared with previous ovine studies may reflect organ specific differences in urocortin-2 actions. SNA may be regionally differentiated. In this study, muscle SNA was obtained from the peroneal nerve whilst in the sheep study direct cardiac sympathetic activity was measured. Finally, the difference could simply reflect interspecies differences. With what we have learnt from the current study, future directions for investigation include:

1. The possible SNA modulation by urocortin-2 could be evaluated by a comparative study that achieves similar magnitude of hypotension as urocortin-2 using a vasodilator such as nitroprusside.

2. To determine the effect of urocortin-2 on human cardiac SNA, an alternative technique would be required as direct cardiac microneurography is not feasible in humans. Other alternative methodologies include the invasive norepinephrine spillover technique, which requires coronary sinus cannulation, or non-invasive single-photon emission computed tomography using MIBG tracer imaging. However the use of MIBG tracer imaging in HF application is limited due to the reduced MIBG uptake and increased

likelihood of ventricular arrhythmia in HF.[191]

The second hypothesis, that urocortin-2 would produce a more pronounced inhibition of SNA in HF patients, was also rejected. However, only four heart failure patients had completed the study and all were minimally symptomatic at the time of study (NYHA functional class I). It is possible that a larger study group with more symptomatic HF patients may be required to show a different SNA response.

6.3 zLAP-CRT

This study aimed to characterise the effect of CRT optimisation on LAP and waveform. The feasibility of using LAP and waveform parameters to guide CRT optimisation was then assessed. This small cohort confirmed LAP and waveform morphology tracked changes in CRT setting. The changes were more pronounced with changes in AVD than with alterations in VVD. The study showed good correlation of LAP-derived optimal AVD with echocardiographically-derived optimal AVD, the current gold standard. Thus it is feasible to utilise LAP measurements in the ambulatory setting to guide CRT optimisation.

The potential application of the findings in this study depends on the ongoing debate on the necessity of CRT optimisation. In this study, the optimal CRT setting varied greatly between individuals and supports a role of CRT optimisation in particular the non-responders. For future possible studies:

1. To investigate the long-term effect of LAP-guided CRT optimisation, a large-scale three-arm randomisation comparing LAP-guided CRT optimisation to echocardiography-guided CRT optimisation and no optimisation is required to address this question.

2. The LAP sensor would also allow for studies on CRT optimisation in other physiological conditions such as exercise.

6.4 Final Summary

This thesis examined novel therapies for HF in three different target populations. In the UNICORN study, urocortin-2 administered in ADHF resulted in beneficial haemodynamic effects, and suggested a potential therapeutic role. In the TOUCHÉ study, urocortin-2 resulted in an increase in muscle SNA in healthy volunteers and patients with stable HF. In the zLAP-CRT, directly measured LAP provided valuable insights in the haemodynamic effects of CRT optimisation. The findings from these studies provide the foundations for future investigations for clinically meaningful applications.

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